

Akhlaq A. Farooqui

Phytochemicals, Signal Transduction, and Neurological Disorders

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Akhlaq A. Farooqui
Department of Molecular and Cellular Biochemistry
Ohio State University
Columbus, OH, USA

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*This monograph is dedicated to the memory
of my late parents as a token of affection
and respect! I am still walking through
the doors they opened for me.*

Akhlaq A. Farooqui

Preface

Phytochemicals are a heterogeneous non-nutritive group of chemical compounds with numerous biological effects in animals and men. They are derived from plants and form the backbone of traditional medicine, which uses plant preparations (seeds, fruits, leaves, stems and roots) as a source of drugs. Phytochemicals have been classified into five major families, carotenoids, alkaloids, nitrogen-containing phytochemicals, sulfur-containing phytochemicals and phenolics.

Phytochemicals have been used in various ancient medicinal systems (Indian, Chinese, Egyptian, Babylonian, and Greek), as potential drugs against numerous diseases. They exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals. In recent years, research on phytochemicals has increased all over the world and new terms such as “functional food” and “nutraceutical” have been introduced. These terms illustrate the high expectations associated with current phytochemical research. However, the precise molecular mechanisms through which specific phytochemicals exert their beneficial biological effects still remain the subject of intense investigations. Health benefits of phytochemicals on visceral tissue and brain are due to their anti-inflammatory, antioxidant, anticarcinogenic, antiproliferative, hypocholesterolemic, and cellular repair properties. In addition, effects of phytochemicals are mediated through signal transduction processes, which not only involve various transcription factors, growth factors and inhibition of inflammatory cytokines expression, but also regulation of enzymes, such phospholipases, cyclooxygenases, protein kinases, and protein phosphatases. Phytochemicals also mediate their effects through the modulation of immune function. Regular consumption of phytochemicals from childhood to adulthood may be associated with reduced risks of neurotraumatic (stroke, traumatic brain injury, and spinal cord injury), neurodegenerative (Alzheimer disease, Parkinson disease, and cataracts), neuropsychiatric (depression, Schizophrenia, and bipolar disorders) diseases, osteoporosis, diabetes, and some of the functional decline associated with normal aging. Antioxidant and anti-inflammatory properties of phytochemicals mitigate the damaging effect of oxidative stress, neuroinflammation, and apoptosis. The chemical structures of phytochemicals are often used as “privileged structures”

for creating their synthetic analogs, which have improved pharmacological activities through optimized bioavailability and pharmacokinetic profiles. Recently, there have been considerable developments in defining the molecular mechanisms associated with beneficial effects of phytochemicals on neurological disorders. The effects of phytochemicals on visceral and brain tissues can be conductive, additive, synergistic, and antagonistic. Through these properties, phytochemicals regulate neuronal and glial cell differentiation, proliferation, and apoptosis. Among phytochemicals, polyphenols, phenolic acids, and flavonoids scavenge reactive oxygen species (ROS), singlet molecular oxygen, and peroxy radicals generated during lipid peroxidation. In addition, the use of polyphenols and flavonoids may not only result in improvements of memory acquisition and consolidation, but also in storage and retrieval of memory. These phytochemicals are highly effective in reversing age-related declines in memory via their ability to interact with the cellular and molecular architecture of the brain responsible for memory related processes. Phytochemicals produce their effects through their ability to modulate signal transduction pathways critical in controlling synaptic plasticity, and inducing neurogenesis in the hippocampus. The ability of many phytochemicals to activate the extracellular signal-regulated kinase (ERK1/2) and the protein kinase B (PKB/Akt) signaling pathways, leading to the activation of the cAMP response element binding protein (CREB), a transcription factor responsible for increasing the expression of a number of growth factors (neurotrophins) important in defining memory, a process by which knowledge is encoded, stored, and later retrieved.

Although, many original papers, reviews, and edited books have been published on the effects of phytochemicals on visceral organs, but information on the effect of phytochemicals on brain is scattered throughout the literature in the form of original papers, and reviews. I have decided to provide readers with a comprehensive and cutting-edge description on metabolism and molecular mechanism associated with the beneficial effects of phytochemicals in neurological disorders in a manner that is useful not only to students and teachers but also to researchers and physicians. This monograph has 11 chapters. The first chapter describes the effect of lifestyle, aging, and phytochemicals on the onset of neurological disorders. Chapters 2 and 3 cover beneficial effects of extra virgin olive oil and flaxseed oil on signal transduction processes in neurological disorders. Chapter 4 provides information on the beneficial effects of flavonoids in neurological disorders. Chapter 5 describes beneficial effects of green tea catechins on neurological disorders. Chapters 6 and 7 present beneficial effects of curcumin and resveratrol in neurological disorders, respectively. Chapters 8 and 9 discuss the beneficial effects of Ginkgo biloba and garlic in neurological disorders. Chapter 10 describes beneficial effects of propolis in neurological disorders. Finally, Chapters 11 focuses on my view on the importance of phytochemicals in diet and direction for future research on neurological disorders. Studies on the effect of phytochemicals on brain fall in a fast-paced research area of neurological disorders. This monograph presents information on the metabolism, bioavailability, and proposed molecular mechanism of action in the brain, along with some pharmacokinetics. This monograph also provides information on delaying the onset and target-based treatment of neurological disorders by

using phytochemicals. This monograph can be used as supplemental text for a range of phytotherapeutics courses. Clinicians and pharmacologists will find this book useful for understanding molecular aspects of phytochemicals in neurological disorders.

I have tried to ensure uniformity in mode of presentation along with extensive bibliography. For the sake of simplicity and uniformity a large number of figures with chemical structures of phytochemicals that produce beneficial effects in neurological disorders and signal transduction diagrams showing the site of action of phytochemicals have also been included. I hope that my attempts to integrate and consolidate the knowledge on beneficial effects of phytochemicals on signal transduction processes associated with pathogenesis of neurological disorders will initiate more studies on molecular mechanisms associated with beneficial effects of phytochemicals in neurotraumatic, neurodegenerative, and neuropsychiatric diseases.

Columbus, OH, USA

Akhlaq A. Farooqui

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Akhlaq A. Farooqui

About the Author

Akhlaq A. Farooqui is a leader in the field of signal transduction, brain phospholipases A₂, bioactive ether lipid metabolism, polyunsaturated fatty acid metabolism, glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, glutamate-induced neurotoxicity, and modulation of signal transduction by phytochemicals. Akhlaq A. Farooqui has discovered the stimulation of plasmalogen-selective phospholipase A₂ (PlsEtn-PLA₂) and diacyl- and monoacylglycerol lipases in brains from patients with Alzheimer disease. Stimulation of PlsEtn-PLA₂ produces plasmalogen deficiency and increases levels of eicosanoids that may be related to the loss of synapses in brains of patients with Alzheimer disease. Akhlaq A. Farooqui has published cutting-edge research on the generation and identification of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators in kainic acid-mediated neurotoxicity by lipidomics. Akhlaq A. Farooqui has authored seven monographs:

Glycerophospholipids in Brain: Phospholipase A₂ in Neurological Disorders (2007); *Neurochemical Aspects of Excitotoxicity* (2008); *Metabolism and Functions of Bioactive Ether Lipids in Brain* (2008); and *Hot Topics in Neural Membrane Lipidology* (2009); *Beneficial Effects of Fish Oil in Human Brain* (2009); *Neurochemical Aspects of Neurotraumatic and Neurodegenerative Diseases* (2010); and *Lipid Mediators and their Metabolism in the Brain* (2011). All monographs are published by Springer, New York. In addition, Akhlaq A. Farooqui has edited six books (*Biogenic Amines: Pharmacological, Neurochemical and Molecular Aspects in the CNS* Nova Science Publisher, Hauppauge, N.Y (2010), *Molecular Aspects of Neurodegeneration and Neuroprotection*, Bentham Science Publishers Ltd. (2011); *Phytochemicals and Human Health: Molecular and pharmacological Aspects* (2011), Nova Science Publisher, Hauppauge, N.Y.; *Molecular Aspects of Oxidative Stress on Cell Signaling in Vertebrates Invertebrates* (2012), Wiley Blackwell Publishing Company, New York, 2012); *Beneficial effects of propolis on Human Health in Chronic Diseases* (2012) Vol 1, Nova Science Publishers, Hauppauge, New York (in press); and *Beneficial effects of propolis on Human Health in Chronic Diseases* (2012) Vol 2, Nova Science Publishers, Hauppauge, New York (in press).

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List of Abbreviations

AD	Alzheimer disease
AGE	Aged garlic extract
ALA	α -Linolenic acid
ALS	Amyotrophic lateral sclerosis
ARA	Arachidonic acid
BBB	Blood–brain barrier
CAPE	Caffeic acid phenethyl ester
COX	Cyclooxygenase
DADS	Diallyl disulfide
DAS	Diallyl sulfide
DHA	Docosahexaenoic acid
EGb761	Ginkgo. biloba leaves extract
EGCG	(-)-Epigallocatechin-3-gallate
EPA	Eicosapentaenoic acid
HD	Huntington disease
Ins-1,4,5- P_3	Inositol-1,4,5-trisphosphate
LA	Linoleic acid
LOX	Lipoxygenase
MS	Multiple sclerosis
PD	Parkinson disease
PKC	Protein kinase C
PLA ₂	Phospholipase A ₂
PLC	Phospholipase C
PlsCho	Choline plasmalogen
PlsEtn	Ethanolamine plasmalogen
PtdCho	Phosphatidylcholine

PtdEtn	Phosphatidylethanolamine
PtdIns	Phosphatidylinositol
PtdIns(4,5)P ₂	Phosphatidylinositol 4,5-bisphosphate
PtdIns4P	Phosphatidylinositol 4-phosphate
SAC	<i>S</i> -Allylcysteine

Chapter 1

Effect of Lifestyle, Aging, and Phytochemicals on the Onset of Neurological Disorders

1.1 Introduction

Neurological disorders include neurotraumatic and neurodegenerative diseases. Common neurodegenerative diseases include Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and multiple sclerosis (MS), whereas common neurotraumatic diseases include strokes, traumatic brain injury (TBI), and spinal cord injury (SCI) (Farooqui 2010). Among neurotraumatic diseases, stroke is a metabolic insult induced by severe reduction or blockade in cerebral blood flow. This blockade not only causes deficiency of oxygen and reduction in glucose metabolism, but also results in ATP depletion and accumulation of toxic products. TBI and SCI due to motor cycle and car accidents are major cases of disability among young people. Neurotraumatic and neurodegenerative diseases share excitotoxicity, oxidative stress, and neuroinflammation as a common mechanism of cell death. In addition to excitotoxicity, oxidative stress, and neuroinflammation, neurodegenerative diseases are accompanied by the accumulation of misfolded proteins, mitochondrial and proteasomal dysfunction, loss of synapses, and premature and slow death of certain neuronal populations in brain tissue (Graeber and Moran 2002). For example in AD, neuronal degeneration occurs in the nucleus basalis, whereas in PD, neurons die in the substantia nigra. The most severely affected neurons in HD are striatal medium spiny neurons (Farooqui 2010).

The most important risk factors for stroke and neurodegenerative diseases are old age, race/ethnicity, a positive family history, unhealthy lifestyle, and endogenous factors (Fig. 1.1). The onset of stroke and neurodegenerative diseases is often subtle and usually occurs in mid to late life and their progression depends not only on genetic, but also on environmental factors (Graeber and Moran 2002). The onset of neurological diseases may occur when neurons fail to respond adaptively to age-related increase in oxidative and nitrosative stress and neuroinflammation. Persistence presence of oxidative and nitrosative stress and neuroinflammation induces the accumulation of damaged proteins, DNA, and membrane fragments.

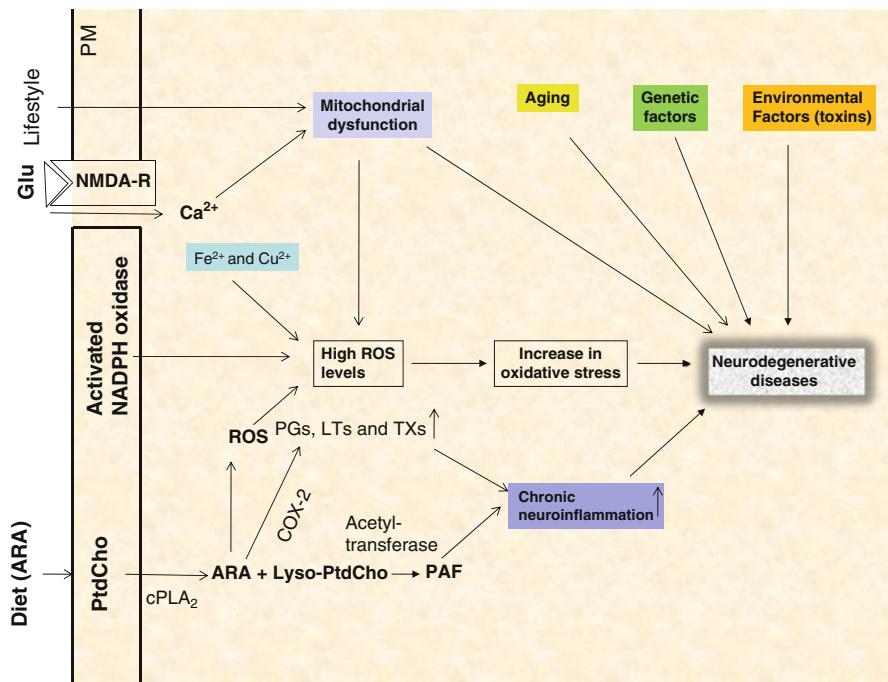


Fig. 1.1 Effect of diet, lifestyle, high oxidative stress, and environmental and genetic factors on the onset of neurodegenerative diseases. Plasma membrane (PM); glutamate (Glu); *N*-methyl D-aspartate receptor (NMDA-R); phospholipase A₂ (PLA₂); arachidonic acid (ARA); phosphatidylcholine (PtdCho); lysophosphatidylcholine (lys-PtdCho); reactive oxygen species (ROS); prostaglandins (PGs); leukotrienes (LTs); and thromboxanes (TXs)

In addition, neurodegenerative diseases are accompanied by the accumulation of disease-specific proteins, such as accumulation of A β and its aggregates in the cerebral cortex and hippocampal region in AD, α -synuclein in the brain stem in PD, and huntingtin in striatal medium spiny neurons in HD. Furthermore, abnormalities in signal transduction processes along with elevated levels of lipid mediators and disturbance in stress resistance mechanisms have also been reported in both types of neurological disorders (Farooqui and Horrocks 2007; Farooqui and Farooqui 2011, 2012). Both stroke and neurodegenerative diseases lead to progressive cognitive and motor disabilities with devastating consequences to patients. In older individuals and animals, age-related alterations in interplay (cross-talk) among excitotoxicity, oxidative stress, and neuroinflammation may cause abnormalities in motor and cognitive performance. An enhanced rate (upregulation) of interplay among excitotoxicity, oxidative stress, and neuroinflammation may be a common mechanism of brain damage in stroke and neurodegenerative diseases (Farooqui and Horrocks 2007; Farooqui et al. 2007; Farooqui 2010). In addition, diet, genetic, lifestyle, and environmental factors may also be associated with the increased vulnerability of neurons in stroke and neurodegenerative diseases (Kidd 2005; Farooqui 2010).

1.2 Factors Influencing the Onset of Stroke and Neurodegenerative Diseases

As stated earlier, the most important risk factors for stroke and neurodegenerative diseases are old age and a positive family history. The pathogenesis of age-related diseases is complex and it is often difficult to identify causal risk factors, especially if their relative effects are weak. Stroke and neurodegenerative diseases are multifactorial illnesses caused by complex interactions among genetic factors, environmental factors, aging, and lifestyle (an expression of individual choices and their interaction with the environment). Environmental and dietary risk factors, such as heavy metals, hormones, cholesterol, high-fat diet, high alcohol intake, diet deficient in n-3 fatty acids, antioxidants and vitamins, and reduced levels of physical activity (exercise) may promote the onset and progression of stroke and neurodegenerative diseases (Fig. 1.1). Other factors, such as too much cigarette smoking, exposure to secondhand smoke, midlife high blood pressure, and chronic diseases (e.g., obesity, diabetes, traumatic brain injury, and cerebrovascular lesions) may also promote the early onset of stroke and neurodegenerative diseases. Stroke prevention guidelines developed and endorsed by the American Heart Association and American Stroke Association emphasize the benefits of adopting healthy lifestyle choices—such as quitting smoking; eating a low-fat diet, which is enriched in n-3 fatty acids, fruits, and vegetables; drinking in moderation; exercising regularly; and maintaining a normal body weight—to reduce the risk of stroke (Mitka 2011) (Fig. 1.2). The risk

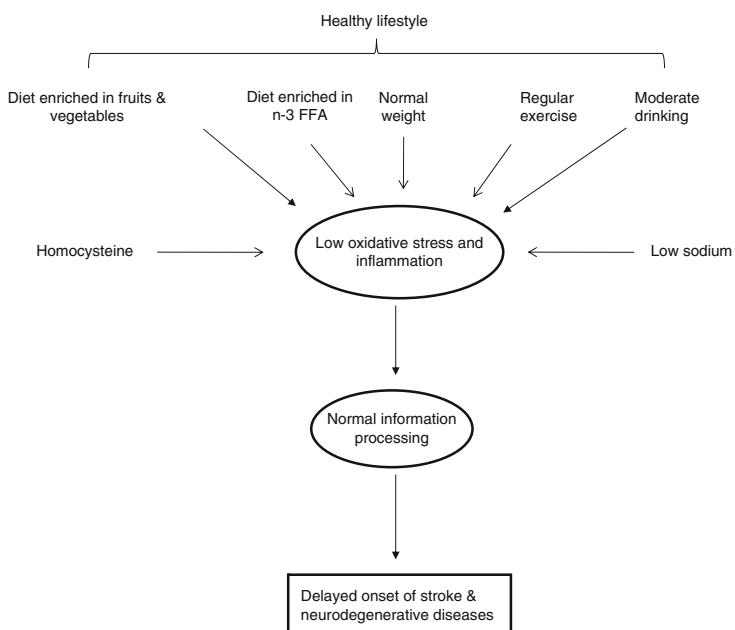


Fig. 1.2 Effect of healthy lifestyle on stroke and neurodegenerative diseases

factors for neurodegenerative diseases also include diet deficient in n-3 fatty acids, environmental factors, and lifestyle (consumption of processed food and lack of exercise) (Santana-Sosa et al. 2008; Pasinetti and Eberstein 2008). Thus, humans consuming fatty fish twice per week have lower risk of developing neurodegenerative diseases compared with those who consumed fatty fish less than once per month. Modest alcohol intake (1–6 drinks per week) provides in the fewest sub-clinical cerebrovascular abnormalities. Compared with little activity, moderate and high leisure-time activity results in 28 % and 44 % lower mortality, respectively, while compared with nonexercisers, low, moderate, and high exercise intensity predicted 30 %, 37 %, and 53 % more years of healthy life, respectively. Former and current smokers have 25 % and 44 % fewer years of healthy life than those who never smoked; lifetime smoking (pack-years) predicts higher mortality (Boden-Albala and Sacco 2000; Mozaffarian et al. 2004; King et al. 2009).

A healthy lifestyle keeps interplay among excitotoxicity, oxidative stress, and neuroinflammation to a level, which is necessary for optimal health (Farooqui 2009). This lifestyle must be maintained throughout the life (from childhood to old age) to delay or prevent stroke and neurodegenerative diseases. Changes in lifestyle after the onset of stroke or neurodegenerative disease may not have any effect on the disease process. This is tempting to speculate that adherence to a healthy lifestyle may either directly protect against stroke and neurodegenerative diseases or may delay these neurological diseases (Fratiglioni and Qiu 2009).

1.2.1 Effect of Natural and Processed Food on Human Health

Fresh natural food contains a higher proportion of naturally occurring vitamins, fibers, and minerals than processed food. Many constituents of natural fresh food are destroyed during food processing (Griep et al. 2011). For example, vitamin C is destroyed by heat and therefore canned fruits have a lower content of vitamin C than fresh ones. Often nutrients are deliberately removed or added to the processed food for improving its “shelf-life,” appearance, and taste (Levenstein 2003). This process is widespread in foods such as bread, pasta, and premade meals. Processed foods contain many additives, such as sugar, salt, flavorings, and texture-enhancing agents (Pollan 2008). As a result, eating large amounts of processed foods can lead to excessive intake of these substances, which can then lead to a variety of health complications including high blood pressure, weight gain, and diabetes. Preservatives added to extend the “shelf-life” of commercially available products, such as nitrites or sulfites, may cause adverse health effects. In vivo chemical reactions between nitrites and secondary amines or proteins can generate nitrosamine, which may exert their toxic effects by alkylating N-7 of guanine, leading to increased DNA damage (Swann and Magee 1968) and generation of reactive oxygen species such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Consequences include increased lipid peroxidation, protein adduct formation, and pro-inflammatory cytokine activation (Espey et al. 2002). These molecular and biochemical pathogenic cascades

have been proposed for the induction of human insulin-resistance diseases, such as type 2 diabetes, nonalcoholic steatohepatitis, and AD (Pasquier et al. 2006; Nicolls 2004; Yeh and Brunt 2007; de la Monte et al. 2009). Additionally, processed food has higher calories than fresh natural food (Levenstein 2003; Pollan 2008). Studies on the effect of raw and processed vegetables and fruits on humans indicate that high intake of raw fruit and vegetables may protect against stroke (Griep et al. 2011). High salt and sugars, which are present in processed food, are also major risk factors for stroke, heart disease, diabetes, and renal diseases. Salt intake is not the only determinant of high blood pressure associated with cerebrovascular, cardiovascular, and renal diseases, but other modifiable risk factors include relative mass, physical activity, overall dietary quality, and alcohol consumption. Consumption of processed meats, but not red meats, is not only associated with higher incidence of coronary heart disease, diabetes mellitus, and stroke, but also several types of cancers. These results highlight the need for better understanding of potential mechanisms of effects and for particular focus on processed meats for dietary intake (Linseisen et al. 2006). Presence of added sugar in processed food raises blood pressure (Nguyen et al. 2009; Bremer et al. 2009). Animal studies in rats and human studies, such as the Framingham Heart Study, indicate that consumption of ≥ 1 soft drink per day significantly increased the odds of developing high blood pressure (Rebello et al. 1983). Processed food is stored and sold in iron, tin, aluminum, and plastic containers. Storage of processed food in metal and plastic containers may result in toxicity due to leakage of chemicals and metal ions (Borchers et al. 2010).

1.2.2 Effect of Soft Drinks on Human Health

Soft drinks contain large amounts of high-fructose corn syrup (55 % fructose and 45 % glucose), which is enriched in fructose. Consumption of a high-fructose diet for 4 weeks in rats not only produces systemic insulin resistance and reduces tyrosine phosphorylation of the insulin receptor in liver, but also impairs insulin receptor substrate-1 (IRS-1) phosphorylation and IRS-1 association with phosphoinositol-3-kinase in both liver and skeletal muscle (Bezerra et al. 2000), supporting the view that specific points in the insulin signal transduction pathway that are affected by dietary fructose. These processes may lead to hepatic insulin resistance, increased total and visceral fat mass, and accumulation of ectopic fat in the liver and skeletal muscle. Thus, in humans, monkeys, and rodents, diet containing high fructose may lead to the development of obesity, diabetes, high blood pressure, and high triglyceride levels (Malik et al. 2006; Havel 2005; Stanhope and Havel 2008). Insulin resistance along with visceral obesity, dyslipidemia, and hypertension is a major component of the metabolic syndrome (Fig. 1.3) which is strongly associated with an increased risk for cardiovascular disease. Unhealthy lifestyle (a lack of regular physical activity and consumption of processed food rich in highly saturated fats, sugars, and salt) results in higher levels of risk factors (hypertension, dyslipidemia, diabetes, and obesity that act independently and synergistically) and are

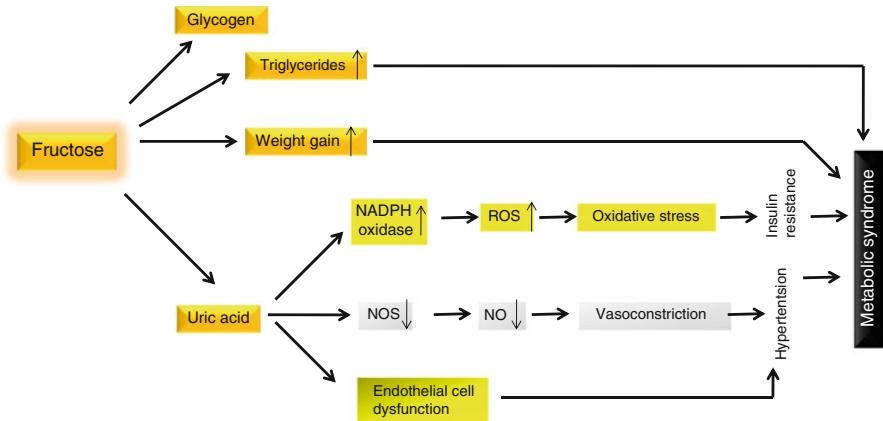


Fig. 1.3 Effect of consumption of fructose (high-fructose corn syrup) on human body. Upward arrow indicates increase and downward arrow indicates decrease

closely associated with the pathogenesis of metabolic syndrome (Wiernsperger et al. 2010).

Insulin is involved in the regulation of body adiposity via its actions in the brain to inhibit food intake and increase energy expenditure (Schwartz et al. 2000). Insulin receptors are highly expressed in the hypothalamus, a brain region associated with the control of food intake and energy homeostasis. Insulin inhibits food intake by activating phosphatidylinositol 3-kinase (PtdIns-3-kinase) in specific hypothalamic nuclei, a signaling pathway that is shared with leptin (a 16-kDa protein hormone) and ghrelin (an acylated peptide) in its effects to reduce food intake (Havel 2002) (Fig. 1.4). Insulin regulates, at least in part, the hormones leptin and ghrelin. Leptin and ghrelin are two key factors in the control of energy balance (Havel 2002), and therefore, lack of normal physiological regulation of these endocrine signals may have a role in overweight and obesity in Type 1 diabetes Melitus. Leptin is produced by adipocytes in proportion to adipose tissue mass and recent energy intake. It acts as a signal of long-term energy status to the brain, acting to reduce food intake and enhance energy expenditure (Havel 2002). In contrast, ghrelin is secreted from endocrine cells in the stomach and upper intestine (Date et al. 2000). Ghrelin's actions on energy balance are opposite to those of leptin. Ghrelin mediates the activation rather than inhibition of hypothalamic neuropeptide Y/agouti gene-related peptide signaling (Shintani et al. 2001). Thus, ghrelin enhances appetite and increases food intake (Wren et al. 2001). Accumulating evidence suggests that leptin and ghrelin serve as vital regulators of energy homeostasis. Consumption of high-fructose corn syrup containing drinks may alter leptin and possibly ghrelin signaling in the brain resulting in overeating, which may lead to weight gain, obesity, and diabetes (Bray et al. 2004).

Unlike glucose, which is utilized by all organs, fructose is solely metabolized in the liver. Fructose metabolizing enzyme, fructokinase, is not regulated by negative feedback, so all fructose that enters the liver cells is rapidly phosphorylated by ATP.

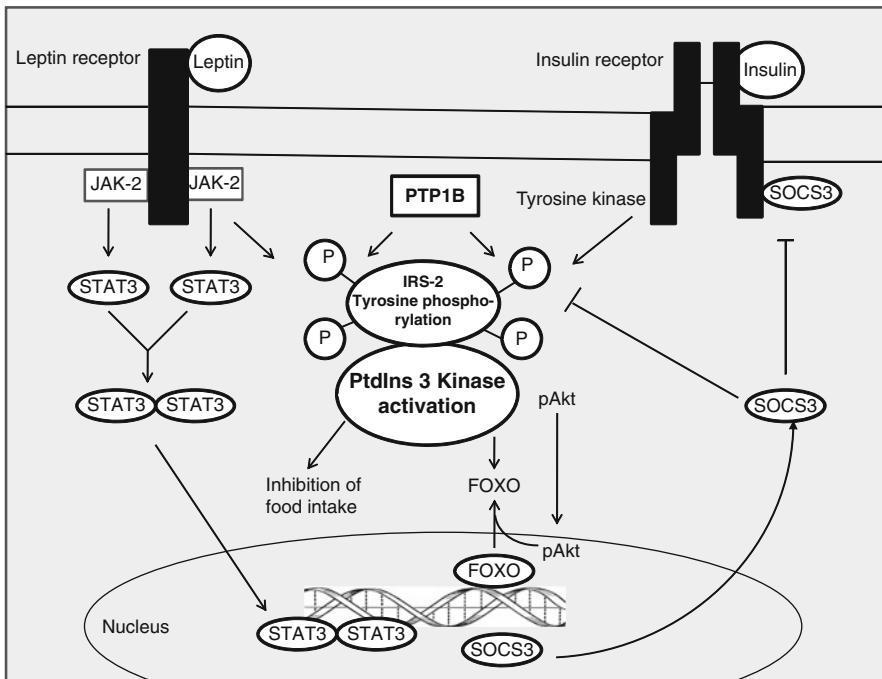


Fig. 1.4 A hypothetical diagram showing interactions between leptin and insulin receptors. Insulin receptor substrate 2 (IRS2); protein kinase B (Akt); Janus kinase 2 (JAK2); suppressor of cytokine signaling (SOCS3); forkhead box O number 1(FOXO); signal transducer and activator of transcription factor 3 (STAT3); phosphatidylinositol 3-kinase (PtdIns 3 kinase); and protein tyrosine phosphatase 1B(PTP1B)

This process results in ATP depletion, which has been well documented in vitro and in vivo in animal models of diabetes and humans. ATP depletion activates enzymes of purine metabolism (AMP deaminase-1), which degrades adenine nucleotides to uric acid via xanthine oxidoreductase with the development of hyperuricemia (Hallfrisch 1990; Nakagawa et al. 2005) (Fig. 1.3). Serum uric acid levels, even within the normal range, are associated with other cardiovascular risk factors and predict cardiovascular events in adults (Gao et al. 2007; Gagliardi et al. 2009). Serum uric acid levels have been associated with cardiovascular risk factors like hypertension and the metabolic syndrome in adolescents (Alper et al. 2005). Metabolism of fructose in liver also results in production of many products (triose phosphate, glucose, glycogen, lactate, and fat). Uric acid, which is derived from fructose not only increases blood pressure through the inhibition of nitric oxide synthase, but also cause gout (Yoo et al. 2009). In vitro, uric acid increases NADPH oxidase activity and oxidative stress in adipocytes, leading to increased p38 MAP kinase activity and insulin resistance (Sautin et al. 2007). High insulin levels have been shown to reduce renal uric acid excretion leading to hyperuricemia, which results from a combination of stimulated formation and reduced elimination in uric acid.

In addition to producing uric acid, fructose enhances the production of tumor necrosis factor (TNF) (Kanuri et al. 2011), a potent pro-inflammatory cytokine that induces insulin resistance and lipoprotein production. In addition, fructose not only evokes alteration of postreceptor insulin signaling, but also decreases insulin receptor activation and phosphorylation of IRS-1. Fructose also produces oxidative stress and mitochondrial dysfunction leading to the stimulation of peroxisome proliferator-activated receptor γ coactivator 1- α and β (PGC1- α and PGC-1 β) inducing insulin resistance and lipogenesis (Nagai et al. 2009).

Another important molecular mechanism associated with the deleterious effects of high fructose consumption is formation of nonenzymatic advanced glycation through Maillard reaction, which starts from Schiff bases and the Amadori product, a 1-amino-1-deoxyketose (Suárez et al. 1989). This product is generated through the reaction of the carbonyl group of a sugar, like glucose, with proteins or lipids amino groups. Glycation of proteins and lipids induces molecular rearrangements that lead to advanced glycation end-products (AGEs). The generation of AGEs may be harmful for cells in several ways (1) it promotes the formation of cross-linkages between key molecules in the basement membrane; (2) it alters intracellular proteins; and (3) interactions of AGEs with specific receptors on the cell surfaces result in abnormal intracellular signaling and disrupting cell function (Suárez et al. 1989).

Consumption of soft drinks is associated with dental caries and enamel erosion (Majewski 2001). Soft drinks contain inherent acids and high-fructose corn syrup. These substances have both acidogenic and cariogenic potential. Many studies show a positive relationship between caries and dental erosion and the consumption of soft drinks (Sayegh et al. 2002; Harding et al. 2003; Luo et al. 2005). Compared with caries, dental erosion seems to have much stronger relationship with soft drinks. The erosive potential of drinks is mainly represented by their pH and the buffering capacity. Carbonated drink can reduce surface hardness of enamel, dentine, microfilled composite, and resin-modified glass ionomer (Sayegh et al. 2002; Harding et al. 2003; Luo et al. 2005).

1.2.3 Effect of Diet on Stroke and Neurodegenerative Diseases

Consumption of processed food containing high fat is known to produce neuroinflammation and oxidative stress in cardiovascular and cerebrovascular systems. Maintaining animals on processed food containing high fat for an extended period not only promote stroke, but has detrimental effect on ischemic outcome. Processed food has marked effect on insulin sensitivity and risk factors for cardiovascular and cerebrovascular diseases and diabetes. Consumption of processed food with high fat contents promotes inflammation and insulin resistance, whereas monounsaturated, polyunsaturated, and longer chain n-3 fatty acids not only block inflammation, but also improve insulin sensitivity (Farooqui 2009, 2010). It is also reported that less-refined and less-starchy carbohydrates (such as fresh fruit, whole grains, green leafy vegetables, nuts, and legumes), in contrast to fruit juice, refined grains, and starchy

vegetables, have direct and independent beneficial effects on both glucose and insulin metabolism leading to less incidences of stroke (Kurth et al. 2006; Bernal-Pacheco and Roman 2007; Galimani et al. 2009).

In aged brain and neurodegenerative diseases, elevation in plasma fatty acids due to high-fat diet induces not only the activation of Luchsinger redox cycling of the copper–albumin complex and excessive lipid peroxidation, but accumulation of AGEs through the Maillard reaction and subsequent activation of the receptors for AGEs (RAGE) (Luchsinger and Mayeux 2004; Farooqui and Farooqui 2009). These receptors belong to multiligand receptor in the immunoglobulin superfamily, and act as a cell surface binding site for A β , and mediate alterations in the phosphorylation state of mitogen-activated protein kinase (MAPKs), supporting the view that MAPKs are associated with neurodegenerative processes (Origlia et al. 2009). In particular, alterations in the phosphorylation state of various MAPKs by aggregated proteins (A β , synuclein, huntingtin) cause synaptic dysfunction and cognitive decline as well as development of inflammatory responses in AD.

Although generation and accumulation of AGEs have been reported to occur during normal aging with lower rate, in neurodegenerative diseases, heart disease, diabetes, and cancers, consumption of food with high fat and salt leads to an accelerated rate of AGE accumulation (Ghosh et al. 2007). Abnormalities in cholesterol transporter apolipoprotein E4 (apo E4) is a major genetic risk factor for hypercholesterolemia, vascular dementia, and sporadic AD (Corder et al. 1994). Inheriting one or two alleles for apo E4 increases the risk of AD by 17 % and 43 %, respectively, compared to subjects, who are hetero- or homozygous for apolipoprotein E2 (APOE2) and APOE3 isoforms (Strittmatter and Roses 1996). A greater impact of APOE4 on brain dysfunction (both in AD and vascular dementia) relative to peripheral effects may be due to its pleiotropic functions including amyloid generation and clearance and maintenance of synaptic and cerebrovascular integrity. These observations support the relationship between cholesterol and AD (Solfrizzi et al. 2010). Cholesterol-enriched diet and subsequent hypercholesterolemia not only alter the insulin-like growth factor-1 (IGF-1) signaling pathway and decrease insulin degrading enzyme, but also increases active p-Tyr276 GSK-3 α levels leading to increase in levels of A β in rabbit hippocampus. These changes may be associated with the phosphorylation of CREB and the upregulation of the antiapoptotic protein Bcl-2, events that may represent a defensive mechanism to prevent neurodegeneration (Sharma et al. 2008). High-fat diet also produces significant upregulation of gp91(phox) subunit of NADPH oxidase and downregulation of superoxide dismutase isoforms, glutathione peroxidase, and heme oxygenase-2 in various body tissues (Roberts et al. 2006). These processes elevate plasma levels of malondialdehyde and impair vasodilatory response to acetylcholine. These observations support the occurrence of oxidative stress and endothelial dysfunction in rats consuming high-fat diet (Roberts et al. 2006).

Collective evidence suggests that dietary lifestyle negatively impacts brain function. In particular, consumption of high calorie “Western diet” rich in n-6 fatty acids and cholesterol is associated with the development of Alzheimer-like cognitive impairment (Pasinetti and Eberstein 2008). Studies on animal models of AD have

also shown that diet enriched in n-6 fatty acids and cholesterol can cause increase in amyloidosis, alterations in synaptic plasticity and behavior. These are essential features of Alzheimer pathology. In addition, n-6 fatty acid and cholesterol-enriched diet (Julien et al. 2010) and excessive sucrose intake (Cao et al. 2007) enhance tau pathology in Tg mice. Hyper-cholesterolemia can cause tau hyperphosphorylation in the brain, which has been shown in apoE-deficient mice fed a high-cholesterol diet (Rahman et al. 2005).

Status of n-3 fatty acids also influences the risk of neurodegenerative diseases. According to many investigators, intake of 3 g of fish oil/day may reduce the risk of developing AD and other neurodegenerative diseases (Morris et al. 2003a, b; Freund-Levi et al. 2006; Schaefer et al. 2006; Farooqui 2009). In addition, consumption of fish oil may also reduce cognitive decline (Farooqui 2009). A recent trial shows positive effects of DHA supplementation on cognition in patients with very mild AD (Freund-Levi et al. 2006). Similarly, studies on the effect of fish oil on three different transgenic models of AD indicate that animal models of AD are more vulnerable to DHA depletion than controls and that fish oil exerts a beneficial effect against pathological signs of AD, including A β accumulation, cognitive impairment, synaptic marker loss, and hyperphosphorylation of tau (Lim et al. 2005; Calon and Cole 2007).

Phytochemicals (small, nonenergetic molecules of vegetal origin) are naturally occurring bioactive compounds found in edible fruits, plants, vegetables, and herbs. Unlike vitamins and minerals, phytochemicals (curcumin, green tea, blueberries, flavonoids, and garlic) are not needed for maintaining cell viability, but they play a vital role in protecting neural cells from oxidative stress and neuroinflammation associated with normal aging and chronic age-related diseases. Diet enriched in phytochemicals reduces the risk of developing neurodegenerative diseases (Farooqui and Farooqui 2011, 2012). Phytochemicals constitute a heterogeneous group of substances that modulate antioxidant activity and detoxifying enzymes, stimulate the immune system, decrease platelet aggregation, and regulate hormone metabolism. Phytochemicals have also been shown to possess antiproliferative, anti-inflammatory, antiviral, and hypocholesterolemic properties. Intake of colored fruit and vegetable extracts reduces the age-enhanced vulnerability to oxidative stress and inflammation. In addition, resveratrol, a red wine polyphenol, also exert its beneficial effects through the modulation of signal transduction and neuronal communication, and delays the onset of dementia (Lau et al. 2007; Joseph et al. 2007). The consumption of extra-virgin olive oil, which contains tyrosol [2-(4-hydroxyphenyl)ethanol], hydroxytyrosol, oleuropein, and oleocanthal, retards or delays the onset and progression of neurodegenerative diseases (Lopez-Miranda et al. 2007). It is proposed that greater adherence to olive oil containing Mediterranean diet results in a significant improvement in health status, as seen by a significant reduction in overall mortality (13 %) in PD and AD patients (Sofi et al. 2008). Accumulating evidence suggests that fruits (pomegranate, purple grapes, and berries) containing relatively high concentrations of flavonols, anthocyanins, and procyandins produce beneficial effects for cardiovascular and cerebrovascular diseases, particularly with respect to antihypertensive effects, inhibition of platelet aggregation, and increasing endothelial-dependent vasodilation.

Phytochemicals may have an impact on brain pathology and aging; however, neither their mechanisms of action nor their cell targets are completely known. Most of the physiological benefits of phytochemicals are generally thought to be due to their antioxidant and free-radical scavenging effect. However, emerging evidence supports the hypothesis that the mechanism of action of phytochemicals may not only be due to modulation of enzyme activities and regulation of gene expression (González-Gallego et al. 2010), but also due to the activation of adaptive cellular stress response pathways that can protect cells against a variety of adverse conditions (Son et al. 2010). Additionally, phytochemicals may also bind membrane or nuclear receptors as elective ligands and their signaling effects may occur at concentrations much lower than that required for effective antioxidant activity (Virgili and Marino 2008).

Excessive calorie intake increases the risk of stroke and chronic visceral and neurodegenerative diseases. Caloric restriction (CR) or intermittent fasting increases lifespan and protects brain against neurodegenerative diseases due to increase in cellular stress resistance (Lee et al. 2000; Mattson 2008). CR not only lowers plasma insulin levels, but also induces greater sensitivity to insulin; lowers body temperatures; and reduces cholesterol, triglycerides, and blood pressure. It also elevates HDL and slows age-related decline in circulating levels of dehydroepiandrosterone sulfate. CR induces the synthesis of cellular stress response-stimulating proteins (neurotrophic factors, neurotransmitter receptors, protein chaperones, and mitochondrial biosynthesis regulators) and enhances neuronal plasticity and resist oxidative and metabolic insults (Lee et al. 2000; Fontan-Lozano et al. 2008). CR also upregulates levels of IGF-1 resulting in prolonged life in mice. Modulation of aging by IGF-I may involve reduction in insulin signaling, enhancement of sensitivity to insulin, reduction in generation of ROS, and improvement in antioxidant defenses resulting in reduced oxidative damage (Bartke et al. 2008). Based on earlier discussion, it can be proposed that CR as well as an active and stimulating lifestyle in late life as well as an optimal control of vascular and other chronic diseases (both at middle age and late life) may facilitate prevention or postponement of the onset of neurodegenerative diseases.

1.2.4 Effect of Genetic and Environmental Factors on Stroke and Neurodegenerative Diseases

It is well known that genes regulate the onset and frequency of neurodegenerative diseases (Coppede et al. 2006). Candidate genes involved in the etiology of familial and sporadic neurodegenerative diseases regulate functioning of neurotransmitter receptors, including cholinergic, dopaminergic, and glutamatergic receptors. Some of these genes interact with environmental factors and increase the risk of neurodegenerative diseases. For example, P450 2D6 gene may increase the risk of PD among persons exposed to pesticides. Familial PD is associated with mutations in Parkin, PINK-1, or DJ-1 genes. These mutations are related to increased oxidative stress.

Pathogenesis of sporadic PD may also involve exposure to metal ions, infections, stress, poor nutrition that may cause an increase in oxidative stress (Nunomura et al. 2007).

Three causative genes, namely, amyloid precursor protein gene (APP gene), presenilin-1 and -2 genes (PS-1 and PS-2), and APOE are involved in the pathogenesis of AD (Selkoe 2001; Siman and Salidas 2004; Priller et al. 2007). The human ApoE gene has three alleles (epsilon2, epsilon3, epsilon4), which are products of the same gene. The epsilon3 allele accounts for the majority of the APOE gene pool (approximately 70–80 %), the epsilon4 allele accounts for 10–15 %, and the epsilon2 allele for 5–10 %. Inheritance of the epsilon4 allele strongly elevates the risk for developing AD at an earlier age. APOE is associated with cholesterol transport, neuronal repair, dendritic growth, and anti-inflammatory activities. APOE4 gene also encodes a protein with crucial roles in cholesterol metabolism. APOE4 modulates the pathogenesis of AD by modulating the metabolism and aggregation of A β peptide and by directly regulating brain lipid metabolism and synaptic functions through APOE receptors (Bu 2009). Early life events such as infections, stress, and poor nutrition may influence the pathogenesis of AD and PD. There are similarities between neurochemical changes in infectious diseases and AD. These diseases are characterized by an increased synthesis of lipid mediators and elevation in production and secretion of cytokines, chemokines, and complement proteins (Urosevic and Martin 2008).

Epidemiological studies indicate that climate and geographic disparity influence cerebrovascular risk factors. In the USA, stroke incidence and mortality are highest in some southeastern states, referred to as the “stroke belt” (Gillum and Ingram 1996). In the UK and Finland, the northern parts of both countries have a higher incidence of stroke than in the south (Morris et al. 2003a; Havulinna et al. 2008). In Europe, northern countries have a higher stroke incidence than those in the south (Bejot et al. 2007). Although these relationships are arbitrary, they may be important because they may yield some public health strategies to help protect vulnerable subjects from the increased stroke-mediated death rates arising during extreme cold and heat waves. Although the underlying mechanisms for the association between climate and stroke is not fully understood, low temperature is known to cause an increase in many cerebrovascular risk factors, including coagulation-related factors such as fibrinogen and factor VII (Woodhouse et al. 1994), elevation in blood pressure (Alpérovitch et al. 2009), exacerbation of hemoconcentration (Neild et al. 1994), and increase in plasma lipids (Woodhouse et al. 1993), which can cause thromboembolic diseases, including stroke. It is possible that changes during extreme cold and heat may affect the circadian cycle, which may play an important role in modulating coagulation-related factors. Some studies suggest that lower temperature increases stroke risk and others suggest the converse, while changes in atmospheric pressure may link with increased intracranial hemorrhage risk. Thus, data on the effect of climate and geographic disparity on stroke are confusing and conflicting and well-conducted prospective studies are required to help clarify these potentially important relationships (McArthur et al. 2010).

The occurrence of chemicals (pesticides, air pollutants, industrial chemicals, and smoking) and heavy metals in water, food, and air may lead to human exposure.

These chemicals and metals may cross the blood–brain barrier (BBB) and produce a neurotoxic threat. Heavy metals (cadmium, aluminum) and toxic chemicals (paraquat, pesticides, and herbicides) can induce degeneration of dopaminergic and noradrenergic neurons, a characteristic of PD (Lai et al. 2002; Frisardi et al. 2010). These chemicals and metals contribute to the pathogenesis of neurodegenerative diseases not only by mediating mitochondrial and proteasomal dysfunctions, but also by chemically inducing changes in gene regulation which are associated with neurodegenerative disorders such as PD and AD (Edward and Myers 2008; Horowitz and Greenamyre 2010).

1.3 Effect of Exercise on Stroke and Neurodegenerative Diseases

Regular physical exercise ameliorates age-related neuronal loss and produces positive effect on neurodegenerative diseases (Trejo et al. 2002). In the brain, exercise induces both acute and long-term beneficial alterations, such as increased levels of various neurotrophic factors and enhanced cognition. Although the molecular mechanisms associated with exercise-induced changes in the brain are not yet well understood, it is becoming increasingly evident that physical exercise increases the expression of IGF-1 and BDNF in the brain. These neurotrophins are important for synaptic plasticity and learning and memory (Carro et al. 2001; Vaynman et al. 2004; Vaynman and Gomez-Pinilla 2006). BDNF exerts its neurochemical effects through two types of receptor: the tyrosine kinase receptor (TrkB) receptor and the pan-neurotropin receptor p75 (p75NTR) (Reichardt 2006). Pro-BDNF preferentially interacts with the p75NTR, whereas mBDNF selectively binds and activates the TrkB (Chao and Bothwell 2002; Ibanez 2002). BDNF acts through the protein tyrosine kinase receptor (TrkB) (Soppet et al. 1991) and regulates a number of processes including memory formation, learning and behavior, synaptic plasticity (Monteggia et al. 2004; Cunha et al. 2010), synaptic efficacy, and neuronal connectivity. It promotes the development of immature neurons and enhances the survival of adult neurons (Tyler et al. 2002). In contrast, endogenous pro-BDNF-mediated activation of p75NTR is associated with long-term depression (LTD) in the hippocampus (Rosch et al. 2005; Wu 2005) and induces apoptosis in peripheral neurons (Teng et al. 2005).

TrkB-IgG blocks beneficial effects of exercise on cognitive function (Vaynman et al. 2004). BDNF-TrkB signaling is associated with mitogen-activated protein kinase (MAPK), the phospholipase C γ (PLC γ), and the phosphatidylinositol 3-kinase (PtdIns3-K) pathways. MAPK and PtdIns3K play crucial roles in both translation and/or trafficking of proteins induced by synaptic activity, whereas PLC γ modulates intracellular Ca $^{2+}$ that can drive transcription via cAMP and a protein kinase C. BDNF secreted from active synapses and neurons recruits TrkB from extrasynaptic sites into lipid rafts. Neuronal activity promotes BDNF-induced TrkB endocytosis. This signaling event is important for long-term BDNF events (Ji et al. 2005).

Detailed investigations on the molecular analysis show that exercise significantly upregulates proteins downstream to BDNF activation that are important for synaptic function such as synapsin I, and phosphorylated calcium/calmodulin protein kinase II and phosphorylated mitogen-activated protein kinase II (Ding et al. 2006). Exercise also stimulates the expression of several key intermediates of the PtdIns-3K/Akt pathway, which contribute to neuronal survival (Chen and Russo-Neustadt 2007). Additionally, cAMP/PKA-mediated activation of synapsin I phosphorylation facilitates regenerative growth of neurons and promote neuronal survival. Inhibition of IGF-I receptor retards the exercise-induced increases. These results explain the molecular mechanisms by which IGF-1 modulates the BDNF system and induce exercise-mediated synaptic and cognitive plasticity. BDNF not only facilitates long-term potentiation, an electrophysiological correlate of learning and memory, but also increases the activities of free radical scavenging enzymes and hence protect neurons against oxidative stress (Pelleymounter et al. 1996). Thus, interplay between IGF-1 and BDNF provides protection to neurons in brain, where IGF-1 performs several functions, including modulation of APP processing, expression of BDNF, suppression of apoptosis through decrease in expression of bax in neurons and bcl-X in astrocytes (Hoyer 2004; Carro and Torres-Aleman 2004). In addition, exercise also upregulates the expression of the mitochondrial uncoupling protein 2, an energy-balancing factor involved in ATP formation and free radical management (Vaynman and Gomez-Pinilla 2006). These observations support the view that in brain physical exercise promotes a fundamental mechanism by which key elements of energy metabolism may modulate the substrates of hippocampal synaptic plasticity.

1.4 Effect of Aging on Human Brain

Aging is defined as a time-dependent progressive functional impairment process that ultimately leads to mortality. The most prominent characteristics of aging are progressive decrease in physiological capacity, cognitive impairments, defects in learning and memory, reduced ability to respond adaptively to environmental stimuli, increased susceptibility to diseases, and increased mortality (Farooqui 2010). Aging is accompanied not only by the shrinkage of human brain (particularly in the frontal cortex), but also by decrease in brain weight and volume due to loss of neurons and myelinated axons. Neurochemically, these changes in brain are accompanied by the overexpression of 2', 3'-cyclic nucleotide 3'-phosphodiesterase and calpain-mediated proteolytic fragmentation (Hinman et al. 2008). Decrease in neurotransmitter has been reported to occur in aging brain. Thus, dopamine levels decline by around 10 % per decade from early adulthood and have been associated with declines in cognitive and motor performance (Nyberg and Backman 2004). This may be due to either decline in the dopaminergic pathways between the frontal cortex and the striatum during aging or may be caused by reduction in binding between dopamine and dopamine receptor. Levels of serotonin and brain-derived

neurotrophic factor levels also fall with increasing age and may be implicated in the regulation of synaptic plasticity and neurogenesis in the adult brain (Mattson et al. 2004). Other factors that are altered in the aging brain include calcium dysregulation (Toescu et al. 2004), mitochondrial dysfunction, and the production of reactive oxygen species (ROS) (see below).

Aging also results in elevation of microglial activation in several brain regions including hippocampus (Finch and Cohen 1997). Aging is accompanied by induction of oxidative stress, which not only refers to increased production of ROS, but also cytotoxic consequences caused by ROS in a cell by processes that utilize molecular oxygen. ROS is a collective term, which includes superoxide anions (O_2^-), hydroxyl ($\cdot OH$), alkoxy ($RO\cdot$), and peroxy radicals ($ROO\cdot$), and hydrogen peroxide (H_2O_2) (Halliwell 2006). At low levels, ROS are needed for signal transduction processes necessary for fundamental cell activities such as growth and adaptation responses. However, higher concentrations of ROS contribute to neuronal membrane damage. The major sources of ROS include mitochondrial respiratory chain, xanthine/xanthine oxidase, myeloperoxidase, cytochrome P450, arachidonic acid (ARA) cascade involving phospholipase A₂ (PLA₂), cyclooxygenase (COX), lipoxygenase (LOX), and activation of NADPH oxidase (Sun et al. 2007). The presence of redox-active metals, such as Fe²⁺ and Cu²⁺ also contributes to ROS generation. ROS-mediated activation of transcription factors (AP-1, NF-κB, HIF-1) results in their translocation to the nucleus leading to signal transduction pathways that modulate neural cell death through direct oxidative modifications of neural membrane components and generation of lipid mediators (Farooqui 2012) (Fig. 1.5). In addition aging also results in the accumulation of AGEs, which also activate NFκB and increase the expression of pro-inflammatory cytokines (May and Ghosh 1998). Thus, age-dependent alterations in gene expression cause disruption of metabolic homeostasis (Mattson 2002; Mocchegiani et al. 2006).

Accumulation of oxidative-damage products (lipid mediators) and failure of cells to neutralize ROS-mediated stress may result in excessive cell death as occurs not only in neurotraumatic and neurodegenerative diseases, but also to some extent in normal aging (Qin et al. 2002; Zekry et al. 2003; Farooqui and Farooqui 2009; Farooqui 2010, 2012). Levels of ARA-derived 4-hydroxynonenal (4-HNE) and isoprotanes and nucleic acid-derived 8-hydroxy-2'-deoxyguanosine (Fig. 1.6) are increased in aging brain. 4-HNE reacts with nucleophiles to form Michael adducts and Schiff base resulting in modification of many enzymes and cytoskeletal proteins (Musiek et al. 2005; Farooqui and Horrocks 2006; Farooqui 2012).

During brain aging, astrocytes generate large amounts of nitric oxide (NO), which may be deleterious to the neighboring neurons and oligodendrocytes. The exact molecular mechanism involved in NO-mediated neuronal damage is not known. However, reaction between NO and superoxide generates peroxynitrite ($ONOO^-$) (Fig. 1.6), which not only interacts with sulphydryl groups, but can hydroxylate the aromatic rings of amino acid residues (Beckman et al. 1992). S-Nitrosylation cysteine thiols contribute to NO-mediated neurotoxicity by triggering misfolding of proteins, a process that may contribute to the pathogenesis of neurodegenerative diseases in old age (Nakamura and Lipton 2008). Furthermore,

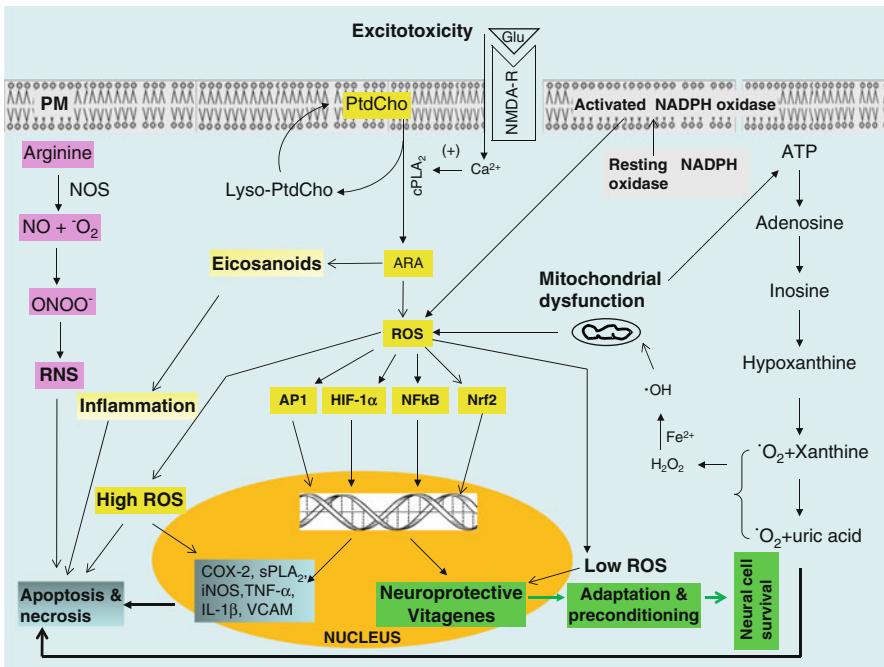


Fig. 1.5 A hypothetical scheme showing the effect of oxidative stress on transcription factors. Phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (PLA₂); arachidonic acid (ARA); reactive oxygen species (ROS); reactive nitrogen species (RNS); nitric oxide synthase (NOS); peroxynitrite (ONOO⁻); activator protein 1 (AP-1); Hypoxia-inducible factor-1 (HIF1); Nuclear factor κB (NF-κB); nuclear factor erythroid 2-related factor 2 (Nrf2); cyclooxygenase (COX); secretory phospholipase A₂ (sPLA₂); tumor necrosis factor-α (TNF-α); and interleukin-1β (IL-1β)

generation of NO also leads to S-nitrosylation of wild-type parkin and initially to a marked increase followed by a decrease in the E3 ligase-ubiquitin-proteasome degradative pathway (Yao et al. 2004). The inhibition of parkin's ubiquitin E3 ligase activity by S-nitrosylation could contribute to the degenerative process in neurodegenerative disorders by impairing the ubiquitination of parkin substrates. In addition, ONOO⁻ reduces mitochondrial respiration, inhibits membrane pumps, depletes cellular glutathione, and damages DNA, thus activating poly (ADP-ribose) synthase, an enzyme that leads to cellular energy depletion (Pryor and Squadrito 1995). Thus, ONOO⁻ reacts with lipids, proteins, and DNA (Radi et al. 1991). It is also reported that ONOO⁻ interferes with key enzymes of the tricarboxylic acid cycle, the mitochondrial respiratory chain, and mitochondrial Ca²⁺ metabolism (Bolanos et al. 1997). All these processes may contribute to neuronal energy deficiency and oxidation of protein sulphhydryls caused by changes that occur during aging as well as neurotraumatic and neurodegenerative situations (Thomas and Mallis 2001).

Age-related changes can be detected by functional Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) studies not only in older adults,

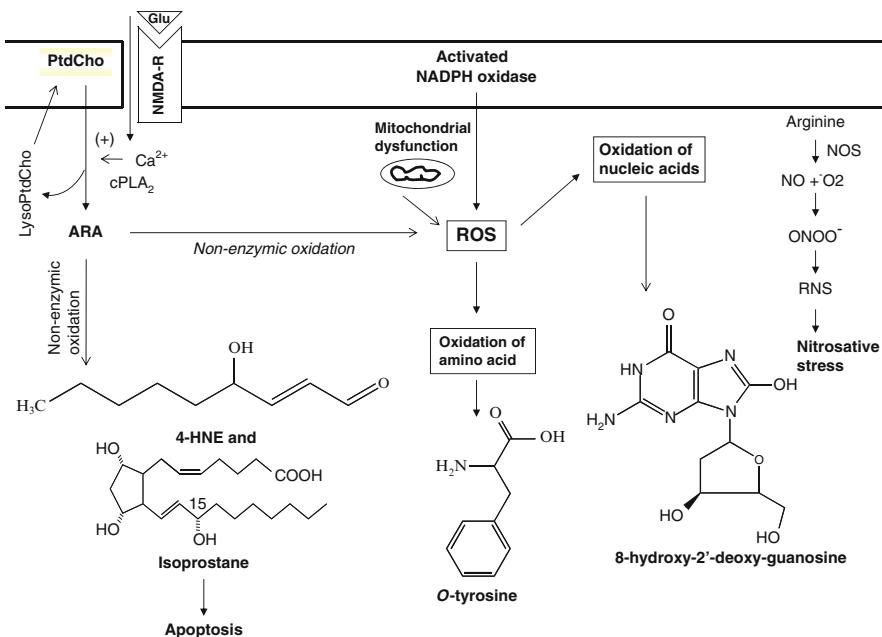


Fig. 1.6 ROS-mediated damage to phospholipids, proteins, and nucleic acids. Phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (cPLA₂); arachidonic acid (ARA); reactive oxygen species (ROS); and 4-hydroxynonal (4-HNE)

but also in AD, where they are associated with changes in the prefrontal cortex and related neural circuitry, which are linked to decline in integrative function between different brain regions (Rossini and Dal Forno 2004; Bano et al. 2011). These changes involve the loss of synaptic connectivity, which is a feature common to many neurodegenerative disorders. In humans, the morphological and functional changes in neurons, such as reduction of spine numbers and synaptic dysfunction, precede the first signs of cognitive decline and likely contribute to progression of neuropathology. Although earlier studies have indicated that aging is accompanied by slow deterioration triggered by accidental environmental factors, the concept of signal transduction supports the view that aging is a biological process tightly controlled by evolutionary highly conserved signaling pathways (Rossini and Dal Forno 2004; Santana-Sosa et al. 2008; Pasinetti and Eberstein 2008; Bano et al. 2011). Importantly, genetic mutations that increase longevity significantly delay the loss of synaptic connectivity and, therefore, the onset of age-related brain disorders, such as cognitive decline dementia and neurodegenerative diseases. Therefore, modulation of aging by diet, lifestyle changes, and physical exercise may be an attractive approach to delay or prevent cognitive decline caused by age-related synaptic dysfunction and progression of diseases.

1.5 Bioavailability, Side Effects, and Safety of Phytochemicals

Edible fruits, plants, vegetables, and herbs consist of widely varied phytochemicals including polyphenols, fibers, vitamins, minerals, and carbohydrates, all of which have beneficial effect in the body. The health effects of phytochemicals depend on both their respective intakes and their bioavailability, which can vary greatly. Phytochemicals are categorized into polyphenols, carotenoids, proanthocyanidins, anthocyanins, stilbenes, lignans, hydrolyzable tannins, naphthoquinones, and phytosterols. Polyphenols are the most bioactive agents within plant-based foods. Polyphenols are categorized into several classes, namely hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes, and lignans (Manach et al. 2005; Scheepens et al. 2010). Other phytochemicals, such as proanthocyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. This particular nature may limit their absorption through the gut barrier, and oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms. In contrast, ingestion of free form of hydroxycinnamic acids results in its rapid absorption in the small intestine. Hydroxycinnamic acids are then transformed into glucuronidated derivatives, which are excreted (see below) (Clifford 2000). However ingestion of esterified hydroxycinnamic acid in plant products does not result in its absorption. Human tissues (intestinal mucosa, liver) and biological fluids (plasma, gastric juice, duodenal fluid) do not possess esterases capable of hydrolyzing hydroxyl or chlorogenic acid to release hydroxycinnamic and caffeic acids (Rechner et al. 2001).

Most polyphenols penetrate the gut wall by passive diffusion due to their hydrophilic nature. Quercetin is able to enter BBB epithelia through passive diffusion mediated by its hydrophobicity (Crespy et al. 2002). However, occurrence of a unique active transport mechanism has been described for the absorption of cinnamic and ferulic acid in the rat jejunum (Ader et al. 1996). Most polyphenols are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. Esterified polyphenols are either hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed. Involvement of microflora in absorption reduces the efficiency of absorption because the microflora is capable of degrading aglycones that it releases and produces various simple aromatic acids in the process. During the course of absorption, polyphenols are conjugated through methylation, sulfation, and glucuronidation in the small intestine and later in the liver (Manach et al. 2004, 2005; Scheepens et al. 2010). They are treated by the body as xenobiotics as they are rapidly removed from the bloodstream. This detoxification is common to many phytochemicals and restricts their potential toxic effects and facilitates their biliary and urinary elimination by increasing their hydrophilicity. Circulating polyphenols bind to albumin and penetrate tissues, particularly those in which they are metabolized (Manach et al. 2004). However, very little is known about their ability to accumulate within specific target tissues. It is likely that bioavailability polyphenols may differ greatly not only among various tissues, but also among various polyphenols, and the most abundant polyphenols in diet are not

necessarily those that have the best bioavailability profile. Polyphenols and their derivatives are eliminated in urine and bile. Polyphenols are secreted via the biliary route into the duodenum, where they are subjected to the action of bacterial enzymes, especially β -glucuronidase, in the distal segments of the intestine, after which they may be reabsorbed. This enterohepatic recycling may lead to a longer presence of polyphenols within the body (Manach et al. 2004, 2005; Scheepens et al. 2010). The bioavailability of polyphenols can be influenced by intrinsic factors in food. Absorption of polyphenols in humans is very low. They are largely metabolized and rapidly eliminated. For this reason continuous consumption of polyphenol containing fruits and vegetable is necessary from childhood to the old age to produce their beneficial effects on human health.

1.6 Mechanism Associated with Beneficial Effects of Phytochemicals on Human Brain

As stated earlier, phytochemicals not only act as antioxidants, and antibacterial or antiviral agents, but also stimulate detoxifying enzymes, immune and hormonal response in humans, who consume colored edible fruits, plants, vegetables, and herbs. Phytochemicals exert their beneficial effects through several common mechanisms, which can be conducive to additive, synergistic, and antagonistic. Thus, phytochemicals not only modulate cellular differentiation, proliferation, oxidative stress, inflammation, and apoptosis, but also regulate activities of proteins and enzymes that are involved in above processes at the molecular level. Phytochemicals have been reported to reverse age-related declines in memory formation by their ability to interact with the cellular and molecular architecture of the brain responsible for memory. These interactions include their ability to upregulate signaling pathways, critical in controlling synaptic plasticity, and have a potential to induce vascular effects by inducing new nerve cell growth in the hippocampus (Spencer et al. 2009). Their ability to activate the extracellular signal-regulated kinase (ERK1/2) and the protein kinase B (PKB/Akt) signaling pathways, leading to the activation of the cAMP response element-binding protein (CREB), a transcription factor responsible for increasing the expression of a number of neurotrophins that are important in mediating memory formation (Spencer et al. 2009; Spencer 2010).

One of the major cellular antioxidant responses is the induction of antioxidative and anti-inflammatory enzymes through the cytoplasmic oxidative stress system (Nrf2-Keap1) activated by a variety of phytochemicals (Fig. 1.7) (Wakabayashi et al. 2010). Under normal conditions, Keap1 keeps the Nrf2 transcription factor within the cytoplasm targeting it for ubiquitination and proteasomal degradation to maintain low levels of Nrf2 that mediate the constitutive expression of Nrf2 downstream genes. When cells are exposed to age or neurodegenerative disease-mediated oxidative stress, a signal involving phosphorylation and/or redox modification of critical cysteine residues in Keap1 blocks the enzymic activity of the Keap1-Cul3-Rbx1 E3 ubiquitin ligase complex, leading to decrease in Nrf2 ubiquitination and degradation.

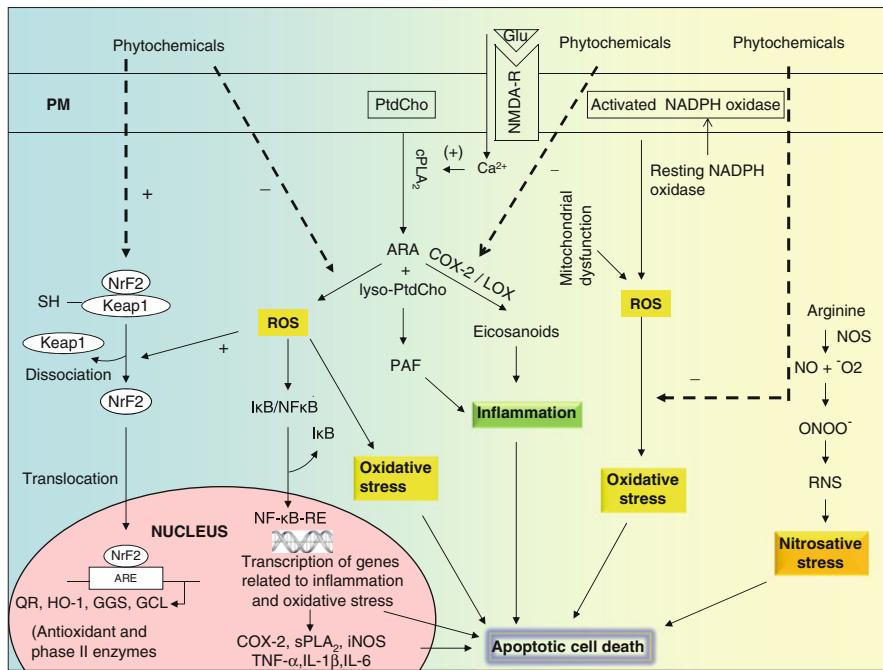


Fig. 1.7 Effect of phytochemicals on signal transduction processes in the brain. Plasma membrane (PM); phosphatidylcholine (PtdCho); lysophosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (cPLA₂); platelet activating factor (PAF); reactive oxygen species (ROS); reactive nitrogen species (RNS); nitric oxide synthase (NOS); peroxynitrite (ONOO⁻); Nuclear factor-kappa B (NF-κB); nuclear factor erythroid 2-related factor 2 (NrF2); cyclooxygenase-2 (COX-2); lipoxygenase (LOX); secretory phospholipase A₂ (sPLA₂); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); kelch-like erythroid Cap'n'Collar homologue-associated protein 1 (Keap1); antioxidant response-element (ARE); NFE2-related factor 2 (NrF2); heme oxygenase 1 (HO-1); NADH quinone oxidoreductase, γ-glutamylcysteine ligase (γ-GCL)

As a result, free Nrf2 migrates into the nucleus, where in cooperation with other transcription factors (e.g., sMaf, ATF4, JunD, PMF-1), it transactivates the antioxidant response elements (AREs) of many cytoprotective genes, as well as Nrf2 itself. Upon recovery of cellular redox status, Keap1 travels into the nucleus and facilitates the dissociation of Nrf2 from the ARE. Subsequently, the Nrf2–Keap1 complex is exported out of the nucleus by the nuclear export sequence (NES) in Keap1. Once in the cytoplasm, the Nrf2–Keap1 complex associates with the Cul3-Rbx1 core ubiquitin machinery, leading to degradation of Nrf2 and termination of the Nrf2/ARE signaling pathway (Wakabayashi et al. 2010).

Other effects of phytochemicals include the activation of adaptive cellular stress-response pathways in neurons. Examples of such adaptive stress response or preconditioning (is “neurohormesis”). These pathways involve oxidant-mediated neural cell survival signaling along with histone deacetylases of the sirtuin family (sirtuin-FOXO pathway) and chaperones, such as the heat-shock proteins (HSPs),

antioxidant enzymes (superoxide dismutases and glutathione peroxidase), and growth factors, such as insulin-like growth factors and brain-derived neurotrophic factor (Mattson et al. 2004; Mattson and Cheng 2006; Mattson 2008).

Maintenance of optimal health involves a complex network of longevity assurance processes that are modulated by vitagenes, a group of genes involved in preserving cellular homeostasis during stressful conditions. Vitagenes encode for HSP32, HSP70, the thioredoxin, and the histone deacetylases of the sirtuin protein systems. Phytochemicals, such as polyphenols have recently been shown to have protective effects through the activation of neurohormetic pathways, including vitagenes (Calabrese et al. 2007, 2008, 2010). HSPs have also been shown to delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span (Calabrese et al. 2007, 2008, 2010). Accumulating evidence suggests that consumption of phytochemicals from childhood to old age may not only promote good health by retarding the motor and cognitive behavioral deficits that occur during normal aging, but also by delaying the onset of stroke, AD, and PD (Mattson et al. 2004; Mattson and Cheng 2006; Mattson 2008; Farooqui and Farooqui 2010; Joseph et al. 2009). Above suggestion is based on epidemiological studies. It is proposed that consumption of diet rich in phytochemicals may lower the risk of developing cardiovascular diseases (heart disease), stroke, age-related neurodegenerative diseases, and many types of cancers (Farooqui and Farooqui 2010, 2011, 2012).

1.7 Conclusion

Aging produces alterations in the brain size, vasculature, and cognition. The brain shrinks with increasing age and there are changes at all levels from molecules to morphology. Incidence of stroke, white matter lesions, neurodegenerative diseases, and dementia also rise with age. This is accompanied by changes in levels of neurotransmitters and hormones and impairment in memory. Age-dependent changes in brain do not occur to the same extent in all brain regions. Unhealthy lifestyle (consumption of high-fat diet enriched in n-6 fatty acids, soft drinks containing high-fructose corn syrup, and lack of exercise) produces oxidative stress and neuroinflammation and may promote onset of stroke and neurodegenerative diseases. In addition to stroke and neurodegenerative diseases, unhealthy lifestyle may also be associated with obesity and may act as an independent risk factor or may affect other heart disease and stroke risk factors such as hypertension, diabetes, and hyperlipidemia. The onset of neurodegenerative diseases occurs when neurons fail to respond adaptively to age-related increases in oxidative and neuroinflammation thereby inducing the accumulation of damaged proteins, DNA, and membrane fragments. Lifestyle changes that reduce the risk of stroke and neurodegenerative diseases include regular exercise, a healthy diet with n-3 fatty acids and colored fruits and vegetables, low to moderate alcohol intake, and consumption of natural food. Collective evidence suggests that a healthy lifestyle may be the best defense against the changes of an aging brain.

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Chapter 2

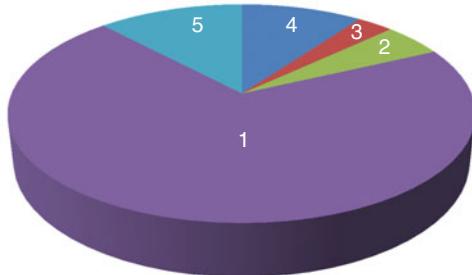
Beneficial Effects of Extra Virgin Olive Oil (n-9 Fatty Acids) on Neurological Disorders

2.1 Introduction

Olive oil is used by humans for food since prehistoric times. Olive oil not only contains oleic acid (18:1n-9), but also small amounts of other fatty acids, such as palmitic, palmitoleic, stearic, linoleic, and α -linolenic acids and squalene (Fig. 2.1). In addition to fatty acids, olive oil also contains phenolic compounds. Oleic acid, a monounsaturated nonessential fatty acid, belongs to n-9 family of fatty acids. It is found in animals and plants and represents a large proportion of human dietary intake with low uptake by liver and brain. Beef and poultry contain 30–45 % oleic acid, while oils such as palm, peanut, soybean, grape seed oil, and sunflower contain 25–49 % oleic acid (Waterman and Lockwood 2007). Other sources of oleic acid include avocado fruit (50 %), Macadamia nuts (45 %), apricot seeds (35 %), almonds (33 %), and olive oil (55–80 %). Among various cooking oils, olive oil is unique because it has high oleic acid content. In contrast, majority of other cooking oils (palm, peanut, soybean, and sunflower) are composed primarily of n-6 polyunsaturated fatty acids. The presence of one double bond makes oleic acid not only less susceptible to oxidation, but also contributes to the high stability and long shelf life of olive oil (Owen et al. 2000a, b).

Dietary fat intake modulates fatty acid composition of membranes, which in turn regulates activities of many membrane proteins, receptors, and ion channels in various body tissues. Thus, Mediterranean diet, which is rich in olive oil, increases the levels of oleic acid in plasma membrane phospholipids from various tissues in rat and human cells (Escudero et al. 1998; Vicario et al 1998). Conversion of stearic acid into oleic acid is catalyzed by stearoyl-CoA desaturase (SCD). This enzyme in association with NADPH, cytochrome b5 reductase, and cytochrome b5 and in the presence of molecular oxygen inserts a single double bond (between carbons 9 and 10) into stearoyl-CoAs (Ntambi and Miyazaki 2004). Oleic acid then becomes a major substrate for the synthesis of various lipids including phospholipids, triglycerides, and cholesteryl esters. Other fatty acids (linoleic acid, α -linolenic acid, arachidonic acid, and docosahexaenoic acid, which are also components of the membrane

Fig. 2.1 Proportions of various fatty acids found in extra virgin olive oil. Oleic acid (1); stearic acid (2); palmitoleic acid (3); linoleic acid (4); and palmitic acid (5)



phospholipids) are not synthesized by human cells and must be taken in through the diet. Studies on fatty acid composition in human brain indicate that in the younger humans, the polyunsaturated fatty acids are generally decreased with age, with the exception of docosahexaenoic acid that shows a significant increase. In humans, levels of monounsaturated fatty acids, such as oleic acid are increased to the age of 18 years. Several other polyunsaturated fatty acids particularly arachidonic acids are also decreased with age in the older subjects. The levels of linoleic acid, however, are increased significantly with age in the older humans. In the older human subjects, there is a significant relationship between brain and erythrocyte levels for several fatty acids, particularly hexadecanoic acid, suggesting that levels of cerebral cortex fatty acids change from early childhood through late adulthood; late adulthood erythrocyte fatty acid levels may be useful in predicting brain fatty acid levels in adults (Carver et al. 2001). Alterations in fatty acid composition of membrane are known to influence the localization and activity of G proteins and protein kinase C (PKC) (Escribá et al. 1997). PKC and G proteins play an important role in signaling and in regulating blood pressure (Escribá et al. 2003). Although the molecular mechanism associated with above processes is not fully understood, but elevation in oleic acid levels increases hexagonal phase propensity and induces fluidification effect in the lamellar phase resulting into densely packed membrane, which is more receptive to signals that reduce blood pressure. In contrast, elaidic acid and stearic acid produce no such effect on membrane propensity and fluidification (Prades et al. 2003; Funari et al. 2003). Collective evidence suggests that membrane fatty acid composition not only modulates physical properties, such as structure, fluidity/viscosity, permeability, microdomain formation, and shear stress, but also gene expression and activities of enzymes (adenylyl cyclase and phospholipase C), receptors, ion channels, and generation of second messengers (Khan et al. 1992; Ntambi and Bené 2001).

2.2 Chemical Composition and Biochemical Activities of Components of Olive Oil

As stated earlier, oleic acid is a major component of olive oil, which unlike most dietary oils that are manufactured from the seeds of plants by means of solvent extraction and refined before being edible, olive oil is obtained from the whole fruit

of *Olea europaea* L. only by physical pressure. This method makes extra virgin olive oil unique because several chemical components that cannot be found in other dietary oils are transferred from the leaves and skin of olives to the olive oil. The hydrocarbon composition of extra virgin olive oil is different from other edible oils. Thus in the unsaponifiable fraction, extra virgin olive oil contains high levels of squalene, a polyunsaturated triterpene, which is a precursor for the biosynthesis of cholesterol and steroid hormones (Perona et al. 2006). The main sterol component of extra virgin olive is β -sitosterol, which makes 95 % of sterol fraction. In addition, minor quantities of campesterol, D7-stigmastenol, stigmasterol, spinasterol, and avenasterol are also found in olive oil. Olive oil also contains α , β , γ -tocopherols, which account for more than 85 % of the total tocopherols (Perona et al. 2006).

It is well known that neurons utilize glucose as the primary energy source (Sokoloff et al. 1977). However, some hypothalamic neurons also utilize long-chain fatty acids as signaling molecules (Migrenne et al. 2006; Jo et al. 2009). In vivo, the ability of hypothalamic neurons to sense fatty acids affects insulin secretion, hepatic glucose production, and food intake (Migrenne et al. 2006). Thus, studies on the utilization of oleic acid by VMN neurons indicate that dissociated ventromedial hypothalamic nucleus (VMN) neurons utilize oleic acid and physiological concentrations of hypothalamic glucose (Silver and Erecinska 1994; De Vries et al. 2003) as a potential means of sensing and regulating energy homeostasis in the body. Although the molecular mechanism associated with this process is not fully understood, interactions between oleic acid and fatty acid transporter CD36, a member of class B scavenger receptor proteins, may play an important role (Le Foll et al. 2009). It is proposed that CD36 interactions with oleic acid alters neuronal activity in a manner analogous to that utilized for fat perception by taste receptor cells (Laugerette et al. 2005). The binding of oleic acid with CD36 induces the phosphorylation of protein tyrosine kinases, leading to generation of inositol 1,4,5-trisphosphate, recruitment of calcium from the endoplasmic reticulum, followed by influx of calcium via opening of store-operated calcium channels, membrane depolarization, and neurotransmitter release (El-Yassimi et al. 2008). Based on detailed investigation, it is suggested that VMN metabolic sensing neurons respond to glucose and fatty acid through two distinct and largely unrelated mechanisms (Le Foll et al. 2009). One involving fatty acid sensing through binding to cell surface receptors with activation of downstream signaling cascades, and the other requires glucose sensing primarily by intracellular glucose metabolism and influx of calcium through voltage-dependent calcium channels (Le Foll et al. 2009).

Oleic acid directly regulates the electrical activity of pro-opiomelanocortin (POMC) neurons in hypothalamus, enhancing the anorexigenic tone exerted by the melanocortinergic system. These neurons not only respond to circulating signals—such as glucose, leptin, insulin, ghrelin, and peptide YY (Jo et al. 2009), but also contribute to the regulation of energy expenditure by releasing the anorexigenic melanocyte-stimulating hormones (α -MSH and γ -MSH) through the activation of centrally expressed melanocortin-3 (MC3R) and melanocortin-4 receptors (MC4Rs) (Mountjoy and Wong 1997). The mitochondrial β -oxidation of oleic acid is a critical step in the regulation of the excitability of POMC neurons. The regulation of K_{ATP} channels in POMC neurons by both acute and long-term treatment with oleic

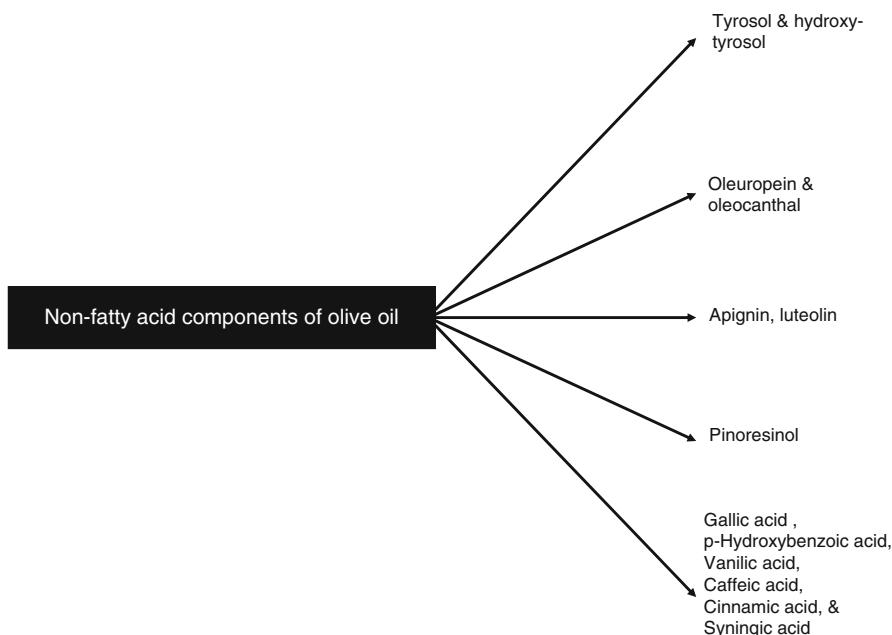


Fig. 2.2 Nonfatty acid components of olive oil

acid and nutrient-related hormones may contribute to the control of food intake and to the maintenance of weight balance (Jo et al. 2009).

The phenolic compounds of olive oil can be divided into three categories: simple phenols, secoiridoids, and lignans. All these components inhibit autooxidation. Major nonfatty acid components of olive oil include hydroxytyrosol, tyrosol, oleuropein, apigenin, luteolin, pinoresinol, caffeic acid, vanillic acid, syringic acid, p-coumaric acid, o-coumaric acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, and 3,4-dihydroxyphenylacetic acid (Owen et al. 2000a, b; Alarcón de la Lastra et al. 2001; Perona et al. 2006) (Fig. 2.2). The olives mainly contain the polar glycosides oleuropein and ligstroside. Oleuropein is the ester of elenolic acid with 3,4'- dihydroxyphenylethanol (hydroxytyrosol), and ligstroside is the ester of elenolic acid with 4-hydroxyphenylethanol (tyrosol). Oleuropein and ligstroside are the parent compounds of the less polar oleuropein- and ligstroside-aglycones. Oleuropein- and ligstroside-aglycones are produced by the removal of the glucose moiety from the oleuropein- and ligstroside-glycoside by β -glucosidase during olive ripening process (Fig. 2.3) (Martinez-Dominguez et al. 2001; Granados-Principal et al. 2010). Those aglycones and their various derivatives are the most abundant phenols in olive oil. Thus, hydroxytyrosol and tyrosol are simple phenols and oleuropein is a secoiridoid (Fig. 2.4).

In cardiovascular system, studies on the effect of monoacylglycerols containing palmitic acid (MAG-P), stearic acid (MAG-S), or oleic acid (MAG-O) at the sn-2 position of glycerol moiety indicate that not only MAG-O shows the strongest

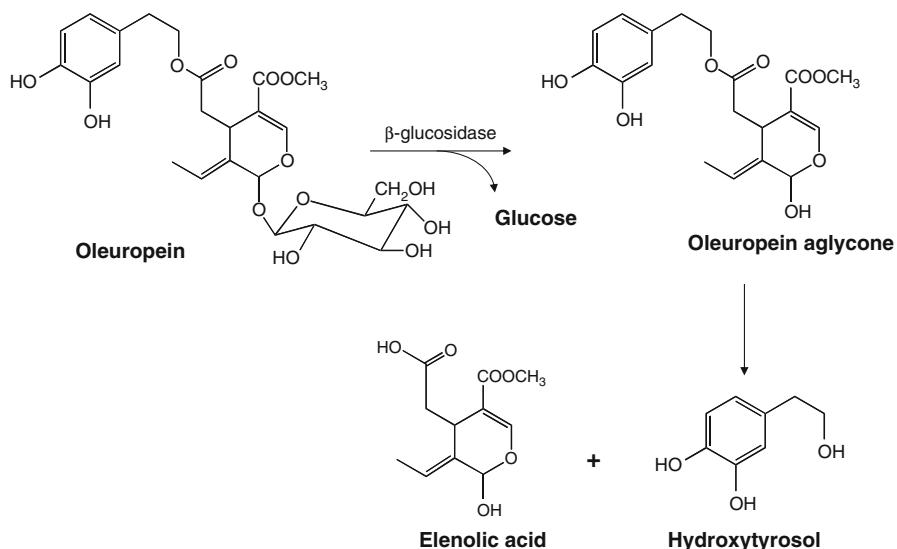


Fig. 2.3 Scheme showing the generation of hydroxytyrosol from oleuropein

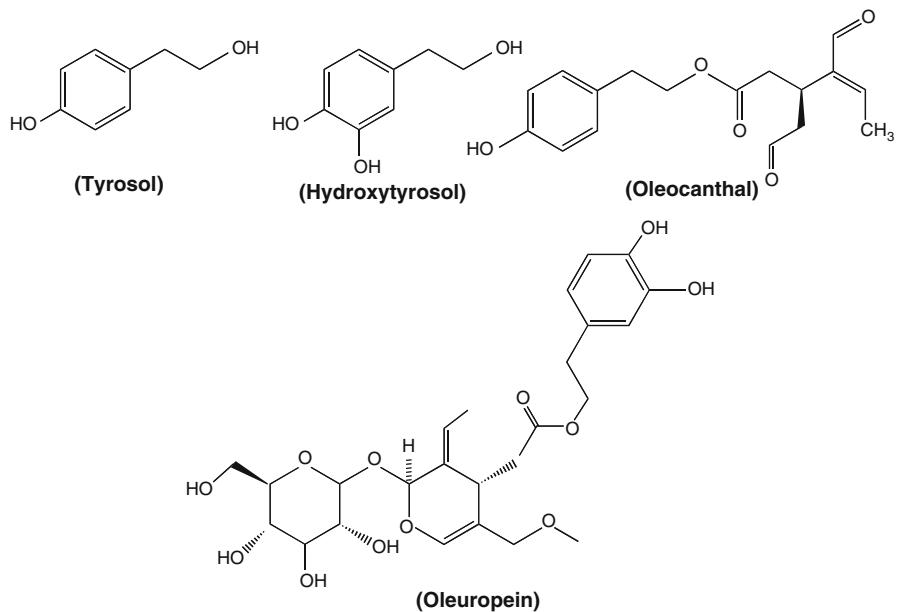


Fig. 2.4 Chemical structures of polyphenols found in extra virgin olive oil

inhibition of LDL-PLA₂ and antiatherogenic activities, but also MAG-S and MAG-P show adequate inhibitory activity (Cho et al. 2010). In contrast, MAG-S and MAG-P show stronger paraoxonase (PON)-enhancing activity than MAG-O. In recent years, PON has emerged as the component of HDL most likely to explain its ability to attenuate the oxidation of LDL. Thus, PON might be a major defense barrier against lipid peroxides from oxLDL. High intake of oleic acid is associated with significantly increased HDL-cholesterol concentrations and PON activity (Tomas et al. 2001). It is also reported that HDL rich in oleic acid was less prone to be oxidized, indicating that dietary oleic acid in MUFA prevents the oxidative modification of HDL (Sola et al. 1997). Although the exact mechanism of MAG-O-mediated atherogenic effect is not fully understood, it is proposed that inhibition of LDL-PLA₂ may be closely associated with atherogenic effect. In addition, MAG-O also shows the strongest antioxidant activity against copper-mediated LDL oxidation, indicating that oleic acid-containing MAG enhances the antioxidant ability against copper-mediated LDL oxidation. Oleic acid has been reported to exert beneficial effects on the pathogenesis of vascular disease via protection of LDL from oxidation (Mata et al. 1997) and to induce less monocyte chemotaxis and adhesion on exposure to oxidative stress (Tsimikas et al. 1999; Reaven et al. 1991). Collective evidence suggests that above activities may contribute to increased anti-atherogenic potential of MAG-O and are associated with beneficial effects of oleic acid on human health.

2.2.1 Bioavailability and Metabolism of Olive Oil Components

Many studies have shown that the phenolic compounds (hydroxytyrosol, tyrosol, oleuropein, and oleuropein-aglycone) are absorbed after ingestion in a dose-dependent manner (Vissers et al. 2002; Edgecombe et al. 2000; Tuck et al. 2001). The mechanism underlying absorption of olive oil phenolic compounds remains unclear. However, different polarities of various phenolics have been postulated to play an important role in the absorption of these compounds (Vissers et al. 2002). For example, tyrosol and hydroxytyrosol are polar compounds and their absorption takes place through the passive diffusion (Manna et al. 2000). The polar but larger phenolic, oleuropein-glycosides are absorbed via a glucose transporter-mediated process involving carrier Na-dependent glucose transporter 1. In addition, oleuropein-glycoside can also be absorbed via the paracellular route or transcellular passive diffusion (Edgecombe et al. 2000). Among phenolic compounds of olive oil, oleuropein-glycoside and oleuropein and ligstroside-aglycones are metabolized to hydroxytyrosol or tyrosol and excreted in urine (Vissers et al. 2002). In addition, hydroxytyrosol and tyrosol can also be conjugated to glucuronic acid and excreted in urine as glucuronides (Vissers et al. 2002; Visioli et al. 2001). Hydroxytyrosol may also be O-methylated in vivo as judged by the presence of homovanillic acid (a well-known metabolite of dopamine) and homovanillyl alcohol in human and animal plasma and urine after olive oil ingestion (Visioli et al. 2003). Even in

moderate dose (25 ml/day), which is lower than the traditional daily dietary intake in Mediterranean countries, around 98 % of these phenolics are present in plasma and urine in conjugated forms, mainly as glucuronides. This suggests the existence of an extensive first-pass intestinal/hepatic metabolism of the ingested tyrosol and hydroxytyrosols in the extra virgin olive oil. Tyrosol also binds to low density lipoproteins (LDL). This binding is directly related to an increase of the LDL resistance to oxidation (Covas et al. 2000). Ingestion of olive oil rich in phenolic compounds for 1 week leads to an increase in the total phenolic content of LDL in human subjects (Covas et al. 2000). Collective evidence suggests that once absorbed, olive oil phenolic compounds undergo extensive metabolism in liver and kidney, where they may play an important role in the prevention of oxidative stress and inflammation.

Hydroxytyrosol is known to enter brain tissue (Wu et al. 2009). Studies on hydroxytyrosol-fed mice brain slices indicate that hydroxytyrosol exerts a dose-dependent decrease in the efflux of lactate dehydrogenase demonstrating the neuroprotective potentials of hydroxytyrosol in rodent model of hypoxia (González-Correa et al. 2008). Treatment of PC12 cells with olive mill waste water extract, which is enriched in hydroxytyrosol indicates that this component of olive oil has cytoprotective effect on PC12 cells (Schaffer et al. 2007, 2010). Hydroxytyrosol acts by inducing the nuclear transcription factor erythroid 2p45-related factor (Nrf2), a transcription factor implicated in the expression of several antioxidant/detoxificant enzymes. Thus, hydroxytyrosol activates two important signaling proteins involved in Nrf2 translocation, the protein kinase B and the extracellular regulated kinases. Studies on the effect of specific inhibitors support the involvement of both molecular pathways for the nuclear translocation of Nrf2. Accumulating evidence suggests that in addition to inherent radical scavenging activity, hydroxytyrosol acts through an additional mechanism, namely, Nrf2 pathway to prevent oxidative stress damage (Martin et al. 2010). It should also be noted that hydroxytyrosol exists in the brain as an endogenous neurotransmitter, such as dopamine and norepinephrine, which are formed via monoamino oxidase-catalyzed deamination and subsequent reduction. Therefore, it is likely that hydroxytyrosol exerts endogenous antioxidant activity in the brain tissue by interacting selectively within signaling cascades, such as tyrosine kinase, PtdIns 3-kinase/Akt, PKC and MAP kinase pathways. These pathways regulate cell survival following exposure to oxidative stress (Visioli et al. 2000).

Health benefits of Mediterranean diet are not only related to the presence of oleic acid and phenolic compounds, but also other components, such as cereals, grains, fish, nuts, fruits, vegetables, and red wine, which are rich in phenols, flavonoids, isoflavonoids, phytosterols, and phytic acid—essential bioactive compounds providing health benefits. Oleic acid is converted into nitrated oleic acid (nitro-oleic acid) in the presence of nitric oxide (NO[•]). Nitrated oleic is found in the plasma and several tissues including brain, where it not only inhibits neuroinflammation, but also promotes blood vessel relaxation through modulation of macrophage activation and prevention of leukocyte and platelet activation (Trostchansky and Rubbo 2008). Phenolic components of olive oil contribute to lower rates of cardiovascular disease,

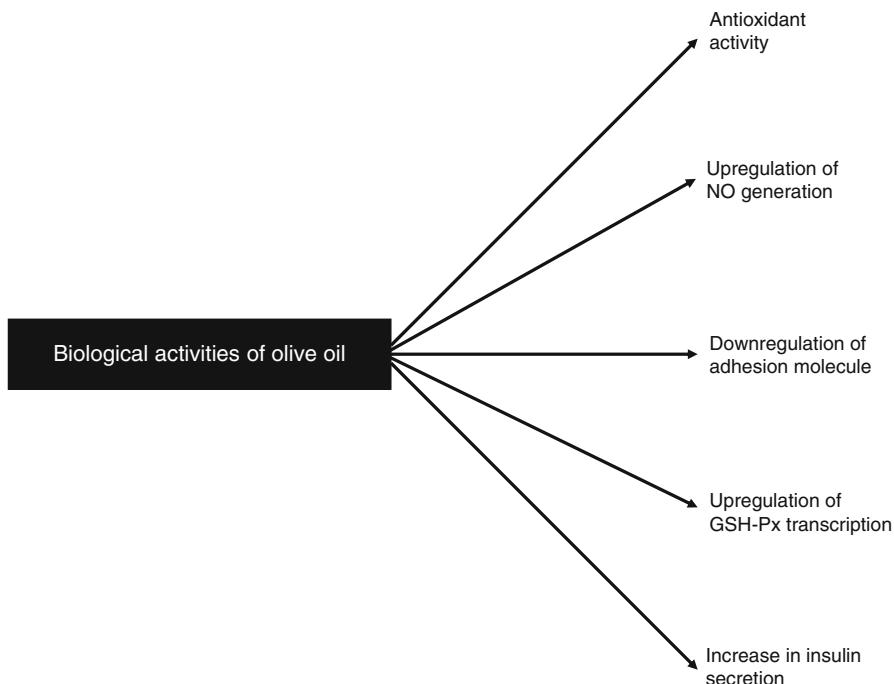


Fig. 2.5 Biological activities of extra virgin olive oil

cancer, and age cognitive decline (Perez-Jimenez et al. 2005; Lopez-Miranda et al. 2007). Olive oil also decreases blood pressure and protects from diabetes. Olive oil not only provides the higher percent of energy but the presence of bioactive polyphenolic compounds promotes human health not only by their antioxidant activities, but also through upregulation of nitric oxide synthase and glutathione peroxidase and increase in insulin secretion (Fig. 2.5). As mentioned earlier, the presence of one double bond and polyphenolic antioxidants increases the shelf life of olive oil compared to other vegetable oils. These phenols have many beneficial effects on human neurovascular and cardiovascular systems.

2.2.2 *Biochemical Effects of Olive Oil Phenolic Compound on Heart*

As stated before, olive oil contains tyrosol [2-(4-hydroxyphenyl)ethanol], hydroxytyrosol, oleuropein, and oleocanthal (Fig. 2.3), and has a balanced ratio of monounsaturated and polyunsaturated fatty acids (Ruano et al. 2005; Covas et al. 2006; Carluccio et al. 2007). Supplementation of olive oil in human diet improves the

Table 2.1 Fatty acid and nonfatty acid components of extra virgin olive oil

Saponifiable components and reference	Nonsaponifiable components and reference	Nonfatty components and reference
Oleic acid (Granados-Principal et al. 2010)	Nonglycerides (Granados-Principal et al. 2010)	Tyrosol (Bendini et al. 2007)
Palmitic acid (Granados-Principal et al. 2010)	Aliphatic alcohol (Granados-Principal et al. 2010)	Hydroxytyrosol (Bendini et al. 2007)
Linoleic acid (Granados-Principal et al. 2010)	Triterpene alcohol (Granados-Principal et al. 2010)	Oleuropein (Bendini et al. 2007)
Stearic acid (Granados-Principal et al. 2010)	Sterols (Granados-Principal et al. 2010)	Oleocanthal (Bendini et al. 2007)
Palmitoleic acid (Granados-Principal et al. 2010)	Carotenoids (Granados-Principal et al. 2010)	Apignin (Bendini et al. 2007)
Linolenic acid (Granados-Principal et al. 2010)	Squalene (Granados-Principal et al. 2010)	Luteolin (Bendini et al. 2007)
Myristic acid (Granados-Principal et al. 2010)	Pigments (Granados-Principal et al. 2010)	Pinoresinol (Bendini et al. 2007)
–	–	Gallic acid (Bendini et al. 2007)

major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism, and antithrombotic profile (Perez-Jimenez et al. 2005). In addition, olive oil contains many other components, such as phenolic acids, lignans, and flavonoids (Table 2.1), which may promote many beneficial effects on human health. Multiple mechanisms have been proposed to explain beneficial effects of Mediterranean diet. These mechanisms include: decrease in LDL-cholesterol, increase HDL-cholesterol, and reduction of oxidative stress due to polyphenols and flavonoids, which may act as scavengers and protect heart tissue and LDL from free radical damage. Components of Mediterranean diet diminish NF-κB activation in mononuclear cells compared to Western diet (Pérez-Jiménez et al. 2007). Thus, extra virgin olive oil extracts inhibit the translocation of NF-κB subunits in both unstimulated and phorbol-myristate acetate (PMA)-stimulated monocytes and monocyte-derived macrophages (Brunelleschi et al. 2007) (Fig. 2.6). This effect occurs at concentrations of tyrosol and hydroxytyrosol found in human plasma after nutritional ingestion of extra virgin olive oil and is quantitatively similar to the effect exerted by ciglitazone, a PPAR-γ ligand. However, extra virgin olive oil extract has no effect on PPAR-γ expression in monocytes. These results support the view that the beneficial effects of extra virgin olive oil are due to its ability to inhibit NF-κB activation in human monocyte/macrophages (Brunelleschi et al. 2007). In RAW 264.7 macrophages, tyrosol not only inhibits exogenous ROS-mediated [³H]arachidonic acid (ARA) release, but also alters PMA-mediated nitric oxide generation (Moreno 2003). Oleuropein, an antioxidative and anti-ischemic compound found in extra virgin olive oil inhibits the adhesion of monocyte cells to the blood vessel lining, a process closely associated with the development of atherosclerosis. Another beneficial component of olive oil is oleuropein, a member of the secoiridoid family, which is hydrolyzed into the hydroxytyrosol and functions as a

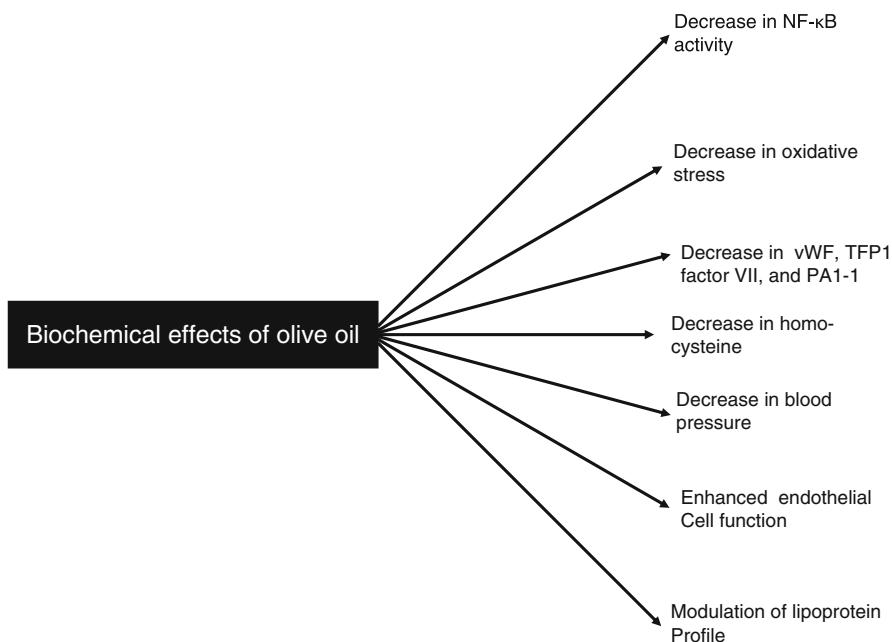


Fig. 2.6 Neurochemical activities of extra virgin olive oil

hydrophilic phenolic antioxidant that is oxidized to its catechol quinone during redox cycling (Cornwell and Ma 2008). Little is known about the biological properties of the catechol hydroxytyrosol quinone, a strong arylating electrophile that forms Michael adducts with thiol nucleophiles in glutathione and proteins. These properties may contribute to the unique nutritional benefits of olive oil. Oleocanthal is another olive oil component that inhibits cyclooxygenases, enzymes responsible for the oxidation of ARA into prostaglandins and thromboxanes. This property is similar to ibuprofen, a nonsteroidal drug with anti-inflammatory, analgesic, and antipyretic properties (Beauchamp et al. 2005; Smith et al. 2007). Diet enriched in virgin olive oil reduces the sensitivity of platelets to aggregate by decreasing von Willebrand and thromboxane B₂ plasma levels (Perez-Jemenez et al. 2006; Ruano et al. 2007). Olive oil components not only decrease activities of vWF, TFP1, and PA1-1 factors, but also decrease homocysteine levels, enhance endothelial cell function, and modulate lipoprotein profiles (Fig. 2.6). The ability of extra virgin olive oil to decrease fasting factor VII (proconvertin) in plasma results in the modulation of postprandial activation, a process which is important in relation to its heart protective effect (Lopez-Miranda et al. 2007). In addition, extra virgin olive oil also prevents inflammation by inhibiting platelet activating factor, a lipid mediator that plays an important role not only in clotting process, but also by activating immune cells and their binding to the endothelial wall (Karantonis et al. 2006). Hydroxytyrosol and oleuropein are potent scavengers. They scavenge superoxide anion and other reactive

species (peroxynitrite, hypochlorous acid) possibly implicated in the onset of heart disease. Moreover, hydroxytyrosol and oleuropein are also capable to modulate enzymic processes (Visioli et al. 2002). For example, hydroxytyrosol has been shown to inhibit platelet aggregation, suggesting it has antithrombotic potentials (Correa et al. 2009). Accumulating evidence indicates that olive oil components interfere with the inflammatory response within atherosclerotic lesion by blocking endothelial activation and inhibiting inflammatory cytokines production and secretion in macrophages, and modulating matrix-degrading enzymes. Accumulating evidence suggests that olive oil in Mediterranean diet not only improves the endothelium-dependent vasodilatory response and stabilizes local vascular tone, but also produces antithrombotic and anti-inflammatory effects through the oleocanthal-mediated inhibition of COX-1 and COX-2 (Bogani et al. 2007; Beauchamp et al. 2005). In addition, olive oil intake also modulates immune function. These processes improve vascular stability (Carluccio et al. 2007; Ruano et al. 2005; Fuentez et al. 2008).

NO^{\cdot} is a free radical signaling mediator generated by the healthy endothelium to maintain vascular homeostasis through the regulation of blood pressure and leukocyte–platelet activation. Elevation of NO^{\cdot} levels is accompanied by its rapid transformation into potent nitrating and nitrosating species, including peroxynitrite (ONOO^{-}), nitrogen dioxide (NO_2), and nitrous acid (HONO). These species can react with unsaturated lipids, forming both oxidized and nitrated products, including nitro, nitrito, and nitroepoxy derivatives (Rubbo et al. 1994). The reaction of oleic acid with NO^{\cdot} - and nitrite (NO_2^{-})-derived species yields nitrated oleic acid (Villacorta et al. 2007). Although the mechanisms of biological fatty acid nitration remain incompletely characterized, recent studies reveal that during oleic acid nitration, vinyl nitro regiosomers represent a component that displays distinctive chemical reactivity and receptor-dependent signaling actions (Villacorta et al. 2007). Similarly, the molecular mechanisms associated with biochemical actions of nitrated oleic acid remain unknown. However, it is becoming increasingly evident that nitrated derivatives of oleic and linoleic acid inhibit leukocyte and platelet activation (Coles et al. 2002), vascular smooth muscle proliferation (Villacorta et al. 2007), lipopolysaccharide-stimulated macrophage cytokine secretion (Cui et al. 2006) (17), activate peroxisome proliferator-activated receptor- γ (Schopfer et al. 2005a), and induce endothelial heme oxygenase 1 expression (Wright et al. 2006) (Fig. 2.7). NO_2 -fatty acid also potently modulates nuclear factor-erythroid 2-related factor 2/Kelch-like ECH-associating protein 1 (Nrf2/Keap1) (Villacorta et al. 2007; Cui et al. 2006) and nuclear factor κB (NF κB)-regulated inflammatory signaling (Cui et al. 2006). Nrf2 governs the expression of ARE-regulated genes (Nguyen et al. 2003). Under physiological conditions, Nrf2 is normally retained in the cytoplasm by the repressor protein Keap1 (Nguyen et al. 2003). Keap1 contains highly reactive sulphydryl groups and acts as a cellular sensor that recognizes electrophilic inducers (Wakabayashi et al. 2004). In response to oxidative stress or glutathione depletion or nitrosative stress, such as generation of nitrated oleic acid, Nrf2/Keap1 complex dissociates, and Nrf2 translocates to the nucleus where it interacts with ARE and coordinates transcription of a collection of cytoprotective and detoxification genes, such as heme oxygenase-1 (Prester et al. 1995), glutathione-S-transferases (GSTs),

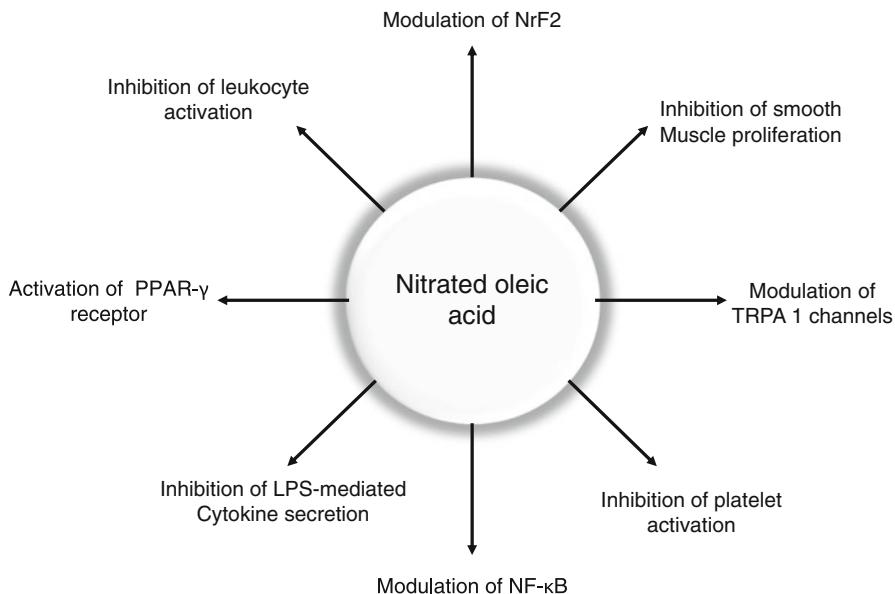


Fig. 2.7 Biolchemical activities of nitrated oleic acid

NADH quinone oxidoreductase, γ -glutamylcysteine ligase (γ -GCL) (Prestera and Talalay 1995), and NAD(P)H:quinone oxidoreductase 1 (Li and Jaiswal 1992). These enzymes provide efficient cytoprotection, in part, by regulating the intracellular redox state. Their induction contributes to protection from a variety of toxins in a variety of cells including neuronal and astrocytic cultures (Shih et al. 2003). It is also reported that micellar and membrane stabilization of nitrated fatty acid prevents NeF-like aqueous decay reactions and consequent NO^{\bullet} release, supporting that the predominant signaling actions mediated by nitrated fatty acids are NO^{\bullet} and cGMP independent (Schopfer et al. 2005b; Lima et al. 2005) (Fig. 2.8). In addition, xanthine oxidoreductase (XOR), a molybdoflavin protein that serves as the rate-limiting enzyme in the terminal steps of purine degradation in humans, catalyzing the oxidation of hypoxanthine to xanthine and finally to uric acid is irreversibly inhibited by nitrated oleic acid. During inflammatory process, reversible oxidation of critical cysteine residues or limited proteolysis converts XDН to xanthine oxidase (XO), which reduces O_2 to superoxide and hydrogen peroxide (H_2O_2) (Harrison 2002). Conversion to XO is not required for reactive oxygen species (ROS) generation because xanthine dehydrogenase displays partial oxidase activity (Harris and Massey 1997). The generation of ROS in the vascular compartment enhances redox-dependent signaling supporting a key role of XOR in oxidative stress and inflammatory processes. It should be noted that despite the significant advances on health benefits of olive oil in heart disease, the molecular mechanism(s) associated

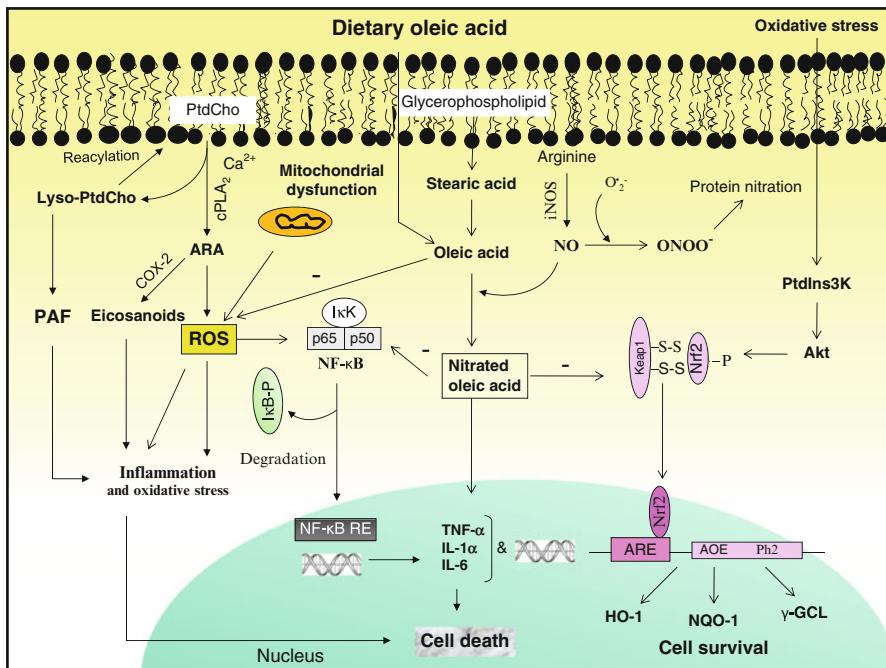


Fig. 2.8 Regulation of oxidative stress and inflammation along with modulation of NF-κB and Nrf2 by nitrated oleic acid. Phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); arachidonic acid (ARA); platelet-activating factor (PAF); cytosolic phospholipase A₂ (cPLA₂); cyclooxygenase-2 (COX-2); reactive oxygen species (ROS); nuclear factor kappaB (NF-κB); nuclear factor κB-response element (NF-κB-RE); inhibitory subunit of NF-κB (IκB); phosphorylated IκB (IκB-P); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); superoxide (O_2^-); and NFE2-related factor 2 (Nrf2); heme oxygenase 1 (HO-1); NADH quinone oxidoreductase, γ-glutamylcysteine ligase (γ-GCL)

with beneficial effects of olive oil still remain(s) speculative. This suggests that more studies are needed on the health benefits of olive oil.

2.2.3 Biochemical Effects of Olive Oil Phenolic Compound on the Brain

Most fatty acids enter the brain from the blood through blood-brain barrier (BBB), which is a complex cellular system formed by specialized endothelial cells that line cerebral capillaries, together with perivascular elements such as closely associated astrocytic end-feet processes, perivascular neurons, and pericytes. The primary function of the BBB is to create ionic homeostasis for neuronal and glial cells functions, supplement the brain with nutrients, and protect it from toxic insults by

sophisticated transport systems. Studies on the transport of [$1-^{14}\text{C}$]oleic acid in primary human brain microvessel endothelial cells (HBMEC) indicate that transport of oleic acid is increased significantly in the presence of bovine serum albumin (Mitchell et al. 2009). The inclusion of nonspecific fatty acid uptake inhibitor, phloretin significantly decreases [$1-^{14}\text{C}$]oleic acid uptake by HBMEC. Similarly, knocking down of fatty acid transport protein-1 or fatty acid translocase/CD36 significantly also decreases [$1-^{14}\text{C}$]oleic acid transport across the HBMEC monolayer from either apical as well as basolateral sides. These findings support the view that a fatty acid acceptor is also associated with the oleic acid transport across HBMEC monolayers (Mitchell et al. 2009). Thus, fatty acids (including oleic acid) enter brain through BBB as fatty acid/albumin complex, fatty acid transport protein-1, and to lesser extent from circulating lipoproteins. Acyl-CoA synthetases trap fatty acid by forming acyl-CoA, which cannot diffuse out of the cell. Selection and incorporation of fatty acid into phospholipids is controlled largely by enzymes of Lands cycle, which begins with the acyl-CoA synthetase (Farooqui et al. 2000; Hamilton and Brunaldi 2007). Albumin not only activates the sterol regulatory element-binding protein-1, but also upregulates stearoyl-CoA 9-desaturase mRNA (Tabernero et al. 2002; Polo-Hernandez et al. 2010). Furthermore, when the activity of sterol regulatory element-binding protein-1 is inhibited by the overexpression of a truncated form of this protein, albumin produces no effect on stearoyl-CoA 9-desaturase mRNA, indicating that the effect of albumin is mediated by this transcription factor. The stimulation by albumin can be prevented either by retarding traffic to the endoplasmic reticulum or adding albumin–oleic acid complex. In addition, oleic acid also induces the expression of microtubule associated protein-2 (MAP-2), a marker of dendritic differentiation (Rodríguez-Rodríguez et al. 2004; Polo-Hernandez et al. 2010). The time course of MAP-2 expression during brain development coincides with that of stearoyl-CoA desaturase, the limiting enzyme of oleic acid synthesis, suggesting that both phenomena coincide during development. The effect of oleic acid on MAP-2 expression is most probably independent of autocrine factors synthesized by neurons (Rodríguez-Rodríguez et al. 2004), and exogenous or endogenous oleic acid by astrocytes exerts its neurotrophic effect through a protein kinase C-dependent mechanism. This effect can be prevented by sphingosine or two myristoylated peptide inhibitors of protein kinase C (Rodríguez-Rodríguez et al. 2004). Thus, during brain development, the presence of albumin plays an important role by triggering the synthesis and release of oleic acid by astrocytes, which induces neuronal differentiation through its interactions with transcription factor NeuroD2 (Tabernero et al. 2001, 2002; Rodríguez-Rodríguez et al. 2004).

In developing rat brain, oleic acid is synthesized from stearic acid by astrocytes via stearoyl-CoA desaturase catalyzed reaction. It is released from astrocytes and utilized by neurons for the synthesis of neural membrane phospholipids. It specifically incorporates into growth cones. In developing brain, oleic acid promotes axonal growth, neuronal clustering, and expression of the axonal growth-associated protein-43 (GAP-43), indicating that this fatty acid facilitates neuronal differentiation. The effect of oleic acid on GAP-43 synthesis is mediated through the activation of protein kinase C (PKC) and is blocked by PKC inhibitors, such as H-7, polymyxin,

or sphingosine. The expression of GAP-43 is significantly increased when neurons are co-cultured with astrocytes in the presence of albumin (Tabernero et al. 2001; Polo-Hernandez et al. 2010).

Long-term consumption of refined- (ROO) and pomace- (POO) olive oil not only modulates brain fatty acid composition in apolipoprotein E (apoE) knockout (KO) mice, but also reduces the level of arachidonic and eicosapentaenoic acid, suggesting a decrease in the generation of pro- and anti-inflammatory eicosanoids (Alemany et al. 2009). The consumption of ROO and POO also influences the levels of pivotal membrane proteins implicated in the activation of PKA and PKC, supporting the view that ROO and POO produce positive effects on neuroinflammation and brain function. The combination of these two molecular effects might convert ROO and POO oils into valuable functional foods in diseases involving apoE deficiency (Alemany et al. 2009). The beneficial effects of olive oil on brain function are not only due to its antioxidative phenolic compounds, but also due to its high content of monounsaturated fatty acids, i.e., oleic acid (Perez-Jimenez et al. 2006), which has only one double bond, therefore an oleic acid-rich neural membrane will be less fluid than a membrane rich in linoleic acid, which has two double bonds. The main mechanism by which the components of olive oil modulate neural membrane and endothelial cell function involves inhibition and/or scavenging of ROS. Oleic acid and β -sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. β -Sitosterol may also enhance SOD activity, hence decreasing superoxide levels (González-Correa et al. 2007). In addition, oleuropein and oleanolic acid and minor components of olive oil may act directly on cyclooxygenases and lipoxygenases, which are inhibited at different points by phenolics and triterpenoids. Similarly, IL-1 β expression is also inhibited by phenolic components of olive oil, contributing to neuroprotection and protection of endothelium against vasoconstriction, platelet aggregation, and monocyte adhesion (González-Correa et al. 2007).

In the brain, oleic acid is also converted into nitrated oleic acid. This metabolite is a highly reactive electrophilic compound that can modulate a variety of cellular targets, including thiol residues and peroxisome proliferator-activated receptor γ (Freeman et al. 2008; Jain et al. 2008; Trostchansky and Rubbo 2008). It is proposed that esterified nitrated fatty acids represent a sink of bioactive mediators, which are produced during nitritative stress leading to cellular dysfunctions after its release from the membrane by phospholipase A₂ (Jain et al. 2008). Free nitrated oleic acid is a stimulator of somatosensory and visceral nociceptors. It acts through the selective and direct activation of Transient Receptor Potential A1 (TRPA1) channels in a concentration-dependent manner (Taylor-Clark et al. 2009; Andersson et al. 2008). Although the role of nitrated oleic acid in neurodegenerative diseases is not fully understood, several studies indicate that 9- and 10-nitro-9-*cis*-octadecenoic acid is a potent ligand for peroxisome proliferator activated receptors (PPAR) at physiological concentrations (Taylor-Clark et al. 2009; Baker et al. 2005). PPAR- γ agonists prevent A β neurotoxicity in hippocampal neurons. In addition, based on concentration-response analysis in both neurons and hTRPA1-HEK cells, it is suggested that nitrated oleic acid is the most potent endogenous Transient receptor

potential cation channel, subfamily A1 (TRPA1) agonist. Oleoylethanolamide (OEA), the naturally occurring amide of ethanolamine and oleic acid interacts with peroxisome-proliferator-activated receptor alpha (PPAR α), which is involved in feeding regulation and it has been proposed to play a role in sleep modulation (Soria-Gómez et al. 2010). The peripheral administration of OEA reduces food intake and increases waking with a concomitant reduction of rapid eye movement sleep. In addition, OEA treatment produces deactivation of the lateral hypothalamus, as inferred from the c-Fos expression and intralateral hypothalamus injections of OEA produce effects similar to the peripheral administration (Soria-Gómez et al. 2010). Oleic acid and OEA inhibit LPS-mediated production of NO and prostaglandin E₂ as well as expression of iNOS and COX-2 by blocking LPS-mediated NF- κ B activation and phosphorylation of inhibitor κ B kinase (IKK) in BV2 microglia (Oh et al. 2009, 2010). OEA inhibits LPS-mediated phosphorylation of Akt, p38 MAPK, and ERK; activation of PtdIns 3-kinase; and accumulation of reactive oxygen species (ROS). The effect of OEA can be blocked by AM630, a specific antagonist of the CB2 receptor. It is proposed that oleic acid and OEA show an anti-inflammatory effect through inhibition of NF- κ B activation in LPS-stimulated BV2 microglia. Emerging evidence suggests that oleic acid, nitrated oleic acid, and OEA comprise a novel class of lipid mediators, which interact with various signal transduction pathways to modulate cell signaling. Although it is not known, but it is likely that their levels are altered in neurological disorders, which are accompanied not only by a higher degree of ROS and NO[•] production, but also by diminished functions of mitochondria, endoplasmic reticulum, and the proteasome system, which are responsible for the maintenance of the normal protein homeostasis of neural cells (Moncada and Bolanos 2006; Farooqui 2009).

2.3 Effect of Oleic Acid on Neurological Disorders

With an increase in lifespan and changing population demographics, the incidence of neurological disorders is expected to increase significantly in the twenty-first century. The most challenging neurological disorders are neurodegenerative diseases, which are accompanied by age-related gradual decline in neurological function and characterized by neurodegeneration in specific area of the human brain (Farooqui 2010). Examples of neurodegenerative disorders include Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS). Although considerable information is available on potential mechanisms and pathology of neurodegenerative diseases, successful treatment strategies for neurodegenerative diseases have so far been limited because most available treatments are symptomatic and not directed towards the cause of neurodegeneration (Farooqui 2010). Most of neurodegenerative diseases are multifactorial and accompanied by oxidative stress, neuroinflammation, abnormalities in

immune system, BBB abnormalities, and accumulation of disease proteins, such as τ , β -amyloid in AD, α -synuclein in PD, and huntingtin in HD. The dysfunction of BBB is accompanied by the disruption of tight junctions, alterations in transport of molecules (plasma proteins) between blood and brain and brain and blood, aberrant angiogenesis, vessel regression, brain hypoperfusion, and changes in inflammatory responses. These processes may contribute to a “vicious circle” that leads to progressive synaptic loss and neurodegeneration in neurodegenerative diseases (Zlokovic 2008). A common feature of neurodegenerative diseases is a long course until sufficient protein accumulates, followed by a cascade of symptoms over many years with increasing disability leading to death (Jellinger 2009; Farooqui 2010).

Ingestion of olive oil not only modulates oxidative stress, with but also influences immune function, particularly the inflammatory processes associated with the immune system. Olive oil is a nonoxidative dietary component, and the attenuation of the inflammatory process by olive oil can be explained by its modulatory effects on oxidative and inflammatory stresses, which are closely associated with the pathogenesis of neurodegenerative diseases in man. The antioxidant effects of olive oil are probably due to a combination of its high oleic acid content (low oxidation potential compared with linoleic acid) and its content of a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, and tyrosol. The generation of nitrated oleic acid under nitrosative stress can inhibit neuroinflammation by blocking NF- κ B activation and prevent oxidative stress by stimulating transcription factor NrF2. In addition, neuroinflammation in neurological disorders may elicit pain, which may be mediated by the activation of somatosensory and visceral nociceptive sensory nerves. Recently, a number of phytochemicals, such as allyl isothiocyanate, cinnamaldehyde, and phytocannabinoids have been shown to activate the transient receptor potential (TRP) ion channel family of receptors (TRPA1 receptors) (Bandell et al. 2004; De Petrocellis et al. 2008). Ingestion of olive oil and the synthesis of nitrated oleic acid may decrease the intensity of pain through the activation of nociceptive neurons via TRPA1 receptor-mediated process (Bautista et al. 2006). It is also possible that high oleic acid content and a proportionate reduction in linoleic acid intake would allow a greater conversion of α -linolenic acid (18:3n-3) to longer-chain n-3 PUFA, which may produce beneficial effects in neurodegenerative diseases (Farooqui 2009, 2010).

2.3.1 Beneficial Effects of Olive Oil Components in Alzheimer Disease

It is well known that AD is accompanied by dementia that typically begins with subtle and poorly recognized failure of memory and slowly becomes more severe. Age is the most important factor that predisposes persons to the nonfamilial form of the disease. Brain from AD patient contains fewer synapses and reduced levels of synaptic proteins and neural membrane phospholipids (Farooqui 2010).

The two classical pathological hallmarks of AD include the deposition of aggregated A β peptide and neurofibrillary tangles (NFTs) composed of hyperphosphorylated τ protein, a microtubule (MT) associated protein, which fibrillizes and aggregates into neurofibrillary tangles. Multiple mechanisms, such as genetic mutations, posttranslational modifications, and intracellular environmental changes have been described to produce tau misfolding and fibrillization to form NFTs, which bear the properties of amyloid deposits (Lee et al. 2001). NFTs accumulation also occurs in tauopathies. Olive oil component, oleocanthal, the dialdehydic form of (-)-deacetoxy-ligstroside aglycone, inhibits tau fibrillization by interacting with the T40 and MT-binding region K18 of tau protein (Li et al. 2009). Using PHF6 consisting of the amino acid residues VQIVYK, a hexapeptide within the third repeat of tau that is essential for fibrillization, it is shown that oleocanthal forms adduct with the lysine residues via initial Schiff base formation and thereby inhibits tau fibrillization (Li et al. 2009). Based on detailed investigation using Fourier transform infrared (FTIR) spectroscopy, it is demonstrated that oleocanthal reacts with tau in the random coil form and prevents its conversion to the β -pleated sheet conformation. Structural activity studies of a series of oleocanthal analogues suggest that both aldehyde functional groups are essential for the inhibitory activity of oleocanthal (Li et al. 2009). Oleocanthal also inhibits the fibrillization of both A β 40 and A β 42 in vitro (Li et al. 2009). The similarities between the amyloidogenic proteins A β and tau in forming β -sheet strands and inhibition of fibrillization by oleocanthal further support the existence of a common mechanism of fibril formation. Other NSAIDs, including aspirin, naproxen, flurbiprofen, and indomethacin failed to block tau fibrillization at concentration up to 100 μ M. Thus, the inhibitory effect of oleocanthal on tau fibril formation may represent a novel activity not shared by other NSAIDs (Li et al. 2009). It is also shown that oleocanthal has the capacity to alter the oligomerization state of β -amyloid (A β) oligomers while protecting neurons from the synaptopathological effects of A β . Thus, oleocanthal protects neurons from A β -induced synaptic deterioration (Pitt et al. 2009). Oleic acid also reduces secreted A β levels in amyloid precursor protein (APP) 695 transfected Cos-7 cells (Amtul et al. 2010). These findings are supported by results obtained in an early onset AD transgenic mouse model expressing the double-mutant form of human APP, Swedish (K670N/M671L) and Indiana (V717F) fed with a high-protein, low-fat (18 % reduction), cholesterol-free diet enriched with oleic acid. These mice have been reported to show an increase in A β 40/A β 42 ratio, reduced levels of β -site APP cleaving enzyme (BACE), and reduced presenilin levels along with reduced amyloid plaques in the brain. The decrease in BACE levels is accompanied by increase in levels of a nonamyloidogenic soluble form of APP (sAPP α). Furthermore, the low-fat/+OA diet produces an augmentation of insulin-degrading enzyme and insulin-like growth factor-II. These results support the view that oleic acid supplementation and cholesterol intake restriction in a mouse model of AD reduce AD-type neuropathology in early onset AD transgenic mouse model (Amtul et al. 2010).

2.3.2 Beneficial Effects of Olive Oil Components on Hypoxic Injury

Studies on the effect of olive oil components (Hydroxytyrosol) in a model of hypoxia–rexygenation in rat brain slices indicate that hydroxytyrosol (5 and 10 mg/kg per day p.o.) reduces lactate dehydrogenase (LDH) efflux by 37.8 % and 52.7 %, respectively (González-Correa et al. 2008), supporting the view that this olive oil components modify processes related to thrombogenesis in brain hypoxic injury. These components reduce oxidative stress and modulate the inducible isoform of nitric oxide synthase, diminishing platelet aggregation, and protecting the brain from the effects of hypoxia–rexygenation (González-Correa et al. 2007). Similarly, in the transient middle cerebral artery occlusion rat model (2 h of occlusion, 22 h of reperfusion), tyrosol treatment results in a dose-dependent neuroprotective effect (Bu et al. 2007). In an in vivo study of rat cerebral ischemia–reperfusion injury, oral administration of olive oil reduces infarct volume, brain edema, BBB permeability, and improves neurologic deficit scores after transient middle cerebral artery occlusion in rats (Mohagheghi et al. 2010).

2.3.3 Beneficial Effects of Olive Oil Components on Atherosclerosis

Atherosclerosis disrupts neuronal signaling pathways by altering lipid composition of neural membranes. This process may facilitate neuroinflammation and oxidative stress. Studies on the effect of refined olive oil (ROO) and pomace- (POO) olive oil in the brain of apolipoprotein E (apoE) knockout (KO) mice for 11 weeks indicate that incorporation of these oils increase the proportions of oleic acid neural membranes while levels of the saturated fatty acids (palmitic and stearic acid) are decreased (Alemany et al. 2010). This results in a higher MUFA:SFA ratio in apoE KO mice brain. Furthermore, both oils reduce the level of arachidonic and eicosapentaenoic acid, indicating a decrease in the generation of pro- and anti-inflammatory eicosanoids. In addition, refined olive oil and pomace olive oil increase the density of membrane proteins, implicating both the G α s/PKA and G α q/PLC β 1/PKC α signaling pathways (Alemany et al. 2010). The combined long-term effects of consumption of refined olive oil and pomace olive oil on neural membrane fatty acid composition and the level of signaling proteins associated with PKA and PKC activation suggest positive effects on neuroinflammation and brain function in apoE KO mice brain, and conversion of these oils into promising functional foods in diseases involving apoE deficiency (Alemany et al. 2010).

2.3.4 Beneficial Effects of Olive Oil Components on Brain Tumor

Astrocytomas are among the most common and aggressive type of primary malignant tumors in the neurological system lacking effective treatments despite the use of multimodal drug regimens. Studies on the effect of olive oil components (uvaol and erythrodiol) on the human 1321N1 astrocytoma cell line indicate that uvaol and erythrodiol inhibit 1321N1 cells proliferation in a time- and dose-dependent manner (Martin et al. 2009). This inhibition is associated with the induction of apoptosis. The effect of uvaol and erythrodiol on 1321N1 cells is accompanied by the appearance of apoptosis-specific hallmarks, such as redistribution of cells into the subdiploid phase of the cell cycle, translocation of phosphatidylserine to the outer leaflet of the cellular membrane, fragmentation of nuclei, and production of ROS, which are accompanied by the fall in $\Delta\Psi_m$ (Martin et al. 2009). Emerging evidence suggests that natural alcoholic triterpenes (uvaol and erythrodiol) are powerful inhibitors of cell growth and efficient apoptotic killing agents. These components can be used to develop the treatment of astrocytomas. Similarly, studies on the antitumoral effects of oleanolic acid and maslinic acid on human astrocytoma cell lines also indicate that oleanolic acid and maslinic acid inhibit DNA synthesis and induce apoptosis in human 1321N1 astrocytoma cells in a dose-dependent manner (Martin et al. 2007). This conclusion is based on typical apoptotic morphologic features, such as translocation of plasma membrane phosphatidylserine and activation of caspase-3 supporting the view that this type of cell death is caused by apoptosis.

2.4 Conclusion

The phenolic compounds of olive oil and oleic acid have bioavailability in humans. The high bioavailability of oleic acid and phenolic compounds lends support to the evidence that nitrated oleic acid and phenolic components exert beneficial effects on human health. Although the beneficial health effects of virgin olive oil ingestion are well known, it is only recent that the biological properties of nitrated oleic acid and olive oil phenolic compounds have been investigated. Olive oil phenolic compounds have been shown to beneficially alter lipid composition, platelet and cellular function, as well as reduce oxidative stress and neuroinflammation. Their beneficial effects are supported by the low rate of brain-related diseases among populations residing in the Mediterranean region. For example, the antiatherogenic effects of olive oil may explain the low rate of heart disease and stroke in Mediterranean populations. The anti-inflammatory effects that arise from the ingestion of olive oil phenolic compounds have been shown to provide protection against diseases marked by an inflammatory component. These biological properties may have a significant impact on population health through the reduction in incidence of chronic neurodegenerative disease development.

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Chapter 3

Beneficial Effects of Flaxseed Oil (n-3 Fatty Acids) on Neurological Disorders

3.1 Introduction

Flaxseeds are not only the richest plant source of α -linolenic acid (ALA, 18:3n-3) and the phytohormone lignans, but also an essential source of high-quality protein and dietary fiber. Whole flaxseed contains 41 % oil by weight, of which 70 % is polyunsaturated; more than half of the total fatty acid is ALA (Fig. 3.1) (Bhatty 1995). ALA is also found in soybeans, rapeseed, walnuts, and dark green leafy vegetables (kale, spinach, broccoli, and Brussels sprouts). Potential health benefits of flaxseeds for cardiovascular disease, neurological disorders, and cancer are related to high ALA contents, vegetable protein, soluble fiber, and flavonoids and related compounds, which may not only possess cholesterol-lowering, hyperlipidemic, and antioxidant properties, but may also produce sex hormone agonistic and antagonistic activities (de Lorgeril et al. 1994; Jenkins et al. 1999). Flaxseed lignans also promote the reduction of serum total cholesterol and low-density lipoprotein cholesterol and elevates serum high-density lipoprotein cholesterol. ALA in flaxseed oil does not have antioxidant activity except it suppresses oxygen radical production by white blood cells. In cardiovascular system, flaxseeds and flaxseed oil have variable effects on inflammatory mediators/markers (interleukin, IL-1 β , IL-2, IL-4, IL-6, IL-10, tumor necrosis factor- α (TNF- α), interferon- γ , C-reactive protein, and serum amyloid protein). Although doses of ALA less than 14 g/d do not affect inflammatory mediators/markers, 14 g/d or greater reduce inflammatory mediators/markers. ALA in flaxseed oil decreases soluble vascular cell adhesion molecule-1 but has no effect on soluble intracellular adhesion molecule-1, soluble E-selectin, and monocyte colony-stimulating factor. ALA in flaxseeds has a very small hypotensive effect, but it does not lower blood pressure. However, secoisolariciresinol diglucoside (SDG), a component of flaxseed oil, is a very potent hypotensive agent. Flaxseed oil also decreases platelet aggregation and increases platelet activating inhibitor-1 and bleeding time. A meta-analysis of observational studies indicates that increased consumption of ALA may reduce coronary heart disease mortality by 21 % (Brouwer et al. 2004). In the Lyon Diet Heart Study, a randomized controlled

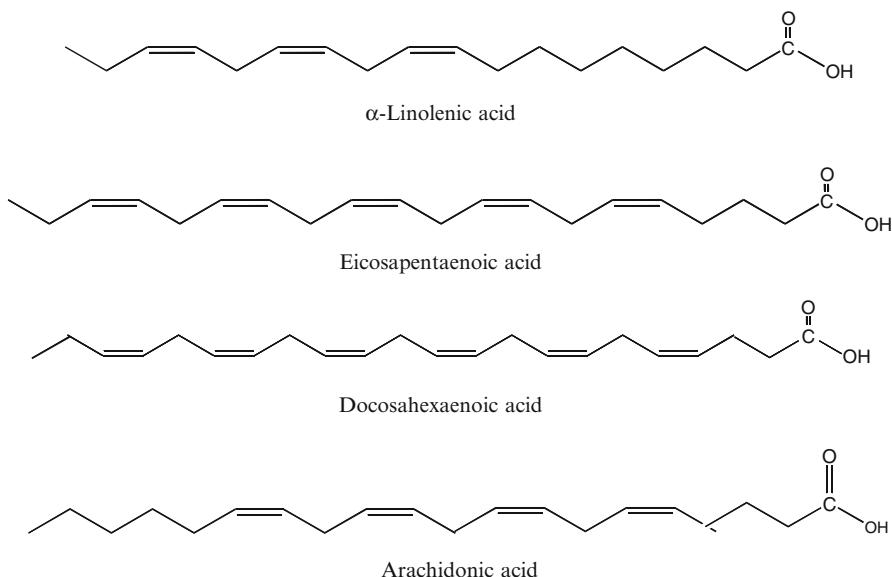


Fig. 3.1 Chemical structures of n-3 fatty acids

trial in coronary patients, consumption of a Mediterranean-type diet that included an additional daily intake of roughly 1 g of ALA significantly reduces the risk of cardiac death and nonfatal myocardial infarction by more than 60 % (Lorgeril et al. 1994). This study, however, was not specifically designed to assess the effect of ALA supplementation, and many dietary factors were included, which differed between the experimental and control group. Collective evidence suggests that ALA status has been inversely associated with cardiovascular disease events, although data are less consistent than for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The biologic mechanisms of ALA action may include platelet function, inflammation, endothelial cell function, arterial compliance, and arrhythmia.

3.2 Absorption of α-Linolenic Acid in the Gut and Transport into the Brain

After ingestion, the overall process of lipid absorption involves lipolysis, solubilization, uptake into the enterocyte, reesterification, and transport into the lymph or portal blood. The relative importance of each step not only depends strongly on the dietary fatty acid species, but also on the membrane structure of the intestine. Very little information is available on the absorption of ALA under physiological conditions in man. In general, the uptake of essential fatty acids (EFA) and long-chain fatty acids (LCPUFA) involves membrane bound fatty acid binding protein(s),

namely FABP and/or a fatty acid translocase (Carey and Hernell 1992; Stremmel 1988; Abumrad et al. 1993; Goré and Hoinard 1993; Burdge et al. 2002). Studies on the administration of [^3U - ^{13}C]ALA indicate that approximately 33 % of administered [^{13}C]ALA is recovered as $^{13}\text{CO}_2$ on breath over the first 24 h. [^{13}C]ALA can be mobilized from enterocytes primarily as chylomicron triacylglycerol (TAG), while [^{13}C]ALA incorporation into plasma phosphatidylcholine (PtdCho) occurs later, probably by the liver (Burdge et al. 2002), which plays a critical role in providing essential fatty acids to the brain, with secretion of LCPUFA in very low density lipoprotein (VLDL). Fatty acids associate themselves either with albumin or lipoproteins in liver, from where fatty acids are transported in the blood either bound to albumin or in the form of triacylglycerol associated with lipoproteins (Cupp et al. 2004). Total fatty acid concentration and fatty acid/albumin ratio regulate the levels of free fatty acids, but at physiological conditions (ratio 0.4:1.4) the effect is negligible. Understanding the mechanisms by fatty acids cross the blood–brain barrier (BBB) and their utilization by neurons and glia is critical for not only understanding normal brain development and function, but also for the diagnosis and therapy of human neurological disorders and planning the delivery of optimal levels of the fatty acids for normal human health. The rate of free fatty acid crossing through BBB is higher from fatty acid–albumin complexes than from circulating fatty acid lipoproteins complexes (Hamilton and Brunaldi 2007). The transport of fatty acids across BBB and other nonneuronal cellular membranes most likely occurs through passive diffusion. PUFA transport is facilitated by a number of membrane-associated and cytoplasmic proteins. These include membrane proteins fatty acid translocase (FAT/CD36), plasma membrane fatty acid-binding protein (FABPpm), and fatty acid-transport protein (FATP) (Utsunomiya et al. 1997). For net fatty acid influx, these fatty acids must be desorbed from the inner leaflet of the neural membrane and should bind with FATP/FABP_{pm}/acyl-CoA binding protein (ACBP) to prevent their repartitioning back into the membrane. If these events do not occur, fatty acids are repartitioned back into the outer leaflet and are desorbed back to the plasma to bind once again with serum albumin.

Brain phospholipid composition studies by HPLC/ESI/MS on two groups of guinea pigs that are raised from birth to 16 weeks of age on either an n-3 deficient diet containing 0.01 g ALA/100 g diet or n-3 sufficient diet containing 0.71 g ALA/100 g diet indicate that proportions of phospholipid classes and of the diradyl GroPEtn subclasses are not altered by dietary changes (Kurvinen et al. 2000). The main polyunsaturated molecular species of diradyl GroPEtn subclasses and of phosphatidyl choline and phosphatidylserine (PtdSer) contain 16:0, 18:0, or 18:1 in combination with DHA (22:6n-3), docosapentaenoic (DPA, 22:5n-6), or arachidonic acid (ARA, 20:4n-6). A significant proportion of DPA-containing species are present in both diet groups. In n-3 fatty acid deficiency, the proportions of DPA-containing molecular species are increased and DHA is primarily replaced by DPA. The combined value of main DHA- and DPA-containing species in the n-3 deficient group ranges from 91 to 111 % when compared with the n-3 sufficient group, indicating a nearly quantitative replacement (Kurvinen et al. 2000). The n-3 fatty acid deficiency does not lower the content of ARA-containing molecular species of

PtdSer of the guinea pig brain. The molecular species of phosphatidylinositol (PtdIns) are not altered by n-3 fatty acid deficiency. Collectively, these studies indicate that the main consequence of a low ALA diet is the preferential replacement of DHA-containing molecular species by DPA-containing molecular species in alkenylacyl- and diacyl GroPEtn and PtdSer of guinea pig brain (Kurvinen et al. 2000). These results are supported by phospholipid molecular species composition studies in the developing postnatal cortex after maternal diet α -linolenic acid deprivation (Brand et al. 2010). Thus, phospholipid molecular species analysis of frontal cortex from 1- to 4-week-old rats shows that DHA is exclusively replaced by DPA. However, molecular species are conserved with respect to the combination of specific polar head groups (i.e., ethanolamine and serine) in sn-3 and defined saturated/monounsaturated fatty acid in sn-1 position even when the sn-2 fatty acid moiety underwent diet-induced changes. It is suggested that substitution of DHA by DPA is tightly regulated for maintaining a proper biophysical characteristic of neural membrane phospholipid molecular species supporting the view that this substitution may modulate certain functions in the developing brain (Brand et al. 2010).

3.2.1 Metabolic Fate of ALA

In mammalian brain the presence of ALA is particularly crucial during periods of active growth around birth (Innis 1991; Green et al. 1999). Many studies in humans have indicated that the conversion of ALA to EPA and DHA occurs in numerous mammalian tissues, including liver and brain, but at a restricted rate (Fig. 3.2) (Igarashi et al. 2007). The use of ALA labeled with radioisotopes suggests that with a background diet high in saturated fat conversion of ALA to long-chain metabolites is approximately 6 % for EPA and 3.8 % for DHA (Gerster 1998; Talahalli et al. 2010). This low rate of ALA to DHA conversion is not only because of high percentage of ALA is directed toward β -oxidation (Poumes-Ballihaut et al. 2001), but also due to lower activities of enzymes responsible for the conversion of ALA to DHA in humans than rats. Fractional oxidation of [^{13}C]ALA in men varies between 24 % and 33 % of administered dose (Burdge et al. 2002). Fractional oxidation of ALA in women (22 % of administered dose) (Burdge and Wootton 2002) is lower than in men (33 %) (Burdge et al. 2002) under identical experimental conditions, which may reflect differences in the muscle mass.

In addition, there is some evidence of gender differences in efficiency of the elongation–desaturation pathway suggesting that sex hormones may play a regulatory role, but this has not been widely studied (Williams and Burdge 2006). It is suggested that the limited conversion of ALA to DHA in HepG2 cells is due to a competition of ALA and tetracosapentaenoic acid (TPA; 24:5n-3) for $\Delta 6$ desaturase (Portolesi et al. 2007). The dietary supply of DHA is more efficient than ALA to increase the DHA level in brain (cerebrum plus cerebellum) glial cell phospholipids in rat neonates during lactation (Bowen and Clandinin 2005). Glial cells are important support to neuronal cells and especially for neurotransmission, neuroprotection,

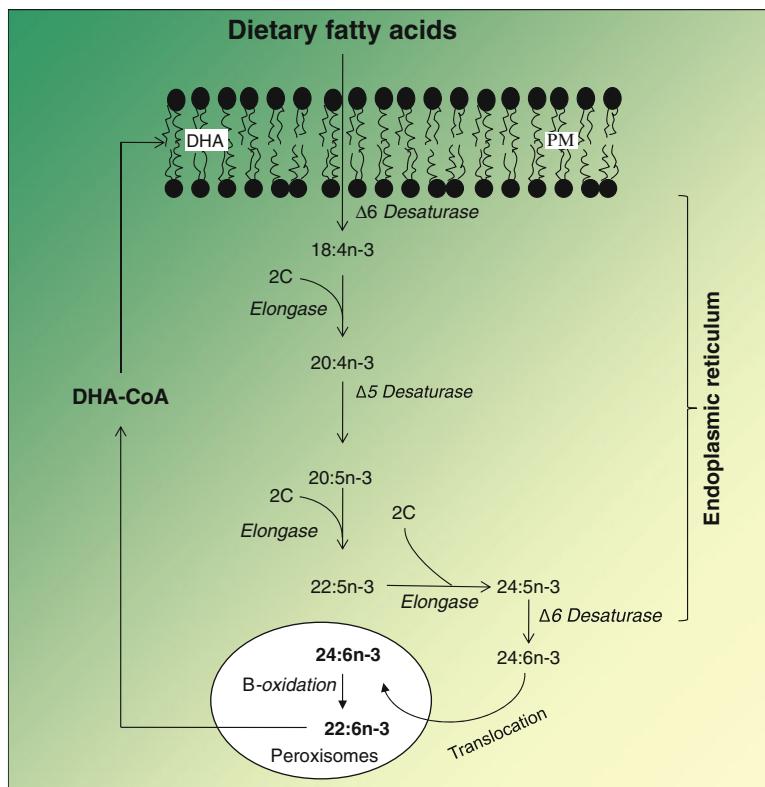


Fig. 3.2 Synthesis of DHA and EPA from ALA. All reactions occur at the endoplasmic reticulum except generation of DHA, which take place in peroxisomes

energy maintenance, and supply of key elements (Morgane et al. 1993). These observations suggest that the most effective way to increase plasma and tissue concentrations of a particular n-3 fatty acid is to feed that specific fatty acid (Arterburn et al. 2006). It should be noted that if ALA is infused directly into the brain ventricles, a much smaller percentage undergoes β -oxidation suggesting that the ability of the brain to distinguish between the metabolic processing of fatty acids coming in the brain from the plasma compared to that derived directly from the cerebral spinal fluid (CSF) (Rosenberger et al. 2002). Metabolic processing of ALA through β -oxidation in CSF is not exclusive for ALA as other dietary derived PUFA are β -oxidized in similar percentages.

ALA also undergoes free radical oxidation, forming compounds known as F_1 -phytoprostanes, which are found in all plants and are in high concentrations in plant pollens (Barden et al. 2009). Studies on metabolism of ALA in men indicate that flaxseed supplementation for 4 weeks results in higher levels of plasma and urinary F_1 -phytoprostanes and F_2 -isoprostanes than olive oil consuming group (Barden et al. 2009). The greater plasma F_1 -phytoprostane concentration in the flaxseed oil group

most likely resulted from the increased plasma concentration of the ALA substrate and/or the E_1 -phytoprostane content of the flaxseed oil. Nothing is known about the biological effects of E_1 -phytoprostanes in the brain. In vitro studies on nonneuronal cells indicate that E_1 -phytoprostanes, but not F_1 -phytoprostanes, dose dependently inhibits interleukin-12 synthesis in dendritic cells, promoting these cells to mature to form Th-2 cells (Karg et al. 2007). However, the E_1 -phytoprostanes and F_1 -phytoprostanes have been recently shown to directly downregulate both Th-1 and Th-2 cytokines (Gutermuth et al. 2007; Mariani et al. 2007). It is likely that consumption of ALA in acute coronary disease may result in decrease in inflammation and reduction in reduced risk for myocardial infarction (Cheng et al. 2005).

3.2.2 Metabolic Fate of EPA

In rat brain, EPA and DHA can be synthesized from ALA. The conversion of ALA into DHA occurs primarily in liver. Thus, ALA is metabolized by $\Delta 6$ desaturation, elongation, and $\Delta 5$ desaturation to yield EPA. DPA is formed by addition of C2 to EPA, which is converted subsequently to 24: 5n-3 and 24: 6n-3 by further chain elongation and $\Delta 6$ desaturation. These reactions occur in the endoplasmic reticulum (ER). However, DHA is synthesized from 24: 6n-3 by peroxisomal β -oxidation, which shortens the carbon chain by C2 (Fig. 3.3). This pathway has also been demonstrated in pigs (Li et al. 2000) and baboons (Su et al. 1999a, b). While $\Delta 6$ -desaturase appears to be the rate-limiting step for this pathway, its overall regulation is unclear and may contain several loci of metabolic control including translocation of 24: 6n-3 and DHA between the endoplasmic reticulum and peroxisomes (Sprecher 2000).

EPA is oxidized by cyclooxygenases and lipoxygenases into 3-series prostaglandins and 5-series leukotrienes. These metabolites (TXA_3 , prostacyclin PGI_3 , and LTB_5) inhibit platelet aggregation and vasoconstriction and promote vasodilation. In addition, 15-LOX-mediated oxidation of EPA generates resolvins of E series (Fig. 3.3), including resolin E_1 (RvE_1 ; (5S,12R,18R)-trihydroxy-6Z,8E,10E,14Z, 16E-eicosapentaenoic acid) and resolin E_2 (15S,18R-dihydroxy-EPE) (Fig. 3.4). These metabolites not only act as anti-inflammatory mediators by assisting in the resolution of inflammatory events, but also assist in the clearance of cellular debris from the site of inflammation (Arita et al. 2005). They also suppress IL-1, IL-2, IL-6, and TNF- α production by T cells (Serhan et al. 2008), thus functioning as endogenous anti-inflammatory agents. The oxidation of RvE_1 to 18-oxo- RvE_1 inactivates its biochemical effects. This oxidation is catalyzed by NAD $^+$ -dependent hydroxyprostaglandin dehydrogenase. Based on detailed pharmacological investigations on the effect of RvE_1 , it is suggested that the binding of RvE_1 to BLT $_1$ receptor and resolin E receptors (resoER $_1$) mediates the resolution of inflammation (Arita et al. 2006, 2007). In vasculature, EPA is oxidized to 18R-hydroxyeicosapentaenoic acid (18R-HEPE) by endothelial cell cyclooxygenase-2 (COX-2). Aspirin acetylates COX-2. The acetylated enzyme no longer produces

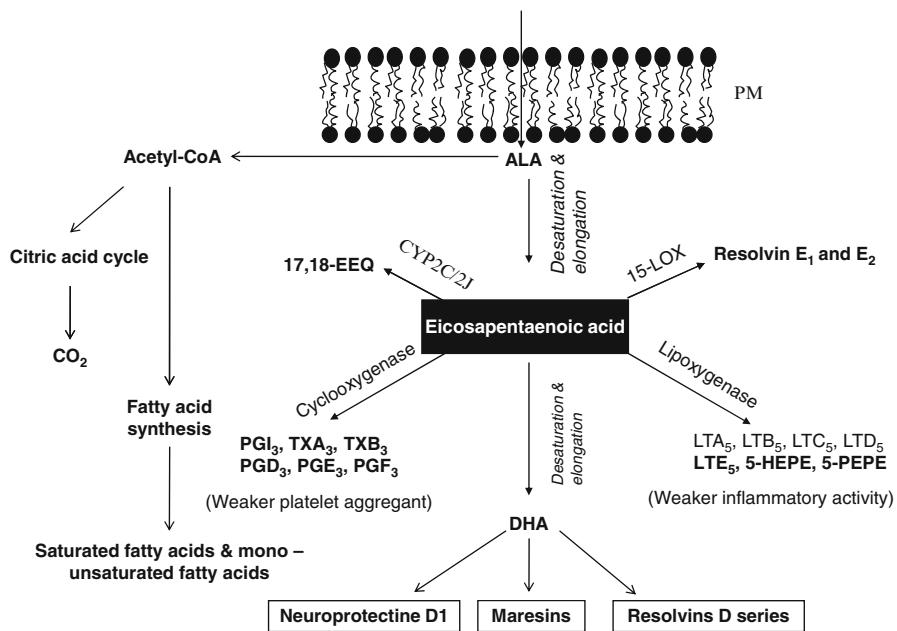


Fig. 3.3 Metabolic fate of EPA and DHA in the brain. Plasma membrane (PM); 15-lipoxygenases (15-LOX); 3-series prostaglandins and thromboxane (PGI₃; PGD₃; PGF₃; TXA₃; and TXB₃); 5-series leukotrienes (LTA₅; LTB₅; LTC₅; LTD₅; and LTE₅); 17,18-epoxy Eicosatetraenoic Acid (17, 18-EEQ) and cytochrome P450 (CYP) enzymes

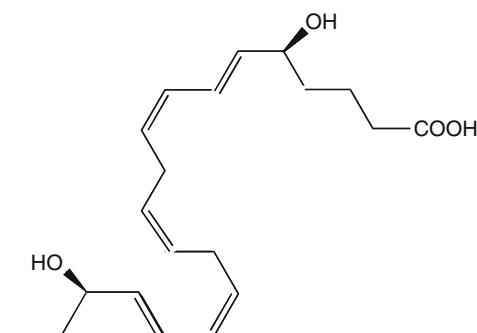
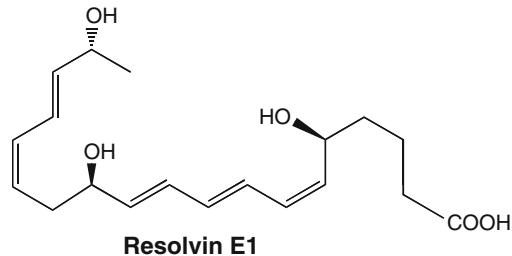


Fig. 3.4 Chemical structures of resolvins E1 and E2

PGs, but can still convert EPA to 18R-HEPE. During cell–cell interactions, 18R-HEPE is released to neighboring leukocytes for subsequent conversion by 5-LOX to RvE₁ via a 5(6) epoxide-containing intermediate.

3.2.3 Metabolic Fate of DHA

15-LOX catalyzed oxidation of DHA produces neuroprotectin D₁ and resolvins of the D series (RvDs) and maresins (Fig. 3.5). These lipid mediators not only antagonize the effects of ARA-derived PGs, LTs, and TXs, but also modulate leukocyte trafficking and downregulate the expression of cytokines in glial cells. They possess potent anti-inflammatory, neuroprotective, and pro-resolving properties (Hong et al. 2003; Marcheselli et al. 2003, 2010; Serhan et al. 2008). In addition, DHA-derived NPD1 targets upstream events of brain cell apoptosis, as well as promote and maintain cellular homeostasis by restoring neural and retinal cell integrity. DHA-derived D series resolvins, like EPA-derived resolving E₁ and E₂ produce potent anti-inflammatory effects (Dangi et al. 2010). D series resolvins exert potent agonist actions on macrophages and vascular endothelial cells. They control the magnitude of the local inflammatory response. They act through specific receptors found in neural and nonneuronal cells. These receptors are called as resolin D receptors (resoDR₁) (Serhan and Chiang 2008). These receptors have neither been fully characterized in nonneuronal tissues nor in brain.

3.2.4 Possible Adverse Effects of ALA Consumption

Consumption of ALA produces very few adverse effects aside from mild gastrointestinal symptoms (Balk et al. 2007). However, concern has arisen from one meta-analysis that reported an increased incidence of prostate cancer risk in men with high intake or high blood levels of ALA (Brouwer et al. 2004; De Stéfani et al. 2000). The molecular mechanism associated with ALA-mediated increase in prostate cancer is not known. So, more studies are needed on the relationship of ALA with prostate cancer.

3.2.5 Possible Adverse Effects of EPA and DHA Consumption

The best source of EPA and DHA is fish oil. High doses of fish oil increase the bleeding time. Individuals who bruise easily or are taking blood-thinning medications should be careful in taking fish oil. The most common adverse effects of fish oil include the fishy odor and aftertaste in the mouth, nausea, and gastrointestinal discomfort with belching and bloating, diarrhea, and flatulence (Farooqui 2009). Occasional nosebleeds are also observed in some individuals taking high doses of fish oil. All these symptoms can be resolved within 1 week of discontinuation of fish

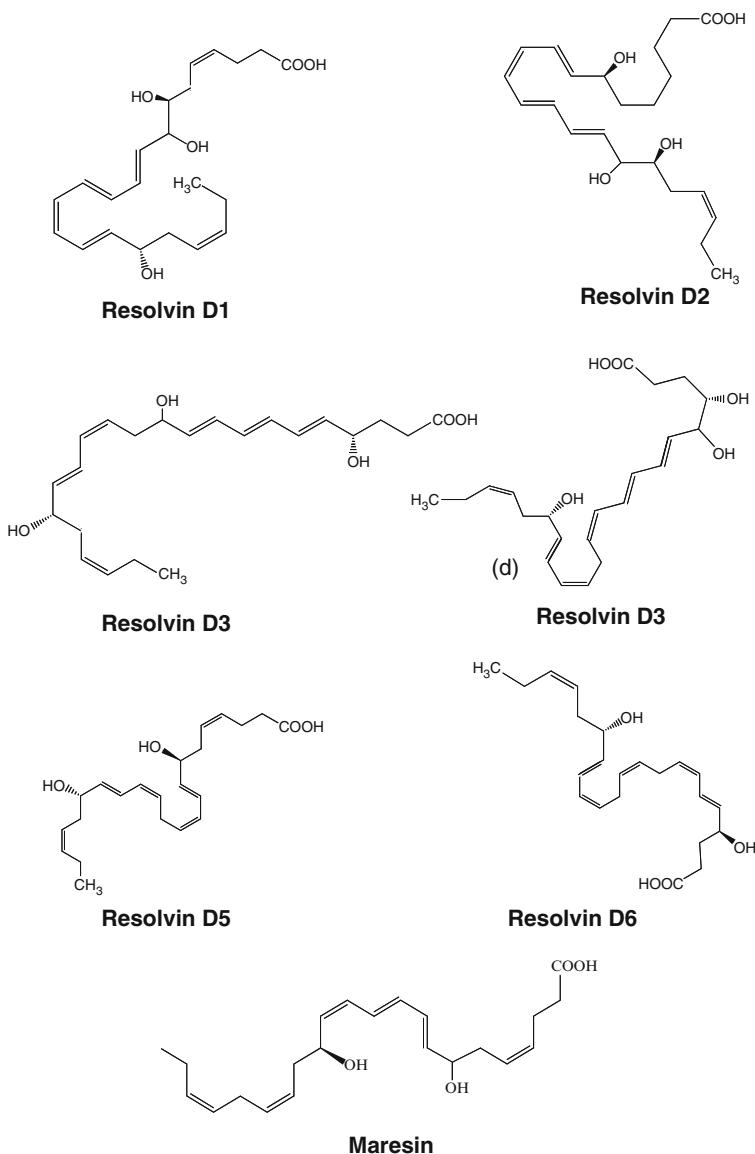


Fig. 3.5 Chemical structures of docosatrienes and maresins

oil supplement. Another important safety concern is the susceptibility of n-3 fatty acid preparations to undergo oxidation, which contributes to rancidity and patient intolerance to potential toxicity. Large amounts of crude fish oil consumption may result in adverse side effects due to the potential presence of environmental toxins such as mercury, polychlorinated biphenyls, dioxins, and other contaminants (Bays 2007).

3.3 Roles of n-3 Fatty Acids in the Brain

It is well known that n-3 family of fatty acids provides important nutrients throughout the life cycle in animals as well as humans (Farooqui 2009). Infants require n-3 fatty acids for visual and cognitive development. n-3 fatty acids provide cardiovascular benefits from childhood to old age. Thus, n-3 fatty acids provide protection not only against cardiovascular, neurotraumatic (stroke, spinal cord trauma, and traumatic brain injury), and neurodegenerative diseases (Alzheimer disease, Parkinson disease, and Huntington disease), but also against peroxisomal disorders, multiple sclerosis, and macular degeneration as well as nonneuronal diseases (Chronic Obstructive Pulmonary Disease, Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, and cystic fibrosis) (Farooqui 2009).

In brain, n-3 Fatty acids are incorporated exclusively into phospholipids (plasmalogens and phosphatidylserine). These fatty acids modulate the biophysical properties of neural membranes (e.g., fluidity, thickness, and deformability) and therefore affect activity of transmembrane proteins (Rajamoorthi et al. 2005; Horrocks and Farooqui 2004; Farooqui 2009). n-3 fatty acids particularly, DHA are highly flexible within the neural membrane and is particularly effective at accommodating transitional changes associated with transmembrane protein activation (Salem et al. 2001; Gawrisch et al. 2003). DHA, EPA, and ALA, as well as the arachidonic acid (ARA), compete for the sn-2 position on membrane phospholipids. The relative proportion of these fatty acids also determines their availability following phospholipase A₂-mediated cleavage of phospholipids and available substrates for cyclooxygenases and lipoxygenases, and hence the balance between eicosanoids and docosanoids (neuroprotectins, resolvins, and maresins) (Farooqui 2009). In addition, these fatty acids are also ligands for nuclear receptors such as peroxisome proliferator-activated receptors and retinoid X receptor, and ions channels. These fatty acids can also modulate gene expression (Radominska-Pandya and Chen 2002; de Urquiza et al. 2000; Guizy et al. 2008; Farooqui 2009). Collectively, these studies suggest that overall membrane fatty acid composition can have a large impact on cell and organ function as well as a wide variety of biological processes.

3.3.1 *Roles of ALA in the Brain*

As stated earlier, ALA is a precursor for the synthesis of EPA and DHA, but the rate of this conversion in human is very low. Moreover, ALA is metabolized by the same enzymes that are responsible for metabolizing linoleic acid (LA). The consumption of ALA is a good strategy to decrease elongation of LA leading to reduction in ARA levels. Levels of ARA are very high levels in the Western diet (Simopoulos 2008) and may contribute to its proinflammatory nature.

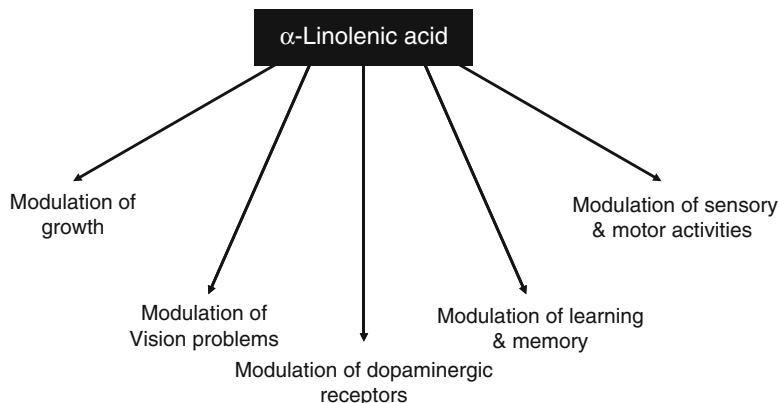


Fig. 3.6 Roles of α -linolenic acid in the brain

Furthermore, ALA produces its beneficial effects by modulating ion channels (Guizy et al. 2008) or nuclear receptors such as PPAR or RXR (de Urquiza et al. 2000). ALA has been reported to stabilize the mitochondrial membrane potential and suppression of nitrosative stress in dissociated cell cultures from guinea pig brain (Eckert et al. 2010). In addition, dietary deprivation of ALA not only compromises behavior responses and promotes learning disabilities (Delion et al. 1996; Moriguchi et al. 2000; Catalan et al. 2002), but also decreases sensory and motor activities and may cause problems with vision in animal models (Neuringer 2000; Birch et al. 1998). ALA deficiency may also produce impairment in neurotransmitters synthesis and release (Kodas et al. 2002; Zimmer et al. 2000) and decrease development of dopamine receptors in the postnatal rat (Kuperstein et al. 2005, 2008) (Fig. 3.6). The deficiency of ALA may also produce decrease in levels of DHA. This fatty acid may be responsible for maintaining the size of neuronal cell body in the hippocampus, hypothalamus, and parietal cortex and supporting the complexity of cortical dendritic arborization (Ahmad et al. 2002). Direct DHA supplements promote an increase in dendritic spine density in the weaning rat hippocampus (Cansev et al. 2009), enhance hippocampal neuritogenesis in transgenic mice (He et al. 2009), and in tissue culture it accelerates neuritic outgrowth (Calderon and Kim 2004). In addition, recent studies indicate that lifelong ALA dietary insufficiency specifically ablates long-term synaptic depression mediated by endocannabinoids in the prelimbic prefrontal cortex and accumbens. It is proposed that in n-3-deficient mice, presynaptic cannabinoid CB₁ receptors (CB₁Rs) are coupled to endocannabinoids, which are linked with effector G(i/o) proteins in the membrane. The diet-mediated reduction in CB₁R function is closely associated with emotional behavior. These observations identify a plausible synaptic substrate for the behavioral alterations caused by the ALA deficiency that is often observed in western diets (Lafourcade et al. 2011).

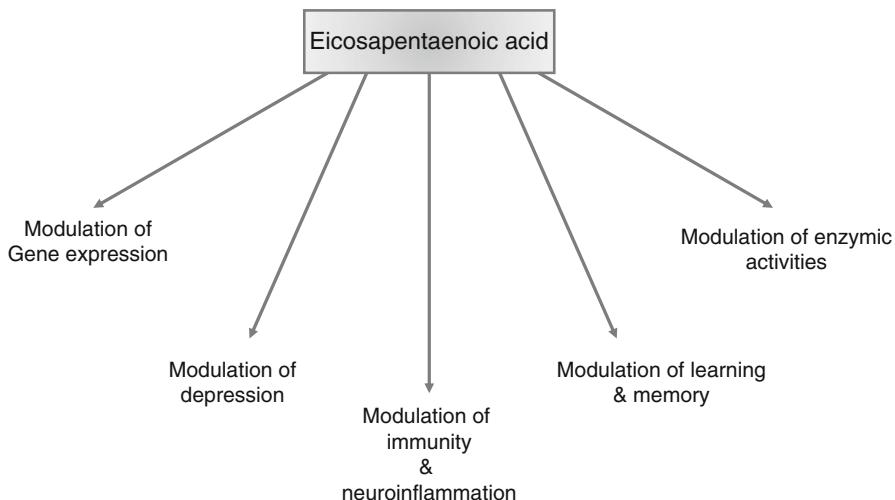


Fig. 3.7 Roles of eicosapentaenoic acid in the brain

3.3.2 Roles of EPA in the Brain

As stated earlier, EPA competes with ARA for cyclooxygenases and lipoxygenases. The free EPA is metabolized to less active eicosanoids (3-series PG and 5-series LT), which retard the action of ARA-derived eicosanoids. EPA optimizes the function of immune cells by modulating inflammatory processes and immune cell activation (Phillis et al. 2006; Farooqui 2009) (Fig. 3.7). EPA suppresses sympathetic nerve activity without inducing any parasympathetic nerve activity. EPA has been used to treat several psychiatric and neurodegenerative diseases due to its anti-inflammatory and neuroprotective effects (Peet 2003; Song and Zhaq 2007). The molecular mechanism associated with beneficial effect of EPA is not known. However, recent evidence suggests that EPA-mediated improvement in motor function not only correlates with downregulation of ARA metabolism and reduction in prostaglandin-mediated signaling, but is also related with alterations in NF- κ B pathway signaling and decrease in brain-derived neurotrophic factor levels and/or alterations in blood flow to the brain tissue (Song and Zhaq 2007). EPA-derived RvE1 and RvE2 inhibit neuroinflammation. In addition, the nonenzymic peroxidation of EPA produces J₃-IsoPs both in vitro and in vivo. These mediators induce Nrf2-directed gene expression (Gao et al. 2007). Under the normal conditions, Nrf2-dependent transcription is repressed by a negative regulator Keap1. When tissues or cells are subjected to oxidative stress, Nrf2 escapes Keap1-mediated repression and migrates to the nucleus where it activates antioxidant responsive element (ARE)-dependent gene expression to maintain cellular redox homeostasis. In addition, Nrf2 has recently been recognized as a key factor regulating an array of genes that defend cells against the deleterious effects of environmental insults (Zhang 2006). The

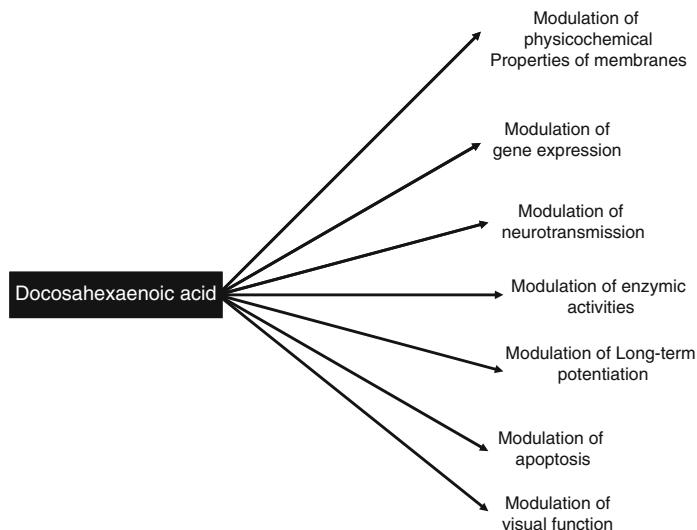


Fig. 3.8 Roles of docosahexaenoic acid in the brain

intake of purified EPA significantly reduces blood pressure without altering heart rate during the 6-month treatment (Matsumura 2007; Farooqui 2009); this effect may be mediated through an alteration in the balance between vasoconstrictive prostaglandins and increasing production of vasodilatory prostacyclin from EPA.

3.3.3 Roles of DHA in the Brain

Dietary intake of DHA results in the suppression of pro-inflammatory cytokines and adhesion molecule expression. These effects occur at the gene expression level and may be responsible for antagonism of the effects of ARA-derived mediators or through more direct actions on the intracellular signaling pathways, which lead to activation of transcription factors such as nuclear factor- κ B (NF- κ B). DHA modulates neurotransmission in the brain (Aid et al. 2005) and inhibits neuronal apoptosis (Kim et al. 2000). Dietary DHA maintains functional maturation of the retina and visual cortex resulting in optimal visual acuity and brain development. DHA deficient animals have reduced visual acuity and impaired learning ability. During brain development DHA induces neurite outgrowth and promotes optimal signal transduction (Farooqui 2009). In hippocampus it improves long-term potentiation, learning ability of aged rats, and promotes cognitive function of humans with memory deficits (Fig. 3.8). A low-DHA diet has been associated with the development of dendritic pathology and behavioral deficits in an Alzheimer disease (AD) mouse model (Calon et al. 2004), whereas DHA can decrease the amyloid toxicity in transgenic AD mice, reducing the brain plaque burden by 40 % (Lim et al. 2005).

This neuroprotective activity may be due, at least in part, to the generation of DHA-derived lipid mediator, neuroprotectin D1 (Lukiw et al. 2005).

3.4 Effect of ALA on Neurological Disorders

ALA has more influence on cardiovascular system than cerebrovascular system. Thus, ALA supplementation reduces total cholesterol (Pang et al. 1998). It significantly decreases the incidence of arrhythmias and cardiac mortality in rats (McLennan and Dallimore 1995), enhances arterial compliance in obese subjects (Nestel et al. 1997), and reduces C-reactive protein, IL-6, and other inflammatory markers implicated in atherogenesis in males with dyslipidemia (Rallidis et al. 2003). Although effects of ALA on platelet aggregation and thrombosis are inconsistent (Knapp 1997), there seems to be an overall protective effect of this fatty acid on cardiac outcomes in humans and rodents that is not explained solely by modest reductions in cholesterol levels. Adhesion molecules including intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin, upon upregulation, facilitate the movement of immune cells into tissue and promote inflammation. ALA not only reduces plasma concentrations of soluble E-selectin and VCAM-1 (Thies et al. 2001), but also downregulates NF- κ B in healthy human subjects. Collective evidence suggests that ALA in dietary flaxseed exerts antiarrhythmic effects during ischemia–reperfusion in rabbit hearts, possibly through shortening of the action potential (Ander et al. 2004).

The increased appreciation of the involvement of microglial cell-mediated oxidative stress and neuroinflammation in neurological disorders, such as AD, Parkinson disease (PD), stroke, traumatic brain, and spinal cord injuries, has attracted considerable interest in treatment with anti-inflammatory drugs (Kempermann and Neumann 2003). A substantial body of biochemical and clinical data supports the use of n-3 fatty acids as anti-inflammatory agents (Mori and Beilin 2004). Support for dietary use of n-3 fatty acids for the treatment of neurological disorders is increasing (Farooqui 2009). n-3 fatty acids reduce oxidative stress and neuroinflammation in several ways. First, they decrease the formation of ARA by blocking the activity of $\Delta 5$ -desaturase; second, they inhibit the synthesis of eicosanoids; and last, they induce the synthesis of resolvins, neuroprotectins, and maresins (Farooqui 2011). Collective evidence suggests that the ratio of ARA to n-3 fatty acid is an important dietary factor in reducing oxidative stress and inflammation in brain tissue (Fig. 3.9). ALA has been used for the treatment of ischemia, spinal cord trauma, AD, and depression.

3.4.1 Effect of ALA on Ischemic Injury

Stroke (ischemia) is a metabolic insult caused by severe reduction or blockade in cerebral blood flow due to cerebrovascular disease. This blockade not only reduces oxygen and glucose delivery to brain tissue but also produces alterations in the

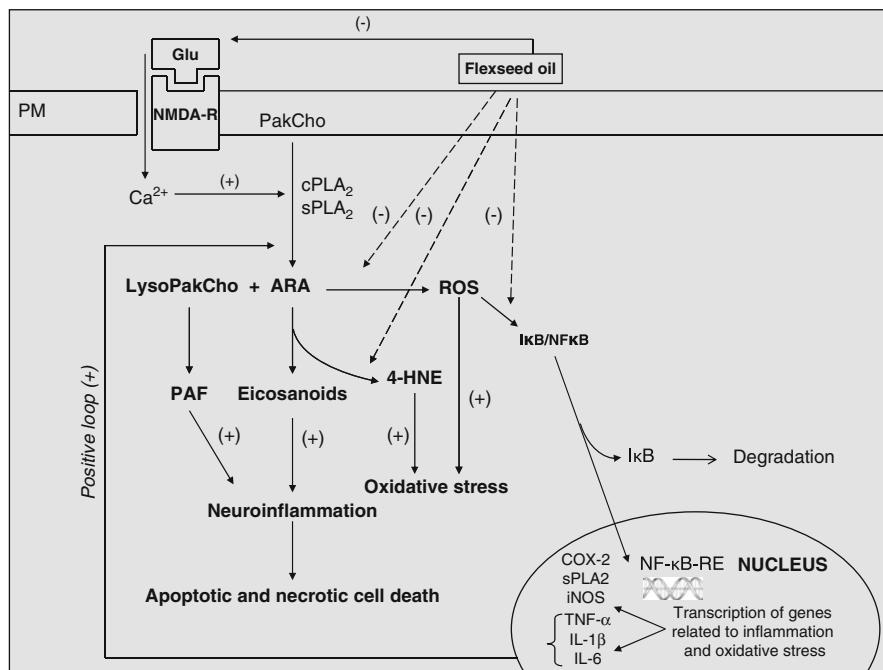


Fig. 3.9 Effect of flaxseed oil on oxidative stress-generating metabolites of phospholipid metabolism. Plasma membrane (PM); Alkylacylglycerophosphocholine (PakCho); lyso-alkylacylglycerophosphocholine (lyso-PakCho); arachidonic acid (ARA); cytosolic phospholipase A₂ (cPLA₂); platelet activating factor (PAF); reactive oxygen species (ROS); inducible nitric oxide synthase (iNOS); nuclear factor-kappa B (NF-κB); nuclear factor; secretory phospholipase A₂ (sPLA₂); cyclooxygenase-2 (COX-2); tumor necrosis factor-α (TNF-α); and interleukin-1β (IL-1β)

breakdown of BBB and buildup of potentially toxic products in brain. Breakdown of BBB integrity in stroke not only results in transmigration of numerous immune system cells including monocytes and lymphocytes, but also cause hyperpermeability mediated by enhanced transcytosis and gap formation between endothelial cells (Farooqui 2009). ALA improves brain resistance against cerebral ischemia. Thus, studies on the effects ALA on the cerebral blood flow and on the tone of vessels that regulate brain perfusion indicate that a single injection of ALA not only increases cerebral blood flow and induces vasodilation of the basilar artery but also reduces ischemic damage by limiting glutamate-mediated neuronal death and reduction in the lipid peroxidation-derived products (Blondeau et al. 2007, 2009; Nguemeni et al. 2010). Three sequential injections of neuroprotective dose of ALA increase neurogenesis and expression of key proteins involved in synaptic functions, such as synaptophysin-1, VAMP-2, and SNAP-25, as well as proteins supporting glutamatergic neurotransmission, namely, V-GLUT1 and V-GLUT2. These effects are correlated with an increase in brain-derived neurotrophic factor

(BDNF) protein levels, both *in vitro* using neural stem cells and hippocampal cultures and *in vivo*, after subchronic ALA treatment. Moreover, repeated injections of ALA induce additive protective benefits as a result of increase in neurogenesis, synaptogenesis, and neurotrophin expression (Nguemeni et al. 2010). The saturated fatty acid palmitic acid has no effect on vasodilation. It is suggested that the target of the polyunsaturated fatty acids effect is the TREK-1 potassium channel associated with basilar but not in carotid arteries (Heurteaux et al. 2004). Polyunsaturated fatty acid-mediated vasodilations in the basilar artery as well as the laser-Doppler flow increase are abolished in TREK-1(−/−) mice supporting the view that TREK-1 activation elicits a robust dilation that probably accounts for the increase of cerebral blood flow induced by polyunsaturated fatty acids such as ALA or DHA. Based on these results it is proposed that the selective expression and activation of TREK-1 in brain collaterals may be associated with the protective mechanisms of polyunsaturated fatty acids against stroke by providing residual circulation during ischemia (Blondeau et al. 2007). It is also reported that a subchronic treatment with ALA promotes several features associated with neurogenesis/synaptogenesis and possesses antidepressant-like activities that may be beneficial for stroke recovery, which is accompanied by the significant increase in synaptophysin. In most synapses of the mammalian brain, synaptic vesicle fusion is executed by a complex called SNARE, which is composed of synaptobrevin-2 (also named VAMP-2), syntaxin-1, and SNAP-25 proteins (Sutton et al 1998). ALA treatment specifically increases the levels of VAMP-2 in cortex and hippocampus, but not in the striatum. SNAP-25 expression is also enhanced in the cerebral cortex. The parallel increase of synaptophysin-1 and VAMP-2, two vesicle-associated synaptic proteins (SV-proteins) and their membrane-associated synaptic protein (PM-protein), SNAP-25, strongly supports the view that ALA promotes synaptogenesis in the cerebral cortex (Blondeau et al. 2009). This functional benefit ALA may be associated not only with increase in vasodilation, but also inhibition of excitotoxicity, as well as long-term enhancement of neurogenesis, synaptogenesis, and neurotransmitter transmission. From a clinical point of view, this “multitarget” effect of subchronic ALA treatment may represent a novel approach for the treatment of ischemic injury and depression (Blondeau et al. 2009).

3.4.2 Effect of ALA on Spinal Cord Trauma

Treatment of rats after 30 min of spinal cord injury with ALA and DHA not only improves locomotor performance, but also results in neuroprotection including decrease in lesion size and reduction in apoptosis, and increase in neuronal and oligodendrocyte survival (King et al. 2006). Evidence showing a decrease in RNA/DNA oxidation suggests that the neuroprotective effect of n-3 PUFAs involves a significant antioxidant function. In contrast, animals treated with arachidonic acid have a significantly worse outcome than controls. This suggests that ALA and DHA

treatment after spinal cord compression greatly increases the survival of neurons and results in significantly better locomotor performance for up to 6 weeks after injury. Given the proven clinical safety of ALA and DHA, these PUFAs have significant therapeutic potential for spinal cord injury (King et al. 2006; Michael-Titus 2007).

3.4.3 Effect of ALA on Alzheimer Disease

Alzheimer disease (AD), the most common cause of dementia, is a progressive neurodegenerative disorder affecting millions of people worldwide. AD is a multi-factorial disease of unknown pathogenesis. The hallmarks of AD include extracellular amyloid beta (senile) plaques ($A\beta$), intracellular neurofibrillary tangles, chronic oxidative stress, neuroinflammation, and disease progression leading to cognitive decline and eventually neurodegeneration. The cognitive decline observed in AD has its roots at the synapse, the space between neurons, through which they communicate. The synapse is also the site at which the $A\beta$ peptide, the characteristic amyloid protein associated with AD, is believed to first deposit (Terry et al. 1991; Farooqui 2010). Activated microglia and astrocytes are usually found to be associated with the amyloid plaques. Activation of microglia leads to uptake and clearance of $A\beta$. However, excessive activation of microglia results in production and release of inflammatory cytokines, nitric oxide, and reactive oxygen species, which contribute to neuronal dysfunction and cell death (Farooqui 2010).

As stated earlier, the conversion of ALA into DHA occurs primarily in liver. Studies on the quantification of polyunsaturated fatty acids in liver and brain samples from control subjects and AD patients indicate that there are statistically detectable differences between the two groups for DHA, EPA, DPA, and tetraicosahexaenoic acid (THA) in liver, but no differences are observed in ALA, EPA, and DPA various regions of the brain. However, statistically significant low levels of DHA are observed in AD brain compared to the age-matched controls (Astarita et al. 2010). Furthermore, levels of DHA-containing phospholipid (1-O-1'-(Z)-octadecenyl,2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine and plasmalogens) are decreased in brain tissue from AD patients compared to age-matched controls. The decrease in DHA-containing phospholipid (plasmalogen) may be due to increase in plasmalogen-selective phospholipase A₂ (PlsEtn-PLA₂) activity and disruption of the expression of the genes that code for the enzymes associated with NPD₁ synthesis (Farooqui et al. 2003; Bazan 2009). These observations may explain, at least in part, why synapses are lost and levels of plasmalogens and NPD₁ are reduced in the hippocampus of AD patients compared to age-matched controls. Although no disease-modifying therapy is available, preventive therapy is available at this time, but decrease in consumption of n-3 fatty acid containing may partly account for increase in number of AD patients throughout the world.

3.4.4 Effect of ALA on Dementia

Dementia is defined as a syndrome that includes not only memory deficits, but disturbances in other higher cortical functions, commonly accompanied by deterioration in emotional control and social behavior (Fratiglioni and Qiu 2009). Studies on n-3 fatty acids content in erythrocytes indicate lower levels of n-3 fatty acids are associated with mild dementia in elderly Koreans (Kim et al. 2010). Multivariate-adjusted regression analysis show that a higher level of α -linolenic acid (ALA; 18:3n-3) significantly decrease the risk of mild dementia after adjusting for age, sex, and height. Mini-Mental Status Examination-Korean (MMSE-K) score are also positively associated with erythrocyte ALA and total n-3 PUFA. However, erythrocyte levels of DHA and EPA are not significantly related with the risk of mild dementia and MMSE-K score. It is suggested that ALA from plant sources, in contrast to fish oil-derived EPA and DHA, decreases the risk for mild dementia among the Korean elderly (Kim et al. 2010).

The causes of the reduction of ALA in participants suffering from dementia are unknown. Earlier longitudinal studies indicate that individuals with dementia have a lower intake of fish. Normally, ALA is for the most part completely oxidized via β -oxidation or partially oxidized for carbon recycling into lipogenesis, whereas only a small percentage is converted to EPA and DHA (Sinclair et al. 2002). Finally, increase in oxidative stress, a process that contributes to dementia, may reduce PUFA levels in the membranes (Urano et al. 1998) and increase the requirement for ALA and DHA intake to compensate for this loss. It is also reported that dementia patients also have higher palmitic acid levels compared to older participants with normal cognitive function. Palmitic acid is a precursor of ceramides and sphingomyelin. Levels of long-chain ceramides are increased in brain tissue samples from demented AD patients compared to age-matched controls (Han et al. 2002). Pharmacological inhibition of ceramide synthesis protected hippocampal neurons from amyloid β -peptide-mediated toxicity and oxidative stress, suggesting increased ceramide levels may be associated with dementia. Thus, alterations in the levels of palmitic acid may contribute to the pathogenesis of dementia in AD.

3.4.5 Effect of ALA on Major Depressive Disorders

It is well known that consumption of n-3 fatty acids is inversely correlated to the prevalence and the severity of MDD, and that an n-3 supplementary diet produces beneficial effects in the depressive states (Hibbeln 1998; Peet et al. 1998, 2005). ALA mediates its beneficial effects through multiple mechanisms. Thus, ALA treatment retards excitotoxicity and enhances vasodilation leading to increase in cerebral blood flow (Blondeau et al. 2007). The vasoactive effect of ALA is mediated by potassium TREK-1 channels, which are activated by ALA. The activation of neuronal potassium TREK 1 prevents excessive release of excitotoxic glutamate while

favoring an Mg²⁺ block at postsynaptic NMDA receptors. This observation is supported by studies on TREK-1-deficient mice, which show marked reduction in neuroprotection during ischemic injury (Heurteaux et al. 2004). In addition, chronic administration of a PUFA-supplemented diet consisting of ALA, linoleic acid, and oleic acid in normal mice induces antidepressant-like effects. These behavioral effects are associated with increases in synaptogenesis, cell number, brain-derived neurotrophic factor (BDNF) gene expression, and hippocampal volume (Venna et al. 2009). Collectively, these studies support the view that mechanism of antidepressant effect of ALA may involve multiple mechanisms, including inhibition of excitotoxicity, enhancement of neuroplasticity, changes in gene expression of BDNF, stimulation of synaptic transmission, increase in neuroplasticity, and neurogenesis (Lemaire et al. 2000; Racagni and Popoli 2008).

3.5 Conclusion

ALA, an essential n-3 fatty acid, is found not only in flaxseed oil, but also in other cooking vegetable oils, such as soybean and canola oils. Intake of ALA in the daily diet is a safe and viable option for meeting dietary requirements and maintaining the suggested n-6: n-3 ratio. Low ALA intake is associated with coronary heart disease and sudden cardiac death. Most of consumed ALA undergoes beta oxidation in the liver and less than 10 % is converted into EPA and DHA, which may reduce the risk of cardiovascular and cerebrovascular diseases. These fatty acids reduce cardiovascular risk not only through inhibition of inflammation and modulation of endothelial cell function, but also through inducing arterial compliance and blockage of arrhythmia. Injections of ALA not only increase cerebral blood flow by inducing vasodilation of the basilar artery but also reduce ischemic damage by limiting glutamate-induced neuronal death and reduction in the lipid peroxidation-derived products. Consumption of ALA may delay or retard the onset of dementia and depression in elder subjects.

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Chapter 4

Beneficial Effects of Flavonoids on Neurological Disorders

4.1 Introduction

Flavonoids are polyphenolic compounds found in fruits, vegetables, and beverages derived from plants. Humans consume about 1–2 g of flavonoids daily. Information about the occurrence of more than 5,000 different flavonoids has been reported in the literature. The six major subclasses of flavonoids include the flavones (e.g., apigenin, luteolin), flavonols (quercetin, myricetin), flavanones (naringenin, hesperidin), catechins or flavanols (epicatechin, gallicatechin), anthocyanidins (cyanidin, pelargonidin), and isoflavones (genistein, daidzein) (Fig. 4.1). Minor dietary flavonoids include dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones, and aurones (Crozier et al. 2009). The basic structure of flavonoids consists of two benzene rings (A and B) linked through a heterocyclic pyran or pyrone (with a double bond) ring (C) in the middle. The carbon atoms are identified with ordinary numerals for A- and C-rings and “primed numerals” for the B-ring, although a modified number system is used for chalcones (Fig. 4.2). In vivo and in vitro studies indicate that flavonoids produce antioxidant and anti-inflammatory effects (Middleton 1998; Havsteen 2002). The antioxidant activity of flavonoids depends on the structure and the substituents of the heterocyclic and B-rings, in particular the presence of an *o*-di-OH structure on the B-ring, a 2,3-double bond in conjugation with a 4-oxo function, and the additional presence of 3- and 5-OH groups on the heterocyclic ring (Croft 1998; Gutierrez-Merino et al. 2011). The antioxidant activity of flavonoid depends on the arrangement of functional groups on its core structure. It is suggested that both the configuration and total number of hydroxyl groups substantially influence the mechanism of the antioxidant activity (Heim et al. 2002). The B-ring hydroxyl configuration is the most significant determinant of ROS scavenging activity (Burda and Oleszek 2001), whereas substitution of the rings A and C has little impact on superoxide anion radical scavenging rate constants (Burda and Oleszek 2001; Amić et al. 2007). Most of the flavonoids present in plants are attached to sugars (glycosides), although occasionally they are found as aglycones.

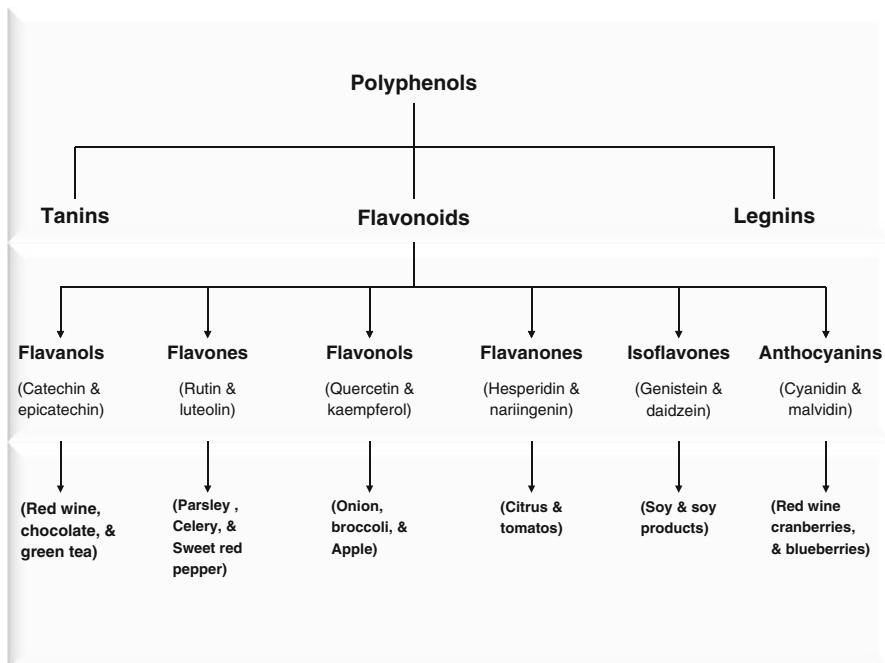


Fig. 4.1 Classification of flavonoids and their plant sources

In vitro studies indicate that flavonoids also have significant anti-inflammatory, antibacterial, antiallergic, antiviral, cytoprotective, and anticarcinogenic properties (Middleton 1998; Loito and Frei 2006; Cushnie and Lamb 2011). Although the molecular mechanism associated with anti-inflammatory effects of flavonoids in vivo is not fully understood, in vitro studies indicate that flavonoids predominantly act through the inhibition of NF- κ B signaling and the downregulation of the expression of proinflammatory markers, such as tumor necrosis factor- α (TNF- α) (González et al. 2011) (Fig. 4.3). The capacity of flavonoids to inhibit TNF- α -induced adhesion molecule expression in human aortic endothelial cells depends on specific structural features of the flavonoids. Thus, 5,7-dihydroxyl substitution of a flavonoid A-ring and 2,3-double bond and 4-keto group of the C-ring are important structural requirements for inhibition of adhesion molecule expression (Loito and Frei 2006). In contrast, hydroxyl substitutions of the B- and C-rings but not the A-ring are essential for antioxidant activity. Therefore, only hydroxyl flavones such as apigenin and chrysanthemum, and flavonols, such as galangin, kaempferol, and quercetin, prevent endothelial adhesion molecule expression, whereas flavone, chromone, the flavanone, naringenin, and the flavanol, (-)-epicatechin, have been ineffectual.

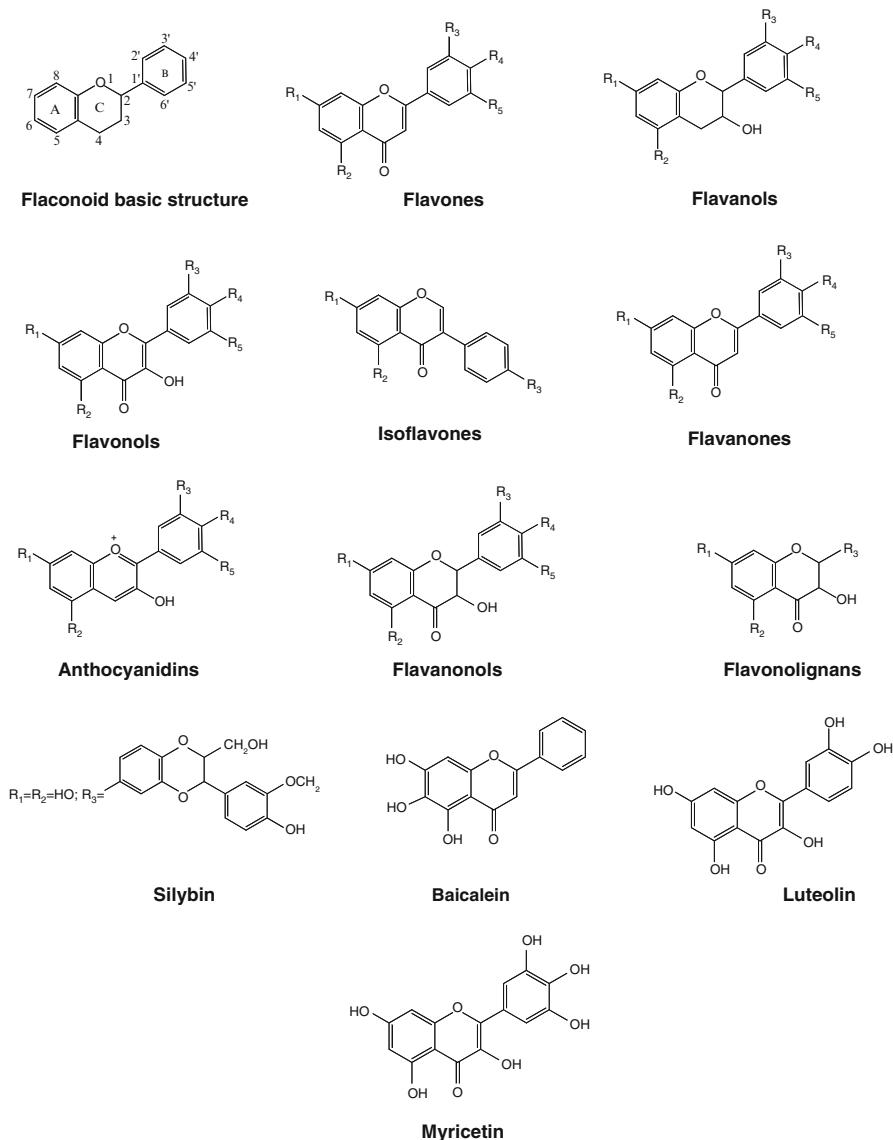


Fig. 4.2 Basic structures of flavonoids and chemical structures of some flavonoids. Flavones [luteolin ($R_1=R_2=R_3=R_4=OH; R_5=H$)]; apigenin ($R_1=R_2=R_4=OH; R_3=H$); flavanols [catechins ($R_1=R_2=R_3=R_4=OH; R_5=H$); gallocatechins ($R_1=R_2=R_3=R_4=R_5=OH$)]; flavonols [quercetin ($R_1=R_2=R_3=R_4=H$); kaempferol ($R_1=R_2=R_4=OH; R_3=R_5=H$); myricetin ($R_1=R_2=R_3=R_4=R_5=OH$)]; isoflavones [genistein ($R_1=R_2=R_3=OH$); daidzein ($R_1=R_3=OH; R_2=H$)]; flavanones [naringenin ($R_1=R_2=R_4=OH, R_3=R_5=H$)]; anthocyanidins [cyaniding ($R_1=R_2=R_3=R_4=OH; R_5=H$)]; flavanonols [taxifolin ($R_1=R_2=R_3=R_4=OH, R_5=H$)]; and silybin [$R_1=R_2=OH; R_3=H$]

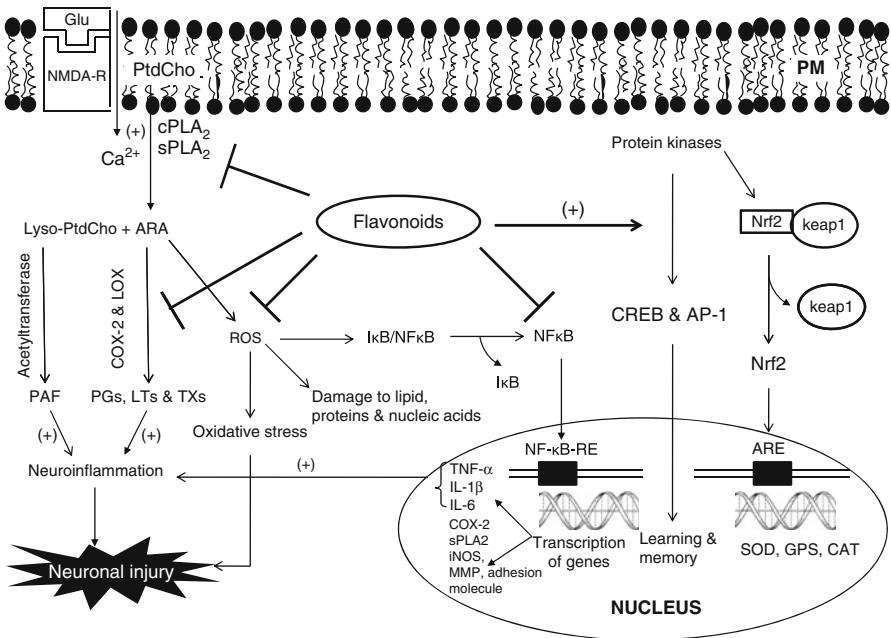


Fig. 4.3 Modulation of protein kinases, PLA₂, COX, LOX, ROS, and NF-κB by flavonoids. *N*-methyl-D-aspartate receptor (NMDA-R); phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); secretory phospholipase A₂ (sPLA₂); arachidonic acid (ARA); lysophosphatidylcholine (Lyo-PtdCho); platelet activating factor (PAF); cyclooxygenase (COX); lipoxygenase (LOX); prostaglandins (PGs); leukotrienes (LTs); thromboxanes (TXs); nuclear factor κB (NFκB); nuclear factor κB-response element (NFκB-RE); inhibitory subunit of NFκB (IκB); IκB kinase (IkK); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); matrix metalloproteinases (MMPs); NF-E2 related factor 2 (Nrf2); kelch-like erythroid Cap'n'Collar homologue-associated protein 1 (Keap1); antioxidant response-element (ARE); cAMP response element binding protein (CREB); activating protein-1 (AP-1); Superoxide Dismutase (SOD); catalase (CAT); glutathione peroxidase (GPS); and \neg (blocked arrow) represents inhibition

Low concentrations of active flavonoids significantly attenuate the expression of E-selectin and intercellular adhesion molecule 1 but not vascular cell adhesion molecule 1 (Loito and Frei 2006). In addition, exposure of apigenin and kaempferol to cultured hepatocytes greatly reduces the inhibitory effect of flavonoids on endothelial intercellular adhesion molecule 1 expression. Based on these results, it is suggested that the effect of dietary flavonoids on endothelial adhesion molecule expression depends on their molecular structure, concentration, and metabolic transformation but not their antioxidant activity (Loito and Frei 2006). Collective evidence from in vitro studies suggests that flavonoids act by downregulating or

suppressing many inflammatory pathways. It should be noted that the antioxidant efficacy of flavonoids *in vivo* is less documented, presumably because of the availability of limited information on the uptake and metabolism in humans.

Some flavonoids not only show antibacterial activity and suppression of bacterial virulence, but show synergism with antibiotics (Cushnie and Lamb 2011). The molecular mechanism associated with these effects of flavonoids is not fully understood, but it is proposed that at the cellular level flavonoids not only inhibit biofilm formation and bacterial attachment to host ligands, but also neutralize toxicity towards cultured human cells (Cushnie and Lamb 2011). In addition, some flavonoids (flavones) have capacity to interact with membrane bilayers due to the presence of lipophilic (nonpolar) as well as hydrophilic (polar) fragments (Oteiza et al. 2005). These interactions not only involve the partitioning of the nonpolar fragment of the flavones between the hydrophobic interior of the lipid bilayers, but also result in the formation of hydrogen bonds between the polar head groups of the lipids and the hydrophilic fragments of flavones at the membrane interface. The induction of alterations in physical properties of neural membranes can affect the rate of oxidation of membrane lipids and proteins (Oteiza et al. 2005).

4.2 Bioavailability and Metabolism of Flavonoids in the Brain

The bioavailability of flavonoids is very low in mammals and information on molecular mechanisms of their action is still poorly understood. The ability of flavonoids to form complexes with reactive metals, such as iron, zinc, and copper, reduces their absorption. Dietary flavonoids enter the gastrointestinal tract in the form of esters and glycosides that are not easily absorbed. Conversion of esters and glycosides into aglycones results in better bioavailability because aglycones are lipophilic and more permeable across the cell membrane than the parent glycoside, and thus are more efficiently absorbed across the gastrointestinal tract wall (Viskupičová et al. 2008; Leonarduzzi et al. 2010). The conversion of glycosides into aglycones mainly occurs in the acidic environment of the stomach in the gastrointestinal tract. Absorption of flavonoids by the intestinal epithelial cells is accompanied by their extensive biotransformation, with the generation of different conjugated products, such as glucuronides, sulfates, and O-methylated derivatives, first in the intestine and then in the liver, where conjugates are secreted into the bile (Felgines et al. 2003; Chyu et al. 2004; Lambert et al. 2007; Leonarduzzi et al. 2010). Favorable absorption across the gastrointestinal tract does not always result in improved bioavailability. Once in the enterocyte, the flavonoids may be subjected to various efflux pumps including the ATP-binding cassette (ABC) transporters, P-glycoprotein (Pgp/ABCB1/MDR1), multidrug resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2), which actively transport flavonoids (or its metabolites) back into the gastrointestinal tract

lumen (Hu et al. 2003; Walle 2004; Dreiseitel et al. 2009). Thus, intestinal and microbial glycosidases and intestinal phase II enzymes make a significant contribution to the disposition of flavonoids via the enteric and enterohepatic recycling scheme (Hu et al. 2003). Flavonoids are metabolized by at least three types of intracellular pathways (a) oxidative metabolism, (b) P450-mediated metabolism, and (c) conjugation with thiols, particularly glutathione (GSH) (Spencer et al. 2003). Accumulating evidence suggests that after oral administration, flavonoids are metabolized in the small intestine, and subsequently undergo metabolic transformation in the presence of enzymes from phases I and II in the liver. In phase I, the major part is played by enzymes involving oxidation and reduction reactions as well as hydrolysis of esters, amides, and other linkages. These reactions introduce more hydrophilic groups such as $-\text{OH}$, $-\text{NH}_2$, $-\text{SH}$, and $-\text{COOH}$. Phase II enzymes are transferases or conjugation enzymes, and they connect the hydrophilic groups to even stronger hydrophilic ligands like glucuronic acid or sulfate. These changes facilitate the absorption of flavonoids in the gut (Hu et al. 2003; Lambert et al. 2007; Leonarduzzi et al. 2010). Collectively, these studies indicate that the intact form of flavonoid and respective metabolites derived from flavonoid biotransformation in the gastrointestinal tract and in the liver are the forms which enter the circulation and ultimately reach the brain (Youdim et al. 2004). In the brain flavonoids improve neural cell health not only through their ability to interact with intracellular neuronal and glial signaling pathways, but also by modulating blood flow in peripheral and cerebral vascular system and reducing neuronal damage and losses mediated by various neurotoxic reactive oxygen species and neuroinflammation (Williams and Spencer 2012).

Brain is protected by the blood–brain barrier (BBB), which not only differs from other vascular barriers in its physical characteristics, but also in the nature and number of transporters it possesses. It is formed by interactions between the highly specialized endothelial cells within brain microvessels and epithelial cells of the choroid plexus. In order for flavonoids to cross the BBB, they must first cross the physical filter, which controls the entry of xenobiotics into the brain. The degree of BBB penetration of flavonoids depends on their lipophilicity (Youdim et al. 2003). Thus, less polar *O*-methylated metabolites may be capable of greater brain uptake than the more polar flavonoid glucuronides. However, it is reported that certain glucuronides may cross the BBB (Aasmundstad et al. 1995) by specific uptake mechanism for glucuronides *in vivo*. Their brain entry may also be modulated by their interactions with specific efflux transporters expressed in the BBB, such as P-glycoprotein (Lin and Yamazaki 2003) which appears to be responsible for the differences between naringenin and quercetin flux into the brain *in situ* (Youdim et al. 2004). Earlier studies indicate that flavonoids, in particular, isoflavones such as genistein produce detrimental effects on memory-related processes in the brain due to their ability to act as tyrosine kinase inhibitors (O'Dell et al. 1991). However, more recent studies show that dietary intervention with isoflavones results in positive effects on neurocognitive function (Lee et al. 2005). Isoflavone supplementation also produces a favorable effect on cognitive function (Casini et al. 2006).

4.3 Biochemical Effects of Flavonoids

Many epidemiological studies indicate that regular flavonoid intake is associated with a reduced risk of cardiovascular and cerebrovascular diseases. In coronary heart disease and stroke, the protective effects of flavonoids include mainly anti-thrombotic, anti-ischemic, antioxidant, and vasorelaxant (Atmani et al. 2009; Fraga et al. 2010). In addition, flavonoids also show antitumoral, antiviral, and antibacterial, as well as a direct cytoprotective effect. It is suggested that flavonoids decrease the risk of cardiovascular and cerebrovascular diseases by three major actions: improving coronary and cerebrovascular vasodilatation, decreasing the ability of platelets in the blood to clot, and preventing oxidation of low-density lipoproteins (LDLs) (Atmani et al. 2009). Studies on anti-inflammatory properties of the flavonoids have indicated that the anti-inflammatory properties of flavonoids are due to its inhibition of the synthesis and biological activities of different proinflammatory mediators, such as eicosanoids (prostaglandins E₂, F₂, and thromboxane A₂) and peroxynitrite (Wang et al. 2006). The antioxidant and anti-inflammatory properties of flavonoids play a key role in their activity against several chronic degenerative diseases and particularly neurological disorders. Experimental animal studies indicate that dietary flavonoids also produce antitumor effects, such as inhibition of cell growth and kinase activity, induction of apoptosis, suppression of matrix metalloproteinases secretion, impairment of angiogenesis, cell cycle arrest, differentiation, and inhibition of tumor invasive behavior. The molecular mechanism associated with antitumor effects is not fully understood. However, hydroxylation pattern of the B-ring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence not only their protein kinase, cyclooxygenase (COX), and lipoxygenase (LOX) inhibitory activities (Fig. 4.4), but also their antiproliferative activities (Kandaswami et al. 2005). Different mechanisms underlying the potential anticancer action of plant flavonoids await further elucidation. Certain dietary flavonols and flavones target cell surface signal transduction enzymes, such as protein tyrosine and focal adhesion kinases, which may control angiogenesis (Kandaswami et al. 2005). Protein kinase C (PKC), the ubiquitous, Ca²⁺- and phospholipid-dependent, multifunctional serine- and threonine-phosphorylating enzyme, is involved in a wide range of cellular activities, including tumor promotion, mitogenesis, secretory processes, inflammatory cell function, and T lymphocyte function (Gamet-Payrastre et al. 1999). Flavonoids modulate growth and proliferation of certain malignant cells in vitro by not only inhibiting activities of PKC isoforms, but also by inhibiting activities of PtdIns 3-kinases. It is reported that inhibition of kinases is due to the competitive binding of flavonoids with ATP at catalytic sites on the enzymes. These modes of inhibition provide the mechanisms by which flavonoids inhibit the tumor and inflammatory responses.

Although the specific receptors by which flavonoids mediate their actions have not been identified, there is some evidence that the flavonoids may trigger their action by interacting with the γ -aminobutyric acid type A (GABA_A) receptors in the central nervous system (CNS) (Marder and Paladini 2002), neurotrophic factor

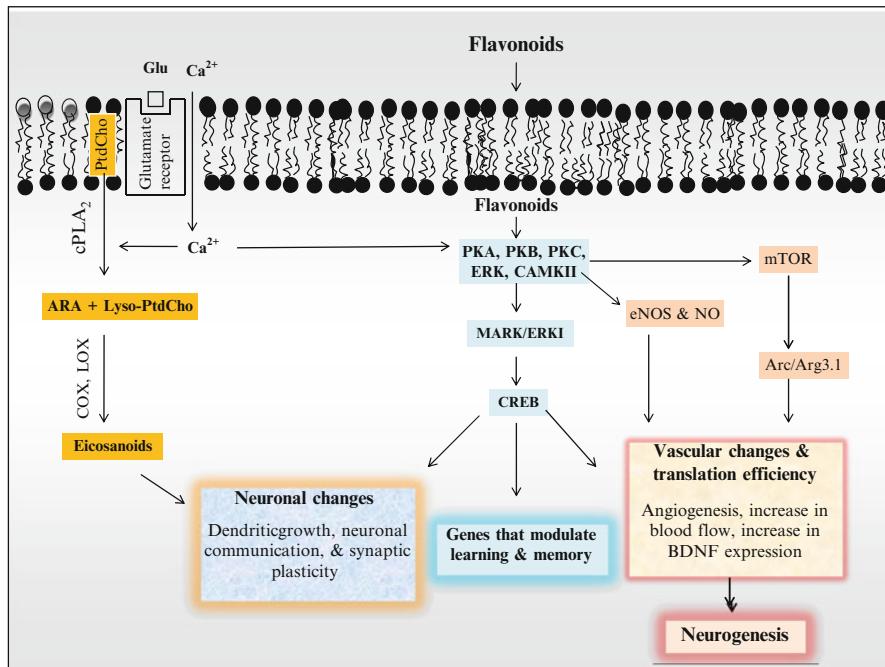


Fig. 4.4 Flavonoid and protein kinase-mediated modulation of neuronal and vascular functions. Glutamate (Glu); phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); protein kinase (PKA); protein kinase B (PKB); protein kinase C (PKC); extracellular signal-regulated kinases (ERK); Ca^{2+} /calmodulin-dependent protein kinases II (CAMKII); mitogen-activated protein kinase (MARK); cAMP response element binding protein (CREB); activity-regulated cytoskeleton-associated protein/activity regulated gene 3.1 (Arc/Arg 3.1); and mammalian target of rapamycin (mTOR)

IGF-1 receptor in hippocampal neurons (Lau et al. 2005), 5-HT1A serotonin receptor (Bodesheim and Holz 1997) and the glutamatergic AMPA receptor or adenosine (type I) receptors (Marder et al. 2003) among others. As mentioned later, many of above receptors are coupled with protein kinases through different signaling pathways such as protein kinase C, tyrosine kinases, serine/threonine kinases, and mitogen-activated protein kinase (MAPK) (Schroeter et al. 2002). In addition, some flavonoids (Flavone) have backbone (2-phenyl-1,4-benzopyrone), which is similar to PD98059 (2'-amino-3' methoxyflavone), a specific pharmacological modulators of ERK signaling. The activation of ERK by flavanols may lead to downstream cAMP response element binding protein (CREB) activation. This process is closely associated not only with long-lasting changes in synaptic plasticity and memory (Finkbeiner et al. 1997), but also with upregulation of neuroprotective pathways including induction of BDNF (Williams et al. 2008). Emerging evidence suggests that flavonoids exert their beneficial health effects through multiple mechanisms.

4.3.1 Antioxidant Properties of Flavonoids

Oxidative stress is defined as a process in which the production of highly reactive oxygen species (ROS) and reactive nitrogen species (RNS) overwhelms antioxidant defenses. Oxidative stress is a feature of many chronic visceral and neurological disorders, which include neurotraumatic and neurodegenerative diseases, such as stroke, spinal cord injury, traumatic brain injury, Alzheimer disease (AD), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS) (Farooqui 2010). ROS and RNS are produced extracellularly and intracellularly by various processes. They initiate and promote neuronal cell death through the oxidative damage to macromolecules such as lipids, proteins, and DNA. Among neural cells, neurons are most susceptible to direct ROS and RNS-mediated oxidative injury (Wang et al. 2006; Farooqui 2010). ROS and RNS can also indirectly contribute to brain injury by activating a number of cellular pathways involving the expression of stress-sensitive genes and proteins to cause oxidative damage. Moreover, oxidative DNA damage leads to the release and nuclear truncation of mitochondrial apoptosis-inducing kinase, which triggers apoptosis-like programmed cell death via cyclophilin A. Thus, it is becoming increasingly evident that oxidative stress plays an important role in pathomechanisms associated with neurodegenerative diseases. Since flavonoids have antioxidant properties, many *in vitro* studies and some *in vivo* studies have shown that flavonoids protect against oxidative stress (Pannala et al. 1997; Ishige et al. 2001; Samhan-Arias et al. 2004; Wang et al. 2006). Based on these studies, it is suggested that consumption of flavonoids may protect from chronic visceral diseases as well as neurotraumatic and neurodegenerative diseases. Flavonoids exert neuroprotective effects not only by decreasing ROS and increasing intracellular glutathione, but also by their selective actions on different components of a number of protein kinase and lipid kinase signaling cascades, such as phosphatidylinositol-3 kinase (PtdIns 3K)/Akt, PKC, and mitogen-activated protein kinase (MAPK) pathways (see below). These processes increase the number and strength of connections between neurons via their specific interactions with the ERK and Akt pathways leading not only to an increase in neurotrophins such as BDNF but also supporting and maintaining cognitive function (Spencer 2008). In addition, interactions of flavonoids with bilayers may also be a relevant mechanism associated with neuroprotective effects of flavonoids. The partitioning of flavonoids in the nonpolar region of the lipid bilayer, their antioxidant activity is not only attributed to their capacity to interact with free radicals, but also their ability to inhibit the propagation of lipid oxidation. Flavonoids also inhibit redox maintaining enzymes, such as NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase (Samhan-Arias et al. 2004; Yazawa et al. 2006; Van Hoom et al. 2002; Mulabagal et al. 2009; Schneider and Bucar 2005), suppress the activation of NF- κ B, and activate adaptive cellular stress responses (Woo et al. 2005; Kim et al. 2010). In addition, flavonoids interact and form chemical complexes with iron and other transition metal ions. This binding of flavonoids with iron retards Fenton reaction thereby inhibiting free radical generation (Mira et al. 2002). Several flavonoids not only stimulate activities of

antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) in humans, but reduce levels of 8-hydroxy-2'-deoxyguanine and DNA damage (Boyle et al. 2000; Huber et al. 2007). It should be noted that clinical trials, as used in pharmaceutical research, may not be appropriate for flavonoids, because most flavonoids are found in foods, but they are not drugs. The activity of flavonoids is generally acute and potent, but rather cumulative and subtle.

4.3.2 Anti-inflammatory Properties of Flavonoids

Activated microglia and astrocytes are known to produce nitric oxide and upregulate the expression of proinflammatory cytokines. These processes involve mitogen-activated protein kinase (MAPK) signaling pathway and the NF- κ B signaling cascade. MAP kinases, which include extracellular signal-regulated kinase (ERK1/2), c-Jun N-terminal kinase (JNK1/2/3), and p38 kinase (p38abcd) are associated with the transfer of signals from outside into inside the cell and cellular responses. During signal transduction process, activated kinases phosphorylate both cytosolic transcription factors (i.e., STAT-1/2/3, NF- κ B, CREB), leading to their nuclear translocation that ultimately result in gene expression (Chang and Karin 2001). The increased expression of both iNOS and cytokines in microglia is partly regulated by signaling MAPK pathway-mediated signaling (Bhat et al. 1998; Culbert et al. 2006). In visceral tissues, dietary flavonoids exhibit anti-inflammatory effects by interacting with different molecular (phospholipase A₂, cyclooxygenase, and lipoxygenase) and cellular targets (macrophages, lymphocytes, epithelial cells, and endothelial cells) (Lin et al. 2003). They predominantly inhibit NF- κ B signaling and downregulate the expression of proinflammatory markers (cytokines and chemokines), which exert their primary effects through the NF- κ B pathway (Fig. 4.3). In brain, ROS and RNS-mediated injury also activates mechanisms that result in a glia cell-mediated inflammation that also causes secondary neuronal damage. Thus, brain damage in neurotraumatic and neurodegenerative diseases is accompanied by the activation of microglial cells and astrocytes at the sites of injury. Activated glial cells are thus histopathological hallmarks of neurodegenerative diseases (Wang et al. 2006; Farooqui 2010). Although direct contact between activated glial cells with neurons per se may not necessarily be toxic, the release of NO and ROS and proinflammatory cytokines and chemokines by activated glial cells may contribute to neurodegeneration. Dietary flavonoids have potential to restore the population of microglial cells in the elderly brain to its youthful state (Jang and Johnson 2010). This suggestion is supported by several studies, which demonstrate that flavonoids significantly suppress the activation of NF- κ B and AP-1 as well as MAPK pathways in activated microglia, resulting in an attenuation of the production of inflammatory molecules (Chang and Karin 2001; Chen et al. 2005; Jang et al. 2008). Collective evidence suggests that neuroinflammation is a highly synchronized series of neural cell activation processes, most of which are linked to eicosanoid and platelet activating factor

Table 4.1 Effects of flavonoids of enzyme activities

Enzyme	Effect
Protein kinases	Inhibitory
Phospholipase A ₂	Inhibitory
Phospholipase C	Inhibitory
Nitric oxide synthase	Inhibitory
Ornithine decarboxylase	Inhibitory
Hyaluronidase	Inhibitory
Sialidase	Inhibitory
ATPases	Inhibitory
Cyclic nucleotide phosphodiesterase	Inhibitory
Adenylate cyclase	Inhibitory
Reverse transcriptase	Inhibitory
Xanthine oxidase	Inhibitory
RNA and DNA polymerase	Inhibitory
Ribonuclease	Inhibitory
Human DNA ligase	Inhibitory
Aldose reductase	Inhibitory
Malate dehydrogenase	Inhibitory
Lactate dehydrogenase	Inhibitory
Glutathione S transferase	Inhibitory
Glutathione reductase	Inhibitory
Glyoxalase	Inhibitory
Aromatase	Inhibitory
Topoisomerase	Inhibitory
HIV-1 integrase	Inhibitory
Monoamino oxidase	Inhibitory

Most of the above-mentioned effects are observed in *in vitro* studies.
Summarized from Middleton et al. (2000) and Fraga et al. (2010)

biosynthesis via arachidonic acid and lysophospholipid metabolism. Flavonoids are nature-derived NF-κB inhibitors. They inhibit TNF-α-induced ICAM-1 expression through an effect on this transcription factor (Rathee et al. 2009).

4.3.3 Regulation of Enzymes by Flavonoids

Most physiological benefits of flavonoids are generally due to their antioxidant and free-radical scavenging activities observed *in vitro*; however, it is becoming increasingly evident that flavonoids may also act through their effect on enzyme activities (Table 4.1). These enzymes include protein kinases, COXs, LOXs, nitric oxide synthases, and ATPases. These reports extend the effects of flavonoids beyond their antioxidant properties. Protein kinases that interact with flavonoids include Akt/protein kinase B (Akt/PKB), Fyn, Janus kinase 1 (JAK1), mitogen-activated protein kinase kinase 1 (MEK1), PtdIns 3K, mitogen-activated protein (MAP) kinase kinase 4 (MKK4), Raf1, and z chain-associated 70-kDa protein (ZAP-70) kinase.

These protein kinases are essential proteins closely associated with intracellular signaling cascades involved in neurodegeneration and neuroprotection. In addition, flavonoids are also known to affect many other enzyme systems such as phospholipase A₂ (PLA₂), phospholipase C (PLC), and lipoxygenase (LOX). These enzymes are closely associated with allergic and inflammatory responses (Middleton and Kandaswami 1992). As stated earlier, inhibition of these enzymes by flavonoids increases the number and strength of neuronal connections between neurons through their specific interactions with the ERK and Akt pathways. ERK and Akt are closely associated with increase in brain-derived neurotrophic factor (BDNF) expression. This process supports and maintains cognitive function (Spencer 2008). In addition, flavonoids also inhibit redox maintaining enzymes, such as NADPH oxidase, xanthine oxidase, myeloperoxidase, COX, and LOX (Samhan-Arias et al. 2004; Yazawa et al. 2006; Van Hoom et al. 2002; Mulabagal et al. 2009; Schneider and Bucar 2005), which suppress the activation of NF-κB and activate adaptive cellular stress responses (Woo et al. 2005; Kim et al. 2010).

It is well known that nitric oxide (NO) is synthesized from L-arginine by three nitric oxide synthases (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Low physiological levels of NO are needed and produced by constitutively expressed eNOS and nNOS for normal neural cell function, whereas iNOS is responsible for prolonged production of larger amounts of NO. In visceral tissues, iNOS is induced by bacterial products and inflammatory cytokines in macrophages along with endothelial dysfunction, whereas in the brain neural cell injury induces the expression of iNOS (Korhonen et al. 2005; Farooqui 2010). Flavanol-rich cocoa reverses endothelial dysfunction via the modulation of the NO pathway (Rassaf and Kelm 2008). Similarly, active flavonoids, such as quercetin, epigallocatechin gallate, morin, apigenin, taxifolin, fisetin, and catechin, attenuate NO production in C6 astrocyte cell cultures by inhibiting NOS (Soliman and Mazzio 1998), and some flavonoids retard NO production in lipopolysaccharide-activated RAW 264.7 cells by inhibiting iNOS expression (Kim et al. 1999). NO not only reacts with several metal centers, molecular oxygen, thiol groups, and some oxygen radicals, but also modulates vasodilation by activating guanylyl cyclase through the reaction with its heme group. In addition, NO reacts with superoxide anion, the one-electron reduction product of oxygen in a near diffusion controlled reaction to form peroxynitrite ($\text{NO} + \text{O}_2^- \rightarrow \text{ONOO}^-$). The production of peroxynitrite results in oxidation of cellular components (Ignarro 2002) and elimination of NO. Collective evidence suggests that flavonoids suppress the inflammatory response by downregulating NO production in response to inflammatory stimuli (Kim et al. 2001; Middleton et al. 2000).

NO is also involved in modulation of blood pressure (BP). The concept of NO as a modulator of BP is based on the bioavailability of NO for normal vasodilation and normal BP and decrease in NO steady-state concentration can lead to a failure in smooth muscle relaxation and the consequent hypertension. BP-lowering effect of flavonoids may be due to (a) increases in levels of NO-derived species in plasma or urine; (b) improvement in NO-mediated flow-mediated dilation as an indicative of vascular function; and (c) reduction in oxidative stress (Fraga et al. 2011).

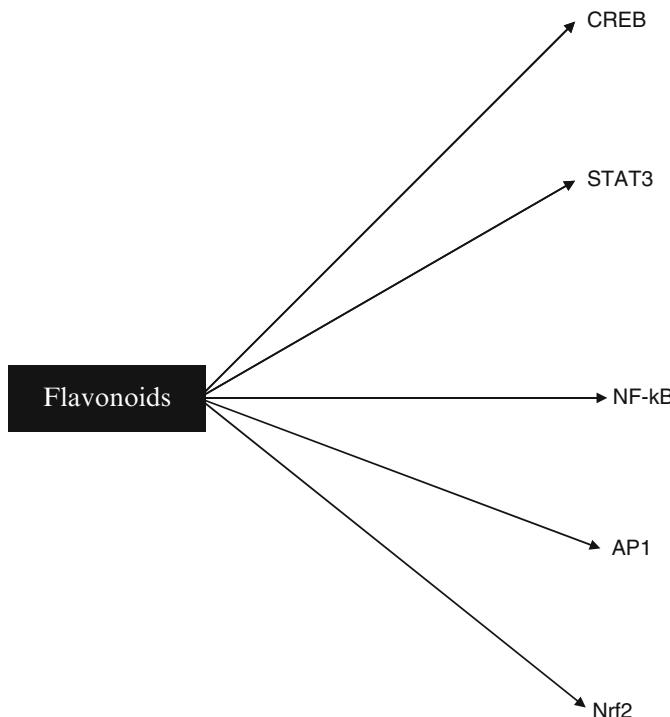


Fig. 4.5 Modulation of transcription factor by flavonoids

Suppression of phosphodiesterases is of particular therapeutic importance in chronic and allergic inflammation (Kusano et al. 1991). Phosphodiesterase inhibition is an important activity associated with a number of flavonoids. It is also shown that flavone aglycones with five or more methoxy substituents (such as citrus polymethoxyflavones) are potent inhibitors of cAMP phosphodiesterase, whereas C-glycosyl flavones are poor inhibitors (Dehmlow et al. 1996; Kusano et al. 1991).

4.3.4 Effect of Flavonoids on Transcription Factors

Flavonoids (EGCG, luteolin, quercetin, chrysin, and eriodictyol) interact with a number of transcription factors (Fig. 4.5), such as CREB, NF-κB, AP-1, STAT3, and Nrf2 and downregulate the expression of proinflammatory cytokines and chemokines as well as proinflammatory enzymes (Kim et al. 2004; Gomes et al. 2008). The stimulation of receptors delivers signals through adaptor molecules, kinases, and ultimately to transcription factors, which trigger target gene transcription in the nucleus. Thus, studies on the effect of flavonoids (genistein, kaempferol, quercetin, and daidzein) indicate that these flavonoids inhibit LPS-mediated STAT-1

and NF-κB activations, and iNOS expression. In addition, flavones (isorhamnetin, naringenin, and pelargonidin) block NF-κB activation and iNOS expression but produce no effect on STAT-1 supporting the view that flavonoids may modulate the expression of different inflammatory mediators associated with different cell signaling pathways (Hämäläinen et al. 2007). Among transcription factors, NF-κB is a ubiquitous transcription factor that regulates many central events in normal neuronal cell function and fate. NF-κB is redox sensitive, and in general, oxidants promote and antioxidants inhibit its activation. Oxidative stress, neuronal injury, infection, and proinflammatory stimuli activate the p50 and p65 proteins subunits of NF-κB and promotes the translocation of NF-κB from cytoplasm to the nucleus where it binds to target sequences in the genome and facilitates the expression of a number of proteins including many enzymes (sPLA₂, COX-2, NADPH oxidase, and inducible nitric oxide synthase), cytokines (TNF-α, IL-1β, and IL-6), chemokines, and adhesion molecules (Fig. 4.3). Activation of NF-κB plays an important role in inflammation because it induces transcription of proinflammatory genes (Jobin et al. 1999). This pathway is activated via cellular stimulation, most often from signals related to oxidative stress and pathogens. Flavonoids not only stabilize NF-κB-I-κB complex in the cytoplasm, but prevent NF-κB translocation to the nucleus (Gomes et al. 2008). It is also reported that flavonoids inhibit NF-κB activation by stopping IκBα degradation. Collective evidence suggests that flavonoids interfere with NF-κB activation not only by counterbalancing changes in cell redox state, but also by specific bonding to proteins involved in the NF-κB pathway.

AP-1 is another redox-sensitive transcriptional factor, which plays important roles in development and stress responses. AP-1 activation is associated with growth regulation, cell transformation, inflammation, and innate immune response. AP-1 has been linked to the regulation of genes involved in apoptosis and proliferation, and may promote cell proliferation by activating the cyclin D1 gene, and repressing tumor-suppressor genes, such as p53, p21cip1/waf1, and p16. Several phenolic compounds, such as green tea flavan-3-ols, quercetin, trans-resveratrol, and curcumin have been shown to suppress the AP-1 activation process (Hammerstone et al. 1999; Burns et al. 2002; Aggarwal and Shishodia 2006).

In addition, flavonoids also modulate the NF-E2 related factor 2 (Nrf2), a member of the cap'n'collar family of basic leucine zipper transcription factors. This transcription factor is a redox-sensitive factor whose nuclear translocation and binding to the antioxidant response elements (ARE) in their promoter regions result in the induction of antioxidant enzymes, which protect neural cells from oxidative stress-mediated cell injury (Brigelius-Flohe and Banning 2006; Banning et al. 2005; Andreadl et al. 2006). Collective evidence suggests that increase in antioxidant defenses through activation of the NF-E2 related factor 2 (Nrf2) also contribute to the anti-inflammatory capacity of flavonoids (González-Gallego et al. 2007) (Fig. 4.3).

In addition, in vitro studies indicate that flavonoids, such as quercetin mediate antifibrotic (Lee et al. 2003), anticoagulative (Bucki et al. 2003), antibacterial (Cushnie and Lamb 2005), antiatherogenic (Perez-Vizcaino et al. 2006), antihypertensive (Duarte et al. 2001), and genotoxic effects (Pacifici 2004), which may lead

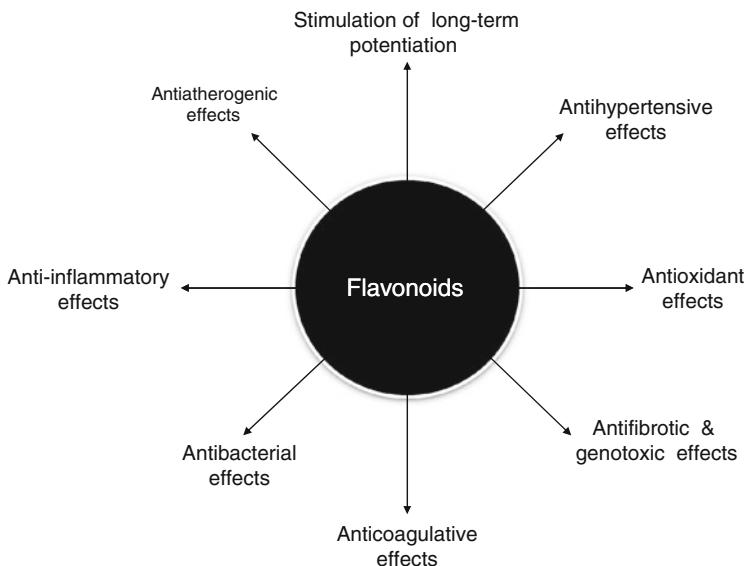


Fig. 4.6 Beneficial effects of flavonoids on human health

to inhibition of proliferation by interacting with estrogen-binding sites (Caltagirone et al. 1997). Collective studies indicate that quercetin may exert health-beneficial capacities via various damage modulating effects (Fig. 4.6). However, most of these studies have been performed with immortalized or cultured cell lines only. They cannot be extrapolated to the *in vivo* human situation.

4.3.5 Flavonoids and Endothelial Cell Dysfunction

The endothelium is the inner lining of all blood vessels. It is associated with selective barrier permeability between blood and tissues. Normal function of endothelial cells is to regulate vasoconstrictor tone, platelet activity, leukocyte adhesion, and vascular smooth muscle cell proliferation through the generation and release of NO, which not only prevents adherence of leukocytes to the endothelial surface and retards platelet adhesion and platelet aggregation but also inhibits expression of leukocyte adhesion molecules at the endothelial surface. In addition, NO inhibits vascular smooth muscle cell proliferation and alters expression of noncellular components that constitute the matrix of the vascular wall, making NO relevant to lesion formation, hypertrophy of the vessel wall, and vascular compliance (Hodgson and Croft 2010).

The development of endothelial dysfunction may be involved in the pathogenesis of stroke. Endothelial dysfunction is characterized by the loss of normal endothelium-dependent and NO-mediated vasodilation in the artery. Green tea flavonoids

are associated with vasorelaxation of rat aortic rings, which is NO and endothelium dependent (Hodgson et al. 2006; Hodgson and Croft 2010). In humans, one of the main methods has been to use ultrasonography to measure flow-mediated dilatation of conduit vessels, such as the brachial artery. This is a noninvasive technique that measures NO-dependent vasodilation of the artery in response to shear stress induced by increased blood flow. Thus, based on 4 human trials in healthy and diabetic subjects, it is proposed that acute and chronic green tea consumption improves endothelial cell dysfunction not only through the generation and *maintenance* of NO status, but also via the reduction of endothelin 1, a potent vasoconstrictor (Loke et al. 2008).

4.3.6 Effect of Flavonoids on Memory

Earlier studies indicate that flavonoids, in particular isoflavones such as genistein produce detrimental effects on memory-related processes in the brain due to their ability to act as tyrosine kinase inhibitors (O'Dell et al. 1991). However, more recent studies show that dietary intervention with isoflavones results in positive effects on neurocognitive function (Lee et al. 2005). Isoflavone supplementation also produces a favorable effect on cognitive function (Casini et al. 2006). Flavonoids found in spinach, strawberry, blueberry, and green, especially in combination with exercise, have been shown to enhance the retention of rat spatial memory in water maze tests (van Praag et al. 2007). This improvement not only involves increase in angiogenesis and neuronal spine density in the dentate gyrus of the hippocampus, but also the upregulation of genes associated with learning in the hippocampus. Studies on beneficial effects of flavonoid-rich foods and beverages on psychomotor activity in older animals have also been performed (Ramirez et al. 2005), and it is reported that flavonols such as quercetin reverse the course of neuronal and behavioral aging (Shukitt-Hale et al. 2006). The molecular mechanism associated with these processes is not fully understood. However, it is suggested that beneficial effects of flavonoids on brain may be due to the presence of flavonoid-binding sites on neurons (Bastianetto et al. 2010). As mentioned earlier, interactions between flavonoids and flavonoid-binding sites may be associated with changes in the activation status of various downstream kinases, including various members of both the MAP kinase and the PtdIns 3-kinase pathways and the nuclear factor- κ B pathway (Woo et al. 2005; Spencer 2008; Kim et al. 2010; Williams and Spencer 2012). It is proposed that the phosphorylation (activation/inhibition) profiles of such downstream kinases in response to flavonoids *in vitro* is highly suggestive of high-affinity receptor agonist-like actions at low concentrations (low to mid nanomolar) and desensitization or direct enzyme inhibition at higher concentrations (high nanomolar to micromolar) (Williams and Spencer 2012). These processes not only modulate neurotransmitter release (Joseph et al. 1999), but also stimulate hippocampal neurogenesis (Casadesus et al. 2004) and neuronal signaling (Goyerzu et al. 2004) leading to alterations in the functional efficiency and/or connectivity of existing

neurons and modulation of memory formation (Spencer 2009). It is also likely that above-mentioned processes may stimulate the differentiation of neuronal stem cells, producing new neurons that are capable of interconnecting with older neurons, thus leading to an increase in the capacity memory formation and storage (Spencer 2009; Williams and Spencer 2012).

4.3.7 Effects of Flavonoids on Microglial Cells

Microglial cells are the resident macrophages of the brain. They play important roles in immune regulation (Hanisch and Kettenmann 2007) and neuronal homeostasis (Streit 2002). Ramified microglia perform a very active and continuous surveillance function with their long protrusions (Nimmerjahn et al. 2005), whereas activated microglia produce inflammatory molecules such as cytokines, superoxide, and nitric oxide. Excessive or sustained activation of microglia often contributes to acute and chronic neuroinflammatory responses in the brain and the retina, which may be responsible for neurodegeneration in neurological disorders (Hanisch and Kettenmann 2007; Farooqui 2010). It is proposed that blocking the production of these molecules may be an important way to mitigate neurological disorders (Amor et al. 2010; Farooqui 2010). As stated above that transcription factors (NF-κB and AP-1) are important transcription factors. These transcription factors along with ERK1/2, p38, and c-Jun-N-terminal kinase (JNK) regulate the expression of inflammatory gene during the inflammatory response. Flavonoids significantly retard the activation of NF-κB and AP-1 as well as MAPK pathways in activated microglia, inhibiting the production of inflammatory molecules (Jang et al. 2008; Chen et al. 2005). Thus, by modulating the microglial transcriptome and inhibiting the production of IL-1 β , TNF α , NO, and prostaglandin E $_2$, luteolin produces anti-inflammatory, antioxidative, and neuroprotective effects (Dirscherl et al. 2010). Quercetin retards LPS-mediated NO generation and iNOS gene transcription by inhibiting activation of IκB kinase (IKK), NF-κB, and AP-1 (Chen et al. 2005). Taken together, these studies indicate that flavonoids may be important bioactive molecules that retard neuronal damage by attenuating microglia activation and inducing microglial homeostasis. Based on these findings, it is suggested that luteolin may be a promising candidate for developing immunomodulatory and neuroprotective therapies for the treatment of neurodegenerative disorders (Dirscherl et al. 2010).

4.3.8 Modulation of Immunity by Flavonoids

Some flavonoids modulate certain immune processes by inhibiting myelin membrane phagocytosis (Hendricks et al. 2003), retarding the formation of PGE $_2$, and

suppressing the production of IgE (Lim 2003). As mentioned earlier, the interaction between the flavonoids and the neural membrane bilayer results in either the binding at the lipid–water interface or the distribution in the hydrophobic core of the membrane. Collective evidence suggests that interactions of hydrophilic group of flavonoids with neural membrane surface may result in providing neuroprotection against different deleterious agents (oxidants) (Oteiza et al. 2005).

4.4 Effect of Flavonoids on Neurological Disorders

Neurological disorders include neurodevelopmental, neurotraumatic, neurodegenerative, and neuropsychiatric diseases. Neurodegeneration in neurotraumatic, neurodegenerative, and neuropsychiatric diseases is a multifactorial process, which is accompanied by oxidative stress, neuroinflammation, reduction in the expression of trophic factors, and accumulation of protein aggregates leading to neuronal death in brain and spinal cord. Degeneration of neurons and loss of synapses in neurological disorders may cause problems with thinking, speaking, swallowing, breathing, skilled movements, decision making, cognition, and memory (Graeber and Moran 2002; Wishart et al. 2006; Soto and Estrada 2008; Farooqui 2010). It is becoming increasingly evident that the incidence of many chronic neurological (AD and dementia) and visceral disorders (heart disease and arthritis) increase with aging. Their etiology may partially involve lifestyle determinants such as obesity, decreased sensitivity to insulin, and the metabolic syndrome (Farooqui et al. 2012). Prominent neurochemical alterations of many neurological disorders are increased degradation of neuronal membrane phospholipids (phosphatidylcholine and plasmalogen), sphingolipids (sphingomyelin), and cholesterol due to the stimulation of PLA₂, sphingomyelinases (SMase), and cytochrome P450 hydroxylases (Farooqui 2010) along with the abnormal accumulation of iron in the degenerating neurons and in the surrounding microglia. Flavonoids (catechins) have been reported to contain not only antioxidant, anti-inflammatory, and divalent metal chelating activities, but also possess PLA₂, PLC, COX, LOX, and protein kinase inhibitory properties to penetrate the brain barrier and to protect neuronal death in a wide array of cellular and animal models of neurological diseases (Marambaud et al. 2005; Rezai-Zadeh et al. 2005). In addition, many flavonoids induce adaptive cellular stress responses, by which neural cells achieve ability to counteract stressful situations (Calabrese et al. 2008). Adaptive cellular stress responses not only require the activation of pro-survival genes, but also induction and expression of antioxidative and antiapoptotic molecules and activities (Calabrese et al. 2008). The vitagene system has emerged as a neurohormetic potential target for novel cytoprotective interventions of polyphenolic phytochemicals (Calabrese et al. 2008). These genes encode for survival proteins such as HSP70 and HO-1 as well as thioredoxin/thioredoxin reductase (Calabrese et al. 2008, 2009). It is becoming increasingly evident that neurohormetic phytochemicals including flavonoids suppress disease processes in animal models of AD (Mattson et al. 2007; Kim et al. 2010).

4.4.1 Flavonoids and Alzheimer Disease

Alzheimer disease (AD) is characterized by the accumulation of beta-amyloid ($A\beta$) peptide to form senile plaques and phosphorylated tau forming *neurofibrillary* tangles leading to the most common form of dementia. The earliest symptoms of AD often appear as subtle and intermittent deficits in the ability to remember minor events of everyday life. At later stages, AD gradually progresses to severe dementia, which affects multiple cognitive and behavioral functions. Compelling evidence supports the view that $A\beta$ plays a central role in the pathogenesis of the disease (Hardy and Selkoe 2002). Accumulation of $A\beta$ is associated with the stimulation of several enzymes associated with the pathogenesis of AD, such as PLA₂, NOS, and protein kinases. In AD, dementia is correlated to neuronal and synaptic loss, rather than directly to pathological burden (Farooqui 2010). Although the molecular mechanism associated with pathogenesis of AD remains unknown, it is becoming increasingly evident that AD is accompanied not only by induction of oxidative stress and neuroinflammation (Farooqui 2010) in area where neurons are degenerating, but also by marked elevations in levels of enzymic (eicosanoids and platelet activating factor) and nonenzymic lipid mediator (4-hydroxynonenal and isopropanes) alterations in redox homeostasis (Farooqui 2010). These processes occur in nucleus basalis, hippocampal, and entorhinal cortical regions of brain from AD patients (Farooqui 2010). It is suggested that certain flavonols and flavones act as BACE-1 inhibitors and mediate the suppression of BACE-1 expression (Shimmyo et al. 2008a, b). This is consistent with some of the observed $A\beta$ lowering effects reported for flavonoid-rich extracts *in vivo* and *in vitro* (Williame et al. 2004; Williams and Spencer 2012). In addition, a number of flavonoids (myrecetin and epicatechin 5-gallate) block heparin-mediated tau aggregation (Taniguchi et al. 2005). Moreover, grape seed polyphenolic extract also inhibits tau fibrillization, promotes the loss of preformed tau aggregates, and disrupts paired helical filaments (Ksieczak-Reding et al. 2010; Wang et al. 2010; Pasinetti et al. 2010; Ho et al. 2009). It is not known which part of the flavonoid ring possesses BACE inhibitory activity (Williams and Spencer 2012). Furthermore, flavonoids may exert their neuroprotective effects not only through decreasing ROS and increasing intracellular glutathione, but also by modulating activities of a number of protein and lipid kinases (PtdIns 3K)/Akt, PKC, and MAPK), PLA₂s, COXs, and LOXs in signal transduction pathways (Mulabagal et al. 2009; Schneider and Bucar 2005). Flavonoids also inhibit NADPH oxidase and xanthine oxidase (Samhan-Arias et al. 2004; Yazawa et al. 2006; Van Hoom et al. 2002), stimulate activities of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), suppress the activation of NF- κ B, and activate adaptive cellular stress responses (Woo et al. 2005; Boyle et al. 2000; Huber et al. 2007; Kim et al. 2010). In addition, flavonoids form chemical complexes with iron and transition metal ions. These interactions inhibit free radical generation (Mira et al. 2002).

Epidemiological studies indicate that moderate red wine intake reduces the risk of developing AD (Lindsay et al. 2002; Truelsen et al. 2002; Luchsinger et al. 2004).

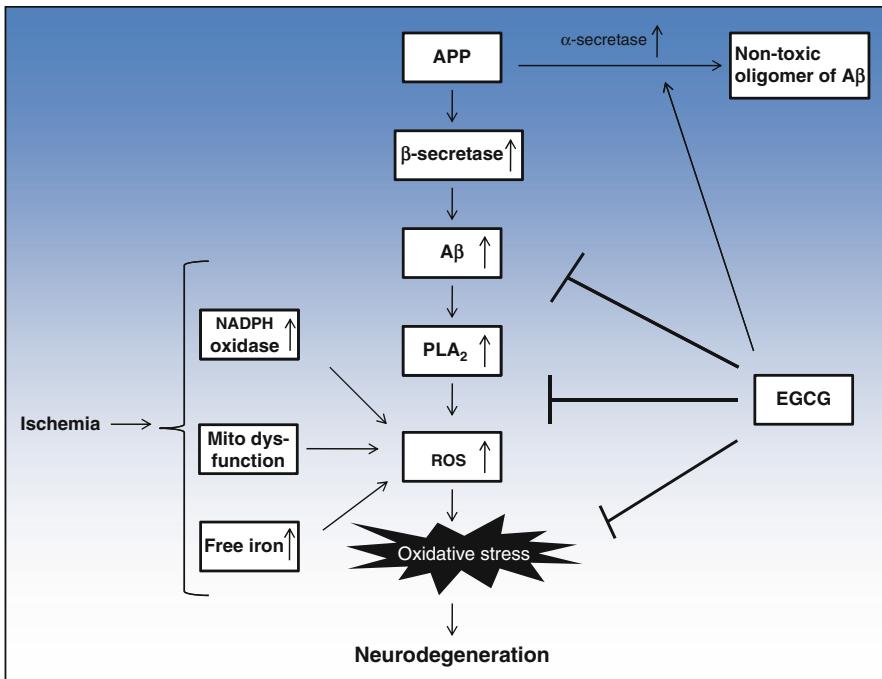


Fig. 4.7 Risk factors for stroke and modulation of signal transduction process and thrombosis by flavonoids. Nitric oxide (NO); arginine (Arg); nitric oxide synthase (NOS); and free fatty acids (FFA). Upward arrows indicate increase; \neg (blocked arrow) represents inhibition

This is due to the presence of resveratrol, a flavonoid found in red grapes and wine. It is also reported that resveratrol not only reduces levels of A β produced in different cell lines expressing wild type or Swedish mutant APP $_{695}$ by its potent antiamyloidogenic activity, but also acts by promoting the intracellular degradation of A β by a mechanism that implicates the proteasome (Marambaud et al. 2005). Similarly, (-)-epigallocatechin-3-gallate (EGCG), the main flavonoid component of green tea, reduces A β generation in both murine neuron-like cells (N2a) transfected with the human “Swedish” mutant amyloid precursor protein (APP) and in primary neurons derived from Swedish mutant APP-overexpressing mice (Tg APP $_{sw}$ line 2576) (Rezai-Zadeh et al. 2005). At the molecular level, EGCG acts by promoting the cleavage of the α -C-terminal fragment of APP and elevating the N-terminal APP cleavage product, soluble APP- α . These cleavage events are not only associated with upregulation of α -secretase activity, but also enhanced hydrolysis of tumor necrosis factor α -converting enzyme, a primary candidate α -secretase (Rezai-Zadeh et al. 2005; Smith et al. 2010) (Fig. 4.7). These effects are associated with increased generation of α -C-terminal fragment (α -CTF) and sAPP- α and elevated α -secretase cleavage activity, showing that EGCG promotes the nonamyloidogenic α -secretase proteolytic pathway both in vitro and in vivo (Rezai-Zadeh et al. 2005).

Oral administration of quercetin, another flavonoid, significantly improves the behavioral performance of old mice fed with high-cholesterol diet in both a step-through test and the Morris water maze task (Lu et al. 2010). This is not only due to decrease in ROS production and protein carbonyl levels, but also because of restoration of Cu–Zn superoxide dismutase activity. In addition, quercetin treatment also results in activation of the AMP-activated protein kinase (AMPK) through the downregulation of protein phosphatase 2C (PP2C), which not only reduces the integral optical density of activated microglia cells and CD11b expression, but also downregulates iNOS and COX-2 expression, and decreases cytokine expression in the brains of high-cholesterol-fed old mice through the suppression of NF- κ B p65 nuclear translocation (Lu et al. 2010). Moreover, AMPK-mediated increase in 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and acetyl-CoA carboxylase (ACC) phosphorylation reduces fatty acid synthase expression in the brains of high-cholesterol-fed old mice. This reduces cholesterol levels, downregulates cholesterol 24-hydroxylase (CYP46A1) and β -amyloid converting enzyme 1 (BACE1) expression, decreases eukaryotic translation initiation factor 2 α (eIF2 α) phosphorylation, and lowers A β deposits (Lu et al. 2010). These results suggest that AMPK activated by quercetin may be a potential target for enhancing the resistance of neurons to age-related diseases (Lu et al. 2010).

Studies in another animal models of AD also show that treatment of 6-month-old Tg2576 and nontransgenic (NonTg) mice with low and high doses of cocktail containing flavonoids and vitamins (curcumin, piperine, epigallocatechin gallate, α -lipoic acid, N-acetylcysteine, B vitamins, vitamin C, and folate) not only improves cognitive function, but also decreases AD neuropathology, suggesting that this treatment may represent a safe, natural treatment for AD-like disease (Parachikova et al. 2010). Collective evidence suggests that curcumin, resveratrol, and EGCG exhibit antioxidant activity and antiamyloidogenic activities, which reduce the formation of neurotoxic A β fibrils in animal models of AD (Youdim et al. 2004; Ono et al. 2004; Marambaud et al. 2005; Rezai-Zadeh et al. 2005; Ehrnhoefer et al. 2008; Singh et al. 2008). It is also reported that EGCG induces the formation of assembly of a new type of unstructured, SDS-stable, nontoxic oligomer.

4.4.2 Flavonoids and Stroke

Stroke is a metabolic insult caused by severe reduction or blockade in cerebral blood flow due to cerebrovascular disease. This blockade not only decreases oxygen and glucose delivery to brain tissue but also results in the breakdown of blood–brain barrier (BBB) and buildup of potentially toxic products in brain (Farooqui 2010). Age is a prominent risk factor for stroke. Other risk factors for stroke include hypertension, diabetes mellitus, abnormal apolipoprotein E metabolism, high alcohol consumption, cigarette smoking, oral contraceptive, and underlying clotting disorders (Farooqui 2010).

Neurochemically, stroke involves the release of excess glutamate in the extracellular space, overstimulation of glutamate receptors, depolarization, and dramatic increase of intracellular calcium (Farooqui and Horrocks 1994). Stroke also triggers alterations in cellular redox status and marked increase in free-radical generation. These processes also lead to the activation of signaling mechanisms involving phospholipases A₂, C, and D (PLA₂, PLC and PLD), calcium/calmodulin-dependent kinases (CaMKs), mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), nitric oxide synthases (NOS), calpains, calcinurin, and endonucleases. Stimulation of these enzymes bring them in contact with appropriate substrates and modulates cell survival/degeneration mechanisms (Hou and MacManus 2002; Farooqui and Horrocks 2007; Farooqui 2010).

Flavonoids provide protection against neurodegeneration in cerebral ischemic injury (Simonyi et al. 2005). Flavonols (flavan-3-ols, flavanones, anthocyanins, and isoflavones) prevent endothelial dysfunction, atherosclerosis, hypertension, and possibly thrombosis. All above mechanisms are associated with the prevention of stroke. With regard to acute treatment, flavonols may produce their effect on different phases of stroke. In the acute phase, flavonols like quercetin, kaempferol, and myricetin have been reported to improve cerebral blood flow, prevent platelet aggregation and thrombosis, reduce excitotoxicity, and inhibit oxidative stress. In the intermediate phase, flavonols reduce inflammation and protect endothelial integrity. For the late phase, flavonols have been shown to interfere with ischemia-induced cell death mechanisms such as apoptosis and necrosis (Hollman et al. 2010; Perez-Vizcaino and Duarte 2010; Edwards et al. 2007). Meta-analysis indicates that high intake of flavonols compared with low intake results in a 20 % lower risk of stroke incidence. Thus, oral administration of catechin in the drinking water for 2 weeks provides protection against hippocampal neuronal death in ischemic injury in gerbils (Inanami et al. 1998) in a dose-dependent manner (Fig. 4.8). Furthermore, injections of EGCG immediately after the onset of ischemia in a rat transient focal cerebral ischemia model also reduce neuronal damage in the hippocampal region (Sutherland et al. 2006). EGCG also reduces matrix metalloproteinase (MMP) an enzyme that plays an important role in the pathophysiology of cerebral ischemia (Park et al. 2010). Similarly, resveratrol, a flavonoid not only reduces the infarct size, but also significantly decreases neuronal death in the hippocampus and also inhibits glial cell activation after common carotid artery ligation in rats.

Studies on neuroprotective effects of flavonoids against cerebral ischemic reperfusion injury indicate that in male Wistar rats intravenous injections of flavonoid (baicalin) after middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion for 24 h results in significant neurological deficit scores and 25 % reduction in the infarction volume (Xue et al. 2010). Western blot analysis and RT-PCR studies indicate that levels of NF-κB p65 are increased in cortex after ischemia–reperfusion injury. Baicalin treatment decreases levels of NF-κB p65 by 73 % indicating that baicalin may be a useful neuroprotective agent in stroke therapy and neuroprotective effects of baicalin may not only relate to inhibition of NF-κB p65, but also with the modulation of angiogenesis through the induction of vascular endothelial

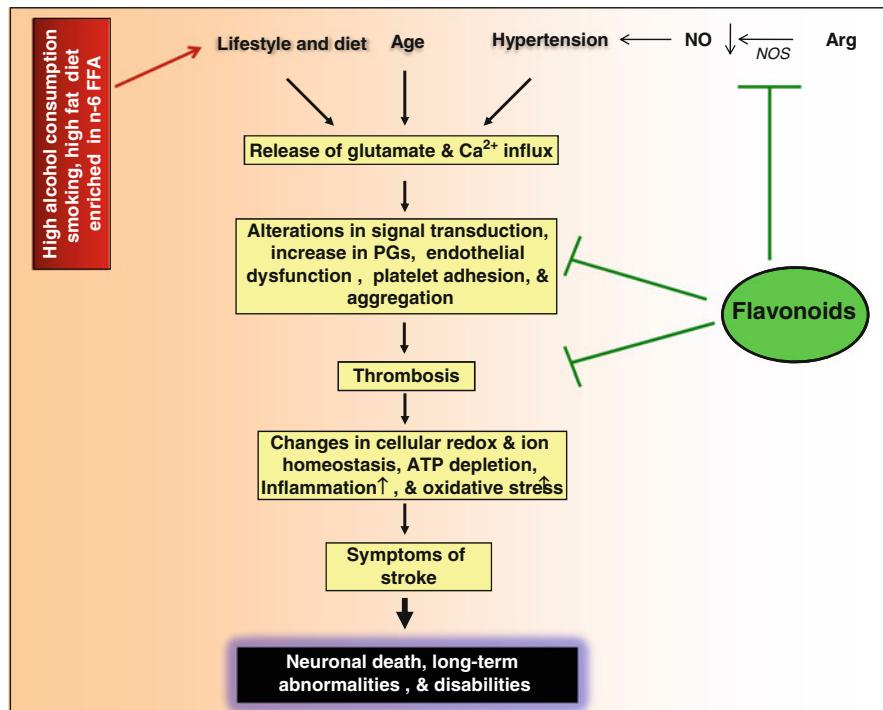


Fig. 4.8 Effect of green tea flavonoids (polyphenols) on risk factors for stroke (age, n-6 enriched food, and hypertension). Nitric oxide (NO); arginine (Arg); nitric oxide synthase (NOS); and free fatty acids (FFA). *Downward arrow* indicates decrease in NO

growth factor (VEGF) expression and activation of the oestrogen-related receptor α (ERR α) pathway (Xue et al. 2010; Zhang et al. 2011). In addition, flavonoids also exert a positive effect on cerebral blood flow (CBF) in humans (Fisher et al. 2006; Francis et al. 2006). Consumption of a flavanol-rich cocoa drink increases blood flow in certain regions of human brain. These observations are supported by “arterial spin-labeling sequence magnetic resonance imaging” (ASL-MRI) studies (Wang et al. 2008), which indicate that cocoa flavanols increase CBF up to a maximum of two hours after ingestion of the flavanol-rich drink (Fisher et al. 2006).

4.4.3 Flavonoids and Parkinson Disease

Parkinson disease (PD) is a chronic and progressive neurological disorder characterized by uncontrolled muscle tremor, rigidity, and bradykinesia. PD is caused by the gradual and selective loss of dopaminergic neurons in the substantia nigra pars

compacta (Beal 1998; Jenner and Olanow 2006). Loss of these neurons causes pathological changes in neurotransmission in the basal ganglia motor circuit. The neuropathologic hallmark of PD is the presence of Lewy bodies composed mostly of α -synuclein and ubiquitin. In addition, *in vivo* brain imaging studies show significant increase of iron levels in the substantia nigra pars compacta in PD (Gerlach et al. 2006). This increase in iron, however, occurs only in the advanced stages of PD, suggesting that this phenomenon may be a secondary rather than a primary initiating event in the disease process. The major metabolic pathways associated with pathophysiology of PD include mitochondrial dysfunction, free radical generation, oxidative and nitrosative stress, glutamate receptor-mediated excitotoxicity, inflammation, oligodendrocytic interaction and neurotrophic factors, accumulation of aberrant or misfolded proteins (α -synuclein), and ubiquitin-proteasome system dysfunction (Beal 1998; Jenner and Olanow 2006). Among the currently used drug treatments for PD, levodopa (L-DOPA, a dopamine precursor) is an effective drug for relieving PD's motor symptoms. Oral administration of EGCG may have significant beneficial effects in PD patients treated with L-DOPA and carbidopa by exerting a modest inhibition of L-DOPA methylation plus a strong neuroprotection against oxidative damage and degeneration (Kang et al. 2010). The molecular mechanism associated with EGCG-mediated neuroprotection includes reductions in ROS accumulation and inhibition of NF- κ B transcriptional activity. In addition, it is also reported that EGCG efficiently inhibits the fibrillogenesis of both α -synuclein and A β by directly binding to the natively unfolded polypeptides and preventing their conversion into toxic moiety in the aggregation pathway (Ehrnhoefer et al. 2008; Meng et al. 2009; Bieschke et al. 2010). Thus, instead of β -sheet-rich formation, EGCG initiates the formation of nontoxic α -synuclein oligomers. Suggestions about neuroprotective effect of EGCG are also supported by recent epidemiological studies, indicating that regular tea or coffee drinking is associated with a reduced risk of PD (Kandinov et al. 2009).

4.4.4 Flavonoids and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease characterized by loss of oligodendrocytes that maintain the myelin sheath as well as oxidative stress-mediated damage to axons with the loss of neurons. Reactive oxygen species (ROS) generated by activated macrophages and microglial cells are thought to play a major role in damaging myelin and myelin-producing cells (oligodendrocytes) in MS (Pedotti et al. 2003). Flavonoids, such as luteolin, have antioxidant and anti-inflammatory properties, including inhibition of activated peripheral blood leukocytes from MS patients. Luteolin also inhibits mast cells, as well as mast cell-dependent T cell activation, recently implicated in MS pathogenesis. Moreover, luteolin inhibits the development of experimental allergic encephalomyelitis (EAE) in rodents, suggesting that luteolin formulation can be used for the

treatment of MS (Pedotti et al. 2003; Theoharides 2009). Similarly quercetin, a flavonoid phytoestrogen, has profound anticancer and anti-inflammatory properties. Treatment of SJL/J mice with quercetin (i.p. 50 or 100 µg every other day) has been reported to ameliorate EAE and its symptoms in association with the inhibition of IL-12 production and neural antigen-specific Th1 differentiation (Muthian and Bright 2004). In vitro treatment of activated T cells with quercetin blocks IL-12-induced tyrosine phosphorylation of JAK2, TYK2, STAT3, and STAT4, resulting in a decrease in IL-12-induced T cell proliferation and Th1 differentiation. These findings suggest that quercetin ameliorates EAE by blocking IL-12 signaling and Th1 differentiation and can be useful in the treatment of MS and other Th1 cell-mediated autoimmune diseases.

4.5 Harmful Effects of Flavonoids

An important aspect of flavonoids therapy is their limited bioavailability due to their low absorption and rapid elimination. Aglycons and glucosides are absorbed in the small intestine, but they are transformed quickly into methylated, sulfated, or glucuronic acid conjugated derivatives, which may or may not have the same biological activity than the original compounds. Many beneficial effects of flavonoids are based on in vitro studies. These effects must be confirmed and validated in humans. Delivery of optimal dose of drugs into the brain is one of the most challenging problems faced in the treatment of neurological disorders, which are commonly accompanied by oxidative stress and neuroinflammation (Farooqui 2010). Very little is known about the relationship between start of oxidative stress, neuroinflammation, and onset of neurological diseases. For flavonoid therapy to work, it is important that these substances should be consumed continuously from childhood to the old age because long-term oxidative stress and inflammatory damage to neurons cannot be compensated and fully or partially reversed by flavonoids that are given after the onset of neurodegenerative process. Therefore, the consumption of variety of fruits and vegetables is recommended for correcting oxidative stress and neuroinflammation in patients with neurological disorders (Farooqui 2010).

In addition, in spite of above-mentioned beneficial effects of flavonoids in neurological and visceral disorders, it is important to realize that at high doses flavonoids may act as prooxidants at high concentrations. Thus, some flavonoids (quercetin and myricetin) increase hydroxyl radical production and DNA damage at concentration >50 µM, (Laughton et al. 1989). In addition, quercetin may also produce genotoxic as well as facilitate the oxidation of NADPH and promote oxidized state (Gonzalez-Gallego 2007; Shih et al. 2004). These prooxidant characteristics may explain why exposure of rat aortic smooth muscle cells to quercetin concentrations >100 µM increases NF-κB activation or the fact that similar quercetin doses increase iNOS and COX-2 expression in parallel to the stimulation of the NF-κB-dependent pathway in liver cells (García-Mediavilla et al. 2007).

4.6 Conclusion

Flavonoids are naturally occurring heterogeneous group of natural molecules differently represented in fruit and vegetables. They play an important role in maintaining human health by delaying or partially preventing the onset of visceral and neurological diseases. A fundamental property of flavonoids responsible for many of their beneficial effects on visceral organs and brain is their antioxidant and anti-inflammatory properties, which not only allow them to interact with transition metals ions (Fe^{2+} , Cu^{2+} , or Zn^{2+}), but also scavenge ROS-like superoxide anion, oxygen singlet, and lipidic peroxyradicals. In addition, flavonoids also inhibit enzymes of oxygen-reduction pathways. Limited absorption and their low bioavailability in the brain indicate that their beneficial effects through antioxidant and anti-inflammatory properties are unlikely. Instead, the effects of flavonoids on human brain may be due to the regulation of signal transduction processes, which involve modulation of PtdIns 3-kinase/Akt, and MAP-kinases pathways and inhibition of PLA₂, COX, LOX, and NADPH oxidase catalyzed reactions to regulate pro-survival gene expression and transcription factors. Collective evidence suggests that flavonoids improve endothelial function and reduce blood pressure, oxidative damage, inflammation, and risk of thrombosis. In addition, flavonoids are not only capable of improving peripheral and cerebral blood flow and triggering angiogenesis, but also have ability to induce neurogenesis in the hippocampus.

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Chapter 5

Beneficial Effects of Green Tea Catechins on Neurological Disorders

5.1 Introduction

Green tea is natural dried leaves of the tea plant, *Camellia sinensis*. This “nonfermented” tea contains more catechins than black tea (oxidized green tea) or oolong tea (partially oxidized tea). The composition of tea leaves depends on a variety of factors, including climate, season, horticultural practices, and the type and age of the plant. Green and black teas contain polyphenols, alkaloids (caffeine, theophylline, and theobromine), flavonols (quercetin, kaempferol, and rutin), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds that contribute to tea flavor, fluoride, aluminum, minerals, and trace elements (Fig. 5.1). Green tea contains gallic acid (GA), chlorogenic acid, and caffeic acid, and flavonols such as kaempferol, myricetin, and quercetin (USDA data base 2003; Wang and Ho 2009). In contrast, black tea mostly has the polymerized catechins such as theaflavins and thearubigins. Collectively, these studies indicate that green tea is the source of catechins—simple flavonoids whereas black tea is rich in theaflavins and thearubigins, which are generated during the process of oxidation (USDA data base 2003; Wang and Ho 2009). Four major theaflavins have been identified from black tea, including theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-digallate. Catechins are strong antioxidants that can quench reactive oxygen species (ROS) such as super oxide radical, singlet oxygen, hydroxyl radical, peroxy radical, nitric oxide, nitrogen dioxide, and peroxynitrite (Feng 2006). Since ancient times, green tea has been considered by the traditional Chinese and Japanese medicine as a healthful beverage. Human studies indicate that green tea not only contributes to a reduction in the risk of cardiovascular disease and some forms of cancer, but also induces antihypertensive effects by suppressing angiotensin I-converting enzyme, body weight control by suppressing the appetite, antibacterial, and antivirasic effects, solar ultraviolet protection, bone mineral density increase, antifibrotic effects, and neuroprotective effects. Green tea also decreases blood pressure (Henry and Stephens-Larson 1984) and blood sugar (Matsumoto et al. 1993). Lipid metabolism studies in animals, tissues, and cells have found that tea extract and catechins

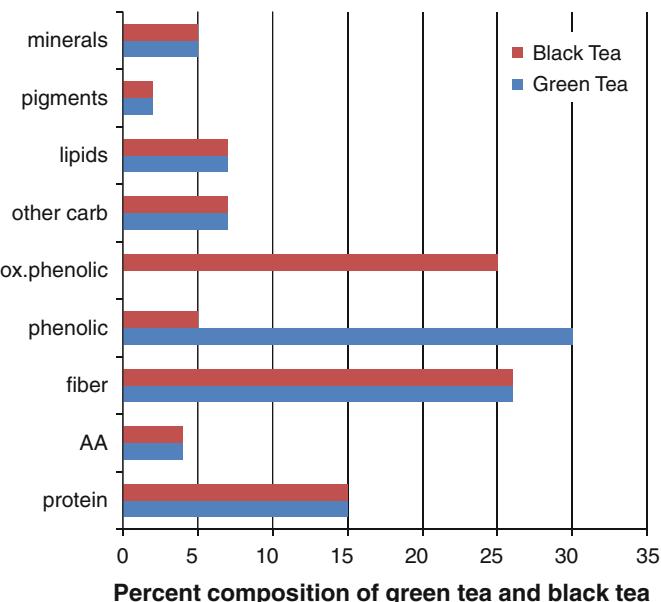


Fig. 5.1 Chemical composition of green tea and black tea. Data are based on the dry weight of tea leaves. Adapted from Chacko et al. (2010). Amino acids (AA), oxidized phenolic compounds (ox-phenolic), and phenolic compounds (phenolic)

reduce triacylglycerol and total cholesterol concentrations (Nanjo et al. 1994; Chan et al. 1999), inhibit hepatic and body fat accumulation (Ishigaki et al. 1991), and stimulate thermogenesis (Dulloo et al. 2000). In addition, green tea boosts metabolism and improves immune function.

Green tea is manufactured by inactivating polyphenol oxidase in the fresh leaves by either firing or by steaming, which prevents the enzymic oxidation of catechins, the most abundant flavonoids found in green tea extracts. For the production of black and oolong teas, the fresh leaves are allowed to wither until their moisture content is reduced to $\approx 55\%$ of the original leaf weight. The withered leaves are then rolled, crushed, and subjected to fermentation. These processes convert catechins into theaflavins and thearubigins. Oolong tea is produced by firing the leaves shortly after rolling to terminate the oxidation and dry the leaves. Normal oolong tea is considered to be about half as fermented as black tea. The fermentation process results in oxidation of simple polyphenols to more complex condensed polyphenols to give black and oolong teas their characteristic colors and flavors.

The major catechins of green tea include (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Mukhtar and Ahmad 2000; Higdon and Frei 2003; Velayutham et al. 2008) (Fig. 5.2). Catechins are polyphenolic compounds with diphenyl propane skeleton. Catechins consist of a polyphenolic ring (A) condensed with six-membered oxygen containing heterocyclic ring (C) that carries another polyphenolic

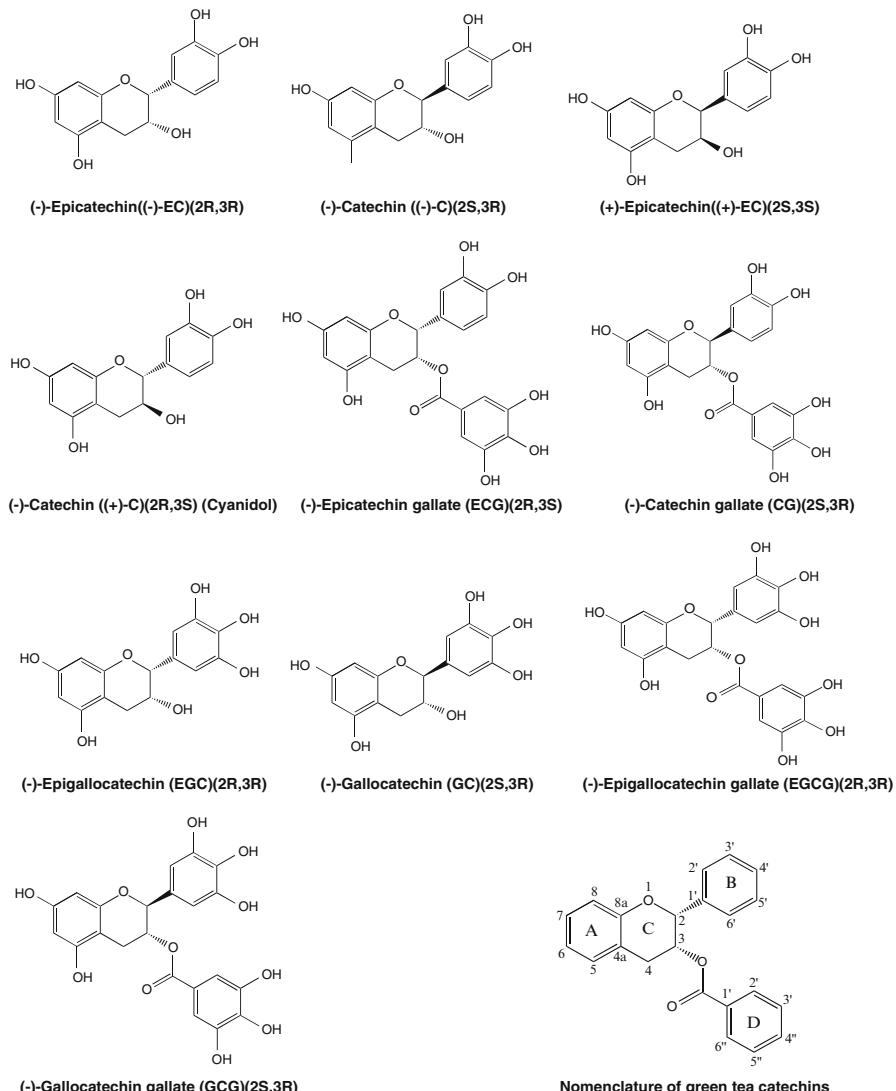


Fig. 5.2 Chemical structures of various catechins found in green tea

ring (B) at the 2 position. Catechins contain multiple hydroxyl groups on the A and B rings. EC is an epimer containing two hydroxyl groups at 3' and 4' position of B-ring and a hydroxyl group at 3 position of the C-ring (Fig. 5.2). The only structural difference between EGC and EC is that EGC possesses an additional hydroxyl group at 5' position of the B ring. ECG and EGCG are ester derivatives of EC and EGC, respectively, through esterification at 3 hydroxyl position of the C-ring

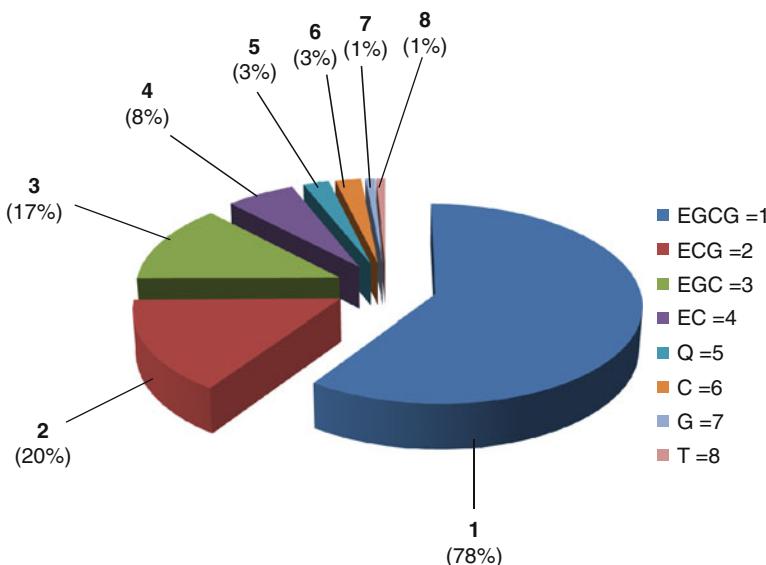


Fig. 5.3 Flavonoid composition of brewed green tea. Proportions are based on amount mg/100 g. Adapted from Rains et al. (2011)

with a gallate moiety (Higdon and Frei 2003; Velayutham et al. 2008). As stated earlier, EGCG is the major catechin in green tea and accounts for 50–80 % representing 200–300 mg in a brewed cup of green tea. Collective evidence suggests that relative concentrations of catechins in green tea are EGCG>ECG>EC>EGC>C (Fig. 5.3).

5.2 Bioavailability and Metabolism of Green Tea Catechins in the Brain

The bioavailability of green tea catechins depends upon their structural diversity. Catechin monomers can be easily absorbed through the gut barrier, whereas the large molecular weight catechins, such as EGCG are poorly absorbed. EGCG is quite stable in the stomach and small intestine. Efflux transporters Pgp, MRP1, and MRP2 play important roles in the absorption and excretion of green tea catechins. Attempts have been made to increase the bioavailability of EGCG through the synthesis of synthetic analogs of EGCG (Zaveri 2001). These analogs contain a trimethoxybenzoyl ester (D-ring) and are equally as potent as natural EGCG for their efficacy as antioxidants and anticarcinogenic agents (Waleh et al. 2005). In addition, it is also reported that bioavailability of EGCG can also be increased by delivering EGCG using lipid nanocapsules and liposome encapsulation (Barras et al. 2009; Siddiqui et al. 2009). Absorbed catechins are biotransformed in the liver

to conjugated metabolites through methylation, glucuronidation, sulfation, and ring fission. Thus, EGCG is methylated into 4"-*O*-methyl(-)-EGCG and 4',4"-*O*-dimethyl(-)-EGCG by catechol-*O*-methyltransferase (COMT) (Lu et al. 2003a). Similarly, EGCG also undergoes glucuronidation to form EGCG-4"-*O*-glucuronide, a major metabolite found in humans, mice, and rats (Lu et al. 2003b). This reaction is catalyzed by human UDP glucuronosyltransferases (UGT1A1, 1A8, and 1A9). These enzymes are located in the endoplasmic reticulum in many tissues and catalyze the transfer of a glucuronic acid from UDP-glucuronic acid to EGCG. Among these enzymes, intestinal UGT1A8 most efficiently catalyzes glucuronidation of EGCG. Sulfation of green tea catechins is catalyzed by sulfotransferases. These enzymes transfer sulfate group from 3-phosphoadenosine-5'-phosphosulfate to hydroxyl groups in EGCG and other catechins in human, mouse, and rat liver cytosol. Methylated EGCG, glucuronidated EGCG, and sulfated EGCG can be further methylated, glucuronidated, and sulfated to form related mixed EGCG metabolites (Sang et al. 2008). Green tea catechins undergo three degradation processes, which include decomposition to smaller molecules, polymerization to larger molecules, and oxidation to oxidized molecules under the natural conditions. The digestive tract plays a very important role in the metabolism and bioavailability of green tea components before they reach the liver. Green tea catechins and their metabolites are formed in the small intestine and transported back into the intestinal lumen and reach the large intestine where they are further metabolized into small phenolic acids and valerolactones by the gut microflora. These metabolites are either reabsorbed or pass out in the feces (Feng 2006).

The development of biological biomarkers in blood and urine is essential for making accurate estimates of green tea intake. However, the relationship between dietary intakes and nutritional biomarkers seems to be highly complex because of limited bioavailability of polyphenol (Spencer et al. 2008). It is suggested that aromatic and phenolic profile of plasma and urine of healthy men after oral consumption of pure polyphenols (quercetin, (-)-epicatechin, and epigallocatechin gallate) may act as useful biomarkers (Loke et al. 2009). Urine analysis indicates that urinary 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid may be potential biomarkers of quercetin intake, and 1,3,5-trimethoxybenzene, 4-*O*-methylgallic acid, 3-*O*-methylgallic acid, and gallic acid may be potential markers of epigallocatechin gallate intake. These urinary biomarkers may provide an accurate indication of polyphenol exposure in human (Loke et al. 2009).

As stated in Chap. 4, brain is protected by the blood–brain barrier (BBB). In order for green tea catechins to cross the BBB, they must first cross the physical filter, which controls the entry of xenobiotics into the brain. The degree of BBB penetration of green tea catechins depends on their lipophilicity (Youdim et al. 2003). Thus, less polar O-methylated metabolites may be capable to greater brain uptake than the more polar glucuronidated catechins. However, it is reported that certain glucuronides may cross the BBB (Aasmundstad et al. 1995) by specific uptake mechanism for glucuronides *in vivo*. Their brain entry may also be modulated by their interactions with specific efflux transporters expressed in the BBB, such as P-glycoprotein (Lin and Yamazaki 2003).

5.3 Beneficial Effects of Green Tea Catechins in the Brain

Green tea catechins are generally amphiphilic molecules, but the different substituents in their chemical structure provide each catechin particular physical characteristics. In general, at the cellular level catechins either interact with lipids at the surface of the bilayer (adsorption) or inserted into the bilayer, where they interact with the hydrophobic chains of lipids. Direct interactions of catechins with lipids induce alterations in membrane physical properties and/or modulate different membrane-associated biological events including the activity of membrane-associated enzymes, ligand–receptor interactions, ion and/or metabolite fluxes, and the modulation of signal transduction (Feng 2006).

Interactions among green tea catechins, lipids, and proteins are accompanied by changes in enzyme activity and ligand/receptor function. During interactions of EGCG with proteins, EGCG or EGC is converted to a catechol-quinone upon autooxidation, and the resultant quinone moiety rapidly reacts with sulphydryl group of a protein to form cysteinyl-flavonoid adducts (Ishii et al. 2008). In addition, EGCG binds with serum proteins such as fibronectin, fibrinogen, histidine-rich glycoproteins, 67-kDa laminin receptor, Bcl-2 proteins, and vimentin (Yang et al. 2006). EGCG also interacts with growth factor receptors, such as epidermal growth factor, platelet-derived growth factor, insulin-like growth factor 1, and vascular endothelial growth factor receptors and alters signal transduction processes (Khan et al. 2008; Spencer 2008; Spencer et al. 2008). It is proposed that the inhibitory effect of EGCG on the activation of epidermal growth factor receptor (EGFR) may be associated with changes in membrane lipid order. In the membrane, EGCG binds with laminin receptor (LamR), a lipid raft protein. Similar to aplidin and edelfosine, EGCG also inhibits epidermal growth factor (EGF) binding to EGFR, dimerization, and relocation of phosphorylated EGFR to lipid rafts (Patra et al. 2008). EGCG not only inhibits the activities of cyclin-dependant kinases 2 and 4, but induces the expression of the Cdk inhibitors p21 and p27, leading to G1 arrest (Lin et al. 1999). EGCG also blocks telomerase, topoisomerase II, DNA methyltransferase 1, DNA polymerase, and cAMP-response element-binding protein (Table 5.1), affecting chromatin maintenance and remodeling (Patra et al. 2008). With ability of EGCG to

Table 5.1 Effect of EGCG on enzyme activities

Enzyme	Effect	Reference
MAP kinase	Inhibition	Kundu and Surh (2007)
Phosphatase	Inhibition	Balasubramanian and Eckert (2004)
DNA methyltransferase	Inhibition	Fang et al. (2003)
Topoisomerase	Inhibition	Sadava et al. (2007)
Dihydrofolate receptor reductase	Inhibition	Navarro-Peran et al. (2005)
DNA polymerase	Inhibition	Kuzuhara et al. (2006)
Matrix metalloproteinase	Inhibition	Fassina et al. (2004)
Urokinase	Inhibition	Jankun et al. (1997)
Monoamine oxidase B	Inhibition	Lin et al. (2010)

interact with so many targets, it is difficult to propose a unifying hypothesis about its action.

EGCG binds to DNA and RNA molecules in cells through polynucleotides and protects against ROS-, ionization-, and ultraviolet radiation-mediated DNA damage caused by DNA methylation (Fang et al. 2003). EGCG not only inhibits the expression of the tumor necrosis factor- α (TNF- α) gene in human cancer cells treated with the tumor promoter okadaic acid (Suganuma et al. 1996), but also affects DNA replication, DNA repair, and transcription (Johnson and Loo 2000). These effects can be explained through the actions of catechin on above-mentioned enzymes (Johnson and Loo 2000; Mizushina et al. 2005). Collectively, these studies indicate that one or two molecules of EGCG bind to double-stranded (AG-CT) oligomers of various nucleotide lengths. Double-stranded DNA (dsDNA) oligomers are detected only as EGCG-bound forms at high temperature, whereas at low temperature both the free and bound forms are detected. This observation supports the view that EGCG protects dsDNA oligomers from dsDNA melting to single-stranded DNA. Because both galloyl and catechol groups of EGCG are essential for DNA binding, both groups seem to hold strands of DNA via their branching structure (Kuzuhara et al. 2006).

Green tea catechins exert neuroprotective effects by interacting with different components of a number of protein kinase and lipid kinase signaling cascades, such as phosphatidylinositol-3 kinase (PtdIns 3K)/Akt, PKC, and mitogen-activated protein kinase (MAPK) pathways (Fig. 5.4). These processes increase the number and strength of connections between neurons via their specific interactions with the ERK and Akt pathways, leading to an increase in neurotrophins such as BDNF that support and maintain cognitive function (Spencer 2008). Catechins also inhibit NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, suppress the activation of NF- κ B, and activate adaptive cellular stress responses (Woo et al. 2005; Kim et al. 2010) (Fig. 5.5). In addition, catechins form complexes with iron and other transition metal ions. This binding of catechins with iron retards Fenton reaction thereby inhibiting free radical generation (Mira et al. 2002). Collective evidence suggests that green tea catechins not only inhibit MAP kinases and PtdIns 3K/AKT pathway, block NF κ B- and AP-1-mediated transcription, but also inhibit growth factor-mediated signaling and retard aberrant arachidonic acid metabolism through cyclooxygenase and lipoxygenase. The end result of these effects may be the inhibition of tumor cell growth, induction of apoptosis, or the inhibition of angiogenesis (see below) (Yang et al. 2006).

5.3.1 Antioxidant Properties of Green Tea Catechins

Presence of di- or tri-hydroxyl groups on the B-ring and the meta-5,7-dihydroxyl groups on the A-ring may be responsible for strong antioxidative activities of green tea. The antioxidative activity is further increased by the presence of trihydroxyl structure in the D-ring (gallate) in EGCG and ECG (Wiseman et al. 1997; Rice-Evans 1999). Green tea components interact with ROS, such as superoxide radical,

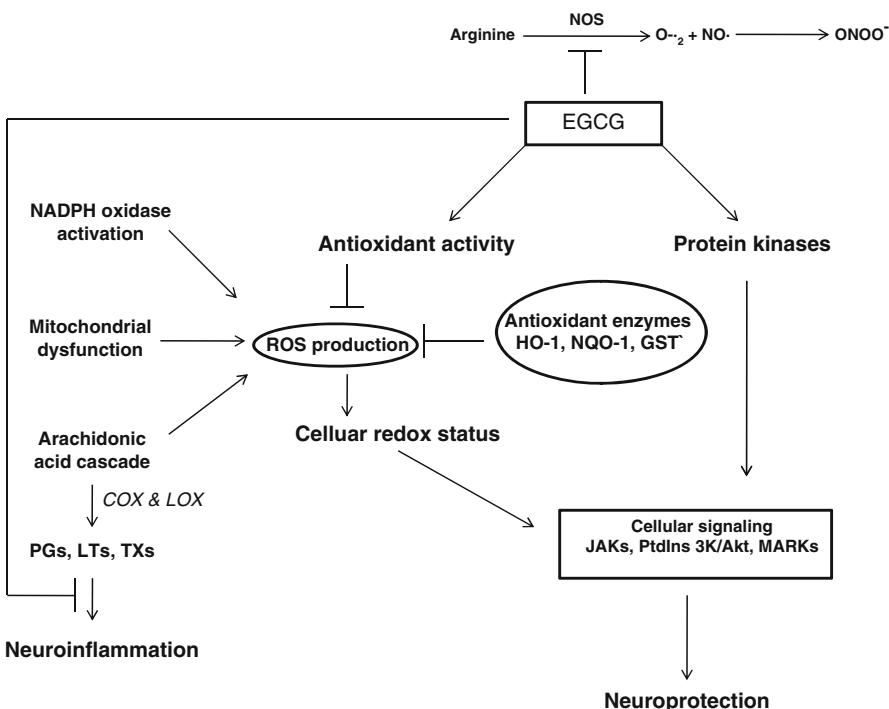


Fig. 5.4 Effect of (-)-epigallocatechin-3-gallate (EGCG) on protein kinases, NOS, and the generation of reactive oxygen species. Reactive oxygen species (ROS); nitric oxide (NO); nitric oxide synthase (NOS); prostaglandins (PGs); leukotrienes (LTs); thromboxanes (TXs); Janus Kinase (JAKs); phosphatidylinositol-3 kinase (PtdIns 3K)/protein kinase B (Akt); protein kinase C (PKC); and mitogen-activated protein kinases (MAPKs); and $\overline{\lvert}$ (blocked arrow) represents inhibition

singlet oxygen, hydroxyl radical, peroxy radical, nitric oxide, nitrogen dioxide, and peroxy nitrite. Among green tea catechins, EGCG is most effective in reacting with most ROS. The B-ring appears to be the principal site of antioxidant reactions (Valcic et al. 2000; Sang et al. 2002). The polyphenolic structure allows electron delocalization, conferring high reactivity to quench free radicals. During the reaction of green tea polyphenols with ROS, several oxidation products are formed (Sang et al. 2007).

The autoxidation of EGCG results in the generation of superoxide anion and H_2O_2 and the formation of dimers such as theasinensins. These reactions occur even during cell culture conditions. It is proposed that this is due to superoxide anion catalyzed chain reactions (Fig. 5.6), because EGCG can be stabilized by the addition of superoxide dismutase (Hou et al. 2005). Reactions of EGCG and other catechins with peroxy radicals lead to the formation of anthocyanin-like compounds (Kondo et al. 1999), as well as seven-member B-ring anhydride dimers and ring-fission compounds (Valcic et al. 2000). Antioxidant activity of green tea may also be due to chelation of metal ion by the dihydroxyl and trihydroxyl structures of

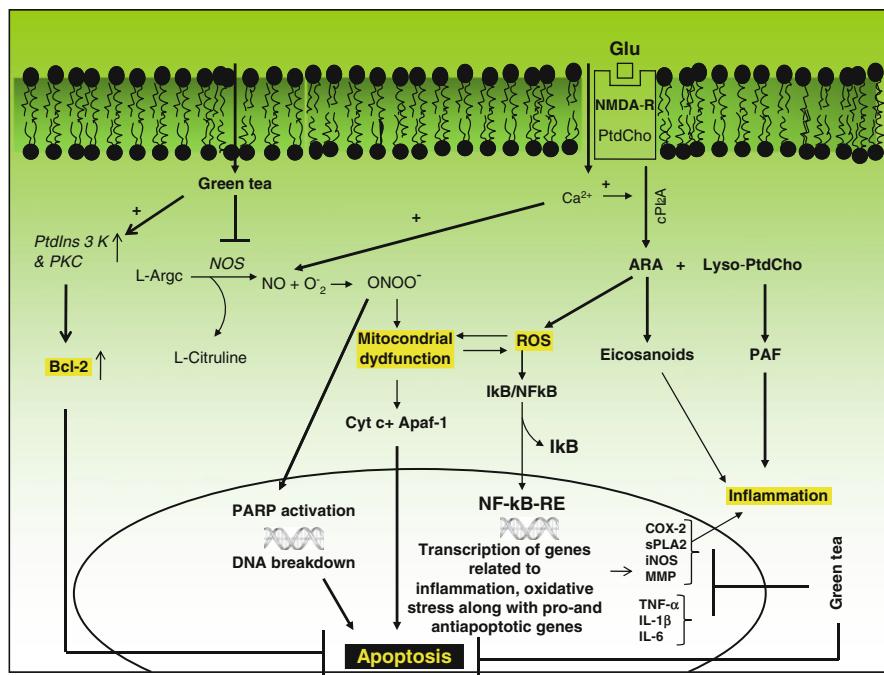


Fig. 5.5 Molecular mechanisms associated with the beneficial effects of green tea. *N*-methyl-D-aspartate receptor (NMDA-R); glutamate (Glu); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (cPLA₂); secretory phospholipase A₂ (sPLA₂); cyclooxygenase (COX-2); matrix metalloproteinases (MMPs); inducible nitric oxide synthase (iNOS); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor kappaB (NF-κB); nuclear factor kappaB response element (NF-κB-RE); inhibitory subunit of NFκB (IκB); tumor necrosis factor- α (TNF- α); interleukin-1 β (IL-1 β); interleukin-6 (IL-6); peroxynitrite (ONOO⁻); Superoxide (O_2^-); matrix metalloproteinases (MMPs); positive sign (+) represents upregulation; ; and \neg (blocked arrow) represents inhibition

green tea components, which inhibit the production of ROS. Green tea catechins not only inhibit ROS synthesizing enzymes, but also block Cu^{2+} -induced oxidation of lipoproteins in vitro (Hashimoto et al. 2000). Pretreatment of macrophages or endothelial cells with green tea polyphenols blocks cell-mediated low-density lipoprotein oxidation (Yoshida et al. 1999).

Other mechanism by which green tea catechins exert their antioxidant effects is through the ultrarapid electron transfer from catechins to ROS-induced radical sites on DNA (Anderson et al. 2001). Another possible mechanism by which catechins scavenge free radicals is by forming stable semiquinone free radicals, thus, preventing the deaminating ability of free radicals (Guo et al. 1996). In addition, after the oxidation of catechins, due to their reaction with free radicals, a dimerized product is formed, which has been shown to have increased superoxide scavenging and iron-chelating potential (Yoshino et al. 1999). Accumulating evidence suggests that green tea catechins reduce the oxidative stress by inhibiting the ROS generating

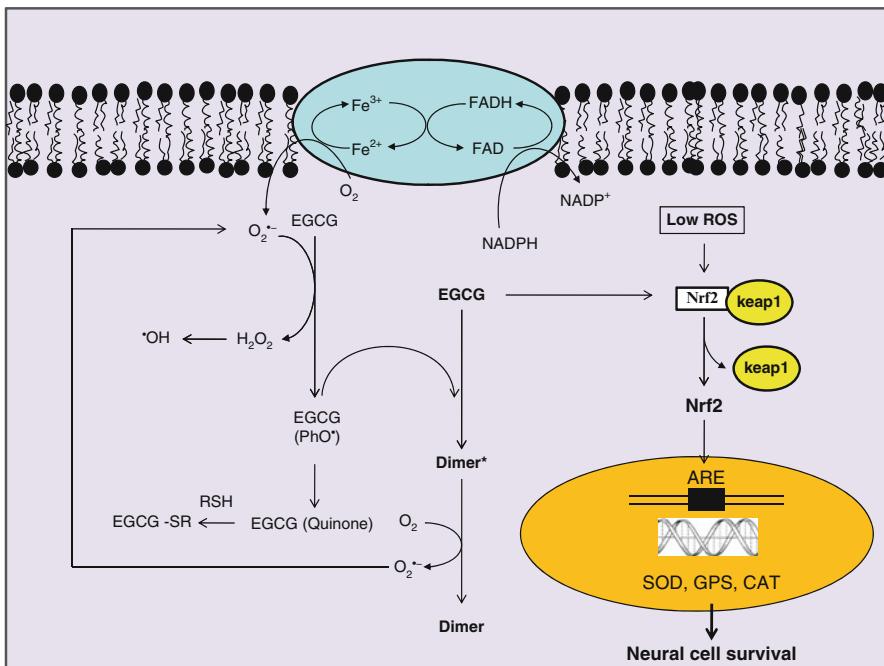


Fig. 5.6 Oxidative reaction between EGCG, superoxide, and ferric iron resulting in the production of oxidative stress, EGCG dimers, and EGCG-cysteine conjugates (EGCG-SR). Ferric (Fe^{3+}); ferrous (Fe^{2+}); super oxide (O_2^-); hydroxyl ion ($\cdot\text{OH}$); flavin adenine dinucleotide (FAD); nuclear factor (erythroid-derived 2)-like 2 (Nrf2); kelch-like erythroid Cap'n'Collar homologue-associated protein 1 (Keap1); superoxide dismutase (SOD); glutathione peroxidase (GPS); catalase (CAT); and PhO (semiquinone radical)

enzymes (iNOS and xanthine oxidase). Although endothelial-derived NO from activation of constitutive NO synthase is important for maintaining vascular tone, vasodilation, and homeostasis, higher concentrations of NO synthesized by iNOS from immune cells such as macrophages can induce oxidative damage. The activated macrophages to a great extent increase the simultaneous production of both NO and superoxide anions. NO reacts with free radicals, thereby producing the highly damaging peroxynitrite that can directly oxidize low density lipoprotein (LDL), resulting in irreversible damage to the cell membranes. In lipopolysaccharide-activated macrophages, EGCG inhibits the expression of iNOS in a dose-dependent manner not only by blocking the interactions of NF- κ B with the iNOS gene promoter, but also by reducing the activity of iNOS, thereby decreasing toxic NO generation (Lin and Lin 1997).

EGCG has also been shown to induce the expression of glutathione S-transferase, glutathione peroxidase, glutamate cysteine ligase, and heme oxygenase-1 (HO-1) in rat neuronal and nonneuronal cells. These enzymes are involved in the elimination or inactivation of ROS. The redox-sensitive transcription factor, nuclear

factor erythroid 2 p45 (NF-E2)-related factor (Nrf2) plays a key role in regulating induction of phase II detoxifying or antioxidant enzymes, such as HO-1 (Na and Surh 2008; Romeo et al. 2009). Induction of HO-1 during physiopathological conditions represents a neuroprotective mechanism, which is potentially active against brain oxidative injury (Calabrese et al. 2003).

5.3.2 Anti-inflammatory Properties of Green Tea Catechins

Inflammation is a neuroprotective mechanism that isolates the injured brain tissue from uninjured area, destroys affected cells, and repairs the extracellular matrix (Correale and Villa 2004). It is characterized by activation/production of at least four classes of bioactive mediators (1) inflammatory cytokines, (2) arachidonic acid (ARA)-derived eicosanoids, (3) inflammatory mediators (e.g., platelet activating factor), and (4) adhesion molecules. Without a strong inflammatory response, brain would be very vulnerable to neurotraumatic and neurodegenerative diseases. Inflammatory response is designed by the evolution to be short-lived and reparative in nature, and chronic sustained elevation of these responses is invariably destructive (Farooqui et al. 2007). All neural cells (microglial cells, astrocytes, neurons, and oligodendrocytes) participate in inflammatory responses, which require the activation of microglial cells and recruitment of polymorphonuclear leukocytes (PMN) from the blood stream into brain tissue. This PMN migration is a co-ordinated multistep process involving chemotaxis, adhesion of PMN to endothelial cells in the area of inflammation, and diapedesis, the penetration of tight junctions and migration through the endothelial monolayer and into the interstitium (Farooqui et al. 2007; Farooqui 2009). These PMNs eliminate invading antigens by phagocytosis and release free radicals and lytic enzymes into phagolysosomes. This is followed by a process called resolution, a turning off mechanism by neural cells to limit tissue injury (Serhan 2005; Farooqui 2009).

Adhesion of PMN to endothelial cells is critically regulated by both chemotactic cytokines and vascular adhesion molecules. Thus, interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) are closely associated with adhesion of monocytes to activated endothelial cells and in monocyte recruitment into subendothelial lesion in atherosclerosis (Gerszten et al. 1999). Adhesion molecules that are involved in neuroinflammation include adhesion molecule-1 (E-selectin), intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1). These molecules are regulated by NF- κ B, and play a pivotal role in attracting binding and transmigration of leukocytes into sites of inflammation (Gerszten et al. 1999) (Fig. 5.5).

In vitro studies indicate that catechins not only inhibit leukocyte endothelial interaction, but also dose dependently reduce cytokine-induced VCAM-1 expression and monocyte adhesion to endothelial cells (Velayutham et al. 2008). The effect depends on the pyrogallol group in catechins because EC and EGC have no effect (Ludwig et al. 2004). EGCG inhibits phorbol 12-myristate 13-acetate-mediated MCP-1

mRNA and protein expression in human endothelial cells and thereby reducing the migration of monocytes, an effect induced through the suppression of p38 mitogen-activated protein kinase (MAPK) and NF- κ B activation (Hong et al. 2007). In addition, EGCG suppresses chemokine production and neutrophil infiltration at the inflammatory site (Takano et al. 2004; Velayutham et al. 2008).

Thus, it is becoming increasingly evident that redox-sensitive transcription factors, NF- κ B and activator protein-1 (AP-1), are closely associated with vascular inflammation and its pathogenesis. NF- κ B activation plays a major role in the expression of proinflammatory molecules, including cytokines, chemokines, and adhesion molecules (Blackwell and Christman 1997). Under physiological conditions, NF- κ B is present in an inactive form (complexed with I κ B α) (Gerritsen et al. 1997; Ghosh et al. 1998). Cellular stimulation with NF- κ B agonists, oxidative stress, and neuronal injury result in the phosphorylation and degradation of I κ B α , allowing the p50/65 heterodimers of NF- κ B to translocate from cytoplasm to the nucleus, where it interacts with NF- κ B-RE and initiates expression of target genes (Ouchi et al. 2000; Velayutham et al. 2008). Green tea catechins inhibit the expression of proinflammatory enzymes (sPLA₂, COX-2, iNOS) as well as proinflammatory cytokines and chemokines (Khan et al. 2008). Administration of EGCG during reperfusion significantly not only inhibits I κ B kinase activity, resulting in the reduction of I κ B α degradation and NF- κ B activity (Yang et al. 2001), but also reduces the AP-1 activity by diminishing phosphorylation of c-Jun (Aneja et al. 2004). These observations support the view that green tea catechins simultaneously modulate multiple signaling pathways in the body. In addition, EGCG can directly inhibit the phosphorylation of I κ B, thereby preventing NF- κ B translocation to the nucleus (Nomura et al. 2000). It is also reported that green tea catechins inhibit I κ B degradation in proteasome, leading to the inhibition of NF- κ B activation (Nam et al. 2001).

Studies on beneficial anti-inflammatory effect of EGCG indicate that both EGCG and the green tea extract suppress COX-2 expression induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin and cultured human mammary epithelial cells (MCF-10A) (Kundu et al. 2003; Kundu and Surh 2007). In addition, EGCG as well as green tea extract leads to a decrease of the catalytic activity of extracellular signal-regulated protein kinase (ERK) and p38 MAPK, which are known as modulators of COX-2 expression in various cell types (Kundu et al. 2003; Kundu and Surh 2007). The ability of green tea components to inhibit the arachidonic acid pathway (phospholipase A₂, COX, and LOX) is particularly important for lowering the production of prostaglandins and leukotrienes, key mediators of the acute inflammatory cascade (Farooqui 2009). Collectively, these studies suggest that green tea components inhibit inflammation by downregulating the expression of proinflammatory enzymes and cytokines (Velayutham et al. 2008).

5.3.3 Antitumor Properties of Green Tea Catechins

Regular consumption of green tea has been shown to exhibit cancer-preventive activities in preclinical and epidemiological studies (Lin et al. 1999; Chen et al. 2011).

Although the exact molecular mechanisms associated with beneficial effects of green tea are not fully understood, it is reported that green tea polyphenols inhibit angiogenesis and metastasis, and induce growth arrest and apoptosis through regulation of multiple signaling pathways, such as metalloproteinases, various protein kinases, and proteins that regulate DNA replication and transformation. EGCG up or downregulates the activity of a number of key enzymes, including mitogen-activated protein kinases (PtdIns 3-K/Akt, Ras/Raf/MAPK) and protein kinase C, and increases or decreases their protein/mRNA levels, including that of cyclins, oncogenes, and tumor suppressor genes. Metastasis is inhibited via effects on urokinase and matrix metalloproteinases. EGCG also inhibits angiogenesis, in part by decreasing vascular endothelial growth factor production and receptor phosphorylation. These processes are closely associated with chemoprevention (Beltz et al. 2006). In addition, (-)-EGCG, potently and specifically inhibits the tumor proteasomal activity (Dou 2009). The methylation on green tea polyphenols under physiological conditions decreases their proteasome-inhibitory activity contributing to decrease in cancer-preventive effects of tea consumption. Since (-)-EGCG is unstable under physiological conditions, prodrug form of (-)-EGCG, Pro-EGCG has been developed. Pro-EGCG has increased bioavailability, stability, and proteasome-inhibitory and anticancer activities in human breast cancer cells and tumors, demonstrating its potential use for cancer prevention and treatment (Dou 2009). EGCG also inhibits telomerase activity that leads to telomere fragmentation. While at high concentrations green tea polyphenols produce pro-oxidative effects, but at lower concentrations these polyphenols induce antioxidative effects. The generation of nitric oxide is reduced by EGCG through the inhibition of inducible nitric oxide synthase. EGCG also blocks the activation of NF- κ B and decreases in I- κ B kinase activity (Beltz et al. 2006). Several recent studies indicate that EGCG inhibits DNA cytosine methyltransferase activity through a direct interaction with enzymes. This may influence methylation status indirectly through metabolic effects associated with energy metabolism (Li and Tollefsbol 2010). Therefore, reversal of hypermethylation-induced inactivation of key tumor suppression genes by dietary DNA cytosine methyltransferase inhibitors can be an effective approach for the prevention of cancer. Collective evidence suggests that green tea catechins may produce their cancer-preventative effects through different mechanisms. These include inhibition of telomerase activity, mitogen-activated protein kinases, active protein-1 mediated transcription, growth factor-mediated signaling, aberrant arachidonic acid metabolism, and other activities (Hou et al. 2004). These events may lead to the inhibition of tumor cell growth or induction of apoptosis as well as the inhibition of angiogenesis.

5.3.4 Antiobesity Properties of Green Tea Catechins

The antiobesity effects of EGCG are supported by *in vivo* as well as *in vitro* studies. EGCG reduces food uptake, lipid absorption, and gluconeogenesis and stimulates energy expenditure (Wolfram et al. 2006). Similarly, *in vitro* studies indicate that EGCG inhibits preadipocyte mitogenesis and adipocyte differentiation, stimulates

fat cell apoptosis and ROS production, and downregulates adipokine expression (Ashida et al. 2004; Dulloo et al. 2000). In addition, EGCG not only stimulates thermogenesis, but also inhibits the activities of pancreatic lipase, gastric lipase, acetyl-CoA carboxylase, fatty acid synthase, and glycerol 3-phosphate dehydrogenase (Lin and Lin-Shiau 2006; Kao et al. 2009). These processes may result in reduction of plasma glucose levels and increase in adipose and muscle glucose uptake in animal-based systems (Kao et al. 2006). However, inconsistent results are obtained on the effect of EGCG on cellular glucose uptake in different tissue- or cell-based systems (Kobayashi et al. 2000; Ueda et al. 2008; Anderson and Polansky 2002). Thus, more studies are needed on this important topic.

5.4 Effects of Green Tea Catechins on Neurological Disorders

It is becoming increasingly evident that green tea catechins interact with neurons and modulate neuronal signaling (Spencer 2009a, b). For example, epicatechin and its in vivo metabolite 30-O-methyl-epicatechin induce neuroprotective effects against oxidized LDL-induced neurodegeneration by inhibiting JNK, c-jun, and caspase-3 activation (Schroeter et al. 2001). Furthermore as stated earlier, the neuroprotective effects of EGCG not only involve modulation of PKC signaling and stimulation of (P_tsIns 3K)/Akt-mediated antiapoptotic pathway, but may also down-regulate glycogen synthase kinase-3 (GSK-3) activity (Levites et al. 2002; Koh et al. 2004). Collectively, these studies suggest that green tea catechins not only inhibit the inflammatory responses, but also retard oxidative stress.

5.4.1 Effect of Green Tea Catechins in Animal Models of Brain Ischemia

As stated earlier, ischemic injury is caused by the blood clot, which lowers or blocks the cerebral blood flow. This blockade not only decreases oxygen and glucose delivery to brain tissue but also results in the breakdown of BBB and buildup of potentially toxic products in brain (Farooqui 2010). Neurochemically, ischemia/reperfusion injury is accompanied by the release of glutamate, overstimulation of glutamate receptors, calcium influx, and stimulation of calcium-dependent enzymes (phospholipases A₂, C, nitric oxide synthase, and calpains) (Farooqui 2010) along with increase in eicosanoids and formation of oxygen free radicals leading to neuroinflammation and oxidative stress-mediated brain damage (Hong et al. 2000). Oral administration of 0.5 % green tea extract to Wistar rats for 3 weeks before induction of ischemia results in reducing ischemia/reperfusion-induced eicosanoid concentration. Thus, leukotriene C₄ is reduced from 245±51 to 186±22 ng/mg protein; prostoglandin E₂ is decreased from 306±71 to 212±43 ng/mg protein, and thromboxane A₂ is depleted from 327±69 to 251±87 ng/mg protein. Ischemia/

reperfusion injury increases levels of hydrogen peroxide, lipid peroxidation products, and 8-oxodG and 0.5 % green tea extracts not only reduce levels of these biomarkers, but also decrease apoptotic cell death in the striatum and cortical regions. In addition, green tea extract pretreatment also promotes recovery from the ischemia/reperfusion-induced inhibition of active avoidance (Hong et al. 2000, 2001). Similarly, EGCG (10, 25, or 50 mg/kg, i.p.) treatment immediately after transient global ischemia in gerbils produces a significant reduction in neuronal cell damage in the hippocampal CA1 region (Lee et al. 2000), supporting the view that EGCG produces beneficial effects in ischemic/reperfusion injury. Astrocyte swelling is another component of brain edema in ischemic injury. Glutamate release, calcium influx, oxidative stress, inflammation, and mitochondrial dysfunction have been reported to contribute to such swelling in neural cell cultures. Studies on neuroprotective effects of green tea extract on ischemic injury in C6 glial cultures indicate that EGCG not only blocks swelling, but also attenuates calcium influx. It is proposed that beneficial effects of EGCG on ischemic injury are due to prevention of neural cell swelling, but also due to protective effects, which may be mediated by its effect on the mitochondria (Panickar et al. 2009).

5.4.2 Effect of Green Tea in Animal Models of Traumatic Brain Injury

Traumatic brain injury (TBI) has two broadly defined components. The primary component, attributable to the mechanical insult itself and is accompanied by mechanical insult-mediated rupture of neural cell membranes leading into the release of intracellular contents, breakdown of the BBB, and intracranial hemorrhage. The secondary component of TBI involves a series of systemic and local neurochemical and pathophysiological changes, such as alterations in signal transduction processes associated with activation of microglial cells and astrocytes, as well as demyelination involving oligodendroglia (Raghupathi 2004; Farooqui 2010). Following TBI, acute neuroinflammation occurs within hours or days after a brain injury, and this brain injury consists of neuron damage and nervous system dysfunction that is associated with specific neurochemical processes. Neuroinflammatory factors that play an important role in secondary brain damage include interleukin1- α , interleukin1- β , prostaglandins, tumor necrosis factor, and PAF (Farooqui 2010). Clinically symptoms of secondary TBI appear slowly (days/week/months) and are accompanied by excitotoxic damage, free radical production, increase in mitochondrial membrane permeability along with changes in mitochondrial membrane permeability transition (mPT), loss of mitochondrial membrane potential (Delta Psi), increase in mitochondrial swelling, alterations in Ca²⁺ homeostasis, and rupture of the outer mitochondrial membrane (Raghupathi 2004; Farooqui 2010).

Studies on the effect of EGCG on cerebral function and morphology following TBI in 6-week-old male rats indicate that EGCG reduces various parameters (increase in immunoreactivity and levels of 8-hydroxy-20-deoxyguanosine-,

4-hydroxy-2-nonenal, increase in levels of malondialdehyde) used to access rat TBI (Itoh et al. 2011). In addition, a significant increase in surviving neurons is observed in EGCG-treated rats compared with water-treated rats. These results support the view that pre- and post-TBI treatment with EGCG may provide neuroprotection by absorbing free radicals resulting in the improvement of cerebral function following TBI (Itoh et al. 2011).

5.4.3 Effect of Green Tea Catechins in Animal Models of Alzheimer Disease

Neurodegeneration in neurodegenerative diseases is a multifactorial process involving oxidative stress, neuroinflammation, reduced expression of trophic factors, and accumulation of protein aggregates, leading to neuronal demise of neurons (Farooqui 2010). Although consumption of green tea has no effect on symptoms of Alzheimer disease (AD), several *in vitro* studies in cell culture and animal models of AD indicate that green tea extract protects neurons from the amyloid β -induced toxicity (Bastianetto et al. 2006; Levites et al. 2003; Ramassamy 2006; Zhao 2009). Amyloid precursor protein (APP) proteolysis and amyloid β ($A\beta$) metabolism has been used as possible targets for the treatment of AD. APP is processed by two pathways: (1) a nonamyloidogenic pathway which involves cleavage of APP to soluble APP (sAPP) by the α -secretase activity and (2) a formation of the amyloidogenic β peptides by the β - and γ -secretases. EGCG has been shown to regulate the proteolytic processing of APP both under *in vitro* and *in vivo* conditions (Levites et al. 2003) (Fig. 5.7). Thus, in neuronal cell cultures, EGCG enhances the nonamyloidogenic α -secretase pathway via PKC-dependent activation of α -secretase (Levites et al. 2003; Singh et al. 2008; Mandel et al. 2008) while EC reduces the formation of amyloid β -fibrils. Similarly, nanolipidic EGCG particles significantly improve the neuronal α -secretase enhancing ability and possess the oral bioavailability more than twofold over free EGCG for the treatment of AD in mouse model (Smith et al. 2010). This is accompanied by a significant reduction in cerebral $A\beta$ levels and β -amyloid plaques. Since sAPP α and $A\beta$ are formed by two mutually exclusive mechanisms, stimulation of the secretory processing of sAPP α may retard the formation of the amyloidogenic $A\beta$. Thus, EGCG may influence $A\beta$ levels, either via translational inhibition of APP or by stimulating sAPP α secretion. It is recently shown that EGCG efficiently inhibits the fibrillogenesis of both α -synuclein and $A\beta$ by directly binding to the natively unfolded polypeptides and preventing their conversion into toxic aggregated intermediates (Wanker 2008). Instead of β -sheet-rich amyloid, the formation of unstructured, nontoxic α -synuclein and $A\beta$ oligomers of a new type is promoted. These observations support the view that green tea catechins produce a generic effect on aggregation pathways in neurodegenerative diseases (Wanker 2008).

Similarly, treatment of murine N2A cells transfected with “Swedish” mutant form of APP (SweAPP N2a) and primary neuronal cells derived from Tg APPsw

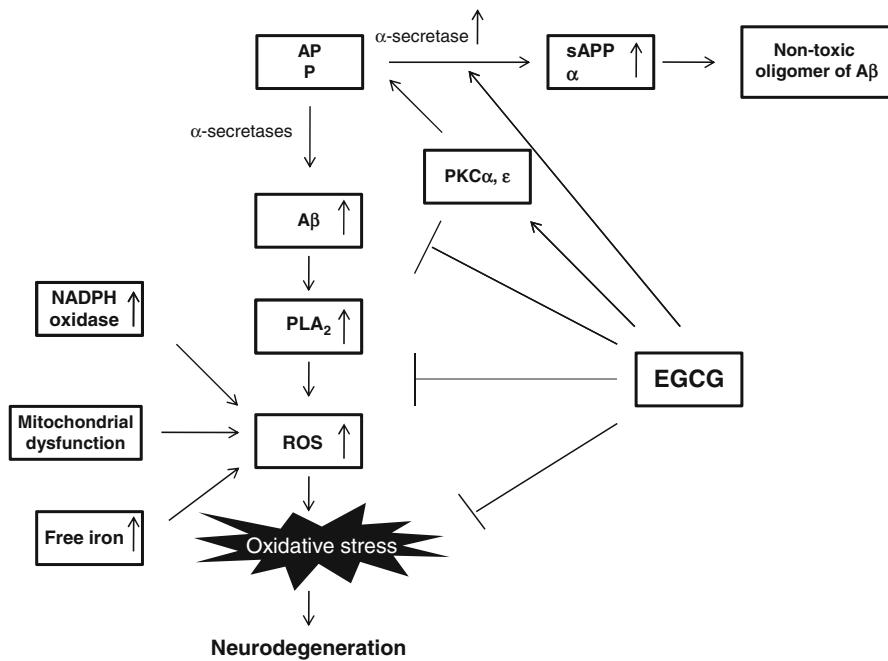


Fig. 5.7 Modulation of A β -induced oxidative stress by EGCG. Amyloid precursor protein (APP); beta amyloid (A β); phospholipase A $_2$ (PLA $_2$); and reactive oxygen species (ROS). Upward arrows indicate increase; and \neg (blocked arrow) represents inhibition

mice with EGCG reduces A β generation in both cell types in a dose-dependent manner (Rezai-Zadeh et al. 2005). Most importantly, EGCG inhibits A β generation in SweAPP N2a cells by 61 % and in primary TgAPPsw-derived neuronal cells by 38 %. Furthermore, at a relatively high concentration (80 μ M), EGC and EC enhance the A β peptide production by 20–30 % and 10–15 % in SweAPPN2a and in primary TgAPPsw-derived neuronal cells, respectively (Ramassamy 2006). The presence of EGC and EC inhibits the ability of EGCG to reduce the A β peptide generation. Interestingly, EGCG alone elicits more profound effects on reduction of the A β peptide generation versus whole green tea. The ability of the purified EGCG to inhibit the amyloid β peptide generation is much greater than that of green tea (Rezai-Zadeh et al. 2005). Moreover, intraperitoneal injections of EGCG daily for 60 days in Tg APPsw mice increase the nonamyloidogenic APP processing and brain α -secretase activity by 40 %. Accordingly, the detergent soluble A β peptide levels are reduced by 47 and 38 %, respectively. The α -secretase pathway is also increased when EGCG is directly injected in the ventricles. It is suggested that EGCG itself, and not one of its metabolites, is able to modulate the α -secretase activity in the brain following the peripheral administration (Ramassamy 2006; Singh et al. 2008). 4G8 immunoreactive and thioflavin S-positive A β deposits are also significantly reduced by 47–54 %, strengthening a decrease of the A β peptide.

Moreover, EGCG-mediated inhibition of the A β peptide generation may also be accomplished through the inhibition of β -secretase (Jeon et al. 2003). Similarly, studies on the effect of green tea on A β -mediated oxidative cell death in cultured rat pheochromocytoma (PC12) cells indicate that A β induces intracellular ROS elevation, production of 8-oxodG (an oxidized form of DNA), and apoptotic cell death in a dose-dependent manner (Lee et al. 2005). A β treatment not only upregulates proapoptotic p53 at the gene level, and Bax and caspase-3 at the protein level, but downregulates antiapoptotic Bcl-2 protein. Co-treatment with green tea extract dose dependently attenuates A β -mediated cell death, intracellular ROS levels, and 8-oxodG formation, in addition to p53, Bax, and caspase-3 expression, but upregulate Bcl-2. Furthermore, green tea extract prevents the A β -mediated activations of the NF- κ B and ERK and p38 MAP kinase pathways. Accumulating evidence from several studies suggests that green tea extract may usefully prevent or retard the development and progression of AD (Lee et al. 2005).

AD is also accompanied by the accumulation of iron at sites where neurons die. Dysregulation of metal ions (Fe $^{2+}$, Cu $^{2+}$, and Zn $^{2+}$) homeostasis not only contributes to induction of oxidative stress, but also induces A β aggregation and neurite plaque formation. Among metal ions, iron has been shown to modulate amyloid precursor holo-protein expression by a pathway similar to that of ferritin L-and H-mRNA translation through iron-responsive elements in their 5'UTRs (Mandel et al. 2007). Two scenarios related to iron chelation therapy in AD have been proposed: one involving the use of novel multimodal brain-permeable iron chelating drugs, possessing neuroprotective-neurorescue and amyloid precursor protein-processing regulatory activities, and the other is focused on the use of EGCG, which possess multifunctional activities, such as metal chelation-radical scavenging, antioxidant, anti-inflammatory, mitochondrial membrane stabilizing, and neuroprotective properties in a wide array of cellular and animal models of neurological disorders (Mandel et al. 2007). Collective evidence suggests that EGCG dose dependently reduces the A β -induced memory dysfunction by increasing brain α -secretase activity and decreasing brain β - and γ -secretase activities. In addition, EGCG not only inhibits the activation of extracellular signal-regulated kinase and nuclear transcription factor-kappaB in the A β -injected mouse brains, but also blocks A β -mediated apoptotic neuronal cell death in the brain. These studies strongly support the view that EGCG may contribute to the prevention of development or progression of AD in cell culture and animal models. These studies set the stage for large, double-blind clinical trials in AD patients.

5.4.4 Effect of Green Tea Catechins in Animal Models of Parkinson Disease

Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by selective dopaminergic neurodegeneration in the substantia nigra pars

compacta (SNpc). Cardinal features of PD include bradykinesia, resting tremor, muscular rigidity, gait disturbances, and postural reflex impairment. Onset of PD is rare before age 50 years, but increases dramatically at older ages, with peak onset occurring during ages 70–85 years. Molecular mechanisms associated with neuronal loss in SNpc remain unknown. So, at present there is no cure for PD. Antioxidants, free radical scavengers, trophic factors, and monoamine oxidase inhibitors have all been identified as potential agents for the treatment of PD (Farooqui 2010). Nutritional studies have shown that the consumption of green tea may have beneficial effects in reducing risk of PD (Checkoway et al. 2002). The molecular mechanisms underlying beneficial effects of EGCG have been investigated in animal model of PD. Thus, pretreatment of mice with either green tea extract (0.5 and 1 mg/kg) or EGCG (2 and 10 mg/kg) prevents dopaminergic neuronal death mediated by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Levites et al. 2001). It is proposed that catechol-like structure in catechins may competitively inhibit the uptake by the presynaptic or vesicular transporters of the metabolite product of MPTP, 1-methyl-4-phenylpyridinium ion (MPP⁺) (Pan et al. 2003), which also has structure similar to catechol. This competition may protect dopaminergic neuronal degeneration against the MPTP/MPP⁺-mediated injury (Pan et al. 2003). As stated earlier, EGCG is not only a potent scavenger of singlet oxygen, superoxide anions, hydroxyl radicals, and peroxy radicals (Nanjo et al. 1996), but also a chelating agent for iron and copper, metals that contribute to ROS production. Detailed investigations on molecular mechanisms indicate that MPTP not only increases the level of phospho-c-Jun, a known substrate of c-Jun N-terminal kinase (JNK), but also stimulates GSK-3β, as evidenced by the decrease in the level of phospho-Ser9 of GSK-3β. However, pretreatment with EGCGs protects dopaminergic neurons in the substantia nigra against MPTP toxicity and restores the depletion of striatal dopamine in mice. EGCG also attenuates the phosphorylation of c-Jun and modulate the phosphorylation of GSK-3beta (Ser9). These results suggest EGCGs protect dopaminergic neurons by inhibiting the JNK/c-Jun and GSK-3β signal pathway (Ruan et al. 2009). A comparison of the beneficial effects of various catechins against iron-induced lipid peroxidation in synaptosomes indicates that the inhibitory effects of catechins decrease in the order of EGCG>ECG>EGC>EC (Guo et al. 1996). EGCG has also been shown to attenuate paraquat-mediated lipid oxidation in mice, a strong redox herbicide that contributes to the formation of ROS and to the toxicity of the nigrostriatal dopaminergic system (Liou et al. 2001). In addition, EGCG could protect cells against 6-hydroxydopamine (6-OHDA), a dopaminergic and adrenergic neurotoxin (Levites et al. 2001, 2002). Once injected, 6-OHDA is taken up by the aminergic transporters and may potentiate neurodegeneration through oxidative stress and inflammation. Collective evidence suggests that EGCG may act through its catechol structure and its free radical scavenging and metal chelator properties in MPTP-, paraquat-, and 6-OHDA-induced animal models of PD (Levites et al. 2001, 2002).

5.4.5 Effect of Green Tea Catechins in Animal Models of Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative and fatal human disorder characterized by progressive loss of upper motor neurons in cerebral cortex and lower motor neurons in brainstem and spinal cord. It is characterized by initial muscle spasticity, cramps, and fasciculations, and then muscle weakness, atrophy, and eventual paralysis and death typically 3–5 years after symptoms begin. Histochemically ALS is characterized by the presence of axonal spheroids and perikaryal accumulations/aggregations comprised of the neuronal intermediate filament proteins, neurofilaments, and peripherin (Beaulieu and Julien 2003; Rowland and Shneider 2001; Eisen 2009). Although the molecular mechanism associated with neurodegeneration in ALS is not known, multiple pathophysiological mechanisms, including oxidative stress, mitochondrial impairment, protein aggregation, axonal dysfunction, reactive astrocytosis, and mutant superoxide dismutase expression, inflammation, and apoptotic cell death, have been suggested to be associated with neurodegeneration (Farooqui 2010).

Studies in SOD1-G93A transgenic mice, a transgenic mouse model of ALS, and wild-type mice indicate that EGCG-treated SOD1-G93A transgenic mice (10 mg/kg, p.o) and vehicle-treated control group show that oral administration of EGCG beginning from a presymptomatic stage significantly delays the onset of ALS and extends life span of animals (Xu et al. 2006). Furthermore, immunochemical studies indicate that EGCG-treated transgenic mice not only show increase in number of motor neurons, diminished microglial activation, and reduction in NF- κ B immunoreactivity, but also display reduction in protein level of iNOS and NF- κ B in the spinal cord (Xu et al. 2006). Similarly, in G93A mutated Cu/Zn-superoxide dismutase (SOD1) gene model of ALS, EGCG treatment not only significantly delays the onset of ALS, but also attenuates neuronal cell death signals. Collective evidence suggests that EGCG treatment may produce beneficial effects in ALS animal models (Koh et al. 2006).

5.4.6 Effect of Green Tea Catechins on EAE

Multiple sclerosis (MS) is a chronic multiphasic inflammatory and demyelinating disease characterized by loss of oligodendrocytes that maintain the myelin sheath as well as oxidative stress-mediated damage to axons with the loss of neurons along with reactive gliosis. Common symptoms include spasticity, fatigue, sexual and bladder dysfunction, cognitive impairments, depression, and weakness. Both genetic and environmental factors contribute to the risk and pathogenesis for developing MS. Although it was initially thought to be a white matter (WM) disease, new studies on extensive pathology indicate that abnormalities are also seen in the gray matter (GM) throughout the CNS. GM pathology is characterized by demyelination

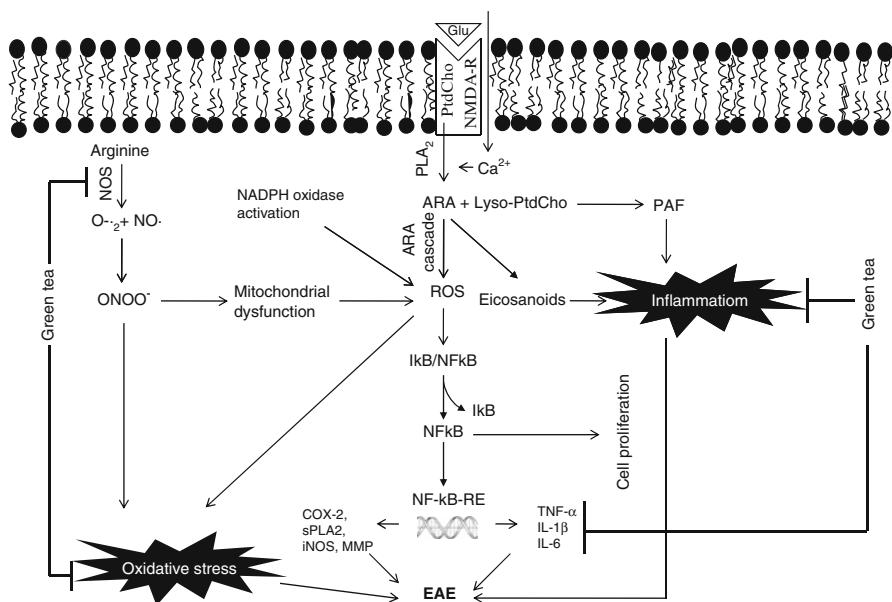


Fig. 5.8 Inhibition of inflammation and oxidative stress by green tea catechins in EAE. *N*-methyl-D-aspartate receptor (NMDA-R); glutamate (Glu); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (cPLA₂); secretory phospholipase A₂ (sPLA₂); cyclooxygenase (COX-2); matrix metalloproteinases (MMPs); inducible nitric oxide synthase (iNOS); matrix metalloproteinases (MMPs); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor kappaB (NF-κB); nuclear factor kappaB response element (NF-κB-RE); inhibitory subunit of NFκB (IκB); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); peroxy nitrite (ONOO⁻); Superoxide ('O₂'); and —(blocked arrow) represents inhibition

in the relative absence of an immune cell infiltrate (Bo et al. 2006). The chronic inflammatory tissue damage involving myelin and axons is driven by autoreactive T cells and represents a key mechanism in the immunopathogenesis of MS. In addition, ROS generated by activated macrophages and microglial cells are thought to play a major role in damaging myelin and myelin-producing cells oligodendrocytes in MS (Raine 2004).

Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS. In MS and EAE (Steinman 1999), early phase of inflammation is accompanied by neuronal pathology that not only involves axonal transaction, but also loss of parental cell bodies. These processes are closely associated with the severity of MS or EAE in rat (Trapp et al. 1998; Bjartmar et al. 2003). It is shown that EGCG dramatically suppresses EAE induced by proteolipid protein 139–151. EGCG decreases the clinical severity of EAE when given at initiation or after the onset of EAE by both limiting neuroinflammation and reducing neuronal damage (Fig. 5.8). Oral administration of EGCG in mice results in inhibition of proliferation and prevention of TNF-α production of encephalitogenic T cells (Aktas et al. 2004). Inhibition of

these processes protects against relapsing brain autoimmune disease. The molecular mechanism of anti-inflammatory action of EGCG includes the downregulation of NF- κ B in T cells. This transcription factor modulates the expression of proinflammatory cytokines, chemokines, immune receptors, and adhesion molecules that play an important role in initiating the recruitment of leukocytes at the site of neuroinflammation and closely associated with the development of EAE. It is shown that EGCG not only protects against *N*-methyl-D-aspartate (NMDA) or TRAIL-mediated neuronal injury in the brain tissue, but also inhibits the production of ROS in neurons.

5.4.7 Effect of Green Tea on Prion Diseases

Prion diseases are fatal neurodegenerative disorders characterized by the accumulation of abnormal isoforms of a host protein known as cellular prion protein (PrP^C), motor dysfunctions, dementia, and neuropathological changes such as spongiosis, astrocytosis, and neuronal loss (Prusiner 2001; Grossman et al. 2003). The cellular prion protein (PrP^C) is abundantly expressed in neurons and glial cells within the brain tissue. PrP^C is associated with cholesterol- and glycosphingolipid-rich lipid rafts through its glycosyl-phosphatidylinositol (GPI) anchor with saturated raft lipids and through interaction of its N-terminal region with an as yet unidentified raft associated molecule. The scrapie prion protein (PrP^{Sc}) is a misfolded and altered β -sheet rich isoform of PrP^C formed by posttranslational modification of the PrP^C . Molecular mechanisms, which lead to the conformational changes in PrP^C are still unknown, but heparan sulfate stimulates conversion of purified PrP^C into PrP^{Sc} in vitro, and heparan sulfate proteoglycan molecules are required for efficient PrP^{Sc} formation in prion-infected cells (Supattapone 2004). In addition, the expression of PrP^C in neuronal cells is required to mediate neurotoxic effects of PrP^{Sc} (Chesebro et al 2005). PrP^{Sc} is relatively resistant to proteinase K digestion. PrP^{Sc} causes prion diseases (transmissible spongiform encephalopathies, TSE), a group of incurable neurodegenerative disorders that affect a wide variety of mammal species. Prion diseases include scrapie, found in goats and sheep, bovine spongiform encephalopathy (mad cow disease) in cattle, and fetal familial insomnia, Creutzfeldt-Jakob disease (CJD), kuru, and Gerstmann–Sträussler–Scheinker syndrome in humans (Prusiner 2001; Grossman et al. 2003).

It is shown that green tea catechins induce the transition of mature PrP^C into a detergent-insoluble conformation distinct from PrP^{Sc} . The PrP conformers induced by EGCG are rapidly internalized from the plasma membrane and degraded in lysosomal compartments. Isothermal titration calorimetry studies indicate that EGCG directly interacts with PrP leading to the destabilizing of the native conformation and the formation of random coil structures. This activity depends on the gallate side chain and the three hydroxyl groups of the trihydroxyphenyl side chain. In scrapie-infected cells EGCG treatment produces beneficial effects through the prevention of PrP^{Sc} formation. However, in uninfected cells EGCG interferes with the

stress-protective activity of PrP^C through the enhancement of vulnerability to stress conditions. Collectively, these results indicate the important role of PrP^C in protecting cells from stress and indicating the occurrence of efficient intracellular pathways to degrade nonnative conformations of PrP^C.

Although large-scale double-blind trial studies using green tea catechins have not been performed in patients with above neurological disorders, based on cell culture and animal model studies, it is proposed that green tea catechins may exert beneficial effects by modulating signal transduction processes in neurological disorders.

5.5 Side Effects of Green Tea

It is well established that low doses of green tea catechins produce many beneficial effects on human health. However, some studies indicate that at high doses green tea (in pill form) can cause several side effects, such as anxiolytic effects, hypoglycemic effects, hypochromic anemia, and liver and kidney failure (Fig. 5.9) (Chantre and Lairon 2002; Javaid and Bonkovsky 2006; Sarma et al. 2008; Mancuso and Barone 2009).

5.5.1 Anxiolytic Effects of Green Tea

EGCG causes some behavioral effects corresponding to a benzodiazepine-like profile, leading anxiolytic activity which may result from an interaction with γ -aminobutyric acid (GABA_A) receptors (Adachi et al. 2006; Vignes et al. 2006). Furthermore, through its interactions with benzodiazepin receptors, EGCG may inhibit spontaneous excitatory synaptic transmission independently of GABA receptor activation. This inhibition may be caused by direct antagonism of glutamate

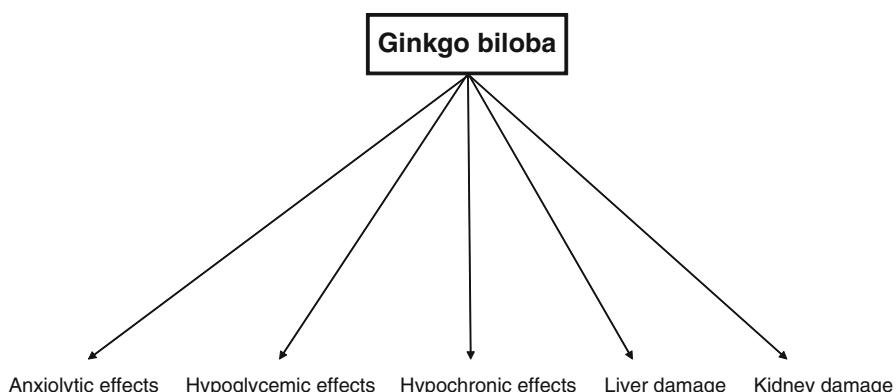


Fig. 5.9 Side effects of high doses of green tea

receptors by EGCG. Alternatively, EGCG may decrease spontaneous synaptic transmission by attenuating neuronal firing by hyperpolarization as shown in medial vestibular neurons (Vignes et al. 2006).

5.5.2 Hypoglycemic Effects of Green Tea

EGCG prevents hepatic gluconeogenesis in hepatocytes at nontoxic concentrations, which are reachable by ingestion of green tea or pure EGCG. This process involves the activation of 5'-AMP-activated protein kinase and suppression of Ca^{2+} /calmodulin-dependent protein kinase along with the generation of ROS (Collins et al. 2007; Mereles and Hunstein 2011). In addition, on one side EGCG mimics insulin response through the involvement of transcription factor Forkhead box protein O1 (FOXO1a) in the absence of insulin (Anton et al. 2007) and on the other side, EGCG prevents β -2-aminobicyclo-(2.2.1)-heptane-2-carboxylic acid stimulated insulin secretion by pancreatic β -cells (Li et al. 2006). It is suggested that these effects along with anxiolytic activity of green tea may partially explain dizziness observed in some patients consuming high concentration or pills of green tea orally (Mereles and Hunstein 2011).

5.5.3 Hypochromic Effects of Green Tea

EGCG interferes with iron absorption and inhibits it in a dose-dependent manner in the gastrointestinal tract making the iron less available for absorption (Zijp et al. 2000). Similarly, green tea tannins reduce the bioavailability of iron (Schöenthal 2011). For this reason, iron supplements should not be ingested together with green tea components, and subjects deficient in iron or susceptible to iron deficiency should not use green tea.

5.5.4 Liver and Kidney Failure and Green Tea

Liver and kidney damage is more pronounced, especially when green tea is consumed under fasting conditions. Although the reported toxicity of green tea extract is sporadic, in the interest of public safety, French and Spanish authorities removed the green tea extract Exolise, a weight loss product containing a hydroalcoholic extract of green tea named AR25 (standardized to 25 % catechins) from their markets in 2003. The molecular mechanism involved in green tea-mediated liver and kidney damage is not fully understood, but elevation in activity of liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] has been reported to occur 3–5 months after a consumption of Exolises. It should be noted

that most cases of toxicity can be self-limited and resolved after discontinuation of the drug (Schöenthal 2011). In addition, the inhibition of CYP3A4 by green tea can be harmful, particularly in case of prolonged consumption of this beverage. EGCG produces augmentation of cyclophosphamide and doxorubicin toxicity not only because it increases the activity of rat and human CYP2B and NADPH-Cyt-P-450 reductase, but also due to the formation of noxious by-products (Park et al. 2009; Dudka et al. 2005).

5.6 Conclusion

Green tea contains catechins (epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin, and (-)-epigallocatechin-3-gallate), alkaloids (caffeine, theophylline, and theobromine), flavonols (quercetin, kaempferol, and rutin), amino acids, carbohydrates, proteins, and chlorophyll.

Green tea catchins contain three heterocyclic rings, A, B, and C, and the free radical scavenging property of green tea is attributed to the presence of trihydroxyl group on the B-ring and the gallate moiety at the 3' position in the C ring. Green tea catechin also chelates transition metal ions like iron and copper. There are two sites where metal ions bind to the catchin molecule (a) o-diphenolic group in the 3',4'-dihydroxy positions in the B ring and (b) keto structure 4-keto, 3-hydroxy in the C-ring of flavonols. Green tea catechins are brain permeable. After oral administration, green tea components have been found in the rat brain as epicatechin glucuronide and 30-O-methyl epicatechin glucuronide. These components exert vascular protective, neuroprotective, and tumor protective effects through multiple mechanisms, including antioxidative, anti-inflammatory, antiproliferative, anti-thrombogenic, antihypertensive, and lipid lowering effects. Thus, green tea catechins mediate beneficial antioxidant effects by scavenging free radicals, chelating redox active transition-metal ions, inhibiting redox active transcription factors (NF- κ B, AP-1), and stimulating Nrf2. Modulation of above transcription factors increases induction of antioxidant enzymes and protein kinases. Green tea catechins inhibit NADPH oxidases, cyclooxygenases, lipoxygenases, and nitric oxide synthases. Green tea catechins regulate vascular tone by activating endothelial nitric oxide and preventing vascular inflammation. The anti-inflammatory activities of green tea catechins are due to their suppression of leukocyte adhesion to endothelium and subsequent transmigration through inhibition of transcriptional factor NF- κ B-mediated production of cytokines and adhesion molecules in endothelial, inflammatory, and neural cells. In addition, green tea catechins also scavenge NO, the peroxynitrite anion, and reduce the activity of NO synthase. This effect of catechins also involves the inhibition of NF- κ B activation. Another mechanism associated with anti-inflammatory effect of green tea catechin is the presence of the antioxidant response element (ARE) on the promoter of the eNOS gene, and catechins bind to the ARE and activate eNOS. These effects of green tea catechins on NOS activities also contribute to the anti-ischemic effect.

Collective evidence suggests that multiple mechanisms (gene expression, growth factor-mediated pathways, the mitogen-activated protein kinase-dependent pathway, and the ubiquitin/proteasome degradation pathway) may be involved in the beneficial effects of green tea. Thus, green tea consumption modulates induction of apoptosis, cell cycle arrest downregulation of telomerase, inhibition of vascular endothelial growth factor, and suppression of aromatase activity. The protective effects of green tea polyphenols have also been attributed to the involvement of Phase II detoxification enzymes during the biological responses. Although green tea catechins produce neuroprotective effects in animal models of ischemia/reperfusion injury, AD, PD, ALS, and MS, there is a lack of well-controlled clinical trials in humans with green tea in neurodegenerative diseases. However, human epidemiological and new animal data support the view that the pharmacological benefits of tea drinking may protect the brain from aging and age-related neurological disorders among Asians than in Europeans or Americans.

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Chapter 6

Beneficial Effects of Curcumin on Neurological Disorders

6.1 Introduction

Curcumin, a hydrophobic polyphenol, is the yellow pigment in the Indian spice turmeric (curry powder). It is derived from the rhizome of the herb *Curcuma longa* (Anand et al. 2008), which belongs to the family Zingiberaceae. Curcumin is a biphenolic compound with hydroxyl groups at the ortho-position on the two aromatic rings that are connected by a β -diketone bridge, containing two double bonds (dienone), which can undergo Michael addition, critical for some of the effects of curcumin (Weber et al. 2006), but contributing to chemical instability in aqueous solution (Pan et al. 1999). Curcumin is also known as diferuloylmethane (bis- α,β -unsaturated β -diketone) that exhibits keto-enol tautomerism, having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline media. Curcumin resembles ubiquinols in its structure. It is insoluble in water, but is readily soluble in organic solvents such as dimethylsulfoxide, acetone, and ethanol. It is stable at acidic pH but unstable at neutral and basic pH, under which conditions it is degraded to ferulic acid and feruloylmethane (Wang et al. 1997). Most curcumin (>90 %) is rapidly degraded within 30 min of placement in phosphate buffer systems of pH 7.2 (Wang et al. 1997).

Turmeric rhizome is crushed into a powder and used in Asian cookery, medicine, cosmetics, and fabric dying for more than 2000 years (Ammon and Wahl 1991). Turmeric powder consist of about 4 % curcuminoids, 70 % carbohydrates, 6 % proteins, 5 % resins, 4 % fat, and 10 % moisture (Fig. 6.1). Curcumin's chemical structure includes two methoxyl groups, two phenolic hydroxyl groups, and three double conjugated bonds. Differing in methoxy substitutions on the aromatic ring, commercially available turmeric powder contains three natural analogs (curcuminoids). Curcumin is the most abundant (77 %) and the less common demethoxycurcumin (18 %), bisdemethoxycurcumin (5 %), and the recently identified cyclocurcumin (Anand et al. 2008; Goel et al. 2008) (Fig. 6.2). These analogs differ from each other in their chemical structures only with regard to methoxy substitutions and, while the overall biological activities of these curcuminoids including

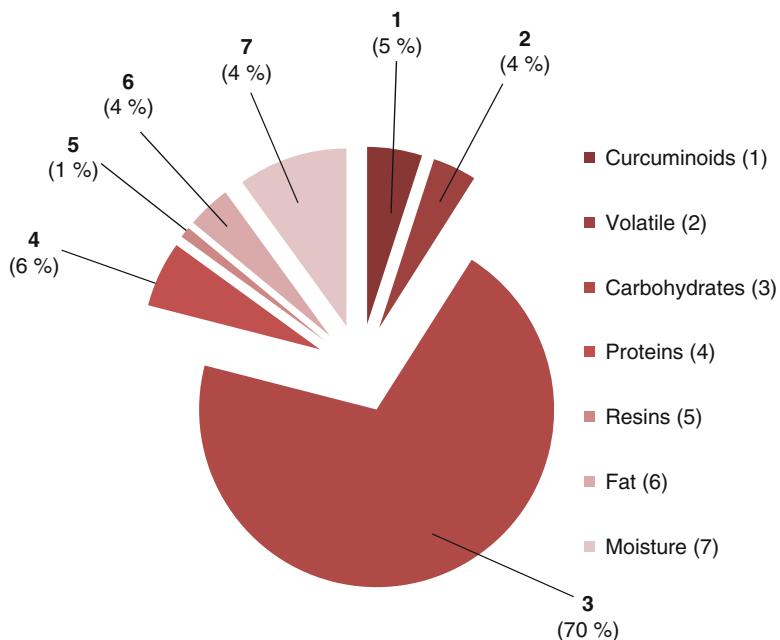


Fig. 6.1 Chemical composition of turmeric powder

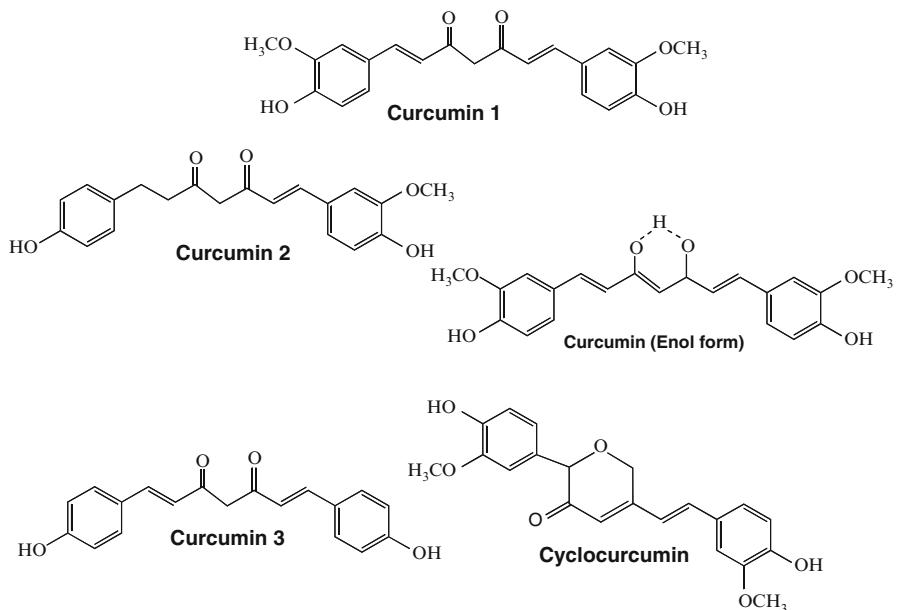


Fig. 6.2 Chemical structures of curcuminoids. Curcumin 1, curcumin 2 (methoxycurcumin), curcumin 3 (demethoxycurcumin), enol form of curcumin, and cyclocurcumin

Table 6.1 Effect of curcumin on enzyme activities in neural and nonneuronal tissues

Enzyme	Effect	References
COX-2	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
5-LOX	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
iNOS	Inhibition	Chan et al. (1998)
MMP9	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
IKK	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
Akt	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
JAK2	Inhibition	Kim et al. (2003)
PKA	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
PKC	Inhibition	Anand et al. (2008), Hatcher et al. (2008)
PLD	Inhibition	Yamamoto et al. (1997)
FTPase	Inhibition	
Histone acetyltransferase	Inhibition	Balasubramanyam et al. (2004)
GST	Activation	Anand et al. (2008), Hatcher et al. (2008)
NQO1	Activation	Shih et al. (2003), Vargas et al. (2008)
Heme oxygenase	Activation	Shih et al. (2003), Vargas et al. (2008)
CAT	Activation	Shih et al. (2003), Vargas et al. (2008)
SOD	Activation	Shih et al. (2003), Vargas et al. (2008)
UGT	Activation	Shih et al. 2003, Vargas et al. (2008)
Epoxide hydrolase	Activation	Anand et al. (2008), Hatcher et al. (2008)

Cycoxygenase-2 (COX-2); 5-lipoxygenase (5-LOX); inducible nitric oxide synthase (iNOS); matrix metallopeptidase9; IκB kinase (I-κK); a serine/threonine protein kinase (Akt or Protein kinase B); NADPH:quinone oxidoreductase (NQO1) Janus kinase 2 (JAK2); protein kinase A (PKA); protein kinase C (PKC); phospholipase D (PLD); glutathione S-transferase (GST); catalase (CAT); superoxide dismutase (SOD); and UDP-glucuronosyl transferase (UGT)

antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, antihyperlipidemic, antiphlogistic, antidiabetic, antipsoriasis, antithrombotic, antihepatotoxic, and neuroprotective properties appear to be comparable, differences in their efficiencies have been observed (Anand et al. 2008). Curcumin has been used extensively in Chinese traditional medicine and Ayurvedic medicine (Indian system of medicine) for centuries as an antinociceptive, anti-inflammatory, and antishock agent to relieve pain and inflammation not only in the skin and muscles (Anand et al. 2008), but also in the treatment of numerous pathological conditions, including rheumatism, digestive and inflammatory disorders, intermittent fevers, urinary discharges, leukoderma, and amenorrhoea as part of traditional medicine (Anand et al. 2008). Beneficial effects of curcumin have been observed both in cultured cells and in animal models, and have paved the way for ongoing present and future human clinical trials (Table 6.1).

6.2 Bioavailability of Curcumin in the Brain

Factors that influence bioavailability of nutrient include gastric acid secretion, gastric emptying time, gastrointestinal blood flow, surface area, and absorption (Martinez and Amidon 2002), along with the effects of presystemic hepatic and gut

metabolism and transport. Curcumin exhibits poor bioavailability (Anand et al. 2008; Goel et al. 2008). There are three major reasons for the low bioavailability of curcumin: (1) its poor absorption, (2) its rapid metabolism, and (3) rapid systemic elimination and short biological half life (Anand et al. 2007, 2008; Bisht and Maitra 2009). In rodents, curcumin undergoes rapid metabolism by conjugation and reduction, and its disposition after oral dosing. Very little information is available on pharmacokinetics of curcumin in humans. First phase I and II clinical trial of curcumin have been performed in patients with advanced colorectal cancer for up to 4 months at several doses (500, 1,000, 2,000, 4,000, 8,000, and 12,000 mg/day) without any toxicity (Sharma et al. 2001; Cheng et al. 2001). The serum concentration of curcumin usually peaks at 1–2 h after oral intake of curcumin and gradually declines within 12 h. The average peak serum concentrations after taking 4,000 mg, 6,000 mg, and 8,000 mg of curcumin were $0.51 \pm 0.11 \mu\text{M}$, $0.63 \pm 0.06 \mu\text{M}$, and $1.77 \pm 1.87 \mu\text{M}$, respectively. In this study serum levels of curcumin peak at 1 and 2 h postdose and declines rapidly. So far, an upper level of toxicity has not been established for curcumin. Studies have shown that a dosage as high as 12 g/day is safe and tolerable by humans with a few reporting mild side effects (Goel et al. 2008; Jiao et al. 2009). Studies on curcumin metabolism in animals indicate that it is rapidly metabolized through glucuronidation (Fig. 6.3) and sulfation, or it is reduced to hexahydrocurcumin (Fig. 6.3) in liver and intestine. Glucuronidation and sulfation of curcumin is catalyzed by UDP-Glucuronosyltransferases (UGTs) and sulfotransferases, respectively, in liver and intestine (Hoehle et al. 2007; Asai and Miyazawa 2000) (Table 6.2).

Curcumin metabolites may not have the same biological activity as the parent compound. Thus, it is reported that conjugated or reduced curcumin metabolites are less effective inhibitors of inflammatory enzyme expression in cultured human colon cells than curcumin itself (Ireson et al. 2001). An oral dose of 0.1 g/kg administered to mice gives a peak plasma concentration of free curcumin (2.25 $\mu\text{g}/\text{ml}$) (Pan et al. 1999). In rats, curcumin completely disappears from plasma within 1 h after a 40 mg/kg i.v. dose (Ireson et al. 2001). When given orally at a 500 mg/kg dose, peak concentrations of 1.8 ng/ml of free curcumin have been detected in plasma (Ireson et al. 2001). Several major and minor metabolites of curcumin (Fig. 6.4) have been separated and identified in rat plasma. These include curcumin glucuronide, curcumin sulfate, hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide. Based on detailed investigation, it is suggested that most of orally consumed curcumin (75 %) is excreted in feces (Wahlström and Blennow 1978), but significant curcumin enters in the circulation and reaches various body tissues including liver, kidney, and brain.

To improve the bioavailability of curcumin, numerous approaches have been used. These approaches include the use of adjuvant like piperine, which interferes with glucuronidation, preparation of curcumin liposomes and nanoparticles, use of curcumin phospholipid conjugates, and the use of structural analogs of curcumin (e.g., FLL32 and EF-24) (Fig. 6.5) (Shoba et al. 1998; Li et al. 2005; Liu et al. 2006; Maiti et al. 2007; Gota et al. 2010; Lim et al. 2011). The possible advantages attributed to such formulations are (1) to provide longer circulation; (2) to increase the

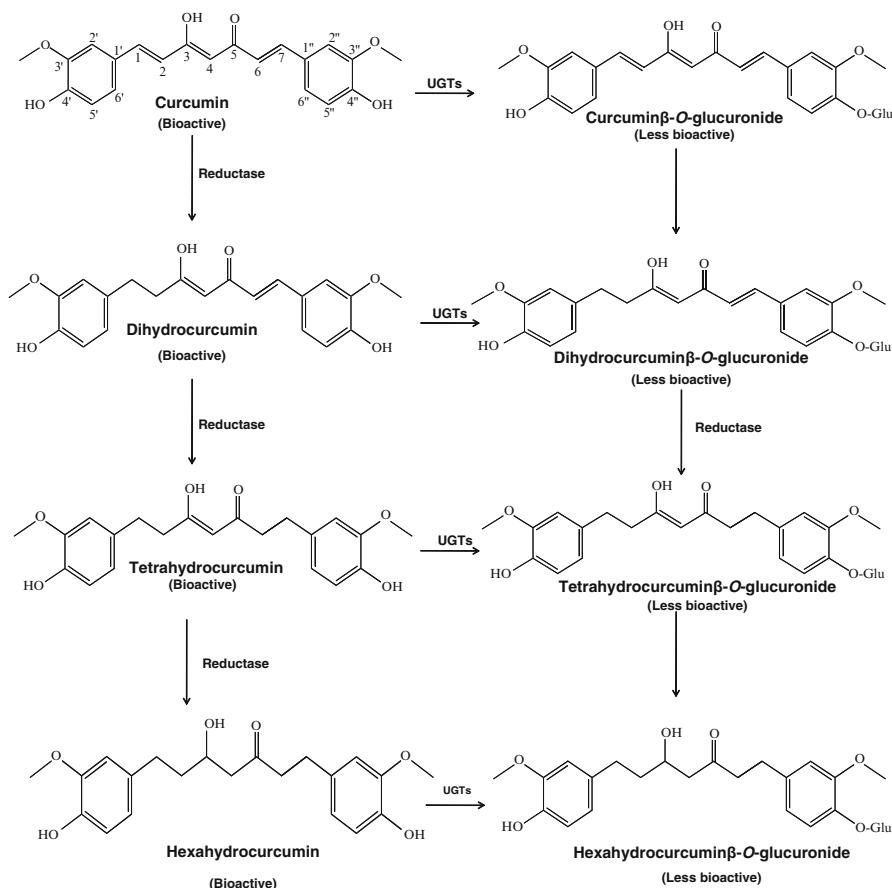


Fig. 6.3 Metabolism of curcumin. Curcumin is transformed into di-, tetra-, and hexahydrocurcumin by reductases. UDP-Glucuronosyltransferases (UGTs) convert curcumin into glucuronides

cellular permeability; and (3) to induce resistance to metabolic processes. Among these approaches, the administration of curcumin as phospholipid conjugates, curcumin nanoparticles, and curcumin analogs has not only attracted considerable attention, but has been reported to greatly increase the bioavailability of free curcumin (Marczylo et al. 2007; Begum et al. 2008; Gota et al. 2010). The low molecular weight and polar structure of curcumin allows it to penetrate the blood–brain barrier (BBB) effectively. It is reported that curcumin can enhance the adult hippocampus neurogenesis by increasing the number of newly generated cells in the dentate gyrus region of hippocampus (Kim et al. 2008). Moreover, curcumin is a potent inhibitor of reactive astrocyte expression, and thus prevents hippocampal cell death induced by kainic acid (Shin et al. 2007).

Table 6.2 Effect of curcumin on neurological disorders

Disease	Disease type	Mechanism of curcumin action	References
Ischemia	Neurotraumatic	Antioxidant, anti-inflammatory, reduction of microglial activation	Ghoneim et al. (2002), Wang et al. (2005), Zhao et al. (2010)
Traumatic brain injury	Neurotraumatic	Antioxidant, anti-inflammatory, reduction of microglial activation	Wu et al. (2006), Laird et al. (2010)
Spinal cord injury	Neurotraumatic	Antioxidant, anti-inflammatory, reduction of microglial activation	Lin et al. (2011a, b)
Epilepsy	Neurotraumatic	Antioxidant, anti-inflammatory	Sumanont et al. (2006), Jyoti et al. (2009), Du et al. (2009)
Subarachnoid hemorrhage	Neurotraumatic	Antioxidant, anti-inflammatory	Wakade et al. (2009)
Alzheimer disease	Neurodegenerative	Antioxidant, anti-inflammatory, reduction of A β burden and microglial activation, induction of memory formation	Yang et al. (2005), Frautschy and Cole (2010)
Parkinson disease	Neurodegenerative	Antioxidant, anti-inflammatory, reduction of microglial activation	Zbarsky et al. (2005)
Creutzfeld–Jakob disease	Neurodegenerative	Antioxidant, anti-inflammatory, reduction of microglial activation	Hafner-Bratkovic et al. (2008)
Multiple sclerosis	Neurodegenerative	Antioxidant, anti-inflammatory	Xie et al. (2011)
Depression	Neuropsychiatric	Antioxidant, anti-inflammatory	Kulkarni et al. (2008), Xu et al. (2005b), Kulkarni and Dhir (2010)
Experimental dementia	Neuropsychiatric	Antioxidant, anti-inflammatory	Rinwa et al. (2010)
Kainic acid neurotoxicity	Neurodegenerative	Antioxidant, anti-inflammatory	Gupta et al. (2009)

Major targets for curcumin are A β , Bcl-2, caspase-3, cytochrome c, GSH, iNOS, NO, and PARP

6.3 Molecular Targets of Curcumin

Curcumin mediates a variety of potentially therapeutic properties, such as anti-inflammatory, antioxidant, antineoplastic, pro- and antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory, and antimicrobial effects (Fig. 6.6) through the modulation of a diverse range of molecular targets including transcription factors, growth factors, and their receptors, cytokines, enzymes, and genes. These targets

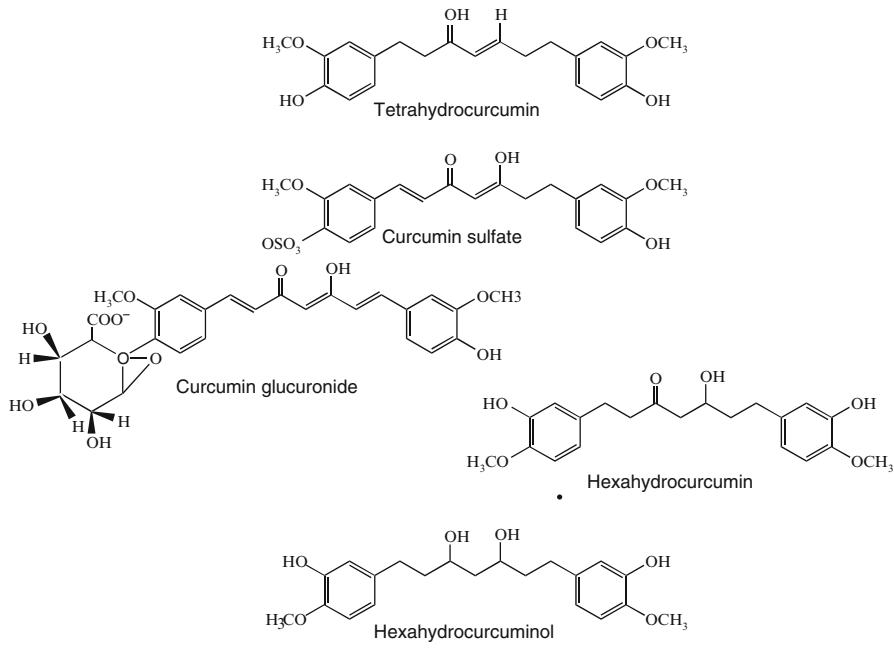


Fig. 6.4 Chemical structures of conjugated products of curcumin

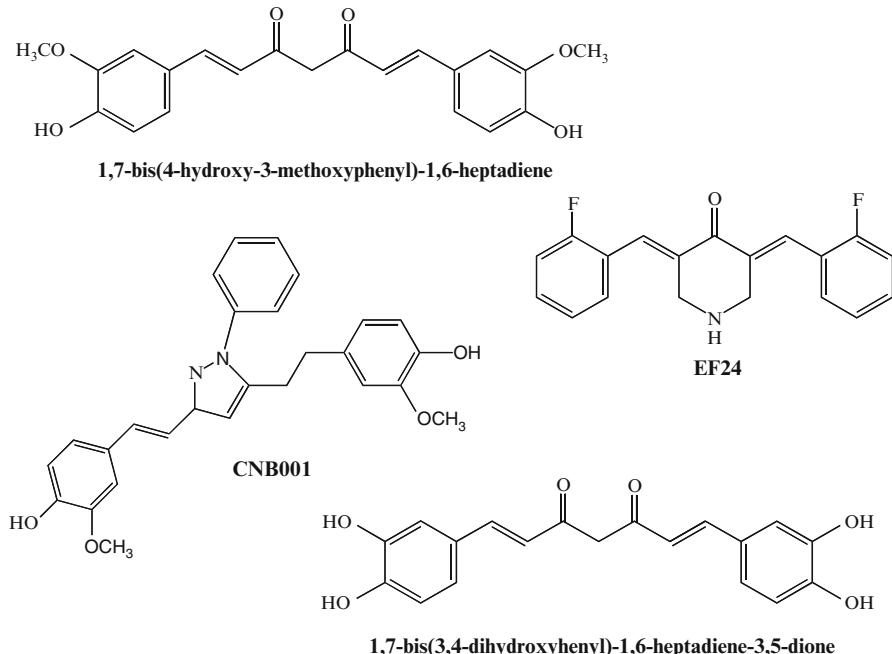


Fig. 6.5 Chemical structures of curcumin analogs

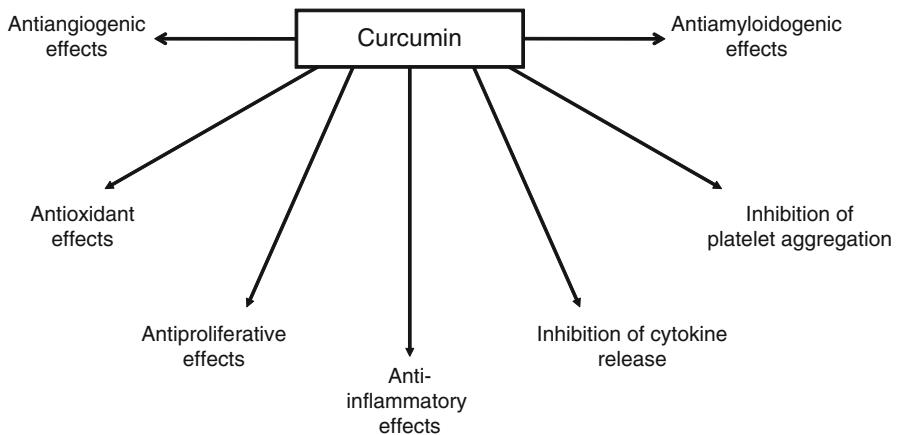


Fig. 6.6 Biological effects of curcumin

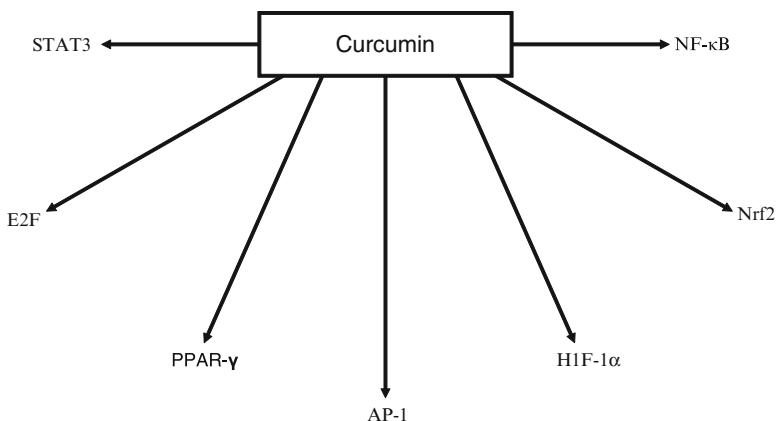


Fig. 6.7 Transcription factors that are modulated by curcumin

are associated with the modulation of many cellular and molecular pathways (Figs. 6.7 and 6.8) (Joe et al. 2004). These targets are associated with cell cycle (cyclin D1 and cyclin E), apoptosis (activation of caspases and downregulation of antiapoptotic gene products), cell proliferation (HER-2, EGFR, and AP-1), cell survival (PtdIns 3K/AKT pathway), invasion (MMP-9 and adhesion molecules), angiogenesis (VEGF), metastasis (CXCR-4), and inflammation (NF-κB, TNF α , IL-6, IL-1, COX-2, and 5-LOX).

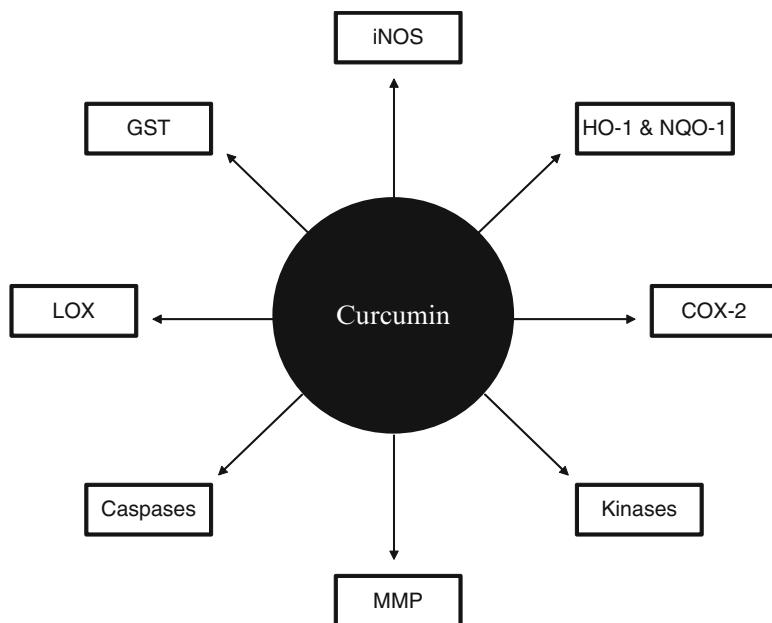


Fig. 6.8 Enzyme activities that are modulated by curcumin. Cyclooxygenase-2 (COX-2); Lipoxygenase (LOX); inducible nitric oxide synthase (iNOS); glutathione S-transferase (GST); heme oxygenase-1 (HO-1); NADPH:quinone oxidoreductase (NQO1); and Matrix metalloproteinases (MMP)

Curcumin also possesses antiplatelet effects. It inhibits platelet aggregation mediated by the platelet agonists (epinephrine, ADP, platelet-activating factor, collagen, and arachidonic acid, ARA). Curcumin preferentially blocks PAF- and ARA-mediated aggregation, whereas much higher concentrations of curcumin are needed to inhibit aggregation induced by other platelet agonists. Pretreatment of platelets with curcumin results in the inhibition of platelet aggregation induced by calcium ionophore A-23187, but curcumin up to 250 μ M has no inhibitory effect on aggregation mediated by the protein kinase C (PKC) activator phorbol myrsitate acetate. Curcumin also inhibits the formation of thromboxane A₂ by platelets. Collective evidence suggests that the curcumin-mediated preferential inhibition of PAF- and ARA-mediated platelet aggregation involves inhibitory effects on thromboxane A₂ synthesis and Ca²⁺ signaling, but without the involvement of PKC (Shah et al. 1999). Curcumin also downregulates the expression of the proinflammatory cytokines IL-1 β , MCP-1, and TNF- α , without altering the expression of anti-inflammatory cytokines in the aorta. In the liver, curcumin increases the expression of IL-10 without altering the expression of the proinflammatory cytokines IL-1 β , MCP-1, and TNF- α or the hepatic enzyme activities. Thus, the complexity of the pleiotropic activity of curcumin may account for its efficacy in combating human diseases such

as cancer, arthritis, diabetes, neurotraumatic, and neurodegenerative diseases, which are not only multifactorial in nature but also involve cellular or molecular defects at more than one level in the signal transduction pathways.

6.3.1 Transcription Factors as a Target for Curcumin

The pleiotropic effects of curcumin include the inhibition of several cell signaling pathways at multiple levels involving several transcription factors, such as NF- κ B, AP-1, STAT3, PPAR γ , HIF-1 α , Nrf2, β -catenin, and E2F. In addition, curcumin also targets growth factors and their receptors, cytokines, enzymes, gene-regulating cell proliferation, and apoptosis. *In vitro* and *in vivo* studies have shown that these transcription factors, receptors, and growth factors not only regulate antioxidant and anti-inflammatory effects, but also modulate cytokines release, apoptotic cell death, and antiangiogenic effects of curcumin (Goel et al. 2008). Due to its efficacy and regulation of multiple targets, as well as nontoxic nature in animals and humans, curcumin has received considerable attention as a potential therapeutic agent for the prevention and/or treatment of various malignant diseases, arthritis, allergies, neurotraumatic, and neurodegenerative diseases.

6.3.1.1 Modulation of NF- κ B by Curcumin

NF- κ B is a master regulator of innate and adaptive immunity, inflammatory responses, organ development, and cell survival (Bonizzi and Karin 2004). This transcription factor controls the expression of a large array of genes that are not only involved in immune function, but also in inflammation and cell survival. Five different proteins of NF- κ B factor, namely, p50, RelA/p65, c-Rel, RelB, and p52, combine differently to form active dimers in response to external stimuli and oxidative stress. RelA is activated by neurotoxic agents while c-Rel produces neuroprotective effects (Sarnico et al. 2009). The neurochemical activity of NF- κ B is regulated by subcellular localization. Inactive NF- κ B is predominantly localized in the cytoplasm because of associated inhibitor proteins, including I κ B α . In response to oxidative stress and proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), I κ B α is phosphorylated by I κ B kinase and ubiquitinated and degraded by the proteasome; simultaneously, the active NF- κ B heterodimer translocates to the nucleus where it initiates gene transcription associated with the expression of a number of proteins including many enzymes (sPLA₂, COX-2, NADPH oxidase and inducible nitric oxide synthase, superoxide dismutase) and cytokines (TNF- α , IL-1 β , and IL-6) (Farooqui 2010). In addition, there are several potential routes through which NF- κ B can act to induce neuronal death, including induction of death proteins and an aborted attempt to reenter the cell cycle.

Curcumin completely blocks both oxidative stress-induced and TNF- α -mediated activation of NF- κ B. Curcumin-mediated inhibition of NF- κ B is not due to the

chemical modification of NF-κB proteins, but because of the inhibition of NF-κB translocation to the nucleus (Goel et al. 2008). Curcumin not only quenches ROS production, but also blocks the activation of NF-κB by inhibiting a protein kinase. In vitro, curcumin has been shown to inhibit both serine/threonine protein kinase and protein tyrosine kinase (Reddy and Aggarwal 1994). Collective evidence suggests that curcumin inhibits IκB kinase β (IKK β) in the cytoplasm and nucleus. This leads to reduction in NFκB activity with no effect on phospho-Akt.

6.3.1.2 Modulation of AP-1 Transcription Factor by Curcumin

AP-1 is a transcription factor assembled from jun-jun, jun-fos, or jun-atf family protein homo- or heterodimers. It belongs to the class of basic leucine zipper (bZIP) transcription factors, which bind to promoters of its target genes in a sequence-specific manner and transactivates or represses them. AP-1 proteins are involved in the modulation of a variety of cellular processes including proliferation and survival, differentiation, growth, apoptosis, cell migration, morphogenesis, and transformation. Depending upon the abundance of dimerization partners, dimer-composition, posttranslational regulation, and interaction with accessory proteins, cells make the decision if AP-1 transcription factor mediates positive or negative effect (Kaminska et al. 2000; Vesely et al. 2009). AP-1 transcription factor is activated by growth factors, neurotransmitters, cellular stress, ionizing and ultraviolet irradiation, cytoskeletal rearrangements, and variety of cytokines (Karin et al. 1997; Kaminska et al. 2000).

Curcumin not only inhibits the DNA binding of c-jun/AP-1 transcription factor but it also downmodulates c-jun level by preventing its transcription (Huang et al. 1991). Curcumin has no effect on the Sp1 transcription factor, but has been reported to inhibit TNF-α-mediated and phorbol ester-stimulated type 1 HIV-LTR-directed gene expression and virus replication (Li et al. 1993). Curcumin has no effect on DNA-binding properties of NF-κB.

6.3.1.3 Modulation of STAT3 by Curcumin

The signal transducer and activator of transcription 3 (STAT3) is a member of the Jak-STAT signaling family. It transduces signals for several cytokines (interferons, interleukin-5, interleukin-6) and growth factors (epidermal growth factor, hepatocyte growth factor, leukemia inhibitory factor), and hormones (leptin) have been implicated in the neural cell injury response. The activation of STAT3 by Janus kinase (JAK)-mediated phosphorylation increases at the tyrosine residue leading to the formation of homo- or heterodimers that translocate to the cell nucleus, where they act as transcription factors and bind to DNA in astrocytes, microglia, endothelial cells, and neurons shortly after CNS insults (Choi et al. 2003; Justicia et al. 2000). It is recently reported that STAT3 modulates the migration of reactive astrocytes to the injury site in the spinal cord trauma (Okada et al. 2006).

The identity of the serine kinase for STAT3 is somewhat controversial, most likely because different activation signals lead to serine phosphorylation by any of various protein kinases, including ERK1, ERK2, p38, JNK, and an H-7-sensitive kinase (Decker and Kovarik 2000). Most evidence suggests a positive role for S727 phosphorylation in STAT3 transcriptional activation, presumably through enhanced recruitment of necessary transcriptional cofactors, as is the case for serine phosphorylation of STAT1 (Zhang et al. 1998). However, there is also evidence for a negative role for serine phosphorylation, although its underlying mechanism is unclear. STAT3 induces the expression of many genes in response to cell stimuli, and thus plays an important role in many cellular processes, including neural cell growth, inflammation, and apoptotic cell death.

Curcumin mediates its anti-inflammatory effects through the modulation of Janus kinase (JAK)-STAT signaling. In both rat primary microglia and murine BV2 microglial cells, curcumin effectively suppresses the ganglioside-, LPS-, or IFN- γ -stimulated induction of cyclooxygenase-2 and inducible NO synthase. These enzymes mediate and support inflammatory processes. Interactions of ligands with receptors result in the formation of an active receptor complex and subsequent phosphorylation of the receptor-associated JAKs (JAK1, JAK2, JAK3) and tyrosine kinase 2. Phosphorylated JAKs lead to the activation of neighboring JAKs, receptor subunits, and several other substrates and provide the docking sites for STATs, which in turn become phosphorylated. Phosphorylated STATs are released from the receptor complex and form homo- or heterodimers. These dimers translocate to the nucleus where they directly bind to the promoter region of specific target genes, thus regulating transcription of inflammation-associated genes (Kovarik et al. 2001). Curcumin inhibits the phosphorylation of JAK1 and JAK2 via the increased phosphorylation of SHP-2 and its association with JAK1/2, thus attenuating inflammatory response. Collective evidence suggests that curcumin acts via a novel anti-inflammatory mechanism and is also a negative regulator of the JAK-STAT pathway by the activation of SHP-2 (Kim et al. 2003).

6.3.1.4 Modulation of HIF-1 α by Curcumin

Hypoxia-inducible factor 1 (HIF1) is a basic helix-loop-helix transcription factor that transactivates genes encoding proteins that modulate and participate in homeostatic responses to hypoxia. It not only facilitates the expression of proteins that control glucose metabolism, but also modulate cell proliferation and vascularization. Several genes involved in cellular differentiation are directly or indirectly regulated by hypoxia, including genes for Epo (Erythropoietin), LDHA (Lactate Dehydrogenase-A), ET1 (Endothelin-1), transferrin, transferrin receptor, Vascular Endothelial Growth Factor, Platelet-Derived Growth Factor-Beta, basic Fibroblast Growth Factor (FGF), and genes for enzymes modulating glycolysis (Jiang et al. 1997). HIF1 is a heterodimer made up of two basic helix-loop-helix PAS (Per-ARNT-Sim) proteins, HIF1- α and HIF1- β . HIF1- α accumulates under hypoxic conditions whereas HIF1- β is constitutively expressed. HIF1- α is closely

associated with the hypoxic response of tumor cells. It controls the upregulation of a number of factors including the angiogenic factor VEGF, where as HIF1- β is the ARNT (Aryl hydrocarbon Receptor Nuclear Translocator), an essential component of the xenobiotic response (O'Rourke et al. 1999).

The hypoxia inducible transcription factor HIF-1 is an important mediator of tumor angiogenesis and survival, and inhibition of HIF levels as well as HIF action is an attractive target for cancer chemotherapy (Semenza 2007). It is shown that both curcumin and EF24, an analog of curcumin, inhibit intracellular levels of both HIF-1 α and HIF-1 β proteins. This in turn results in the subsequent downregulation of HIF's transcriptional activity in various human epithelial cancer cell lines (Thomas et al. 2008). Although the molecular mechanism associated with curcumin-mediated inhibition of HIF protein is not fully understood, based on detailed investigations it is suggested that curcumin inhibits HIF-1 α at the level of transcription, as evidenced by the dose-dependent decrease of HIF-1 α mRNA expression.

6.3.1.5 Modulation of PPAR γ by Curcumin

Peroxisome proliferator-activated receptor gamma (PPAR- γ) is a member of the nuclear receptor family of transcription factors, a large and diverse group of proteins that mediates ligand-dependent transcriptional activation and repression. It regulates fatty acid storage and glucose metabolism. There are three PPAR isoforms (PPAR α , γ , and δ), which are differentially expressed in brain and spinal cord. Among these isoforms, PPAR- γ is the most widely studied form. It regulates cell differentiation, apoptosis, lipid metabolism, and inflammation. Upon ligand binding, PPAR- γ forms heterodimers with the retinoid X receptor and binds to a peroxisome proliferation response element (PPRE) in a gene promoter leading to regulation of gene transcription (Forman et al. 1996). The natural ligands for PPAR receptor family are fatty acids and lipid metabolites, and each PPAR family member displays a distinct pattern of ligand specificity.

PPAR γ isoform is expressed in monocytes, microglia, and macrophages. In these cells, the principal action of PPAR- γ is to suppress the expression of the proinflammatory cytokines IL-1 β , TNF α , and IL-6 and other proinflammatory products (Jiang et al. 1998; Ricote et al. 1998). Importantly, activation of PPAR γ acts to negatively regulate microglial cell and macrophage activation and cytokine expression by antagonizing the activity of the transcription factors NF- κ B, AP-1, and STAT proteins (Lemberger et al. 1996; Ricote et al. 1998). It is reported that gene and protein levels of PPAR- γ in the liver are reduced by approximately 50 % at 20 h after the onset of sepsis. Pretreatment with curcumin for 3 days at 0.24 μ mol/kg body weight in these septic rats causes 45 % and 65 % increase in PPAR- γ mRNA and protein levels, respectively. The mRNA and protein levels of PPAR- γ in the treatment group have been shown to be similar to sham controls (Siddiqui et al. 2006; Jacob et al. 2007). Similarly, pretreatment of RAW 264.7 cells with 50 and 100 μ M curcumin increases PPAR- γ mRNA levels by 86 % and 125 %, respectively, compared to LPS treatment alone. Consistent with these

results, immunohistochemical staining of RAW 264.7 cells with PPAR- γ antibody shows enhancement in nuclear PPAR- γ staining in cells pretreated with 100 μ M curcumin compared to LPS alone. These observations support the view that the beneficial effect of curcumin may be mediated by the upregulation of PPAR- γ (Siddiqui et al. 2006; Jacob et al. 2007).

6.3.1.6 Modulation of Nrf2 by Curcumin

Nuclear factor E2-related factor 2 (Nrf2) is a basic leucine zipper redox-sensitive transcription factor that controls the basal and inducible expression of a battery of antioxidant genes. It induces expression and upregulation of cytoprotective and antioxidant/detoxifying genes that attenuate tissue injury (Lee and Johnson 2004). Under physiological conditions, Nrf2 is localized in the cytoplasm where it binds with the actin-binding protein, Kelch-like ECH-associating protein 1 (Keap1), and is rapidly degraded by ubiquitin-proteasome pathway. Keap 1 acts as negative regulator of Nrf2. Oxidative stress liberates Nrf2 from Keap1 and allows Nrf2 translocation into nucleus, where it binds to stress or antioxidant response elements (ARE) and facilitates expression of cytoprotective genes, numerous protective enzymes and scavengers. Studies with knock-out mice indicate that Nrf2 is part of a transcription factor complex required for regulation of the mouse glutathione S-transferase (GST) and NADPH: quinone oxidoreductase (NQO1) genes (Itoh et al. 1997; Hayes et al. 2000). In addition, Nrf2/small Maf heterodimers bind to the ARE sequence with high affinity during regulation of the GST and NQO1 genes (Venugopal and Jaiswal 1998). These observations support the view that Nrf2 may be the major transcription factor necessary for ARE activation and thus essential for the induction of phase II detoxification enzymes.

In the brain, antioxidant properties of Nrf2-overexpressing glia are more pronounced than neurons. Thus, relatively small number of Nrf2-overexpressing glia protects fully cocultured naive neurons from oxidative stress and glutamate-mediated glutathione (GSH) depletion (Shih et al. 2003; Vargas et al., 2008). Microarray and biochemical analyses indicate that coordinated upregulation of several enzymes including heme oxygenase-1 (HO-1), catalase, superoxide dismutases, epoxide hydrolase, UDP-glucuronosyl transferases (UGTs), and thioredoxin. In addition, Nrf2 also modulates enzymes associated with GSH biosynthesis (xCT cystine antiporter, γ -glutamylcysteine synthetase, and GSH synthase), glutathione S-transferase and glutathione reductase, and multidrug resistance protein 1 overexpression. This regulation may lead to an increase in intracellular GSH contents and decrease in oxidative stress. In addition, several upstream signaling pathways including mitogen-activated protein kinases, protein kinase C, phosphatidylinositol 3-kinase, and transmembrane kinase have also been implicated in the regulation of Nrf2/ARE activity. Thus, Nrf2 plays an essential role in maintaining cellular homeostasis and hence represents a critical target for prevention of oxidative stress- or inflammation-associated with neurotraumatic and neurodegenerative diseases (Farooqui 2010).

Collective evidence suggests that Nrf2/ARE system not only mediates the upregulation of phase II detoxification and antioxidant enzymes expression, but also coordinates the expression of genes required for free radical scavenging and maintenance of redox potential. These processes preferentially occur in activated astrocytes (Kraft et al. 2004). It is proposed that Nrf2/ARE activation in astrocytes confers neuroprotection to neighboring neurons (Shih et al. 2003; Kraft et al. 2004). Curcumin targets the Nrf2-ARE signaling pathway to induce phase II detoxifying enzymes on an event of oxidative stress (Hatcher et al. 2008). Addition of curcumin disrupts the Nrf2-Keap1 complex, leading to elevated Nrf2 binding to ARE and subsequent increase in the expression and activity of HO-1 in neural and nonneuronal cells via activation of p38 MAP kinase and UDP-glucuronosyltransferase (UGT) isozymes. These enzymes are associated with the detoxification of numerous chemical toxins present in our daily diet and environment giving rise to ROS. Curcumin modulates the activity of cellular UGTs.

Curcumin not only activates Nrf2 (Stridh et al. 2010), but also inhibits inflammation by suppressing cyclooxygenases, lipoxygenases, and inducible nitric oxide synthase expression and superoxide and nitric oxide (NO) production by downregulating NF- κ B activity (see below). HO-1 is an important antioxidant enzyme that plays a pivotal role in cytoprotection against noxious stimuli of both endogenous and exogenous origin. Curcumin promotes the expression of HO-1 in neural cell culture leading to neuroprotection (Hatcher et al. 2008).

6.3.1.7 Modulation of E2F Transcription Factor by Curcumin

E2F, a family of transcription factor, plays an important role in controlling development, proliferation, differentiation, and survival. It has been divided into either activator or repressor, based on their ability to induce E2F-responsive gene expression, which causes the quiescent cells to progress into S-phase (Muller et al. 2001; DeGregori and Johnson 2006; Garneau et al. 2009), and involves cyclin-dependent kinase (CDK) and its inhibitors of the INK4 family. The downstream effectors in the pathway are the E2F transcription factors. The E2Fs regulate the timely expression of a series of genes whose products are essential for cell proliferation (Helin 1998; Muller et al. 2001; Polager et al. 2002). Treatment of HCT116 colon cancer cells with curcumin results not only in the expression of E2F4, but also several E2F4 downstream genes, E2F1, c-myc, CDK2, cyclin A, cyclin D1, p21, and p27, which are involved in cell cycle control and related to the inhibition of apoptotic cell death. This inhibition can be restored by pretreatment of *N*-acetylcysteine and doxycyclin-induced E2F4 expression. Moreover, E2F4 overexpression partially restores curcumin-mediated growth inhibition, confirming the role of E2F4 in the cell proliferation. It is noteworthy that partial compensation of curcumin-induced cell death by E2F4 overexpression implies that E2F4 is closely associated with antiproliferative activity.

6.3.2 Effect of Curcumin on Enzyme Activities and Neurochemical Processes

Curcumin exhibits a variety of biological activities including antioxidative, anti-inflammatory, antiamyloidogenic, and anticarcinogenic activities (Anand et al. 2008). The underlying mechanisms of curcumin-mediated effects are diverse and appear to involve the regulation of various molecular targets, including transcription factors (such as nuclear factor-kB), growth factors (such as vascular endothelial cell growth factor), inflammatory cytokines (such as TNF- α , interleukin 1 β , and interleukin 6), protein kinases (Protein kinase C, mitogen-activated protein kinases, and Akt), and other enzymes (such as cyclooxygenase 2, 5 lipoxygenase, and inducible nitric oxide synthase) (Zhou et al. 2011). The molecular mechanism by which curcumin activates protein kinases (ERK and p38 MAP kinases) is unknown. However, it is proposed that curcumin either directly interacts with the kinases or phosphatases upstream of the MAP kinases or the activation of ERK and p38 MAP kinases by curcumin may represent an adaptive response of the cells to stress (Kim et al. 2008). It is speculated that curcumin may increase proliferation and survival of neural stem cells. The latter mechanism is consistent with the hormesis hypothesis for the beneficial actions of phytochemicals on neurons (Mattson and Cheng 2006).

In addition to its direct antioxidant activity, curcumin may function indirectly as an antioxidant by inhibiting the activity of inflammatory enzymes or by enhancing the synthesis of glutathione. The anti-inflammatory activity of curcumin seems to be comparable to steroid drugs and nonsteroidal drugs such as indomethacin and phenylbutazone (Menon and Sudheer 2007). Furthermore, curcumin also modulates adhesion molecules as well as cellular redox status. Thus, due to its efficacy and regulation of multiple targets, as well as its safety for human use, curcumin has received considerable attention as a potential therapeutic agent for the treatment of various malignant diseases, Alzheimer disease, Parkinson disease, and other inflammatory diseases. It modulates above pathological conditions by inhibiting or enhancing several enzymes that are associated with oxidative stress (cyclooxygenase-2, 5-lipoxygenase, matrix metalloproteinases, and inducible nitric oxide synthase) or upregulating phase II detoxification enzymes (HO-1, thioredoxin, GST, and NQO1). These enzymes have been reported to counteract oxidative stress and modulate apoptosis (Yang et al. 2009).

6.3.3 Effect of Curcumin on Biochemical Processes in the Brain

Curcumin is a pleiotropic molecule capable of interacting with numerous molecular targets involved in neuroinflammation, oxidative stress, and neuroplasticity. It is becoming increasingly evident that proinflammatory states are linked with the pathogenesis of neurotraumatic and neurodegenerative diseases (Farooqui 2010).

Consequently, Curcumin's strong anti-inflammatory and antioxidant effects can be better understood through its modulatory response by downregulating cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS), respectively. The stimulation of these enzymes is accompanied with the generation of several inflammatory mediators such as cytokines, leukotrienes, prostaglandins, platelet activating factor, tumor necrosis factor-alpha (TNF- α), interleukin 1 β (IL-1 β), and many others. The inhibition of COX-2 and iNOS involves the curcumin-mediated suppression of NF- κ B, a ubiquitous transcription factor associated with the regulation of cellular proliferation, neuroinflammation, and oxidative stress. Curcumin also suppresses the expression of proinflammatory gene (TNF- α) and (IL-1 β) expression by blocking phosphorylation of inhibitory factor I-kappa B kinase (IkB). Suppression of NF- κ B activation is associated with downregulation of COX-2 and iNOS expression thereby blocking signal transduction processes involved in neuroinflammation and neurodegeneration (Mandlekar et al. 2006; Farooqui 2010; Kannappan et al. 2011).

6.3.3.1 Oxidative Stress and Curcumin

Antioxidant properties of curcumin are due to the presence of chain breaking or hydrogen donating phenolic groups in its molecular structure. It is an effective scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Its protective action against peroxidation-mediated membrane damage is mainly attributed to the oxidation of neural membrane phospholipids and scavenging of the reactive free radicals generated from polyunsaturated fatty acids. The scavenging properties of curcumin are responsible for its protective effect on DNA and proteins (Rajasekaran 2011).

Homocysteine (Hcy) is an independent risk factor for heart disease and stroke (Clarke et al. 1991). This amino acid is readily oxidized in plasma and culture medium, resulting in the generation of ROS. Moreover, Hcy has the ability to retard the expression of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Hankey and Eikelboom 1999). It is also suggested that Hcy is recognized as an independent risk factor for Alzheimer disease and cognitive impairment (Schwartz et al. 1997). Chronic administration of Hcy to rats results in both long- and short-term memory in the Morris water maze task (Streck et al. 2004). Other studies indicate that in patients with Alzheimer disease, high plasma levels of Hcy are associated with neurodegeneration (Agnati et al. 2005). Intracerebral injections of Hcy induce lipid peroxidation and increase malondialdehyde (MDA) and superoxide anion (SOA) levels in whole rat brain. In addition, Hcy induces impairment in memory retention in the passive avoidance learning test (Ataie et al. 2010a, b). Curcumin treatment not only decreases MDA and superoxide anion levels, but also improves learning and memory in rats. These results support the view that Hcy-mediated induction of lipid peroxidation in rat brain can be retarded by curcumin, which also improves learning and memory deficits by protecting the nervous system against oxidative stress (Ataie et al. 2010a, b).

Curcumin not only oxidizes polyunsaturated fatty acids into fatty acid radicals, but also acts as a chain-breaking antioxidant at the 3' position, resulting in an intramolecular Diels–Alder reaction and neutralization of the lipid radicals (Masuda et al. 2001). Curcumin scavenges various reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, and nitrite radicals generated by microglia and macrophages both *in vitro* as well as *in vivo* in the brain and visceral tissues (He et al. 2010; Ray and Lahiri 2009). Thus, curcumin is a natural antioxidant, which protects brain from oxidative stress. Inducible nitric oxide synthase (iNOS) is an enzyme that generates large amounts of NO in brain and visceral tissues. NO reacts with superoxide radicals to form peroxynitrite, which is highly toxic reactive nitrogen species (RNS) to neural and visceral cells. Curcumin inhibits not only ROS generation, but also downregulates iNOS activity in microglia and macrophages, thus reducing the levels of ROS and RNS generated in response to oxidative stress (Chan et al. 1998; He et al. 2010; Ray and Lahiri 2009). These processes constitute to the inhibition of lipid peroxidation.

6.3.3.2 Neuroinflammation and Curcumin

Neuroinflammation is a host defense mechanism that not only isolates the injured brain tissue from uninjured area, but also destroys affected cells and repairs the extracellular matrix (Wood 1998; Correale and Villa 2004). The main mediators of neuroinflammation are microglial cells and astrocytes, which are activated during a CNS injury. Microglial cells and astrocytes mediated response in the injured brain initiates a rapid release of cytokines/chemokines and trophic and/or toxic effects. These cytokines/chemokines stimulate phospholipases A₂ and cyclooxygenases, which degrade neural membrane phospholipids and release of arachidonic acid and lyso-phosphatidylcholine. Arachidonic acid is oxidized to proinflammatory eicosanoid (prostaglandins, leukotrienes, and thromboxanes) by cyclooxygenases and lipoxygenases, and lyso-phosphatidylcholine is utilized for the synthesis of proinflammatory platelet-activating factor. Thus, eicosanoids and platelet activating factor initiate and intensify neuroinflammation. Two types of inflammatory responses (acute and chronic) occur in brain tissue. Acute inflammation response develops rapidly and is accompanied by pain, whereas chronic inflammation develops slowly and remains below the threshold of pain perception (Farooqui et al. 2007; Farooqui 2010). As a result, the immune system continues to attack the brain tissue and chronic inflammation lingers for years, ultimately reaching the threshold of detection (Wood 1998). Inflammatory response also involves recruitment and migration of polymorphonuclear leukocytes (PMN) from the blood stream into brain tissue. This is followed by a process called resolution, a turning off mechanism by neural cells to limit tissue injury.

Resolution involves anti-inflammatory mediators (IL-10, lipoxins, resolvins, protectins) (Farooqui 2011). This process is essential for the termination (resolution) of the beneficial effects of inflammation. An abnormal, ineffective, or absent

resolution contributes to tissue damage and persistent inflammation. Acute inflammation normally resolves spontaneously, but the mechanism associated with this process remains elusive (Serhan and Savill 2005). Curcumin not only retards the synthesis of proinflammatory prostaglandins and leukotrienes by inhibiting cyclooxygenases and lipoxygenases, but also blocks the uptake of arachidonic acid by macrophages, thereby limiting the availability of arachidonic acid for eicosanoid production (Menon and Sudheer 2007; Rajasekaran 2011). In addition, curcumin also reduces the conversion of linoleic acid to arachidonic acid. COXs, LOXs, NOS, and MMP 9 are involved in induction and maintenance of oxidative stress and neuroinflammation leading to neurodegeneration (Farooqui 2010), whereas enhanced activities of protein kinases and glutathione *S*-transferase (GST), catalase (CAT), superoxide dismutase (SOD), UDP-glucuronosyl transferase (UGT), and heme oxygenase-1 (HO-1) are associated with neuroprotection. Activation of above antioxidant pathways is particularly important for brain, which is relatively weak endogenous antioxidant defense (Farooqui et al. 2007). Among above enzymes, HO-1 has been extensively studied in the brain for its potential role in protecting neurons against cell death. HO-1 enzymes provide the first and rate-limiting step in heme degradation, to give biliverdin, gaseous carbon monoxide and free iron. All by-products of HO-1 activity play a significant role in physiological cell functions (Abraham et al. 1996). In the brain, the HO-1 system is very active (Maines 2000), and its stimulation by curcumin may protect neurons from neurodegeneration.

6.3.3.3 Neuroplasticity and Curcumin

Neuroplasticity is a comprehensive term, which is defined as the lifelong ability (capacity) of brain to reorganize or adapt itself by forming new neural connections. Neuroplasticity allows the neuron not only to compensate for injury and disease, but also promote neurons to adjust their activities and metabolism in response to new situations or to changes in their environment (Thickbroom and Mastaglia 2009). Neuroplasticity involves both neuronal and nonneuronal cells, such as neurons, glia, and vascular cells. Neuroplasticity is a substrate for learning and memory formation, cognitive abilities progressively lost in neurotraumatic and neurodegenerative diseases (Farooqui 2010). It is well known that oxidative stress that occurs following traumatic brain or spinal cord injuries and chronic stress that occurs in everyday life may induce impairment in spatial cognition and produce neuroendocrine and plasticity abnormalities (Wu et al. 2006; Xu et al. 2009).

Curcumin not only reduces oxidative stress caused by neurotraumatic injury, but also reverses the chronic stress-induced behavioral deficits in escape from an aversive stimulus. Although the mechanism associated with the beneficial effects of curcumin is not fully understood, recent studies indicate that curcumin acts by restoring memory deficits in a dose-dependent manner. The effects are similar to positive antidepressant effects of imipramine (Xu et al. 2009). Additionally, curcumin blocks adverse changes in the dendritic morphology of CA3 pyramidal

neurons in the hippocampus, as judged by the alterations in branch points and dendritic length. In primary hippocampal neurons, curcumin or imipramine protects hippocampal neurons against traumatic injury and corticosterone-induced toxicity. Furthermore, traumatic brain injury and corticosterone-mediated increase in calcium/calmodulin kinase II (CaMKII) activity and the glutamate receptor subtype [NMDA(2B)] expressions are also blocked by curcumin or imipramine treatment. Furthermore, it is becoming increasingly evident that high-fat diet exacerbates the effects of traumatic brain injury (TBI) on synaptic plasticity and cognitive function. Supplementation of curcumin in the diet dramatically reduces oxidative damage and normalizes levels of BDNF, synapsin I, and cAMP response element-binding (CREB) in rats following TBI. BDNF, synapsin, and CREB are closely associated with modulation of neuroplasticity (Wu et al. 2006). Collective evidence suggests that curcumin may be an effective therapeutic agent treating cognitive dysfunction and learning and memory disturbances following traumatic brain injury and chronic stress (Wu et al. 2006; Xu et al. 2009). Reduced cognitive function is also associated with AD and PD diseases as well as brain trauma and ischemia.

6.3.3.4 Interactions of Curcumin with Abnormal (Misfolded) Proteins

Curcumin has been reported to exist in an equilibrium mixture between keto and enol tautomeric forms, which bind to A β fibrils/aggregates (Yanagisawa et al. 2010). Detailed investigations on interactions between tautomeric forms (keto and enol) of curcumin and A β (monomeric and aggregated forms) indicate that curcumin derivatives with keto-enol tautomerism show high levels of binding to A β aggregates but not to A β monomers (Yanagisawa et al. 2010). The binding activity of the keto form analog of curcumin to A β aggregates is much weaker than that of curcumin derivatives with keto-enol tautomerism. The color of a curcumin derivative with keto-enol tautomerism, which is substituted at the C-4 position, changes from yellow to orange within 30 min of interactions between curcumin and A β aggregates in physiological buffer. Based on these results, it is suggested that curcumin derivatives exist predominantly in the enol form during binding to A β aggregates, and that the enolization of curcumin derivatives is crucial for binding to A β aggregates (Yanagisawa et al. 2010).

Protein disulfide isomerase (PDI) is an endoplasmic reticulum (ER) enzyme that catalyzes maturation of disulfide-bond-containing proteins and contributes to the pathogenesis of both PD and AD. S-nitrosylation of PDI cysteines due to nitrosative stress may be responsible not only for cytosolic debris accumulation, but also for the aggregation and accumulation of Lewy body in PD and AD brains (Pal et al. 2010). It is also shown that curcumin and masoprolac, an antineoplastic drug, can rescue PDI from becoming S-nitrosylated and maintain its catalytic function under conditions mimicking nitrosative stress by forming stable NOx adducts (Pal et al. 2010). Furthermore, curcumin also prevents the formation of PDI-resistant polymeric misfolded protein forms that accumulate upon exposure to oxidative stress. So, curcumin and masoprolac can serve as leading prophylactics for ROS-mediated

chaperone damage, protein misfolding, and neurodegeneration; importantly, they also can play a vital role in sustaining traffic along the ER's secretory pathway by preserving functional integrity of PDI (Pal et al. 2010).

6.3.3.5 Interactions of Curcumin with Nucleic Acids

Curcumin interacts with nucleic acids and modulates their physiological functions. Studies on the binding of curcumin with calf thymus DNA and yeast RNA in aqueous solution under physiological conditions indicate that curcumin binds DNA through thymine O₂ (minor groove) and guanine and adenine N7 (major groove), as well as to the backbone PO₂⁻ group with overall binding constants of $4.3 \times 10^4 \text{ M}^{-1}$. The interactions between curcumin and RNA are mediated by hydrogen bonding with uracil O₂ and guanine and adenine N7 atoms as well as the backbone phosphate group, with overall binding constants of $1.3 \times 10^4 \text{ M}^{-1}$ (Nafisi et al. 2009).

Although major DNA and RNA aggregation are observed at high curcumin concentration, the conformation of nucleic acids remains unchanged; DNA remains in the B, and RNA retains its A-family structure.

6.3.3.6 Interactions of Curcumin with Glutathione

Curcumin contains two electrophilic α, β-unsaturated carbonyl groups, which react with glutathione (GSH) forming the GSH–curcumin conjugates. The reaction is catalyzed by human glutathione S-transferase (GST)P1-1. This enzyme optimally acts at pH 7.0 and has apparent Km value for curcumin of $25 \pm 11 \mu\text{M}$. It should be noted that recombinant human glutathione S-transferase (GST)P1-1 not only generates mono- and di-glutathionyl-adducts of curcumin, but forms cyclic rearrangement products of GSH adducts of feruloylmethylketone (FMK) and feruloylaldehyde (FAL) (Awasthi et al. 2002). The physiological significance of curcumin glutathione interactions in brain remains unknown.

6.4 Therapeutic Importance of Curcumin in Neurological Disorders

Neurological disorders include neurodegenerative, neurotraumatic, and neuropsychiatric disorders. Neurodegenerative diseases are generally characterized by the selective degeneration of particular neuronal populations and the accumulation of abnormal or aggregated (misfolded) proteins within, but occasionally external to, neurons in affected brain regions (Farooqui 2010). Neurodegenerative diseases are also accompanied by cognitive dysfunction, loss of memory, and abnormal movement. Both these features may often coexist in a single disease. In recent years, the

identification of genetic mutations that cause rare monogenic familial disease has revolutionized our understanding of the molecular basis of neurodegenerative diseases (Farooqui 2010). Although ROS are generated by enzymic and nonenzymic reactions in the mitochondria and cytoplasm under normal conditions, excessive production of ROS in neurodegenerative diseases is associated with activation of Ca^{2+} -dependent enzymes including proteases, phospholipases, nucleases, and alterations of signaling pathways which subsequently lead to mitochondrial dysfunction, release of inflammatory factors, and apoptosis (Farooqui and Farooqui 2011).

Curcumin through its antioxidant and anti-inflammatory properties plays an important role by not only blocking oxidative stress and neuroinflammation in neurodegenerative diseases, but also restoring cellular homeostasis and rebalancing redox equilibrium. Curcumin also acts by inducing HO-1 expression and its activity in different brain cells via the activation of Nrf2/antioxidant responsive element (ARE) pathway (Scapagnini et al. 2011). Activation of Nrf2 target genes, and particularly HO-1, in astrocytes and neurons produces strongly protective effects against neuroinflammation, oxidative stress, and apoptotic cell death. In the brain tissue, the HO-1 system is very active, and its modulation plays a crucial role in the pathogenesis of neurodegenerative diseases (Scapagnini et al. 2011). Most of this evidence for the above processes is preclinical, and more clinical trials are needed to further strengthen this evidence. Because chronic diseases associated with brain incubate at least for 50–55 years before they manifest, designing and structuring such clinical trials will be difficult. It remains unknown what amount of curcumin is needed and for how long and whether it is better to consume curcumin in food or if supplements will suffice. The evidence, however, that curcumin is safe, multitargeted, efficacious, and affordable demands further investigation on the use of curcumin in chronic neurodegenerative diseases.

6.4.1 Curcumin and Alzheimer Disease

AD is a progressive neurodegenerative disease marked by the progressive loss of memory and cognitive function. Neuropathologically, AD is characterized by the accumulation of beta-amyloid ($\text{A}\beta$) protein that forms plaques and tau protein phosphorylation that facilitate formation and deposition of neurofibrillary tangles (Farooqui 2010). Both processes result in neuronal atrophy, which initially appears in the entorhinal region and the temporal lobe, before progressing to the limbic system and subsequently to major areas of the neocortex, severely damaging the brain (Braak and Braak 1995). In AD, dementia correlates to synaptic and neuronal loss, rather than directly with the pathological burden. So much attention is focused on understanding the pathways that lead to formation and deposition of senile plaques and neurofibrillary tangles, and then to the molecular mechanism(s) associated with the loss of synapse and induction of neurodegeneration in specific regions of AD brain. Strong genetic evidence supports the view that aberrant accumulation of $\text{A}\beta$ which lies upstream may not only lead to downstream pathologies such

as tangle and plaques formation (Oddo et al. 2003), but also to extensive neuroinflammation (Meda et al. 1995); oxidative stress-mediated damage to lipids, proteins, and DNA (Hensley et al. 1995; Farooqui 2010); and glycation of proteins (Horie et al. 1997). Collective evidence suggests that in AD multiple pathways contribute to cognitive deficits and memory formation through the disruption of neuronal signal transduction pathways. These pathways are altered by aberrant signaling, inflammation, oxidative stress-mediated damage, tau pathology, and neuronal and synaptic loss (Lesne et al. 2006; Farooqui 2010).

Epidemiological studies indicate that in India, where curcumin is widely used in daily diet, there is a significant reduction in the prevalence of AD (in patients between 70 and 79 years of age is 4.4-fold less than that of the United States) (Chandra et al. 2001). In addition, curcumin has been reported to decrease oxidative damage and amyloid deposition in a transgenic mouse model of Alzheimer disease and to reverse A β -induced cognitive deficits and neuropathology in rats (Yang et al. 2005). It is suggested that curcumin acts through its pleiotropic effects. The targets include transcription factors (NF- κ B, AP-1, PPAR γ , and Nrf2), enzymes (COX2, 5-LOX, iNOS, HO-1, and JNK), and cytokines (TNF- α , IL-1 β , IL-6) (Shishodia et al. 2005) (Fig. 6.9). Modulation of above transcription factors, enzymes, and cytokines by curcumin leads to neuroprotection through anti-inflammatory, antioxidant, and antiprotein aggregate and neurogenic effects of curcumin in AD models (Yang et al. 2005; Cole and Frautschy 2007; Ma et al. 2009). Curcumin not only reduces senile plaque deposition, but also suppresses JNK/IRS-1/tau signaling pathway that leads to AD-like p-IRS-1/insulin signaling defects in 3 \times Tg-AD mice (Ma et al. 2009). Thus, treatment of the 3 \times Tg-AD mice on high-fat diet with curcumin for 4 months not only reduces levels of phosphorylated JNK, IRS-1, and tau, but also prevents the degradation of total IRS-1. These neurochemical changes are accompanied by improvement in Y-maze performance (Ma et al. 2009). Mice consuming curcumin for 1 month show more significant effects on Y-maze performance and the combination of curcumin and fish oil shows more significant inhibition of JNK, IRS-1, and tau phosphorylation. These data support the view that JNK induced A β oligomer-mediated inactivation of IRS-1 and phospho-tau pathology and dietary treatment with fish oil, curcumin, or a combination of both may produce improvement in insulin/trophic signaling and cognitive deficits in AD (Ma et al. 2009). This provides an additional mechanism for molecular action and efficacy of curcumin in an AD model with tau pathology. Antioxidant and anti-inflammatory activities of curcumin confer significant protection against neurotoxic effects of A β . In the neurons, A β interacts with p75NTR, activates NF- κ B-mediated proinflammatory signaling in a time- and dose-dependent manner leading to neurodegeneration. Curcumin retards the activation of NF- κ B and prevents A β -induced cell death in a human neuroblastoma cell line, suggesting a possibility of AD treatment with curcumin (Kuner et al. 1998; Ono et al. 2004). Based on several studies, it is proposed that curcumin lowers A β levels by attenuating the maturation of APP in the secretory pathway. In addition, it is also reported that interactions between curcumin and A β produces activation of early growth response-1 (Egr-1), a nuclear transcription factor, which leads to increase in the expression of cytokines (TNF- α and IL-1 β)

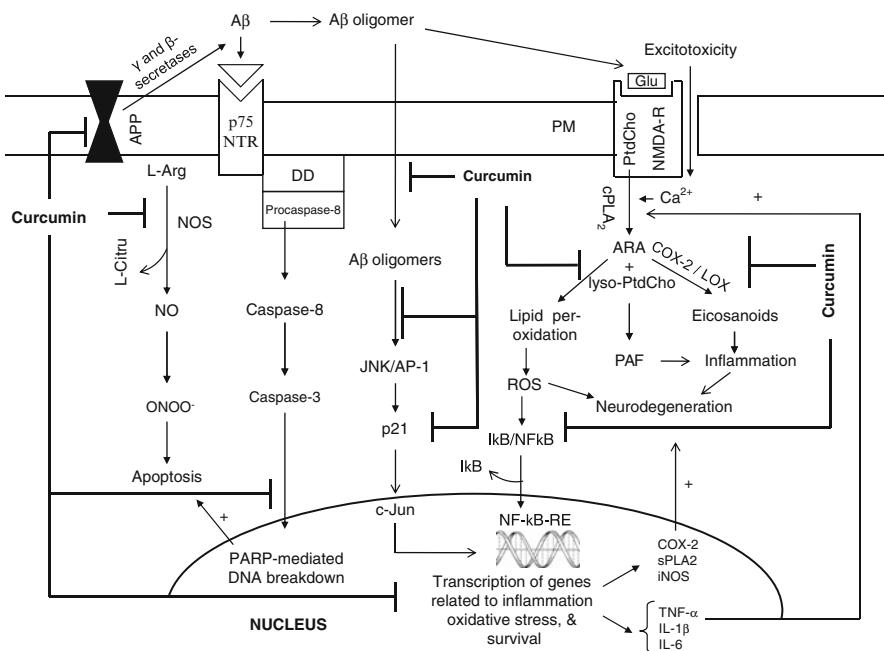


Fig. 6.9 A hypothetical signal transduction diagram showing the effect of curcumin on signal transduction processes in AD. Amyloid precursor protein (APP); monomeric β -amyloid peptide (A_β); N-methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); cyclooxygenase (COX); lipoxygenase (LOX); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor- κ B (NF- κ B); nuclear factor- β B-response element (NF- κ B-RE); inhibitory subunit of NF- κ B (I- κ B); tumor necrosis factor- α (TNF- α); interleukin-1 β (IL-1 β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); secretory phospholipase A₂ (sPLA₂); death domain (DD); nitric oxide (NO); JUN N-terminal kinase (JNK); poly(ADP) ribose polymerase (PARP). Positive sign indicates stimulation; and \neg (blocked arrow) represents inhibition

and chemokines (MIP-1 β , MCP-1, and IL-8) in monocytes (Giri et al. 2003). Curcumin suppresses the activation of Egr-1 DNA-binding activity and abrogates A_β-mediated expression of these cytokines and chemokines. Collective evidence suggests that curcumin or curcumin phospholipid conjugate not only reduces A_β-mediated (1) increase in the level of ROS, (2) decrease in mitochondrial membrane potential, and (3) caspase activation. In addition, curcumin protects human neurons from oligomeric amyloid- β -induced toxicity as well (Zhang et al. 2010; Moshra et al. 2011). Thus, curcumin confers protection against A_β-induced toxicity not only by inhibiting the formation of A_β oligomers and fibrils, but also binding to plaques (Fig. 6.9).

At present, four clinical trials have been conducted to test the usefulness of curcumin for the treatment of AD. Two trials have been performed in China and USA, but no significant differences are observed in cognitive function between placebo

and curcumin groups. No results have been reported on two other remaining clinical trials. Additional trials are necessary to determine the clinical usefulness and efficacy of curcumin in the prevention and treatment of AD.

6.4.2 *Curcumin and Dementia*

Dementia is a major cause of disability. It is clinically defined not only by memory deficits, but also by deterioration of emotional control and social behavior due to disturbances in cortical functions (Sonnen et al. 2009). Very little is known about the molecular mechanisms of dementia. However, it is proposed that misfolding protein-mediated alterations in vascular pathology along with changes in brain insulin receptors, alterations in neurotransmission, and oxidative stress-mediated loss of synapse may be closely associated with the pathogenesis of dementia (Sonnen et al. 2009). Thus, stimulation of brain insulin receptors (IRs), increase in acetylcholinesterase (AChE) activity, and enhancement in oxidative stress along with memory deficit have been reported to occur in streptozotocin (STZ) induced model of dementia in rats (Agrawal et al. 2010). Administration of curcumin in this model results not only in a significant reduction in IR protein level and inhibition of malondialdehyde in both hippocampus and cerebral cortex, but also in restoration of the memory deficit and increase in cholinergic activity. These findings support the view that curcumin can be a potentially beneficial agent for treating dementia (Agrawal et al. 2010, 2011).

6.4.3 *Curcumin and Parkinson Disease*

Parkinson disease (PD) is a chronic and progressive neurodegenerative disorder characterized by depletion of dopaminergic neurons within the substantia nigra pars compacta of the midbrain (Jenner 1998; Jenner and Olanow 2006). Loss of these neurons causes pathological changes in neurotransmission in the basal ganglia motor circuit. The vulnerability of dopaminergic neurons in the substantia nigra pars compacta to oxidative stress is due to monoamine oxidase (MAO)-mediated abnormal dopamine metabolism and hydrogen peroxide generation. One of the pathological hallmarks of PD is the presence of intracellular inclusions called Lewy bodies that consist of aggregates of the presynaptic soluble protein called α -synuclein (Farooqui 2010).

In MPTP-induced model of PD, systemic administration of curcumin and tetrahydrocurcumin significantly reverses the MPTP-induced depletion of dopamine and 3,4-dihydroxy phenyl acetic acid (DOPAC). The MAO-B activity is also significantly inhibited by curcumin and tetrahydrocurcumin. These observations support the view that curcumin and tetrahydrocurcumin exert neuroprotective effects against MPTP-induced neurotoxicity (Rajeswari and Sabesan 2008). Beside

alterations in dopamine metabolism and oxidative stress, PD is accompanied by α -synuclein aggregation (Betarbet et al. 2002; Quilty et al. 2006). Intracellular overexpression of α -synuclein generates excess ROS and causes severe oxidative stress to the cells (Jenner 1998) leading not only to the disruption in redox homeostasis, cell metabolism, free radical generation, but also more lipid peroxidation and protein oxidation (Jenner 1998). Excess ROS not only induces plasma membrane damage and mitochondrial dysfunction, but also produces defects in the glutathione peroxidase expression and reduction in glutathione levels. All processes render the brain more susceptible to oxidative stress and neuroinflammation (Jenner 1998; Mancuso et al. 2007).

Curcumin inhibits oxidative stress and significantly reduces the cytotoxicity mediated by extracellular or intracellular α -synuclein aggregates, suggesting that curcumin may be a useful therapeutic agent for treating PD. Since extracellularly added curcumin provides protection even against intracellularly induced α -synuclein toxicity, suggesting that there is a significant extracellular or cell surface component to α -synuclein-induced neurotoxicity in PD models (Liu et al. 2011). This observation is consistent with recently published reports of interneuronal transmission of extracellular α -synuclein pathology in neuronal cells (Desplats et al. 2009). Curcumin protects against A53T α -synuclein-mediated cell death in a dose-dependent manner.

Curcumin reduces mutant α -synuclein-mediated intracellular ROS levels, mitochondrial depolarization, cytochrome c release, and caspase-9 and caspase-3 activation. In SH-SY5Y neuroblastoma cells, curcumin not only reduces α -synuclein-induced neurotoxicity and generation of ROS generation, but also ameliorates signs of apoptosis (Wang et al. 2010). Studies on the effect of curcumin on α -syn protein aggregation indicate that treatment with 1 mM Fe³⁺ (Fenton reaction) produces aggregation of α -synuclein. The addition of curcumin not only results in a decrease in aggregate formation, but increases the solubility of α -syn aggregates (Pandey et al. 2008). Although these observations support the view that curcumin protects against α -syn-induced cell death via inhibition of oxidative stress and mitochondrial cell death pathway, more studies are needed on the effect of curcumin on α -synuclein-mediated cytotoxicity and subsequent pathogenesis and progression of the disease in in vivo models of PD.

6.4.4 Curcumin and Prion Diseases

Prion diseases, a group of fatal neurodegenerative disorders, are not only characterized by the accumulation of abnormal isoforms of a host protein known as cellular prion protein (PrP^C), but also by motor dysfunctions, dementia, and neuropathological changes such as spongiosis, astrocytosis, and neuronal loss (Prusiner 2001; Grossman et al. 2003). The cellular prion protein (PrP^C), a membrane-bound glycoprotein, is abundantly expressed in neurons and glial cells within the brain tissue. Conversion of the native, predominantly α -helical conformation of prion protein (PrP^C) into the β -stranded conformation is the characteristic of prion diseases, which

include scrapie in goats and sheep, bovine spongiform encephalopathy (mad cow disease) in cattle, and Creutzfeldt–Jakob disease (CJD), kuru, and Gerstmann–Sträussler–Scheinker syndrome in humans (Prusiner 2001; Grossman et al. 2003). Neuronal loss, spongiform degeneration, and glial cell proliferation are other pathological characteristics of prion diseases.

Curcumin not only inhibits the accumulation of PrP^{sc} in scrapie agent-infected neuroblastoma cells (50 % inhibitory concentration, approximately 10 nM), but partially blocks the cell-free conversion of PrP^c to PrP^{sc} (Caughey et al. 2003). It should be noted that curcumin only recognizes the PrP^{sc} both in oligomer and fibril forms, but has no effect on the native form (PrP^c). Circular dichroism studies indicate that curcumin binds to the prion fibrils in the left-handed chiral arrangement manner. In brain sections of a variant of CJD, curcumin labels the plaques similar to PrP^c antibodies (Hafner-Bratkovic et al. 2008), indicating that curcumin binds to the antibody binding site in the PrP^{sc}. However, *in vivo* studies indicate that dietary administration of curcumin has no significant effect on the onset of scrapie in hamsters (Caughey et al. 2003). So, more studies are required on the effect of curcumin on prion diseases.

6.4.5 Curcumin and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the brain (Noseworthy et al. 2000). MS is characterized by a variety of pathophysiological features, including breakdown of the BBB, autoimmune attack, injury of axons and myelin sheaths (Noseworthy et al. 2000). The most common symptoms of MS are weakness in one or more limbs, sensory disturbances, optic neuritis, ataxia, bladder dysfunction, fatigue, and cognitive deficits (O'Connor 2002). The most common clinical progression pattern, affecting about 80 % of patients, is a phase of relapsing and remitting clinical signs and symptoms at the beginning of disease (RRMS), which is characterized by acute attacks followed by complete or partial recovery and a lack of disease progression in between two relapses. The localization and severity of MS lesions within the brain and spinal cord is unpredictable and, therefore a wide range of body systems can be adversely affected to a variable degree. Consequently, there is a myriad of symptoms and comorbidities associated with MS that can impact negatively on patient quality of life (QoL) (Noseworthy et al. 2000; de Sa et al. 2011). The onset of MS occurs between age 20 and 40 years. MS may also develop in children and in persons over age 60 years. Women are affected approximately twice as often as men.

Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS. A number of recent studies indicate that T helper cells that produce IL-17 play a dominant role in the pathogenesis of EAE. Due to its strong anti-inflammatory and antioxidant activities and ability to modulate the expression of transcription factors, cell cycle proteins, and signal transducing kinases, curcumin has been used for the treatment of EAE. The treatment of Lewis rats with curcumin significantly reduces the clinical severity of EAE, causing a dramatic reduction in the number of

inflammatory cells infiltration in the spinal cord (Xie et al. 2009). The proliferation of the MBP-reaction lymphocyte is also reduced in a curcumin dose-dependent manner (Xie et al. 2009). Furthermore, curcumin inhibits the mRNA expression of the cytokines and transcription factors, such as IL-12, IL-17, IL-6, IL-21, TGF- β , NF- κ B, and STAT3. These findings indicate that curcumin produces beneficial effects in amelioration of EAE not only by inhibiting differentiation by downregulating above cytokines and transcription factors, but also through the development of Th17 cells (Xie et al. 2009). It should be noted that very little is known about the effect of curcumin on various facets of the immune response, including its effect on lymphoid cell populations, antigen presentation, humoral and cell-mediated immunity, and cytokine production (Gautam et al. 2007; Bright 2007). So, more studies are required on the molecular mechanism associated with beneficial effects of curcumin in EAE.

6.4.6 Curcumin and Ischemic Injury

Ischemic injury to the brain is caused by reduction in blood flow sufficient to alter normal cellular function (Farooqui 2010). Brain tissue is exquisitely sensitive to ischemic injury because it utilizes about 20 % of respired oxygen for normal function, even though it represents only 5 % of the body weight. Thus, brief ischemic insult to the brain can initiate a complex sequence of events that may ultimately lead to neurodegeneration (Farooqui 2010). Different brain regions have varying thresholds for ischemic injury, with white matter being more resilient than gray matter (Mattson et al. 2001). In addition, certain populations of neurons are selectively more vulnerable to ischemia; for example, in the hippocampus, CA1 pyramidal neurons are highly susceptible to ischemic injury, whereas dentate granule neurons are more resistant (Mattson et al. 2001).

Ischemic injury triggers a complex series of neurochemical events that impairs the neurologic functions through the breakdown of cellular and subcellular integrity mediated by excitotoxic glutamatergic signaling, Ca²⁺-influx, alterations in ionic balance and redox, and free-radical generation (Farooqui and Horrocks 1994; Farooqui et al. 2008; Farooqui 2010). These processes also lead to the activation of signaling mechanisms involving phospholipases A₂, C, and D (PLA₂, PLC and PLD), calcium/calmodulin-dependent kinases (CaMKs), mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), nitric oxide synthases (NOS), calpains, calcinurin, and endonucleases. Stimulation of these enzymes brings them in contact with appropriate substrates and modulates cell survival/degeneration mechanisms (Hou and MacManus 2002; Farooqui and Horrocks 2007). Degenerative mechanisms include apoptosis, necrosis, and autophagy in traumatized neurons following ischemic injury *in vitro* (Farooqui 2010).

A single injection of curcumin (1 and 2 mg/kg, i.v.) 30 min after focal cerebral ischemic/reperfusion injury in rats not only diminishes infarct volume, improves

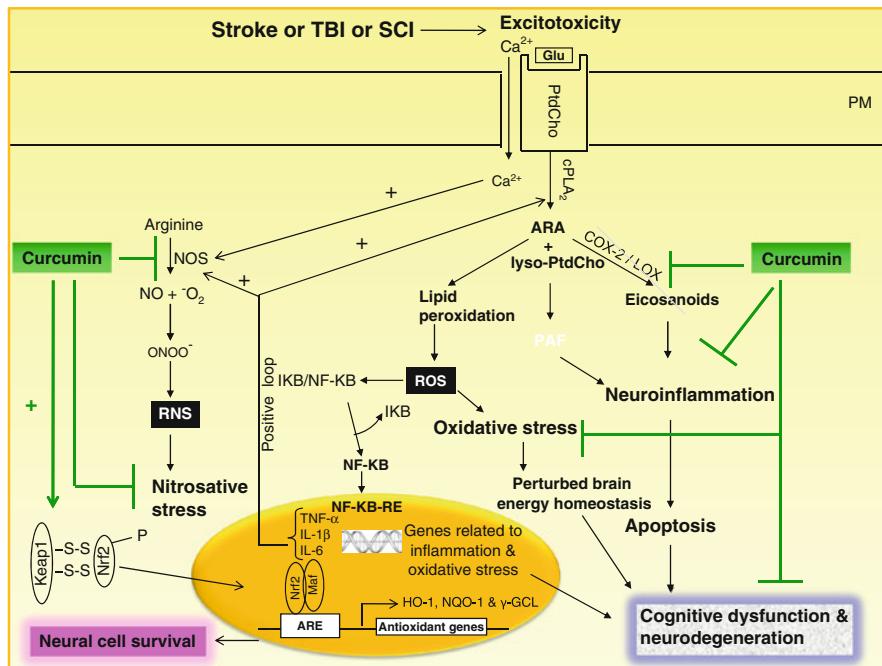


Fig. 6.10 Hypothetical diagram showing beneficial effects of curcumin in neurotraumatic diseases. N-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); cyclooxygenase (COX); lipoxygenase (LOX); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor-κB (NF-κB); nuclear factor-βB-response element (NF-κB-RE); inhibitory subunit of NF-κB (I-κB); tumor necrosis factor-α (TNF-α); interleukin-1β(IL-1β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); secretory phospholipase A₂ (sPLA₂); nitric oxide (NO); peroxynitrite (ONOO⁻); hemoxygenase (HO-1); NADPH quinone oxidoreductase (NQO-1); γ-glutamate cystein ligase (γ-GCL). Positive sign indicates stimulation

neurological deficit, and decreases mortality, but also reduces the water content of the brain and extravasates the Evans blue dye in ipsilateral hemisphere in a dose-dependent manner (Jiang et al. 2007). In cultured astrocytes, curcumin significantly inhibits iNOS expression and NO(x) (nitrites/nitrates contents) formation mediated by lipopolysaccharide (LPS)/tumor necrosis factor-α (TNF-α). Based on in vivo and in vitro observations, it is proposed that curcumin ameliorates cerebral ischemia/reperfusion injury not only by preventing peroxy nitrite mediated BBB, but also by decreasing lipid peroxidation-mediated damage and retarding apoptotic cell death as well as glial cell activation (Jiang et al. 2007). The biochemical changes resulting from curcumin correlate well with its ability to ameliorate the changes in locomotor activity induced by cerebral ischemia/reperfusion (I/R) injury (Wang et al. 2005). In addition, curcumin-mediated stimulation of transcription factor Nrf2 not only coordinates expression of genes and proteins required for scavenging free radical (Fig. 6.10), but also associated with maintenance of redox potential (Yang et al. 2009).

Thus, i.p. injections of curcumin produce $50.96 \pm 6.04\%$ reduction in edema ($p < 0.05$) volume, reduce lipid peroxidation, prevent ischemia/reperfusion-mediated decrease in glutathione peroxide activity, and reduce peroxynitrite production and hence decrease the extent of tyrosine nitration in the cytosolic proteins. Curcumin treatment not only decreases cytochrome c, caspase 3 expression, but increases mitochondrial Bcl-2 expression.

It is well known that in brain, the HO-1 system actively operates as a fundamental defensive mechanism for neurons that are exposed to oxidative stress. Curcumin potently induces HO-1 expression and activity in rat astrocytes (Scapagnini et al. 2004). A significant expression of quinone reductase and glutathione S-transferase, two members of phase II detoxification enzymes, is found in astrocytes exposed to curcumin (Scapagnini et al. 2006). Thus, inhibition of oxidative stress and neuroinflammation with curcumin improves outcomes after focal cerebral ischemia. This neuroprotective effect is likely exerted by antiapoptotic mechanisms (Zhao et al. 2010). Collectively, these studies suggest that neuroprotective activity of curcumin in cerebral ischemia is mediated through its antioxidant activity (Zhao et al. 2010; Thiagarajan and Sharma 2004).

6.4.7 Curcumin and Traumatic Brain Injury

Traumatic brain injury (TBI) results in primary and secondary events (Raghupathi 2004). The primary event is mechanical. It involves rapid deformation of brain tissue and rupture of neural cell membranes leading to the release of intracellular contents, disruption of blood flow, breakdown of the BBB, and intracranial hemorrhage. Secondary event in TBI induces neurochemical alterations, such as activation of microglial cells and astrocytes, and demyelination involving oligodendroglia (Raghupathi 2004). Disruption in cerebral blood flow not only results in mitochondrial damage, but also induces alterations in ion homeostasis and edema. Adult brain responds to TBI not only by activating a program of cell proliferation during which many oligodendrocyte precursors, microglia, and some astrocytes proliferate, but also by inducing reactive gliosis, a process by which dormant astrocytes undergo morphological changes and alter their transcriptional profiles. Inflammation and oxidative stress are major components, which cause neurodegeneration in TBI.

Studies on the effect of curcumin in TBI indicate that this polyphenol dramatically reduces oxidative damage, normalizes levels of BDNF and synapsin I, and decreases the levels of AMP-activated protein kinase (AMPK), ubiquitous mitochondrial creatine kinase (uMtCK), and cytochrome c oxidase II; CREB has also been altered after TBI (Wu et al. 2006; Sharma et al. 2009). Additionally, curcumin and pyrazole curcumin derivative not only reduce the increase in calcium-independent phospholipase A2 (iPLA2) activity and decrease in levels of 4-hydroxynonenal, but increase the fatty acid transport protein (FATP). Furthermore, curcumin supplementation counteracts the cognitive impairment induced by TBI

and effectively restores parameters of membrane homeostasis. These results support the view that in the injured brain oxidative stress acts through the BDNF system to modulate synaptic plasticity membrane homeostasis, and cognition and curcumin can promote protective mechanisms in the injured brain (Wu et al. 2006; Sharma et al. 2009, 2010a) by facilitating endogenous upregulation of molecules that are important for neural repair and plasticity.

6.4.8 Curcumin and Spinal Cord Injury

Spinal cord injury (SCI) is a catastrophic event, which induces autodestructive changes that lead to varying degrees of tissue necrosis and paralysis. Like TBI, it consists of two broadly defined events: a primary event, attributable to the mechanical insult itself, and a secondary event, attributable to the series of systemic and local neurochemical and pathophysiological changes that occur in spinal cord after the initial traumatic insult (Klussmann and Martin-Villalba 2005; Farooqui 2010). Primary event is instantaneous and beyond therapeutic management, but the secondary event develops over the hours and days after SCI, causing neurochemical alterations resulting in behavioral and functional impairments. Neurochemical alterations in SCI include induction of increase in excitatory amino acids, elevation in calcium influx, stimulation of calcium-dependent enzymes, generation of ROS, release of cytokines leading to neuroinflammation (Fig. 6.10). These neurochemical alterations not only affect neuronal activities and glial cell reaction associated with astrocytic activation, and demyelination involving oligodendrocytes, but also modulate leukocyte infiltration and activation of macrophages and vascular endothelial cells (Bramlett and Dietrich 2004; Farooqui 2010). Among nonneuronal cells following SCI, macrophages are present at the injury site in large numbers and for the longer duration.

Due to its antioxidant and anti-inflammatory properties, curcumin not only inhibits oxidative stress, neuroinflammation, and apoptotic cell death in SCI, but also quenches astrocyte activation, and significantly improves neurologic deficit, restores cellular homeostasis, and rebalances redox equilibrium around the injury site 7 days after spinal cord hemisection (Lin et al. 2011a). Thus, by downregulating above neurochemical processes along with GFAP expression, curcumin attenuates astrocyte reactivation, which may be beneficial for neuronal survival (Lin et al. 2011a, b). On one hand at the molecular level, curcumin also inhibits the injury-mediated activation of NF- κ B and induces the expression of HO-1 via the activation of Nrf2/antioxidant responsive element (ARE) pathway (Farooqui 2010). On the other hand, curcumin also increases tissue levels of glutathione and glutathione peroxidase and catalase, which may be beneficial for neuronal survival (Cemil et al. 2010). The activation of Nrf2 target genes, and particularly HO-1, in astrocytes and neurons produces strong protective effects against neuroinflammation, oxidative stress, and apoptotic cell death. In the

spinal cord HO-1 system is very active, and its induction by curcumin may play a crucial role in the survival of neuronal cells surrounding the injury site (Scapagnini et al. 2011).

6.4.9 Curcumin and Epilepsy

Epilepsy is a complex and heterogeneous neurological disorder characterized by the periodic and unpredictable occurrence of seizures, which are sudden bursts of electrical energy produced by the brain (Jacobs et al. 2009). Epileptic seizures are transient signs and/or symptoms of abnormal, excessive, or hypersynchronous neuronal activity in the brain. Epilepsy is caused by complex temporal and spatial abnormalities in neural network structure and activity mediated by posttranslational modifications of proteins, activation of immediate early genes (IEGs), and other alterations in profiles of gene expression and function (e.g., GABA_A receptor subunit, CREB, JAK-STAT, BDNF, and EGR3) that eventually lead to deregulation of neural circuits with a predisposition for synchronous electrical activity (Rakhade and Jensen 2009). At the molecular level, major epigenetic mechanisms include DNA methylation, histone code modifications and chromatin remodeling, the deployment and actions of noncoding RNAs (ncRNAs), and RNA editing along with alterations in BDNF and CREB, which is an important transcriptional activator that has been implicated in modulating the differential expression of GABA_A receptor subunits in the hippocampus (Qureshi and Mehler 2010). GABA receptor subunits are closely associated with epileptogenesis. It is suggested that GABA receptors are predominantly depolarized, whereas AMPA and NMDA receptors display hyperexcitability and have different subunit compositions than in the adult (resulting in enhanced excitability) (Silverstein and Jensen 2007).

Curcumin possesses anticonvulsant properties. Thus, acute administration of curcumin in pentylenetetrazole (PTZ)-induced kindling in mice results in protective activity against kindling in mice (Agarwal et al. 2011; Sharma et al. 2010b). Elevated activities of the antioxidant enzymes (catalase and glutathione S-transferase) and malondialdehyde (MDA) in the cerebrum and cerebellum of epileptic rats induced by PTZ are markedly decreased. Although the exact molecular mechanism associated with beneficial effects of curcumin is not known, it is suggested that curcumin may produce beneficial effects through multiple mechanisms. Thus, it is well known that curcumin significantly decreases lipid peroxidation and increases reduced glutathione levels, superoxide dismutase, and catalase activities in lead-induced neurotoxicity in rats (Shukla et al. 2003; Wu et al. 2006). Hence it is an effective antioxidant which may be responsible for its anticonvulsive activity. Second, the role of Brain Derived Neurotrophic Factor (BDNF) in epileptogenesis is slowly becoming apparent. BDNF exerts a modulatory effect on neuronal excitability in hippocampus. BDNF administration protects against hippocampal kindling with possible carry-over effect (Larmet et al. 1995; Reibel et al. 2000). As mentioned earlier, curcumin reverses TBI-mediated reduction in brain BDNF in rats. Thus the modulation of

BDNF in the brain regions may be responsible for observed anticonvulsant activity of curcumin.

6.4.10 Curcumin and Depression

Major depression is an etiologically severe neurological disorder characterized by irritable mood, decreased interest in pleasurable activities, significant weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feeling of worthlessness or excessive guilt, decreased concentrating power, and increase in suicidal tendencies (Caspi et al. 2003). The etiology of depression is largely unknown, although a joint contribution of genetic, environmental, social factors; decrease in brain serotonin; and elevation in cortisol secretion are widely acknowledged (Caspi et al. 2003; Dinan 2001). L-glutamic acid (glutamate, Glu) and γ -aminobutyric acid (GABA) are the principal excitatory and inhibitory neurotransmitters in the central nervous system (CNS), respectively. Increasing evidence suggests that alterations in both of these neurotransmitter systems along with alterations in biogenic amine (dopamine, norepinephrine, and serotonin) metabolism and levels may contribute to the pathophysiology of depression (Krystal et al. 2002; Cryan and Slattery 2010). Recent studies have led to the hypothesis that alterations in proinflammatory cytokines and production of corticotropin releasing hormone (CRF), adrenocorticotrophic hormone (ACTH), and cortisol may underlie serotonergic dysfunctions and cortisol hypersecretion (Krystal et al. 2002; Cryan and Slattery 2010) (Fig. 6.11). Accordingly neurochemical changes in depression include increase in levels of proinflammatory cytokines (TNF- α , IL-1 β , IL-6, and IFN γ), lower levels of $n-3$ polyunsaturated acids, increase in oxidative and nitrosative stress, and induction of the hypothalamic-pituitary-adrenal (HPA)-axis via stimulated release/production of CRF, adrenocorticotrophic hormone (ACTH) and cortisol; the induction of indoleamine-2,3-dioxygenase (IDO) with decrease in levels of tryptophan and serotonin and the consequent formation of tryptophan catabolites along the IDO-pathway (TRYCATs) (Ma et al. 2009).

In vitro exposure of cortical neurons to corticosterone reduces mRNA levels for three 5-HT receptor subtypes (5-HT1A, 5-HT2A, and 5-HT4), but corticosterone has no effect on mRNA of 5-HT1B, 5-HT2B, 5-HT2C, 5-HT6, and 5-HT7 receptors. Pretreatment of cortical cultures with curcumin not only reverses the effect of corticosterone on mRNA for the 5-HT1A and 5-HT4 receptors, but not for the 5-HT2A receptor, but also exerts a neuroprotective effect against corticosterone-induced neuronal death (Xu et al. 2011). The effect of curcumin can be partially blocked by either 5-HT1A receptor antagonist p-MPPI or 5-HT4 receptor antagonist RS 39604 alone. The simultaneous treatment of cortical cultures with the above antagonists completely reverses the effect of curcumin. In addition, curcumin also regulates corticosterone-mediated morphological changes such as increases in soma size, dendritic branching and dendritic spine density, as well as

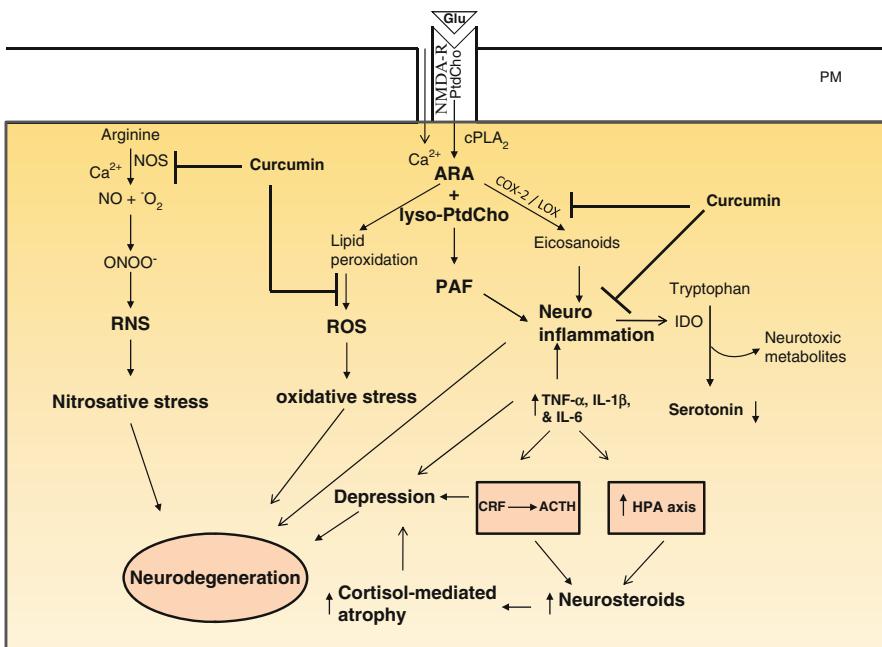


Fig. 6.11 Hypothetical diagram showing neurochemical changes in depression. *N*-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); cyclooxygenase (COX); lipoxygenase (LOX); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); tumor necrosis factor- α (TNF- α); interleukin-1 β (IL-1 β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); nitric oxide (NO); peroxynitrite (ONOO⁻); hypothalamic-pituitary-adrenal (HPA)-axis; corticotropin releasing hormone (CRF), adrenocorticotropic hormone (ACTH). Positive sign indicates stimulation

elevates synaptophysin expression in cortical neurons (Xu et al. 2011). p-MPPI and RS 39604 not only reverse the effect of curcumin-mediated change in neuronal morphology, but also inhibit the expression of synaptophysin in corticosterone-treated neurons. In addition, an increase in cyclic adenosine monophosphate (cAMP) level has been observed after curcumin treatment, which can be further prevented by RS 39604, but not by p-MPPI. Although the molecular mechanism associated with these processes is not fully understood, but it is proposed that modulation of 5-HT receptor-mediated cAMP-PKA-CREB signal pathway by curcumin may be closely associated with these processes (Xu et al. 2011).

Neuroinflammation also induces decrease in neurogenesis in depression, which is characterized by decrease in BDNF, neural cell adhesion molecule (NCAM), and FGF. It is proposed that increase in neuroinflammation may also induce neurodegeneration through elevation in levels of TRYCATs, oxidative and nitrosative stress, glucocorticoids, and some proinflammatory cytokines (Ma et al. 2009). Nonetheless,

it remains to be fully elucidated if these molecular abnormalities are the cause or consequence of depression (Cowen 2002; Maes et al. 2009).

Curcumin acts as antidepressant in animal models of depression. Studies on the effect of curcumin on glutamate-mediated toxicity indicate that curcumin modulates the expression of BDNF, which is closely associated with the etiology of depression and its treatment (Wang et al. 2008). Exposure of rat cortical neurons with glutamate for 24 h not only produces a significant decrease in BDNF level, but also decreases cell viability and enhances cell apoptosis. Pretreatment of neurons with curcumin reverses the BDNF expression and cell viability in a dose- and time-dependent manner. However, K252a, a Trk receptor inhibitor, which is known to inhibit the activity of BDNF, blocks the survival-promoting effect of curcumin (Wang et al. 2008). In addition, the upregulation of BDNF levels by curcumin is also suppressed by K252a, suggesting that the neuroprotective effect of curcumin might be mediated via BDNF/TrkB signaling pathway. Collective evidence suggests that curcumin mediates its antidepressant activity not only by modulating the release of serotonin and dopamine, but also by enhancing the level of neurotrophic factors such as BDNF (Wang et al. 2008). In addition, curcumin may also act by inhibiting the monoamine oxidase (MOA) activity and modulating the release of serotonin and dopamine (Kulkarni et al. 2008, 2009). Moreover, it is also reported that curcumin enhances neurogenesis, notably in the frontal cortex and hippocampal regions of the brain. The use of curcumin in clinics for the treatment of major depression is limited due to its poor bioavailability through gastrointestinal tract, but the use of phospholipid curcumin complex may increase the bioavailability (Kulkarni et al. 2008, 2009). Curcumin also reverses olfactory bulbectomy-induced major depression (Xu et al. 2005a). Olfactory bulbectomized animals display low levels of serotonin and noradrenaline and high levels of 5-hydroxyindoleacetic acid and 4-dihydroxyphenylacetic acid. These changes can be completely reversed by administration of curcumin (Xu et al. 2005a).

6.4.11 Curcumin and Tardive Dyskinesia

Tardive dyskinesia (TD) is a motor disorder of the orofacial region resulting from chronic neuroleptic treatment (Bishnoi et al. 2008). TD is characterized by repetitive involuntary movement in the orofacial regions and sometimes limb and trunk musculature. Features of the disorder may include grimacing, tongue protrusion, lip smacking, and rapid eye blinking. Rapid movements of the arms, legs, and trunk may also occur. Involuntary movements of the fingers may appear as though the patient is playing an invisible guitar or piano. There is circumstantial evidence that orofacial dyskinesia in humans may be caused by hyperfunctioning mesolimbic-pallidal circuitry, in which the mesolimbic region occupies a central role, in contrast to typical Parkinson-like symptoms, which involve hypofunction in the nigrostriatal-nigral circuitry (Koshikawa et al. 2011). It is reported that in rats dopaminergic, cholinergic, γ -aminobutyric acid (GABA)ergic, and glutamatergic systems may be

associated with rhythmical jaw movements, controlling the dorsal and ventral part of the striatum, the shell and core of the nucleus accumbens (Koshikawa et al. 2011). There is no standardized treatment available for the treatment of TD. Although neuroleptic drugs can be used for the treatment of TD, neuroleptics produce their own side effects.

Chronic curcumin treatment (25 and 50 mg/kg) dose dependently inhibits the increase of haloperidol-induced vacuous chewing movements (VCMs), tongue protrusions, and facial jerking (Bishnoi et al. 2008). Moreover, chronic administration of haloperidol is accompanied by increase in lipid peroxidation, decrease in glutathione levels, reduction in superoxide dismutase and catalase activities in different regions of rat brain, which can be reversed by pretreatment with curcumin (Bishnoi et al. 2008). Additionally, haloperidol-mediated decrease in dopamine, norepinephrine, and serotonin levels in cortical and subcortical regions (including striatum) homogenates can be prevented by pretreatment with curcumin (Bishnoi et al. 2008). Based on these results, it is hypothesized that curcumin can be a useful drug therapy for the treatment of TD.

6.4.12 Curcumin and Kainic Acid Neurotoxicity

Kainic acid (KA) is a cyclic and nondegradable analog of glutamate. It is 30–100-fold more potent than glutamate as a neuronal excitant. Intracerebroventricular injections of KA produce dense staining of cPLA₂ and 4-HNE at 1 day postinjection, before there is any histological evidence of neurodegeneration (Farooqui et al. 2008). Degenerating neurons in CA1 and CA3 regions of hippocampus of KA-injected rats are observed at 3 days and 1 week after injection. The increased immunoreactivity remains confined to a cluster of neurons at the edge of the degenerating CA1 and CA3 regions at 2 and 3 weeks after KA injections (Farooqui et al. 2008). Administration of curcumin 30 min before KA not only reduces KA-mediated seizures, but also decreases levels of MDA. Curcumin also enhances glutathione levels, which are decreased in KA-induced neurotoxicity (Gupta et al. 2009). These observations suggest that in addition to antioxidant effects, curcumin also possesses antiepileptic potentials.

6.5 Side Effects of Curcumin

Food and drug administration has declared that curcumin is a safe drug (supplement) that exhibits a wide variety of pharmacological activities in animals and humans. Still, it is proposed that curcumin may alter the effectiveness of radiotherapy and chemotherapy in cancer treatment (López-Lázaro 2008). However, National Toxicology Program (NTP) has conducted the short term as well as long-term toxicity of turmeric oleoresin (79–85 % curcumin) in F344/N rats and B6C3F1 mice.

Animals are fed with diets containing the turmeric extract at different concentrations (1,000, 5,000, 10,000, 25,000, or 50,000 ppm that delivers daily doses of 50, 250, 480, 1,300, or 2,600 mg/kg body weight) for periods of 13 weeks or 2 years. In 13-week study, no deaths are attributed to curcumin and only toxicity noted was relative increase in liver weight, stained fur, discolored faces, and hyperplasia of the mucosal epithelium in the cecum and colon of rats that received 50,000 ppm. No sign of carcinogenic lesions is observed (Aggarwal and Harikumar 2009). In 2 years study, the turmeric administration does not have any effect on the food consumption when compared to controls and no mortality is seen in both male and female rats. In 50,000 ppm group, however, rats developed ulcers, chronic active inflammation, hyperplasia of the cecum, and forestomach, increased incidences of clitoral gland adenomas, the development of hepatocellular adenoma and intestinal carcinoma (NTP 1993; Aggarwal and Harikumar 2009).

Curcumin has been reported to bind iron (Baum and Ng 2004). This may cause iron deficiency in people with low iron stores, cancer, or other chronic diseases (Jiao et al. 2009). Curcumin also has blood thinner properties, thus people who are undergoing surgery should avoid it. High (micromolar) concentrations of curcumin have been reported to induce oxidative stress that may mediate its ability to trigger apoptosis in cancer cells (Salvioli et al. 2007). Curcumin attenuates hepcidin biosynthesis, a regulatory protein involved in iron transport (Jiao et al. 2009).

6.6 Conclusion

Curcumin, the active compound of the rhizome *Curcuma longa*, has anti-inflammatory, antioxidant, and antiproliferative activities. It can cross the BBB and reach the brain, but its bioavailability is very low, since the drug is rapidly metabolized by conjugation in visceral tissues. Curcumin is a highly pleiotropic molecule capable of interacting and modulating a multitude of molecular targets that include transcription factors (NF- κ B, AP-1, STAT3, PPAR γ ; HIF-1 α ; Nrf2, β -catenin, and E2F), inflammatory cytokines (TNF- α , IL-6, IL-1, IL-8, and monocyte inflammatory protein (MIP)-1 α), and enzymes (PKA, PKC, JAK2, JNK, Akt, iNOS, COX-2, 5-LOX, MMP-9, GST, and HO-1). Additionally, curcumin is an inhibitor of proliferation targeting cell-cycle regulatory proteins, including cyclins E and D1 and the CDKs inhibitor targeting p21. Curcumin not only has antioxidant activities, but also inhibits prostaglandin, leukotrienes, and thromboxanes synthesis. Curcumin boosts expression of cytoprotective enzymes such as HO-1 in neural cells, possibly via the induction of the transcription factor Nrf2. This may partly explain its effectiveness against a variety of neurological disorders. However, it is important to distinguish between the primary molecular targets of curcumin and the events caused as downstream effects. For example, curcumin modulates expression and function of COX-2 and 5-LOX, which generate eicosanoids. Eicosanoids are important proinflammatory lipid mediators, which in addition to inflammation also modulate transcriptional and posttranslational activities in neural cells (Farooqui et al. 2006; Farooqui and

Horrocks 2007). Curcumin interacts with more than 30 proteins; of these, many are directly relevant to the signal transduction processes associated with neural cell function. In addition, curcumin binds with metal ions such as Zn²⁺, Cu²⁺, and Fe²⁺, which may also affect neuronal function. With the advances in molecular biology and combinatorial chemistry during the past few years, it is hoped that problems of curcumin absorption, biodistribution, and bioavailability will be solved and curcumin can then be used for clinical trials in human subjects.

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Chapter 7

Beneficial Effects of Resveratrol on Neurological Disorders

7.1 Introduction

Resveratrol (3,4',5-trihydroxystilbene) is a natural compound found in grapes, mulberries, peanuts, and other plants or food products, including raspberries, blueberries, Scots pine, Eastern white pine, and knotweed. It belongs to a class of polyphenolic compounds called stilbenes (Saiko et al. 2008). In plants, stilbenes are produced by the enzyme stilbene synthase in response to environmental stress such as vicissitudes in climate, exposure to ozone, sunlight and heavy metals, and infection by pathogenic microorganisms. Resveratrol is a fat-soluble stilbene that occurs in a trans and a cis configuration (Fig. 7.1). Its trans to cis isomerization is facilitated by exposure to UV radiations and the trans isomer is more stable than cis form. The glycosylated forms of resveratrol also occur in trans and cis configuration. Resveratrol-3-*O*-β-glucoside (5,4'-Dihydroxystilbene-3-*O*-β-D-glucopyranoside) is called piceid (Romero-Perez et al. 1999) (Fig. 7.1). It is probably the most abundant form of resveratrol in nature. Resveratrol derivatives differ from the parent resveratrol not only in their antioxidant and biological activities, but also in their water solubility and bioavailability (Stivala et al. 2001).

Resveratrol produces several beneficial effects in humans. It promotes antiaging, anticarcinogenic, cardioprotective, and cerebroprotective activities, which are attributed to its antioxidant, anti-inflammatory, and gene modulating properties. It also acts as an analgesic. Due to its structural similarity with diethylstilbestrol (a synthetic estrogen), resveratrol produces oestrogenic effects by binding to estrogen receptors and evoking neurochemical effects parallel to those exerted by endogenous estrogen. These oestrogenic properties may also play a role in the beneficial cardiovascular effects. Additionally, resveratrol not only inhibits platelet aggregation and lipid peroxidation, but also blocks eicosanoid synthesis, modulates lipoprotein metabolism, and exhibits vasorelaxing and anti-inflammatory activities (Das and Das 2007) (Fig. 7.2). Collective evidence suggests that resveratrol functions as an antioxidant, vasorelaxing and anti-inflammatory agent by hampering

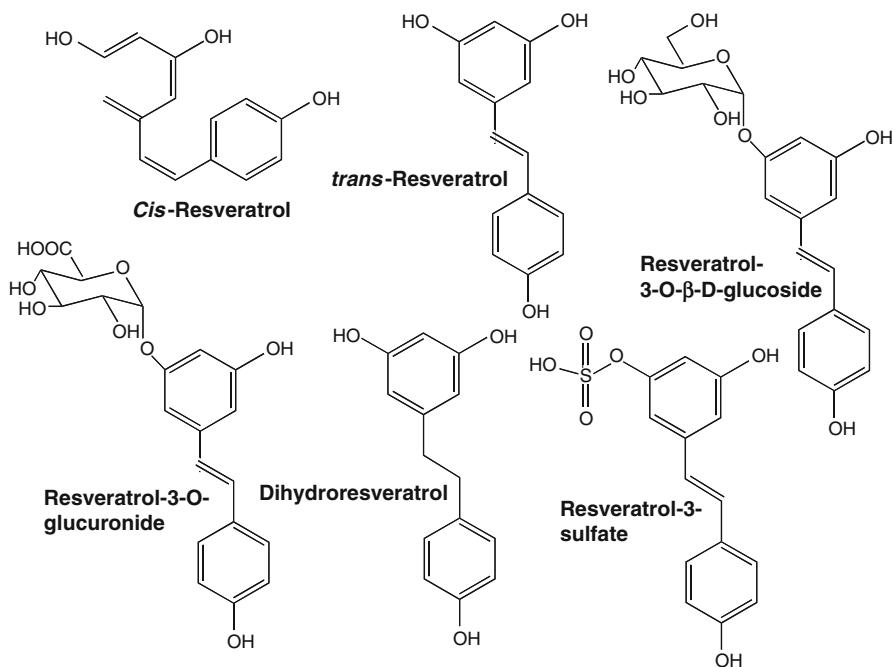


Fig. 7.1 Chemical structures of resveratrol and its metabolites

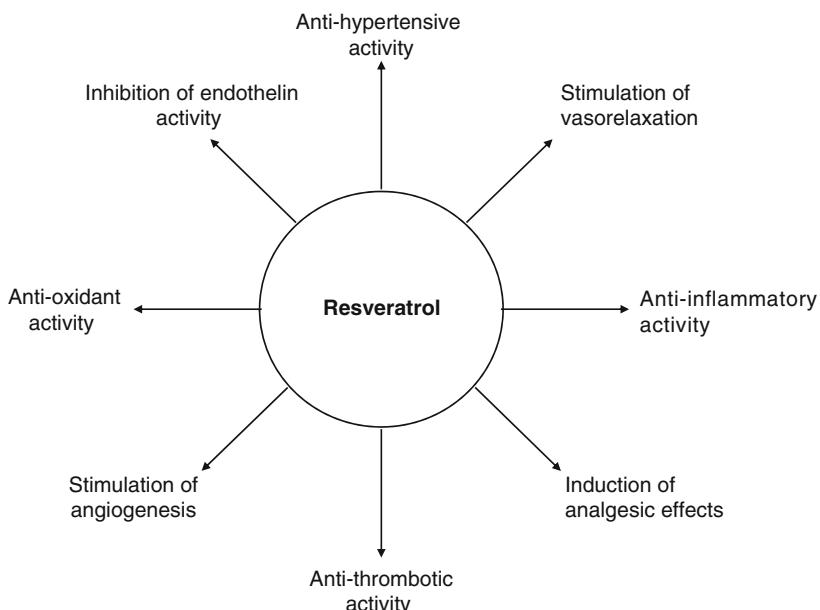


Fig. 7.2 Biological activities of resveratrol

Table 7.1 Effect of resveratrol on enzyme activities

Enzyme	Effect	Reference
Topoisomerase	Inhibition	Jo et al. (2006)
Aromatase	Inhibition	Neves et al. (2007)
Ribonucleotide reductase	Inhibition	Fontecave et al. (1998)
DNA polymerase	Inhibition	Stivala et al. (2001)
PtdIns 3 kinase	Inhibition	Harikumar and Aggarwal (2008)
Extracellular-signal-regulated protein kinases (ERKs)	Activation	She et al. (2001)
c-Jun N-terminal kinases (JNK)	Inhibition	Harikumar and Aggarwal (2008)
Akt (serine/threonine protein kinase)	Inhibition	Aziz et al. (2006)
AMP-activated protein kinase	Activation	Vingtdeux et al. (2010)
Cathepsin D		Lanzilli et al. (2006)
Matrix metalloproteinase	Inhibition	Gagliano et al. (2005)
Catalase	Activation	Harikumar and Aggarwal (2008)
Superoxide dismutase	Activation	Robb et al. (2008)
Heme oxygenase-1	Activation	Zhuang et al. (2003), Li et al. (2009)

free radical generation and scavenging free radicals, but also by inhibiting the release of proinflammatory cytokines.

In addition, resveratrol also induces antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase, and heme-oxygenase-1. The anti-inflammatory effects of resveratrol are mediated through the downregulation of various biomarkers such as TNF- α , COX-2, iNOS, interferon- γ , and various interleukins. Resveratrol interacts with numerous proteins and cell-signaling molecules (Harikumar and Aggarwal 2008). According to these authors, molecular targets for resveratrol can be categorized into those that are modulated by direct physical interactions with resveratrol and others, which are modulated indirectly through changes in the expression levels of various transcription factors, genes, enzymes, and cytokines. Resveratrol interacts with multidrug resistance protein, topoisomerase II, aromatase, DNA polymerase, estrogen receptors, tubulin, and F1-ATPase (Harikumar and Aggarwal 2008) (Table 7.1). It not only activates various transcription factors (NF- κ B, STAT1 and 3, HIF-1 α , β -catenin, and PPAR- γ) and suppresses the expression of antiapoptotic gene products (e.g., Bcl-2, Bcl-X_L, XIAP, and survivin), but also inhibits protein kinases (src, PtdIns 3K, JNK, and Akt) and induces antioxidant enzymes (catalase, superoxide dismutase, and heme oxygenase-1) (Fig. 7.3). Resveratrol also inhibits elevation in mRNA for interferon-1 (IFN-1), suppresses the expression of inflammatory biomarkers (TNF- α , COX-2, iNOS, and C reactive protein), blocks the translocation or activation of interferon regulatory factor (IRF-3), c-Jun, a subunit of AP-1, inhibits the expression of angiogenic and metastatic gene products (MMPs, VEGF, cathepsin D, and ICAM-1), and modulate cell cycle regulatory genes (p53, Rb, PTEN, cyclins, and CDKs) (Harikumar and Aggarwal 2008; Kim et al. 2011). Accumulating evidence supports the view that resveratrol, a

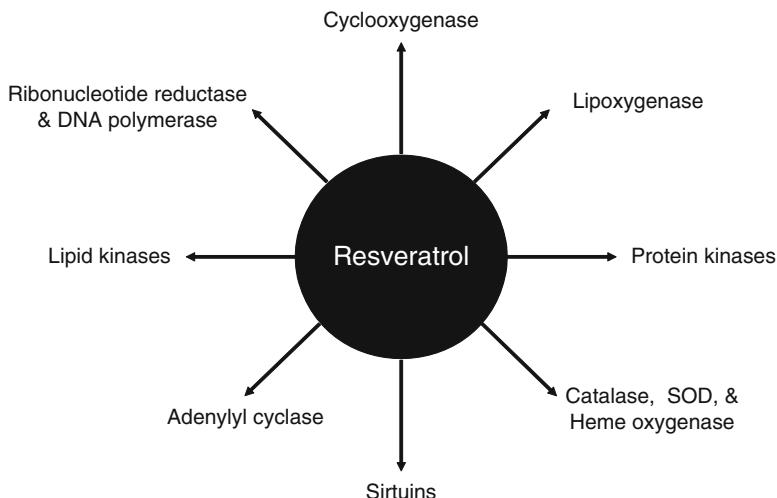


Fig. 7.3 Modulation of enzymes by resveratrol

naturally occurring polyphenolic compound, mediates pleiotropic health benefits through its antioxidant, anti-inflammatory, antiaging, cardioprotective, neuroprotective, anticancer, and chemopreventive activities (Brisdelli et al. 2009) (Fig. 7.2).

7.2 Metabolism and Bioavailability of Resveratrol in the Brain

Although trans-resveratrol is rapidly absorbed and distributed through the blood stream to a number of organs in various animal species including humans, its bioavailability is relatively low due to its rapid metabolism and elimination in the urine (Walle et al. 2004; Wenzel and Somoza 2005). Thus, its oral absorption in humans is about 75 % and is thought to occur mainly by transepithelial diffusion. Its extensive metabolism in the intestine and liver results in an oral bioavailability, which is less than 1 % (Walle 2011). Resveratrol is well tolerated and metabolized by animals and humans through glucuronidation or sulfation reactions in the intestine/liver (Goldberg et al. 2003). Presence of reduced dihydroresveratrol conjugates has also been reported. The major glucuronidation derivatives of resveratrol are trans-resveratrol 3-*O*-glucuronide, trans-resveratrol-*O*-glucuronide, whereas sulfated derivative is trans-resveratrol-3-*O*-sulfate (Yu et al. 2002) (Fig. 7.1). Kinetic analysis of resveratrol metabolism indicates that glucuronidation is favored over sulfation, with similar rates of reaction in the liver. In vivo studies indicate that free trans-resveratrol in plasma is very sparse and short lived.

Information about the bioavailability of resveratrol in various tissues of animals and humans is important. Most studies on this polyphenol have been performed in cultured cells exposed to unmetabolized resveratrol at concentrations that are often

10–100 times greater than peak concentrations observed in human plasma after oral consumption (Gescher and Steward 2003). During *in vivo* studies, visceral tissues are exposed primarily to resveratrol metabolites, such as resveratrol glucuronide or sulfate (Walle et al. 2004) and very little information is available on metabolic effects of resveratrol glucuronide or sulfate on animal and human tissues. In serum, resveratrol interacts with proteins (lipoproteins and albumin) (Belguendouz et al. 1998). Albumin appears to be one of the plasmatic carriers transporting resveratrol in blood circulation in order to deliver the compound at the cell surface before cell membrane uptake and finally allowing its intracellular biological effect (Jannin et al. 2004).

Because of rapid metabolism of resveratrol and its elimination in urine, some information is available on its bioavailability in visceral organs (liver and kidney), but little is known about the bioavailability of resveratrol to the brain. Intravenous administration of 15 mg/kg in rats after 90 min results in wide distribution of resveratrol in various tissues. The highest concentrations (nmol/g tissue) are found in kidney (resveratrol: 1.45 ± 0.35 ; glucuronide: 2.91 ± 0.19), and the lowest occur in the brain (resveratrol: 0.17 ± 0.04 ; glucuronide: not detected; sulfate: 0.04 ± 0.01) (Juan et al. 2010).

Attempts have been made to improve its bioavailability in the brain. Trans-resveratrol can be loaded into lipid-core nanocapsules and analyzed for particle size, polydispersity, and zeta potential. The nanocapsule distribution in brain tissue has been evaluated by intraperitoneal and gavage routes in healthy rats. Lipid-core nanocapsules result in high entrapment of trans-resveratrol and display a higher trans-resveratrol concentration in the brain, liver, and kidneys after daily i.p. or gavage administration than observed with the free trans-resveratrol (Frozza et al. 2010).

7.3 Biological Activities of Resveratrol

Resveratrol possesses many biological activities. It not only produces positive effects on longevity (age) and lipid levels, but has beneficial effects against certain cancers and viral infections. Resveratrol produces apoptotic cell death by upregulating the expression of Bax, Bak, PUMA, Noxa, Bim, p53, TRAIL, TRAIL-R1/DR4, and TRAIL-R2/DR5 and simultaneously downregulating the expression of Bcl-2, Bcl-XL, Mcl-1, and surviving (Shankar et al. 2007). These proteins exert their effect mainly at the level of mitochondria. Furthermore, resveratrol facilitates the translocation of p53 and Bax to mitochondria where these proteins may interact with other Bcl-2 family members to cause permeabilization of outer mitochondrial membrane and release of mitochondrial proteins, leading to caspase activation and apoptosis. In addition, resveratrol regulates G1 and G1/S phases of cell cycle by modulating the expression of CDK inhibitors p21/WAF1/CIP1 and p27/KIP1. It reduces inflammation not only by inhibiting cyclooxygenase-2 activity, blocking synthesis of prostaglandin, and downregulating nuclear factor- κ B (NF- κ B) activity.

Resveratrol is an antioxidant that not only possesses anticarcinogenic properties, antimicrobial, and antiviral effects, but also has the ability to reverse dyslipidemia and obesity. Its ability to attenuate hyperglycemia and hyperinsulinemia promotes,

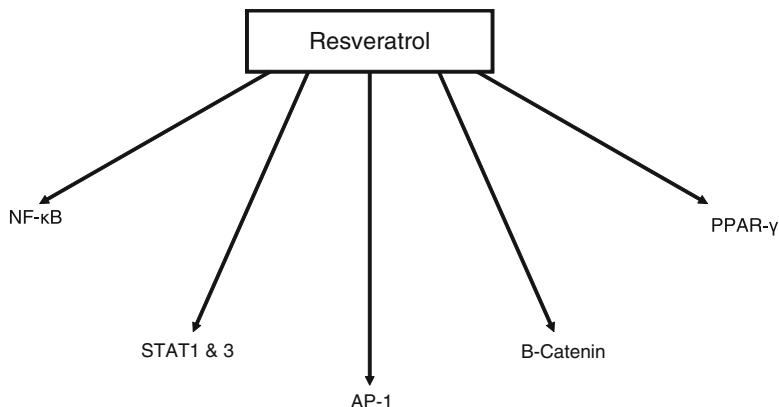


Fig. 7.4 Modulation of transcription factors by resveratrol

protects, and maintains endothelial cell function (Bertelli and Das 2009). Thus, it is becoming increasingly evident that modulation of cell signaling pathway may explain multiple and diverse bioactivities of resveratrol (Shankar et al. 2007).

7.3.1 *Effect of Resveratrol on Transcription Factors*

Transcription factors are proteins involved in the regulation of gene expression that bind to the promoter elements upstream of genes and either promote or block transcription. Through this process they modulate gene expression. Transcription factors consist of two essential functional domains: a DNA-binding domain and an activator domain. The DNA-binding domain consists of amino acids that recognize specific DNA bases near the start of transcription. Transcription factors not only interact with RNA polymerase, but also bind to other transcription factors and cis-acting DNA sequences. Examples of transcription factors include NF-κB, AP-1, and members of STAT family. These transcription factors link nutrient availability, energy metabolism, and stress signaling to changes in transcriptional profiles, thus affecting the pathophysiology of multiple age-associated diseases (Longo and Kennedy 2006). As stated earlier, by interacting with NF-κB, STAT1 and 3, HIF-1 α , β -catenin, and PPAR- γ (Fig. 7.4), resveratrol modulates several signal transduction pathways associated with modulation of oxidative stress and neuroinflammation.

7.3.1.1 Resveratrol and NF-κB in the Brain

NF-κB is a family of redox sensitive transcription factors, which is composed of five DNA binding proteins sharing the N-terminal Rel-homology domain (RHD): NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), cRel, and RelB that recognize

a common sequence motif. NF- κ B is found in neuronal and glial cells, and is involved in activation and modulation of a large number of genes in response to ischemic injury, immune responses, neuroinflammation, macrophage infiltration factors, cell adhesion molecules, cell survival, and other stressful situations requiring rapid reprogramming of gene expression. Five different proteins of NF- κ B factor, namely p50, RelA/p65, c-Rel, RelB, and p52, can combine differently to form active dimers in response to external stimuli. RelA is activated by neurotoxic agents while c-Rel produces neuroprotective effects (Sarnico et al. 2009). Aberrant and sustained NF- κ B activity is closely associated with neurotraumatic (stroke, spinal cord injury, and traumatic brain injury), neurodegenerative (Alzheimer disease, Parkinson disease, and Huntington disease), and neuropsychiatric diseases (depression) (Farooqui 2010). In its inactive state, NF- κ B is located in the cytoplasm, bound by the family of inhibitor proteins called I κ Bs. Following neural cell injury, I κ B kinase (IKK) phosphorylates I κ B α , leading to ubiquitination-dependent degradation of I κ B α . Dissociation of I κ B α from NF- κ B facilitates nuclear translocation of the activated free NF- κ B dimer, where it binds to the specific *cis*-acting sequence in the promoter of target genes, such as sPLA₂, COX-2, MMP, NADPH oxidase, and inducible nitric oxide synthase. Interactions between NF- κ B and DNA motif also modulate the expression of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), which are closely associated with neuronal cell death in neurotraumatic, neurodegenerative, and neuropsychiatric diseases (Hang et al. 2006).

Resveratrol not only suppresses IKK phosphorylation, but also blocks the subsequent degradation of I κ B α , thereby inhibiting the activation and translocation of NF- κ B to the nucleus. Resveratrol also downregulates the expression of NF- κ B-regulated genes (proliferative and antiapoptotic gene products), including interleukin-6, Bcl-2, Bcl-xL, XIAP, c-IAP-2, XIAP, survivin, vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and TNF receptor-associated factor 2 (TRAF2). Collective evidence suggests that retardation of NF- κ B mobilization, which controls the expression of inducible NO synthase (iNOS) and COX, downregulation of proinflammatory cytokines, and upregulation of VEGF, and MMP-9 may be the major targets of resveratrol.

7.3.1.2 Resveratrol and AP-1 in the Brain

The transcription factor AP-1 consists of a number of different dimeric combinations of the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, and Fra-2) families and Jun dimerization partners (JDP1 and JDP2) and activating transcription factor (ATF2, LRF1/ATF3, and B-ATF) subfamilies (Kundu et al. 2006). AP-1 is involved in the control of cell proliferation, differentiation, and death via the regulation of multiple gene families. Neurotraumatic and neurodegenerative injuries are accompanied by significant changes in the expression of AP-1. For example, marked increases are observed in c-fos and c-jun, junB, jun D Krox-24 mRNAs in a rat model of ischemia, spinal cord trauma, and traumatic brain injury (Kiessling et al. 1993;

An et al. 1993; Rafati et al. 2008; Hang et al. 2006). Resveratrol is known to inhibit c-Fos mRNA expression and suppression of AP-1 DNA binding affinity (Kundu et al. 2006). Downregulation of c-Jun and suppression of AP-1 activity by resveratrol involves inhibition of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK)1>ERK1/2 signaling.

7.3.1.3 Resveratrol and STAT in the Brain

Signal transducers and activators of transcription (STATs), a group of novel transcription factors that orchestrate the downstream events propagated by cytokine/growth factor interaction with their cognate receptors (Rane and Reddy 2002). These factors are activated by the Janus Kinase. The dysregulation of this pathway is associated with angiogenesis and immunosuppression. Unphosphorylated STAT proteins are monomers, which are translocated from cytoplasm to the nucleus, where in response to specific stimuli they are phosphorylated and bind to the promoter region of target genes and are thereby involved in regulating the transcription of target genes.

Signal transducers and activators of transcription (STATs) are latent cytoplasmic transcription factors that can be activated by a variety of tyrosine kinases in response to many different cytokines and growth factors. Accumulation of tyrosine phosphorylated STAT dimers in the nucleus is followed by DNA binding, activation of target gene transcription, dephosphorylation, and return to the cytoplasm (Levy and Darnell 2002). Resveratrol not only inhibits nitric oxide production and IFN- γ -mediated transcriptional activity of STAT-1 in macrophages, but also induces IFN- γ -mediated Tyr(701) or Ser(727) phosphorylation of STAT-1 and STAT-3.

Phosphorylation of STAT-1 and STAT-3 induces its dimerization and translocation from the cytoplasm into the nucleus. Staining of resveratrol-treated and untreated cells with anti-STAT-1 and STAT-3 antibody substantially blocks the translocation of STAT3 from the cytoplasm to the nucleus suggesting that resveratrol interacts with STAT transcription factors (Bromberg et al. 1999). Resveratrol also retards IFN- γ -mediated activation of Janus kinase-2 (JAK-2). Taken together, these studies support the view that resveratrol acts by blocking JAK/STAT-1 pathway that controls inflammatory responses in IFN- γ -activated macrophages (Chung et al. 2010).

7.3.2 Regulation of Neuroinflammation by Resveratrol

Neuroinflammation is a protective mechanism that not only isolates the injured brain tissue from uninjured area but also destroys affected cells and repairs the extracellular matrix (Farooqui et al. 2007). Without a strong neuroinflammatory response, brain would be prone to neurotraumatic and neurodegenerative diseases. Glial cells mediate the endogenous immune system within the microenvironment in

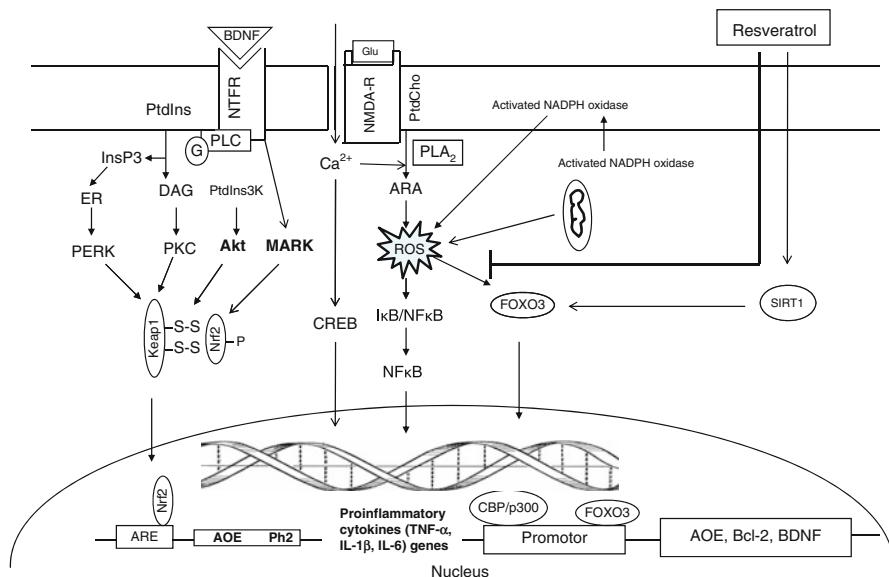


Fig. 7.5 Hypothetical diagram showing signal transduction processes associated with modulation of gene expression by resveratrol. N-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); phospholipase C (PLC); phosphatidylinositol (PtdIns); inositol 1,4,5-trisphosphate (InsP₃); arachidonic acid (ARA); diacylglycerol (DAG); protein kinase C (PKC); endoplasmic reticulum (ER); nuclear factor E2-related factor 2 (Nrf2); kelch-like ECH-associated protein 1 (Keap1); serine/threonine protein kinase (Akt); mitogen activated protein kinase (MARK); cAMP Response Element Binding (CREB); antioxidant responsive element (ARE); CREB binding protein (CBP/p300); Forkhead box O3 (FOXO3); sirtuin1 (SIRT1); reactive oxygen species (ROS); nuclear factor-κB (NF-κB); nuclear factor-κB-response element (NF-κB-RE); inhibitory subunit of NF-κB (I-κB); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); and interleukin-6 (IL-6); and \neg (blocked arrow) represents inhibition

the brain (Kreutzberg 1996) and their activation is the hallmark of neuroinflammation in the brain (Orr et al. 2002). Activated microglial cells produce inflammatory molecules such as proinflammatory cytokines, growth factors, and complement proteins (McGeer and McGeer 1995; Chen et al. 1996). These proinflammatory mediators in turn activate other cells (endothelial cells, PMN, and platelets) to produce additional signaling molecules that further activate microglia in a positive feedback loop to perpetuate and amplify the inflammatory signaling cascade (Floyd 1999; Farooqui et al. 2007). Chronic neuroinflammation is closely associated with pathogenesis of neurotraumatic, neurodegenerative, and neuropsychiatric diseases. In glial and neuronal cells, eicosanoids, the oxidized products of arachidonic acid (ARA) by cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) and proinflammatory cytokines, such as TNF-α, IL-1α, IL-1β, and IL-6 initiate and maintain neuroinflammation (Fig. 7.5). There is growing evidence indicating that neuroinflammation plays a crucial role in the pathophysiology of neurological disorders. During inflammatory process in the brain, microglial cells create an environment that

enhances oxidative stress and the production of various proinflammatory factors, which consequently cause neuronal dysfunction and neurodegeneration.

Resveratrol retards neuroinflammation by downregulating NF-κB activity. This process not only inhibits the release of proinflammatory cytokines, but also modifies eicosanoid synthesis, inhibits activated immune cells, and blocks COX-2 and 5-LOX activities (Farooqui 2010). In addition, neurotraumatic, neurodegenerative, and neuropsychiatric diseases are accompanied by increase in generation of reactive oxygen species (ROS), which contribute to the pathogenesis of above neurological disorders by enhancing neuroinflammation through activation and phosphorylation of stress kinases (JNK, ERK, p38) and redox-sensitive transcription factors in such a way that inflammation develops slowly and remains below the threshold of pain perception. As a result, the immune system continues to attack the brain tissue and chronic inflammation lingers for years, ultimately reaching the threshold of detection (Wood 1998). Activation of NF-κB is also mediated via the activation of intrinsic histone acetyltransferase activity (Rahman et al. 2004). Acetylation by histone acetyltransferase of specific lysine residues on the N-terminal tail of core histones results in uncoiling of the DNA and increased accessibility to transcription factor binding. On the other hand deacetylation by histone deacetylase represses gene transcription by promoting DNA winding, thereby limiting access to transcription factors (Rahman et al. 2004). Accumulating evidence suggests that neuroinflammation plays a major role in the pathophysiology of neurotraumatic, neurodegenerative, and neuropsychiatric diseases. Resveratrol inhibits neuroinflammatory responses not only through the inhibition of synthesis of various proinflammatory cytokines, but also through the modulation of eicosanoid synthesis and via the inhibition of transcription factors such as NF-κB and AP-1 (Farooqui 2010).

7.3.3 Regulation of Oxidative Stress by Resveratrol

Compared to the other tissues, brain has higher probability to be challenged by ROS, because it not only consumes more than 20 % of all the oxygen utilized by other organs during mitochondrial respiration, but also has high levels of polyunsaturated fatty acids in neural membrane phospholipid. The imbalance between intracellular ROS and antioxidant defense mechanisms results in oxidative stress, which damages the biological components (DNA, lipids, sugars, and proteins) of neural membranes. Neurons have reduced capacity to compensate for imbalance between intracellular ROS and antioxidant defense mechanisms. They undergo irreversible injury, which may contribute to the pathogenesis of neurotraumatic and neurodegenerative diseases (Farooqui 2010).

Resveratrol has significant antioxidant properties in a variety of in vitro and in vivo models of neurotraumatic diseases. It reduces damage caused by ischemia/reperfusion injury in rodent brain. Most of the neuroprotective actions of resveratrol are associated with its intrinsic radical scavenger properties. The treatment of HT22 cells with resveratrol alone or resveratrol plus glutamate not only leads to Akt

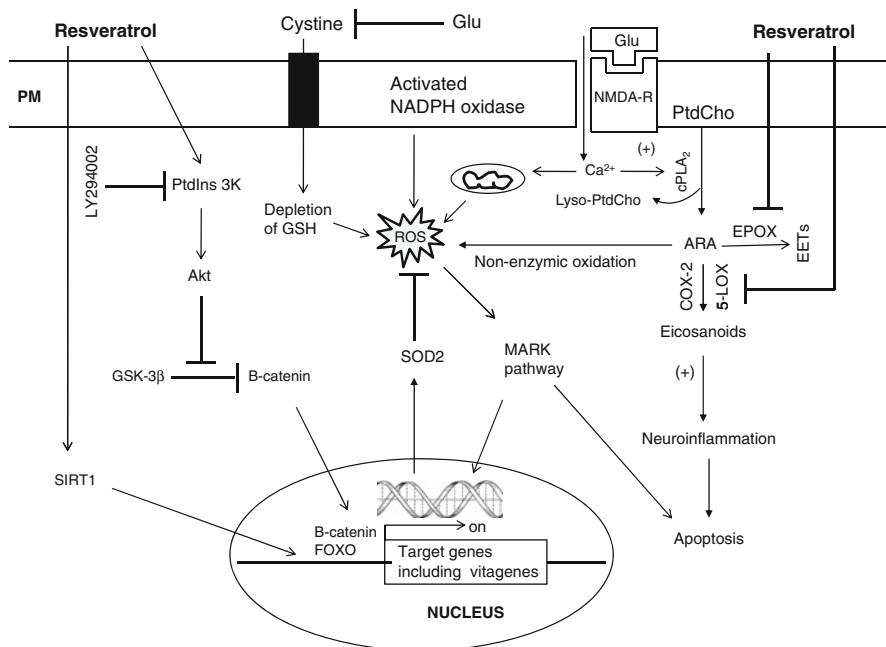


Fig. 7.6 Hypothetical diagram showing glutamate-mediated ROS generation and modulation of SIRT1, COX, LOX by resveratrol. *N*-Methyl-d-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (Lyo-PtdCho); cyclooxygenase-2 (COX-2); 5-lipoxygenase (5-LOX); epoxyenase (EPOX); arachidonic acid (ARA); serine/threonine protein kinase (Akt); mitogen activated protein kinase (MARK); cAMP Forkhead box O (FOXO); sirtuin1 (SIRT1); reactive oxygen species (ROS); reduced glutathione (GSH); phosphatidylinositol 3-kinase (PtdIns 3 kinase); and Glycogen synthase kinase 3 (GSK-3)

activation and GSK-3 β inactivation, but also results in β -catenin stabilization in a time-dependent manner and subsequently enhancement in expression of SOD2 protein, whereas treatment with glutamate alone produces no effect. This effect of resveratrol can be suppressed by the treatment with a PtsIns 3K inhibitor (LY294002) (Fig. 7.6) (Fukui et al. 2010).

Resveratrol also modulates ARE-directed Nrf-2, which upregulates heme oxygenase 1 (HO-1) and several phase II detoxifying and antioxidant enzymes in PC12 cells. At the same time, a transient activation of PtdIns 3K/Akt and ERK1/2 has also been observed. In cell cultures system resveratrol has been shown to exert its neuroprotective activity by inducing and activating heme oxygenase (HO1) (Zhuang et al. 2003). Although the molecular mechanism of resveratrol action is not fully understood, it is becoming increasingly evident that the induction of HO1 stimulates the degradation of pro-oxidant heme into free iron, carbon monoxide, and biliverdin/bilirubin, which acts as an antioxidant. This may possibly explain the neuroprotective effect associated with induction of HO-1 in resveratrol-treated

neural cell cultures (Li et al. 2009). Similarly *in vivo*, resveratrol has been shown to increase plasma antioxidant capacity, decrease lipid peroxidation, and restore the depleting cellular glutathione levels. Collective evidence suggests that resveratrol suppresses pathological increases in the peroxidation of lipids and other macromolecules *in vivo*, and these effects are direct, or the result of upregulating endogenous antioxidant enzymes (Orallo 2006).

7.3.4 Effect of Resveratrol and Other Wine Polyphenols on Angiogenesis

Angiogenesis is defined as the formation of new blood vessels from existing ones. This process begins with a directed and locally limited degradation of the vascular wall and ends with the formation of a network of new blood vessels. Angiogenesis involves several sequential phases during which endothelial cells play a major role. Sprout formation is initiated with the release of proteolytic enzymes from endothelial cells in order to degrade surrounding basement membrane, followed by endothelial cell proliferation and migration (Folkman and Klagsbrun 1987). Endothelial cell migration and proliferation are essential processes involved in angiogenesis. In mammalian adult brain, neurogenesis occurs in the subgranular zone (SGZ) of the hippocampus, subventricular zone (SVZ), and olfactory bulb (OB) (Zhao et al. 2008). In the normal adult brain, SVZ cells migrate along the rostral migratory stream (RMS) to the OB where they differentiate into interneurons. It should be noted that in the adult brain vascular system is stable under normal conditions. However, in response to neurotraumatic injuries in the adult brain, residual stem cells and vascular system are activated (Greenberg 2007; Farooqui 2010). Vascular endothelial growth factor (VEGF), a strong stimulator of angiogenesis and neurogenesis, is colocalized and associated with endothelial cells and microglial cells (brain macrophages) (Lok et al. 2007). In brain, neurovascular units (NVUs) are multicellular complexes consisting of endothelial cells, pericytes, neurons, and glial cells as well as growth factors and extracellular matrix proteins that are in physical proximity to the endothelium (Lok et al. 2007). NVUs are important sites for the development of neural stem/progenitor cells (NSPCs) in the adult brain. Within the NVUs, newly born, immature neurons are involved in the remodeling vasculature. Adult neurogenesis is regulated by interleukins and nitric oxide, which are released by immune cells (Vallieres et al. 2002; Packer et al. 2003). The synthesis of new vasculature and induction of adult neurogenesis is associated with neurorestorative processes, such as increase in neuroplasticity and synaptogenesis (Fig. 7.7), which may in turn promote the improvement in functional recovery following stroke and traumatic brain injury (Beck and Plate 2009; Li and Chopp 2009).

Resveratrol inhibits angiogenesis by regulating expression and secretion of VEGF, basic fibroblast growth factor (bFGF), Matrix metalloproteinase-2, and Matrix metalloproteinase-9 (MMP-2 and MMP-9) in a dose-dependent manner.

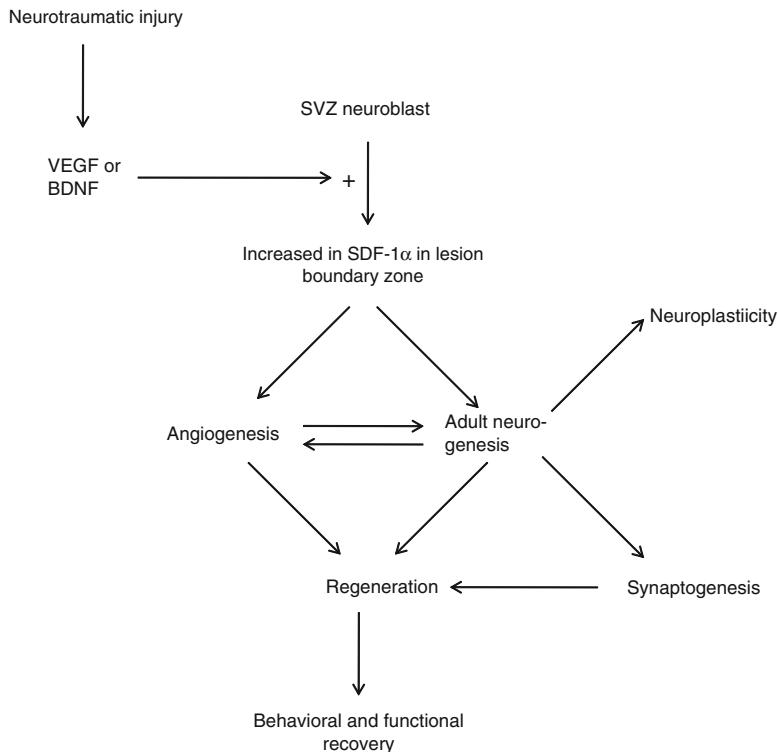


Fig. 7.7 Modulation of neurogenesis and angiogenesis by VEGF and BDNF in the brain. Vascular endothelial growth factor (VEGF); brain-derived neurotrophic factor (BDNF); and subventricular zone (SVZ)

Resveratrol promotes apoptotic cell death in bFGF-stimulated endothelial cells by upregulating p53 protein synthesis and inhibiting bFGF-mediated angiogenesis in the chick chorioallantoic membrane model of angiogenesis (Mousa et al. 2005). MMP-2 and MMP-9 are endopeptidases that hydrolyze protein components of extracellular matrix and, thus contribute to cell migration by eliminating the surrounding extracellular matrix and basement membrane barriers, which play an important role in the process of angiogenesis (Bogaczewicz et al. 2006). Resveratrol modulates abrogating of VEGF-mediated tyrosine phosphorylation of vascular cadherin and its protein complex partner, β -catenin in a dose-dependent manner. The inhibition of VEGF-mediated angiogenesis is also stimulated by the disruption of ROS-dependent Src kinase activation and the consequent vascular endothelial cadherin tyrosine phosphorylation (Lin et al. 2003). VEGF stimulates superoxide generation by activating NADPH oxidase (Arbiser et al. 2002; Sun et al. 2007). Based on detailed investigations, it is proposed that resveratrol acts by blocking generation of superoxides through the inhibition of NADPH oxidase activity in microglia and astrocytes (Ushio-Fukai et al. 2002). Resveratrol induces relaxation

in isolated arteries and rat aortic rings. Resveratrol elicits endothelium-dependent and -independent dilation of isolated retinal arterioles. Endothelium-dependent dilation is mediated by the activation of nitric oxide synthase and release of NO. In addition, resveratrol also upregulates expression of mRNA for endothelial NOS and VEGF and decreases the secretion of endothelin. These effects may be closely associated with vasodilation and blood pressure regulation (Nicholson et al. 2009).

7.3.5 *Effect of Resveratrol on Silent Information Regulator*

Reversible protein acetylation has emerged as a critical posttranslational modification that modulates many biological processes. Histones are acetylated at lysines in their NH₂-terminal tails under the control of two competing enzymes, namely histone acetyltransferases and histone deacetylases (HDACs). Silent information regulator two proteins (SIRTs) belong to a family of histone deacetylases (HDACs), which catalyze a reaction that couples lysine deacetylation to the formation of nicotinamide and O-acetyl-ADP-ribose from NAD⁺ and the abstracted acetyl group (Michishita et al. 2005). These enzymes are widely distributed in all the phyla of life and have been implicated in aging, cell cycle regulation, apoptosis, metabolism, and inflammation (Kaeberlein et al. 1999; Dryden et al. 2003; Tissenbaum and Guarente 2001). Eighteen distinct HDACs have been identified and are grouped in four classes based on their homology to *Saccharomyces cerevisiae* histone deacetylases: RPD3 (class I), HDA1 (class II), silent information regulator (SIR)2 (class III), and deacetylase HDAC119 L class IV). Unlike class I and II HDACs, which consume a water molecule for direct hydrolysis of the acetyl group, sirtuins require NAD⁺ as a cosubstrate for the deacetylation reaction (Fig. 7.8). The stoichiometry between NAD⁺ and the substrate (acetylated protein) is 1:1 and forms the deacetylated product 2-O-acetyl-ADP-ribose, cleaving nicotinamide and deacetylated protein. Thus, sirtuins are fundamentally different in chemical mechanism compared with Zn²⁺-dependent Class I and Class II deacetylases, which are sensitive to class-specific inhibitors such as trichostatin A (Gallinari et al. 2007). The human genome encodes for seven sirtuins designated as SIRT1–SIRT7. Human sirtuins are located in different cellular compartments within neural and nonneuronal cells, with SIRT1, SIRT6, and SIRT7 predominantly nuclear, whereas SIRT3, SIRT4, and SIRT5 are mitochondrial. SIRT2 exhibits a largely cytosolic localization (North and Verdin 2007) but it has been reported to exhibit nuclear localization at distinct parts of the cell cycle and regulates cell cycle progression (North and Verdin 2007; Dryden et al. 2003). SIRT2 (human analog is SIRT1) directly binds to one or more constituent in the chromatin complex, resulting in structural reorganization, and therefore has the ability to establish silent chromatin domains (Grubisha et al. 2005). However, its role in the regulation of expression and release of proinflammatory cytokines in response to environmental stresses is not fully understood. This is especially important in light of a recent study showing that SIRT1 regulates NF-κB by yet unknown mechanisms (Yeung et al. 2004). In addition, SIRT1 also plays important roles in

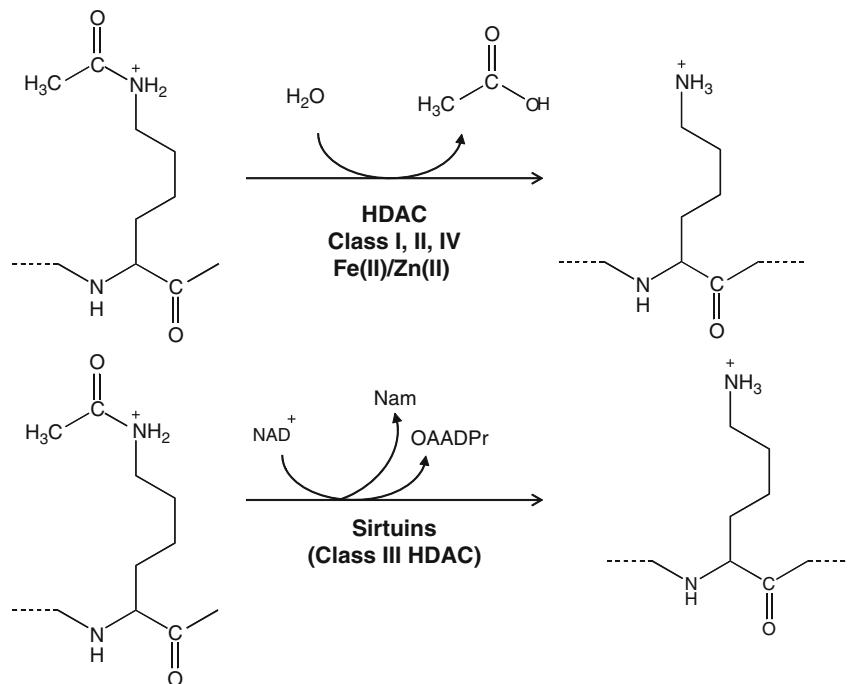


Fig. 7.8 Mechanisms of reactions catalyzed by histone deacetylase. HDAC class I, II, IV mechanism (a). Classical HDAC enzymes use a redox-active metal [Zn(II) or Fe(II)] to coordinate the hydrolysis of acetate from lysine residues. (b) Mechanism of sirtuin catalyzed reaction (b). Sirtuins facilitate the removal of acetyl groups at the expense of NAD, liberating nicotinamide and the unique metabolite O-acetyl-ADP-ribose. Both groups of HDAC and sirtuin enzymes may catalyze the deacetylation of substrates other than histones. Histone deacetylase (HDAC); 20-*O*-acetyl-ADPribose (OAADPr); and nicotinamide (Nam)

many cellular processes including gene silencing, regulation of p53, fatty acid metabolism, cell cycle regulation, and life span extension (Borra et al. 2005).

Resveratrol is a potent activator of the SIRT1/SIRT2 (Howitz et al. 2003). It increases SIRT1 activity by as much as eightfold, lowering the K_m value for acetylated substrate and to a much lesser extent that of NAD⁺, with no effect on the overall turnover rate of the enzyme (Howitz et al. 2003). Several studies have shown that many of the beneficial effects of resveratrol are due to activation of SIRT1, including stress resistance and life span extension (Howitz et al. 2003). It has been hypothesized that the benefits of resveratrol are due either to its antioxidant properties or to a specific activation of SIRT1, which is involved in responding to molecular damage and metabolic imbalances (Borra et al. 2005). In the brain, resveratrol also enhances SIRT1-dependent cellular processes such as axonal protection (Araki et al. 2004) and fat mobilization (Picard et al. 2004).

Resveratrol has been shown to increase life span in three model organisms through a SIRT1/2-dependent pathway. Studies on the molecular mechanism of

SIRT1/2 activation by resveratrol indicate that only SIRT1 exhibits significant enzyme activation (~8-fold). Although resveratrol activates SIRT2 only by 2–3-folds, the activation of SIRT2 requires the presence of a fluorophore covalently attached to the peptide substrate. Using peptide substrate, it is shown that the peptide sequence has no effect on resveratrol-mediated activation. Based on these results, it is proposed that resveratrol binding to SIRT1 promotes conformational changes in the enzyme, which allow tighter binding of the fluorophore, but resveratrol interactions with SIRT2 are not as tight as reported for SIRT1. Accumulating evidence suggests that stimulation of SIRT1/2 by resveratrol increases life span, cell survival, and neuroprotection not only by downregulating p53 activity, but also by deacetylating peroxisome proliferator-activated receptor- γ and its coactivator 1 α , increasing mitochondrial size and number, and restoring energy metabolism in the brain tissue (Borra et al. 2005; Howitz et al. 2003; Lagouge et al. 2006).

7.3.6 Regulation of Cell Cycle Progression by Resveratrol

Cell cycle progression is tightly modulated by interaction among cyclin-dependent kinases (Cdk1, 2, 4, or 6), regulatory cyclin subunits (cyclin A, B, D, or E), and inhibitor proteins (p21WAF1 and p27KIP1) (Peschos et al. 2004). The coordinated activities of cyclin Ds/Cdk4/6, cyclin E/Cdk2, and cyclin A/Cdk2 are involved in G1/S transition and progression through S phase, while Cdk1/cyclin A and B activities are associated with entry into mitosis. Cyclin D1 is a rate-limiting activator for the G1/S transition, a critical cell-cycle checkpoint. The G1/S transition requires the activation of the cyclin D/Cdk4/Cdk6 and cyclin E/Cdk2 complexes, which in turn phosphorylates the retinoblastoma protein (Rb). The subsequent dissociation of E2Fs from Rb activates a series of target genes that are required for entering S phase (Athar et al. 2009).

Resveratrol modulates the major cell cycle mediators at micromolar concentrations, arresting cancer cells at the G1/S phase of the cell cycle. The antiproliferative activity of resveratrol is involved in the induction of p21WAF1 and p27KIP1 and downregulation of cyclins D1/D2/E, Cdks 2/4/6, and hyperphosphorylated pRb (Ahmad et al. 2001). Resveratrol upregulates the p53 tumor suppressor protein (Ahmad et al. 2001) and its posttranslational modification which may be associated with its prooxidant stress response (Zhang et al. 2004). In addition, resveratrol produces the expression of p21WAF1, p300/CBP, APAF1, and Bak and facilitates Bcl2 downregulation (Narayanan et al. 2003).

7.4 Beneficial Effects of Resveratrol on Neurological Disorders

Resveratrol exerts its beneficial effects through the activation of intracellular signal transduction pathways. These effects are similar to those activated by calorie restriction, an intervention long known to enhance health and prolong life span

(Wood et al. 2004). An important target of resveratrol action is the NAD-dependent deacetylases (sirtuins, SIRTs). The pathways that are regulated by SIRTs include gluconeogenesis and glycolysis in the liver, fat metabolism in adipose tissues, and cell survival in the brain. Depending on neural cell type (neurons, astrocytes, oligodendrocytes, and microglial cells) and circumstances, SIRTs activate or suppress members of the forkhead box O (FOXO) group of transcription factors. Four transcription factors of FOXO family occur (FOXO1, FOXO3a, FOXO4, and FOXO6) in mammalian tissues. They are regulated by the Akt/PKB signaling pathway. During cellular stress, SIRT1 is localized in the nucleus irrespective of the cellular stress status while cytoplasmic FOXO3a relocates to the nucleus in response to various stress stimuli (Giannakou and Partridge 2004). SIRT1 interacts with phosphorylated FOXO3a and deacetylates it in the nucleus (Brunet et al. 2004). These interactions increase FOXO3a's capability to induce cell cycle arrest, resist stress, and induce DNA repair mechanisms. FOXO also activates or suppresses specific genes (Fig. 7.5), leading to a decrease in apoptosis, an increase in antioxidant activities, DNA protection, anti-inflammatory effects, and modulation of various other mechanisms so as to promote the health of the cell, and thus the organism (Morris 2005). As stated earlier, SIRT1 deacetylates the acetylated proteins, metabolic regulator, transcriptional coactivator, and peroxisome proliferator-activated receptor- γ co-activator 1 α (PGC-1 α). By doing so it improves mitochondrial function, induces genes for mitochondrial and fatty acid oxidation, and increases mitochondrial membrane potential (Lagouge et al. 2006; Gerhart-Hines et al. 2007; Anderson et al. 2008).

Phosphoinositide 3-kinase (PtdIns 3K) is another direct target of resveratrol action (Frojdo et al. 2007). It is suggested that this pathway may be associated with the control of life span through the modulation of insulin-like signaling that involves PtdIns 3K/protein kinase B cascade and the downstream FOXOs. In addition, resveratrol promotes cell survival, increases life span, and mimics caloric restriction, thereby improving health and survival of mice on high-calorie diet. All of these effects are potentially mediated by the pleiotropic interactions of resveratrol with different enzyme targets including COX-1 and COX-2, and QR2 (quinone reductase 2) (Calamini et al. 2010).

Beneficial underlying mechanisms of resveratrol action in neurological disorders (Table 7.2) are similar and linked to pathways that are important during the aging process (Markus and Morris 2008), characterized by a progressive deterioration of physiological functions and metabolic processes. In normal aging, the loss of neuronal cells in vital regions of the brain (hippocampus) may be related to several factors, among which the production of ROS by mitochondria is a common denominator and one of the leading cause of DNA damage, apoptosis, and death. Through the activation of *SIRT1*, resveratrol affects multiple transcription factors and other protein targets. It indirectly inhibits not only NF- κ B signaling involving expression of proinflammatory cytokines, but also blocks oxidative stress, lipid peroxidation, and decrease in glutathione levels through the induction of HO-1 (Zhuang et al. 2003; Li et al. 2009).

Table 7.2 Effect of resveratrol on neurological disorders

Neurological disorder	Effect	Target	Reference
Ischemia	Beneficial	Scavenging ROS, induction of antioxidant enzymes, inhibition of cytokine expression	Wang et al. (2002), Wang et al. (2004)
Spinal cord injury	Beneficial	Suppression of cytokines expression	Liu et al. (2011)
Traumatic head injury	Beneficial	–	Sönmez et al. (2007), Ates et al. (2007)
Epilepsy	Beneficial	–	Shetty (2011)
Alzheimer disease	Beneficial	Inhibition of A β aggregation, activation of Sirtuin 1	Wang et al. (2006), Vingtdeux et al. (2008), Albani et al. (2009)
Parkinson disease	Beneficial	Inhibition of α -synuclein aggregation	Albani et al. (2009), Chao et al. (2006)
Huntington disease	Beneficial	Activation of Sirtuin 1; antioxidant effect	Ho et al. (2010), Kumar et al. (2006)
Amyotrophic lateral sclerosis	Beneficial	–	
Kainic acid neurotoxicity	Beneficial	Antioxidant and anti-inflammatory effects	Wang et al. (2004)
Experimental autoimmune encephalomyelitis	Beneficial	–	Shindler et al. (2010)
Neuropathic pain	Beneficial	Reduction in allodynia	Bermudez-Ocana et al. (2006)
Diabetic neuropathy	Beneficial	Reduction in oxidative stress	Sharma et al. (2007)

The neuroprotective effect of resveratrol is independent of its direct radical scavenging property, but instead it is also dependent on its ability to selectively induce the expression of mitochondrial superoxide dismutase (SOD2) and, subsequently, reduce mitochondrial oxidative stress and damage (Fukui et al. 2010). The induction of mitochondrial SOD2 by resveratrol is mediated through the activation of the PtsIns 3K/Akt and GSK-3 β /β-catenin signaling pathways (Fig. 7.6). Accumulating evidence suggests that SIRT1 not only modulates mitochondrial oxygen detoxification genes for MnSOD and SOD, but also positively regulates the expression of the DNA repair gene GADD45 through regulatory effects on FOXO transcription factors (Brunet et al. 2004).

7.4.1 *Beneficial Effects of Resveratrol on Ischemia/Reperfusion Injury*

Ischemia/reperfusion injury triggers a complex series of biochemical and molecular mechanisms that impairs neurologic functions through the ATP depletion, breakdown of cellular and subcellular integrity mediated by excitotoxic glutamatergic

signaling, Ca^{2+} influx, alterations in ionic balance and redox, and free-radical generation (Farooqui 2010). These processes are associated with the activation of signaling mechanisms involving phospholipases (PLA₂, PLC, and PLD), calcium/calmodulin-dependent kinases (CaMKs), mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), nitric oxide synthases (NOS), calpains, calcinurin, and endonucleases. Stimulation of these enzymes bring them in contact with appropriate substrates and modulates cell survival/degeneration mechanisms (Hou and MacManus 2002; Farooqui and Horrocks 2007). In addition, deficit in energy metabolism and ATP depletion during ischemic injury may lead to depolarization and failure of energy conduction. The exact molecular mechanism for mitochondrial dysfunction due to cerebral ischemia/reperfusion is not fully understood. However, mitochondrial respiratory chain has strongly been suggested susceptible to cerebral ischemia/reperfusion induced ROS. Thus, during cerebral ischemia/reperfusion injury mitochondrial complexes (I–IV) may be damaged (Allen et al. 1995), and alterations in mitochondrial oxidative phosphorylation may eventually affect ATP production.

Resveratrol treatment efficiently restores the disrupted mitochondrial integrity after ischemic damage in hippocampus (Yousuf et al. 2009). Furthermore, resveratrol treatment not only increases the basal levels of adenosine and inosine, inhibits the elevations of hypoxanthine, and xanthine levels, but remarkably decreases xanthine oxidase activity, malondialdehyde levels (MDA), and restores Na^+ , K^+ -ATPase activity following ischemia/reperfusion injury in male rats (Li et al. 2011; Simão et al. 2011). In addition a marked decrease in cytochrome c release, lactate dehydrogenase (LDH) activity, and DNA damage has also been reported after resveratrol treatment. Resveratrol has been reported to prevent alterations of mitochondrial functions against in vitro hypoxia/re-oxygenation in a concentration-dependent manner by maintaining respiratory control and ROS generation as evidenced by cytochrome c release and membrane potential collapse (Morin et al. 2003). Recently, resveratrol has been shown to inhibit the activation of microglia, decrease the number of reactive astrocytes, and reduce the production of proinflammatory factors through cellular cascade signaling pathways (Wang et al. 2002). Based on above studies, it is proposed that inhibition of transcription factors, downregulation of proinflammatory cytokines, energy depletion, oxidative stress, and apoptosis are prominent features in the pathogenesis of ischemia/reperfusion injury (Farooqui 2010).

Intravenous administration of resveratrol attenuates these deleterious effects of ischemia/reperfusion injury (Shigematsu et al. 2003). Resveratrol has also been shown to attenuate the proinflammatory effects invoked by PAF. Resveratrol also confers vasculoprotection by regulating the expression of proinflammatory and pro-atherogenic genes in endothelial cells of cerebrovascular and cardiovascular systems. Resveratrol decreases neural and endothelial cells VCAM and ICAM-1 expression (Carluccio et al. 2003). Because of the potent anti-inflammatory action of resveratrol, it regulates the expression of inflammatory mediators, such as adhesion molecules, cytokines (e.g. TNF- α , IL-1 β , IL-6), and iNOS through transcriptional mechanisms that include C/EBP, fos/jun, AP-1

(An et al. 1993), and NF-κB. Resveratrol also increases levels of MMP-2 and VEGF, which may contribute to neuroprotective effects by inducing angiogenesis (Dong et al. 2008).

Several lines of evidence suggest that inhibition of NF-κB by resveratrol underlies many anti-inflammatory effects of resveratrol (Carluccio et al. 2003). NF-κB is a redox-sensitive transcription factor that is expressed by both neural and endothelial cells. It is also generally believed that chronic activation of NF-κB predisposes cerebrovascular and cardiovascular arteries to atherosclerosis, which may facilitate stroke (Hajra et al. 2000). Numerous studies have demonstrated that increase in levels of ROS may activate NF-κB in neural and endothelial cells. This may lead to the upregulation of adhesion molecules, iNOS, and TNF-α. Resveratrol treatment significantly decreases iNOS expression in aged vessels and brain. Thus, it is logical to hypothesize that in the circulation of aged animals, the inhibition of NF-κB activation and iNOS expression by resveratrol exerts vasculoprotective and neuroprotective effects. A second proinflammatory transcription factor, AP-1 is also inhibited by resveratrol (Manna et al. 2000). AP-1, similarly to NF-κB, is important in the regulation of many inflammatory genes that are induced by oxidative stress and its inhibition may contribute to the anti-inflammatory properties of resveratrol. It is recently shown that resveratrol pretreatment not only ameliorates neurological scores, reduces infarct volume and brain water content, and decreases MDA levels, but also restores the SOD activity, upregulates the protein and mRNA expression of Nrf2 and HO-1 (Ren et al. 2011). It also downregulates the protein expression of caspase-3. TUNEL-positive cells are significantly decreased compared with the physiological saline-treated group. Collectively, these observations show that resveratrol pretreatment produces neuroprotective effects on cerebral I/R injury. This neuroprotective effect is likely exerted by upregulated expression of transcription factor Nrf2 and HO-1 to ameliorate oxidative damage and decreases the protein expression of caspase-3 (Ren et al. 2011).

7.4.2 Beneficial Effects of Resveratrol on Traumatic Brain Injury

TBI consists of two broadly defined events: a primary event attributable to mechanical insult itself, and a secondary event that involves a series of systemic and local neurochemical and pathophysiological changes that occur in the brain after the initial insult (Raghupathi 2004; Farooqui 2010). The primary event rapidly ruptures neural cell membranes and results in the release of intracellular contents, disruption of blood flow, breakdown of the blood-brain barrier, and intracranial hemorrhage. In contrast, secondary event of the TBI induces neurochemical alterations, activation of microglial cells and astrocytes, and demyelination involving oligodendroglia (Raghupathi 2004). Excitotoxicity, inflammatory

reactions, oxidative stress, and nitrosative stress are major components of secondary injury. All these processes play a major role in regulating the pathogenesis of acute and chronic TBI (Farooqui 2010).

Studies on the effect of resveratrol following traumatic brain injury (TBI) in 7-day-old rat pups indicate that treatment with a single dose of 100 mg/kg resveratrol (i.p.) after the TBI significantly ameliorates the hippocampal neuronal loss at ipsilateral and contralateral hippocampal brain regions of rats (Sönmez et al. 2007). Additionally, resveratrol treatment decreases anxiety and increases cortex/hippocampus dependent memory of animals subjected to blunt TBI. These results support the view that acute resveratrol treatment results in a neuroprotective effect against TBI-induced hippocampal neuronal loss and associated cognitive impairment in rats (Sönmez et al. 2007).

7.4.3 Beneficial Effects of Resveratrol on Spinal Cord Injury

Like TBI, trauma to the spinal cord results in autodestructive changes consisting of two broadly defined events: a primary event, attributable to the mechanical insult itself, and a secondary event, attributable to the series of systemic and local neurochemical and pathophysiological changes that occur in spinal cord after the initial traumatic insult (Klussmann and Martin-Villalba 2005). The primary event occurs instantly and beyond therapeutic management. However, the secondary event develops slowly over the hours and days after spinal cord injury (SCI), causing behavioral and functional impairments. At the core of primary injury site, SCI ruptures neural cell membranes resulting in the release of neuronal intracellular contents (Farooqui 2010). In contrast, secondary injury is accompanied by neurochemical changes (ischemia, edema, increase in glutamate, and ROS). These neurochemical changes not only effect neuronal activities, astrocytic activation, and demyelination, but also modulate leukocyte infiltration and activation of macrophages and vascular endothelial cells (Farooqui 2010).

Resveratrol administration not only restores neural morphology and increases the number of neurons following SCI, but also results in higher Basso Beattie Bresnahan (BBB) locomotor score. It is also shown that resveratrol treatment reverses the decrease in SOD activity and increase in MDA level caused by SCI supporting the view that resveratrol acts as antioxidant. In addition, resveratrol treatment also suppresses immunoreactivity and expression of proinflammatory cytokines including IL-1 β , IL-10, TNF- α , and myeloperoxidase (MPO) after SCI, suggesting an anti-inflammation effect of resveratrol. Finally, resveratrol treatment also inhibits SCI-induced apoptosis and expression of apoptosis-related gene Bax, Bcl-2 and caspase-3, which suggests an antiapoptotic role of resveratrol after SCI. Collective evidence suggests that resveratrol promotes the recovery of rat dorsal neuronal function after SCI (Yang and Piao 2003; Kaplan et al. 2005; Liu et al. 2011).

7.4.4 Beneficial Effects of Resveratrol on Epilepsy

Epilepsy, one of the most prevalent neurologic disease, is characterized by spontaneous recurrent seizures caused by focal or generalized paroxysmal changes in neurologic functions triggered by abnormal electrical activity in the cortex involving hyperexcitable neurons (Dichter 1994). A basic assumption links to the pathogenesis of epilepsy and the generation of synchronized neuronal activity with an imbalance between γ -aminobutyric acid (GABA) inhibitory and glutamate-mediated neurotransmission (Dalby and Mody 2001).

Studies on the effect of resveratrol on pentylenetetrazole (PTZ), sodium valproate, and diazepam-induced seizures in rats indicate that resveratrol administration 20 min prior to convulsive challenge with PTZ dose dependently reduces the percent incidence of generalized tonic-clonic convulsions. These seizures can be partially prevented by adenosine. Nonspecific adenosine receptor antagonist theophylline significantly reverses the resveratrol-mediated neuroprotection, whereas the specific adenosine A₂ receptor antagonist 3,7-dimethyl-1-propargylxanthine reverses the resveratrol-induced protection. These observations suggest resveratrol has antiepileptic potential and that an adenosinergic-mediated mechanism may play a role in its anticonvulsant activity.

Overstimulation of excitatory amino acid is an important mechanism in seizure genesis. Studies on the effect of resveratrol against kainic acid (KA)-induced seizures indicate that pretreatment of single dose of resveratrol does not block convulsions, but multiple doses of resveratrol significantly reduce the incidence of convulsions (Gupta et al. 2002). Resveratrol administration also attenuates KA-mediated increase in MDA levels. The protective effect of resveratrol against KA-induced convulsions and attenuation of increase in MDA level suggest that this stilbene can produce beneficial effects in epilepsy (Gupta et al. 2002).

7.4.5 Beneficial Effects of Resveratrol on Alzheimer Disease

Alzheimer disease (AD) is a complex, progressive, and multifactorial neurodegenerative disease characterized by the presence of neurofibrillary tangles and extracellular amyloid β (A β 1-42) plaques in the cortex and hippocampus areas of the brain that are important for memory and learning. AD is accompanied by irreversible cognitive dysfunction, memory loss, dementia, and behavioral changes due to neurodegeneration in hippocampus and cerebral cortex. A β 1-42 is generated by the cleavage of the amyloid precursor protein by proteinases. A β 1-42 exerts neurotoxic effects through induction of neuroinflammation, immune activation, and oxidative stress and thus plays a critical role in the pathogenesis of AD (Farooqui 2010). However, the mechanism underlying β -amyloid peptide (A β) neurotoxicity remains to be fully understood. A β hypothesis for the pathogenesis of AD states that A β generated from deregulated proteolysis of the APP undergoes accelerated A β oligomerization, fibril formation, and amyloid deposition in a process that initiates the

AD pathology (Hardy and Selkoe 2002). There is increasing evidence that A β 1-42 not only alters Ca²⁺ homeostasis and mitochondrial function, but also induces apoptosis and increases the intracellular level of ROS in the AD brain. Accumulating evidence suggests that the pathogenesis of AD is complex and is driven by both environmental and genetic factors.

The first report on the beneficial effect of moderate wine consumption appeared in 1997, when it was shown that intake of wine reduces the risk of AD (Orgogozo et al. 1997). This suggestion was supported by two later studies (nested case-control study and cohort study), which indicated that wine decreases the risk of AD (Luchsinger et al. 2004; Truelsen et al. 2002). Several epidemiological studies have indicated that moderate consumption of wine lowers the incidence of AD by decreasing levels of secreted and intracellular A β peptides observed in the presence of resveratrol in cell lines expressing wild-type APP or Swedish mutant APP-695 (Orgogozo et al. 1997; Truelsen et al. 2002). Resveratrol also promotes intracellular degradation of A β through a mechanism that involves the proteasome activity (Marambaud et al. 2005). Moreover, studies in neuronal cell models indicate that the neuroprotective action of resveratrol against A β -induced toxicity involves protein kinase C (Han et al. 2004).

Investigations on risk factors for AD in the Canadian population also have indicated that moderate wine consumption is the most neuroprotective variable against AD risk reduction. Moderate wine consumption reduces the risk of AD by 50 % (Lindsay et al. 2002). Interestingly, wine intake in this population was even more protective than the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Lindsay et al. 2002). Nevertheless, the notion that wine intake—and more specifically red wine intake—lowers AD risk is still controversial and must be treated with caution. Solid biochemical data on human population of several ages are needed on this controversial topic.

Studies on the effect of Cabernet Sauvignon (rich in polyphenols, such as resveratrol) in the Tg2576 mouse model indicate that moderate consumption of Cabernet Sauvignon promotes nonamyloidogenic, α -secretase-mediated APP processing and prevents the generation of amyloidogenic A β 1-42-mediated cognitive deterioration (Wang et al. 2006). Similarly, in primary neuron cultures derived from Tg2576 embryos, Cabernet Sauvignon polyphenols exhibit A β -lowering activity through promotion of nonamyloidogenic processing of APP. Resveratrol treatment markedly inhibits polymerization of the β -amyloid peptide (Riviere et al. 2007) by a mechanism that does not involve β -amyloid production because resveratrol has no effect on activity of β - and γ -secretases but stimulates indirectly the proteosomal degradation of β -amyloid peptides (Marambaud et al. 2005). In other types of neuronal cultures, resveratrol also delays A β -induced toxicity. It is also proposed that resveratrol acts as an antioxidant by preventing the formation of toxic A β oligomers and protofibrillar intermediates (Jang and Surh 2003; Savaskan et al. 2003; Han et al. 2004). Binding and autoradiographic studies indicate that effects of resveratrol may be through specific binding sites, which are particularly enriched in the choroid plexus in the rat brain. The choroid plexus secretes transthyretin, a protein that has been shown to modulate A β aggregation, which may be critical for maintaining of normal learning capacities during aging (Bastianetto et al. 2009).

As mentioned earlier, resveratrol mimics caloric restriction by extending the life span of different organisms via activation of deacetylases from the sirtuin family (Guarente 2005). Resveratrol binds to and activates the deacetylase activity of several sirtuin members, including the mammalian ortholog, SIRT1 (Howitz et al. 2003). Sirtuins deacetylate and control the activity of several transcription factors, including peroxisome proliferator-activated receptor (PPAR) γ cofactors, through a mechanism that regulates life span in mice (Nemoto et al. 2005). Recent studies indicate that intracerebroventricular injection of resveratrol reduces neurodegeneration in the hippocampus and prevents learning impairment in the p25 transgenic AD mouse model by a mechanism that may involve a decrease in the acetylation of known SIRT1 substrates (Kim et al. 2007). In addition, caloric restriction attenuates A β deposition and A β -associated neuropathology in different animal models (Patel et al. 2005), and SIRT1 activation may contribute to the antiamyloidogenic properties of this particular intervention (Qin et al. 2006). Although the molecular mechanism of beneficial effects of SIRT1 in AD is not fully understood, *in vitro* studies indicate that resveratrol-mediated overexpression of SIRT1 not only leads to a reduction in oligomerization of A β peptides in a concentration-dependent manner, but also ameliorates oxidative stress in same cells (Albani et al. 2009; Pasinetti G.M. 2010). It is suggested that reduction in oligomerization of A β peptides is mediated by increase in α -secretase-mediated nonamyloidogenic processing of APP (Qin et al. 2006). In other studies, it is demonstrated that overexpression of SIRT1 prevents the activation of microglial cells by fibrillar A β and the consequent production of neurotoxic chemokines, cytokines, and nitric oxide from the activated microglia through inhibition of NF κ B signaling (Chen et al. 2005), which involves the SIRT1-mediated deacetylation of the lysine 310 residue of the RelA/p65 subunit of NF κ B and blocks its transcriptional activity (Fig. 7.9). In line with these studies, resveratrol not only protects against the dysregulation of energy homeostasis observed in mouse models for metabolic syndromes by a mechanism implicating the activation of SIRT1, PGC-1 α , and the energy sensor protein kinase AMPK (AMP-activated protein kinase) (Dasgupta and Milbrandt 2007; Baur et al. 2006), but also enhanced deacetylation of PGC- α . This process improves mitochondrial function and energy balance. In addition, these processes are linked to regulation of the vitagene system (Figs. 7.6, 7.7, and 7.10). These genes include genes for heat shock proteins (Hsps), thioredoxin/thioredoxin reductase system, and heme oxygenase-1 (Calabrese et al. 2008, 2009). Heat shock response contributes to establish a cytoprotective state in a wide variety of neurodegenerative disorders including AD. When appropriately activated, heat shock response not only initiates and restores cellular homeostasis, but rebalances redox equilibrium. Activation of this pathway is particularly important for neural cells with relatively weak endogenous antioxidant defenses (Calabrese et al. 2008, 2009). Their dependence on the oxidized form of NAD, SIRT1, and SIRT2 has been suggested to be important molecular links between redox status and cellular longevity genes which are important in controlling the maintenance and repair processes in the body (Calabrese et al. 2008, 2009).

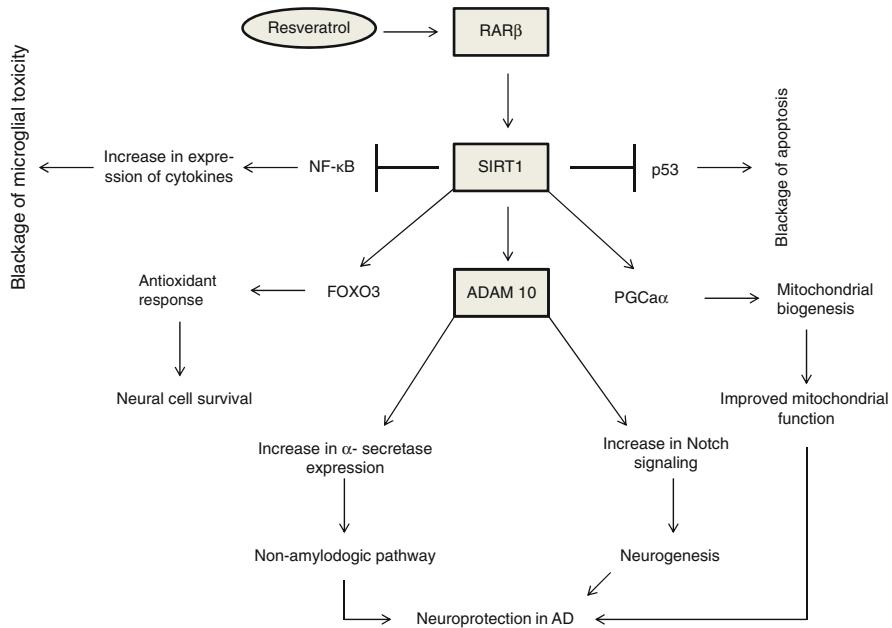


Fig. 7.9 Neuroprotective effect in Alzheimer disease. SIRT1 controls the activation of RAR β . This leads to the induction of nonamyloidogenic processing of APP as well as the activation of Notch signaling (both through transcriptional activation of the ADAM10 gene). In addition, SIRT1 not only regulates proteins and genes associated with the antioxidant response (FOXO3), but also controls anti-inflammatory response (NF κ B), antiapoptotic response (p53), and mitochondrial biogenesis and ROS sequestration (PGC1 α). SIRT1 also blocks p53 and NF κ B signaling, and activates FOXO3, RAR β , and PGC1 α signaling. A SIRT1-activated cell shows decrease in oligomerization of A β , reduction in oxidative stress, and increase in resistance to apoptosis and inflammation-induced toxicity

7.4.6 Beneficial Effects of Resveratrol on Parkinson Disease

Parkinson disease (PD) is a progressive and degenerative disorder caused by the gradual and selective loss of dopaminergic neurons in the substantia nigra pars compacta (Beal 1998; Jenner and Olanow 2006). Loss of dopaminergic neurons not only results in changes in neurotransmission in the basal ganglia motor circuit, but also induces movement disorders such as tremor, rigidity, and akinesia. Histopathologically, PD is characterized by the presence of Lewy bodies (LB), proteinaceous inclusions constituted primarily by α -synuclein (Farooqui 2010). Neurochemically, PD is not only accompanied by oxidative stress, neuroinflammation, selective loss of mitochondrial complex I, and the α -ketoglutarate dehydrogenase complex in the nigral neurons of PD patients, but also by increase in hydroxynonenal and 8-oxo-deoxyguanine, indices of oxidative damage, in the nigral

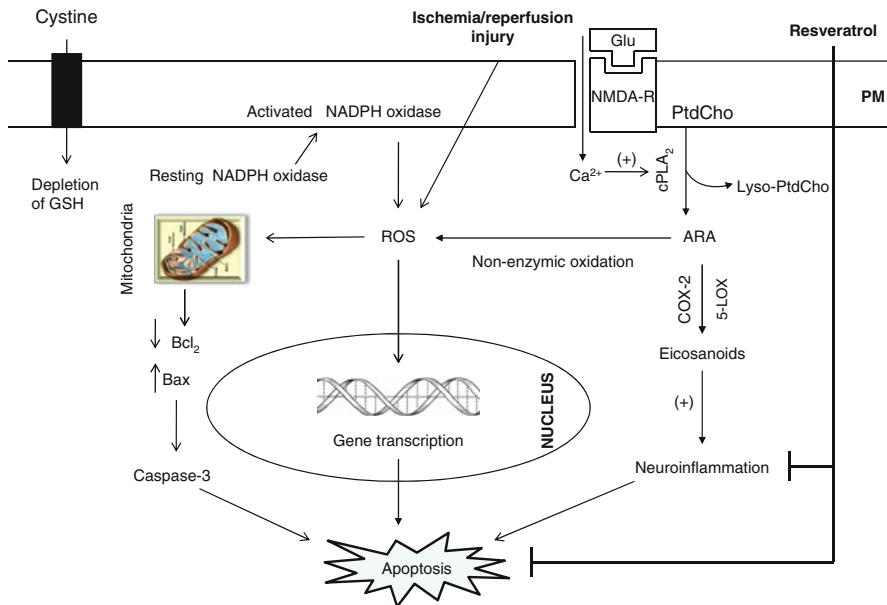


Fig. 7.10 Modulation of ischemia/reperfusion-mediated oxidative stress, neuroinflammation, and apoptosis by resveratrol. *N*-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (Lyso-PtdCho); cyclooxygenase-2 (COX-2); 5-lipoxygenase (5-LOX); epoxyenase (EPOX); arachidonic acid (ARA); reactive oxygen species (ROS); and reduced glutathione (GSH)

neurons of PD. Once the mitochondria are destabilized, above abnormalities can trigger apoptotic cell death. Although primary events which induce mitochondrial failure and oxidative damage are not fully understood, it has been postulated that the interaction of genetic risk factors and environmental factors may initiate the neurodegenerative process (Farooqui 2010).

Resveratrol not only prevents 6-hydroxydopamine (6-OHDA)-mediated oxidative stress in neuroblastoma SH-SY5Y cells, but also protects against rotenone-induced apoptosis and enhances degradation of α -synucleins in α -synuclein-expressing PC12 cell lines via autophagy induction (Chao et al. 2006; Khan et al. 2010; Wu et al. 2011). It is reported that suppression of AMPK and/or SIRT1 induces reduction in protein levels of LC3-II, indicating that AMPK and/or SIRT1 is required in resveratrol-mediated autophagy induction. Moreover, suppression of AMPK results in the inhibition of SIRT1 activity and attenuation of protective effects of resveratrol in rotenone-mediated apoptotic cell death, further supporting the view that AMPK-SIRT1-autophagy pathway is closely associated with resveratrol-mediated neuroprotection in this cellular model of PD (Wu et al. 2011). Accumulating evidence suggests that the beneficial effects of resveratrol are not only limited to its antioxidant and anti-inflammatory action but also include

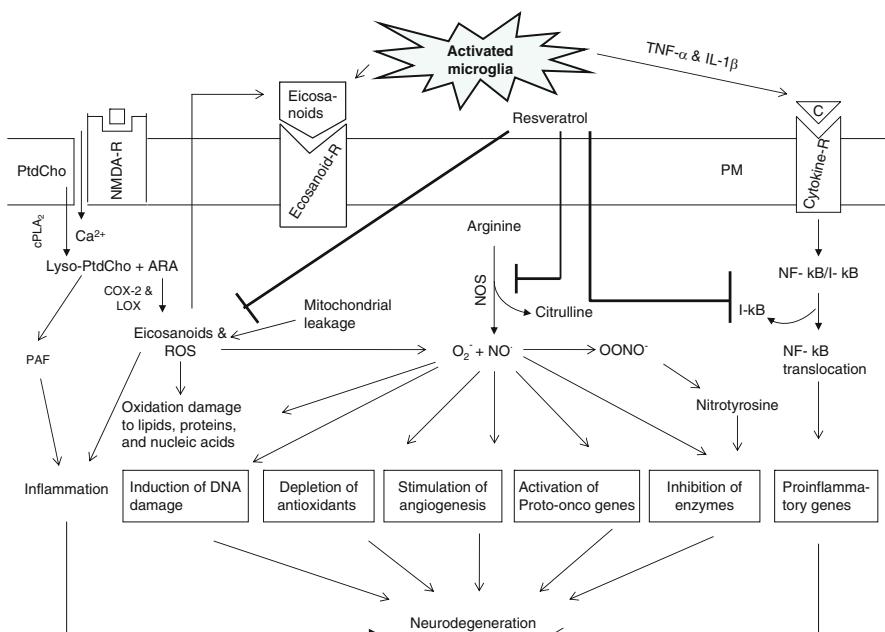


Fig. 7.11 Contribution of microglial cell-mediated neurodegeneration in PD. Microglial cells release nitric oxide, eicosanoids, and cytokines (TNF- α and IL-1 β), which interact with their receptors on neuronal surface and mediate alterations in signal transduction processes resulting in neurodegeneration. Phosphatidylcholine (PtdCho); Lyso-phosphatidylcholine (Lyso-PtdCho); arachidonic acid (ARA); cytosolic phospholipase A $_2$ (cPLA $_2$); cyclooxygenase-2 (COX-2), lipoxygenase (LOX); nitric oxide synthase (NOS); nitric oxide (NO $^-$); peroxynitrite (OONO $^-$); reactive oxygen species (ROS), cyclooxygenase-2 (COX-2), lipoxygenase (LOX); and platelet activating factor (PAF); and \neg (blocked arrow) represents inhibition

activation of sirtuin 1 (SIRT1) and vitagenes, which can prevent the deleterious effects triggered by oxidative stress.

It is becoming increasingly evident that nitric oxide (NO) and its reactive metabolites play a key role in neurodegeneration in PD (Fig. 7.11). The inhibition of NO synthesis or the scavenging of reactive nitrogen species (RNS) may represent a novel and efficient tool against PD progression. It is obvious from above-mentioned studies that resveratrol may be a promising natural antioxidant and anti-inflammatory molecule to retard oxidative/nitrosative stress (Aquilano et al. 2008). Resveratrol not only exhibits the ability to induce the expression of intracellular antioxidants enzymes, but also may mediate anti-inflammatory responses depending on inhibition of the inflammatory genes expression such as eicosanoid generating enzymes and iNOS, mainly through interfering with the activation of the upstream NF- κ B transcription factor (Aquilano et al. 2008). PD is a multifactorial disease in which NO, inflammation, and oxidative stress play important roles. Thus, the use of compounds such as resveratrol, showing poly-pharmacological activities, appears to be greatly attractive.

7.4.7 *Beneficial Effects of Resveratrol on Other Neurodegenerative Diseases*

Huntington disease (HD) is a progressive, neurodegenerative disease characterized by oxidative stress, abnormal body movements called chorea, and a reduction of various mental abilities (Farooqui 2010). It is caused by a polyglutamine repeat expansion in the huntingtin (htt) gene. Studies in transgenic mice and human microarrays indicate that mitochondrial dysfunction, which accompanies HD neurodegeneration, may be due to defective PGC-1 α activity (McGill and Beal 2006). Insoluble aggregates containing huntingtin occur in cytosol and nuclei of HD patients, transgenic animal and cell culture models of HD. The molecular mechanism associated with aggregate formation is not fully understood. However, it is proposed that at the molecular level, mutant huntingtin polypeptides acquire an unusual conformation facilitating their aggregation into inclusion bodies, which impair vesicular and mitochondrial traffic and cause mitochondrial dysfunction leading to an increase in free radicals production and cell death (Scherzinger et al. 1997; Solans et al. 2006). Despite its widespread expression in the brain and body, mutant huntingtin causes selective neurodegeneration in striatal medium-sized spiny GABAergic projection neurons (MSNs), resulting in the appearance of generalized involuntary movements, the main phenotypic alteration in HD. Although the molecular mechanism associated with selective neurodegeneration is not known, the selective nuclear localization of mutant huntingtin in striatal nuclei may contribute to the region specific atrophy in transgenic models of HD (Van Raamsdonk et al. 2007).

Injections of 3-nitropropionic acid (NPA) (an inhibitor of complex II of the electron transport chain) have been used to induce symptoms of HD in rodents (Kumar et al. 2006). Studies on the effect of resveratrol, in NPA-treated rats indicate that this toxin causes significant loss of body weight, a decline in motor function (locomotor activity, movement pattern, and vacuous chewing movements), and poor retention of memory. Repeated treatment with resveratrol significantly improves the NPA-induced motor and cognitive impairment. Biochemical analysis indicates that systemic administration of NPA not only increases lipid peroxidation, nitrite levels, but also depletes reduced glutathione levels and decreases succinate dehydrogenase activity in the brains of rats. Resveratrol treatment reverses NPA-mediated motor and cognitive impairment and support the view that resveratrol antioxidant activity of this stilbene may contribute to neuroprotective effect in HD. In yeast cell model of HD, resveratrol significantly reduces the amount of free radicals generated in the mutant strain but it does not diminish the size of polyQ aggregates, mitochondrial fragmentation, or the rate of growth of the strains control and mutant suggesting that the resveratrol plays a role delaying but not inhibiting disease development (Solans et al. 2006).

Amyotrophic lateral sclerosis (ALS), a major motor neuronal disorder, is characterized by progressive loss of motor neurons leading to muscle loss, paralysis, and death from respiratory failure. Although the molecular mechanism of neurodegeneration in ALS is not known, it is becoming increasingly evident that oxidative stress, mitochondrial impairment, protein aggregation, axonal dysfunction, mutant

superoxide dismutase expression (only in 20 % cases), cytoskeletal disorganization, glutamate toxicity, and apoptotic cell death play an important role (Farooqui and Horrocks 2007). Treatment of neural cell cultures with resveratrol reduces neurodegeneration in animal models of ALS (Kim et al. 2007). The effect of resveratrol is mediated through the activation of SIRT1 and deacetylation of p53 and PGC-1 α (St-Pierre et al. 2006). By modulating above processes, resveratrol protects neuron from oxidative stress and apoptotic cell death in animal models of ALS.

7.4.8 *Beneficial Effects of Resveratrol in Kainic Acid Neurotoxicity*

Kainic acid (KA) is a cyclic and nondegradable analog of glutamate. Its intracerebroventricular injections produce many neurochemical changes including dense staining of cPLA₂ and 4-HNE at one day postinjection, before there is any histological evidence of neurodegeneration (Farooqui et al. 2008). Studies have shown that systemic or intracerebral injections of kainate cause epileptiform seizures in the CA3 region of the hippocampus. These seizures propagate to other limbic structures and are followed by a pattern of cell loss that is similar to that seen in patients suffering from temporal lobe epilepsy. Chronic administration of resveratrol inhibits mossy fiber sprouting and KA receptor expression in hippocampus of epileptic rats, which induces the formation of functional aberrant synapses on granule cells of the dentate gyrus. Furthermore, resveratrol protects neurons against KA-induced neurodegeneration and epileptogenesis in the KA-induced temporal lobe epilepsy animal model (Virgili and Contestabile 2000; Wu et al. 2009). In addition, resveratrol also suppresses KA-induced activation of astrocytes and microglial cells. Since increased oxidative stress is a key factor for KA-induced neurotoxicity, it is suggested that resveratrol acts as free radical scavenger to protect against neuronal damage caused by KA-mediated insult.

7.5 Conclusion

Resveratrol, a naturally occurring trihydroxy stilbene, holds great promise as an antioxidant, anti-inflammatory, cardioprotective, and/or antitumor agent. It is present in high concentration in red grapes and red wines. Resveratrol produces its beneficial effects by acting through multiple mechanisms. It has ability to improve endothelial function. Similar to most polyphenols, resveratrol not only has intrinsic antioxidant capacity, and also induces the expression of a number of antioxidant enzymes. Resveratrol further interacts with a large number of receptors, kinases, and other enzymes. It attenuates the activation of immune cells and the subsequent synthesis and release of proinflammatory mediators through the inhibition of transcription factors such as NF- κ B and AP-1. Resveratrol not only effects eNOS

via SIRT1 to mediate upregulation and deacetylation and perhaps also via ER α to induce eNOS phosphorylation, which is induced by ERK1/2. Resveratrol has been shown to protect against neurotraumatic (stroke, SCI, TBI, and epilepsy) and neurodegenerative diseases (AD, PD, HD, and ALS). The molecular mechanisms by which resveratrol exerts its beneficial effects on neurotraumatic and neurodegenerative diseases is not fully understood. However, it is becoming increasingly evident that resveratrol inhibits enzymes generating ROS as well as the activity of vascular NAD(P)H oxidase, which is one of the major sources of superoxide in vascular system. In *in vitro* studies, acute application of resveratrol produces endothelium-dependent relaxation in animal and human arteries. A short-term incubation with resveratrol enhances nitric oxide production in cultured human endothelial cells, whereas long-term treatment with resveratrol improves flow-mediated vasodilation in hypercholesterolemic rabbits and humans. Besides its anti-oxidant and anti-inflammatory properties, resveratrol also acts by activating sirtuin 1 (SIRT1). SIRT1 has recently emerged as a major therapeutic target for the treatment of age-related neurodegenerative diseases. Studies on the effect of resveratrol on neuronal cultures and animal models of neurotraumatic and neurodegenerative diseases indicate that resveratrol acts as a SIRT1 activator and protects cultured cells from oxidative stress and cytotoxicity induced by A β peptide and α -synuclein. Resveratrol not only mimics caloric restriction by increasing SIRT1 activity, but also modulates proliferator-activated receptor coactivator-1 α (PGC-1 α) deacetylation and mitochondrial biogenesis. These processes are linked to regulation of the vitagene system and longevity genes, which are important in controlling the maintenance and repair processes in the brain tissue. In addition, resveratrol also impacts on the mitochondrial functions through the modulation of respiratory chain, on co-proteins, and p53-mediated gene expression. Collective evidence suggests that resveratrol offers a promising approach for treatment of above-described neurotraumatic and neurodegenerative disorders.

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Chapter 8

Beneficial Effects of *Ginkgo biloba* in Neurological Disorders

8.1 Introduction

Ginkgo biloba (*G. biloba*), which belongs to the family Ginkgoaceae, is an important herb from the Chinese traditional system of medicine (Huh and Staba 1992). Extract prepared from its leaves has been used in traditional medicine for several hundred years. Major component of patented *G. biloba* leaves extract is called as EGb761. It was developed by Beaufour-Ipsen Pharma (Paris, France) and Dr. Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany). EGb761 contains glycosides of the flavonols quercetin, isorhamnetin, and kaempferol (24 %); the terpene-lactones bilobalide and ginkgolides A, B and C, M, J, and bilobalide (6 %); and less than 5 ppm ginkgolic acid (Figs. 8.1 and 8.2) (Drieu 1986; DeFeudis and Drieu 2000). Other minor constituents of *G. biloba* include shikimic, vanillic, ascorbic, p-coumaric acids, steroids (sitosterol and stigmasterol), polyprenols, benzoic acid derivatives, carbohydrates, straight chain hydrocarbons, alcohol, ketones, and 2-hexenol. *G. biloba* constituents possess potent central nervous system (CNS) regulating activity (Di Renzo 2000 including enhancement of memory, concentration, mental alertness, and decrease in mental fatigue. Many of these constituents produce memory boosting and mental altering effects, which are due to antioxidant- and neurogenesis-promoting properties of flavonols of *G. biloba* (Fig. 8.3) (Yoo et al. 2011). In addition, *G. biloba* terpenes are involved in antagonizing platelet activating factor, an endogenous mediator of inflammation and nociception produced by a variety of inflammatory cells, which may improve cardiovascular blood flow (Smith et al. 1996). *G. biloba* flavonoids have also been reported to dilate blood vessels by increasing the release of endothelium-derived relaxing factor and prostacyclin (PGI₂) from vascular endothelial cells and decrease blood viscosity by antagonizing platelet activating factor.

In addition to scavenging free radicals, EGb 761 reduces bromethalin-induced edema (Dorman et al. 1992), decreases neuronal injury following ischemia or electroconvulsive shock (Birkle et al. 1988), and reduces subchronic cold stress effects

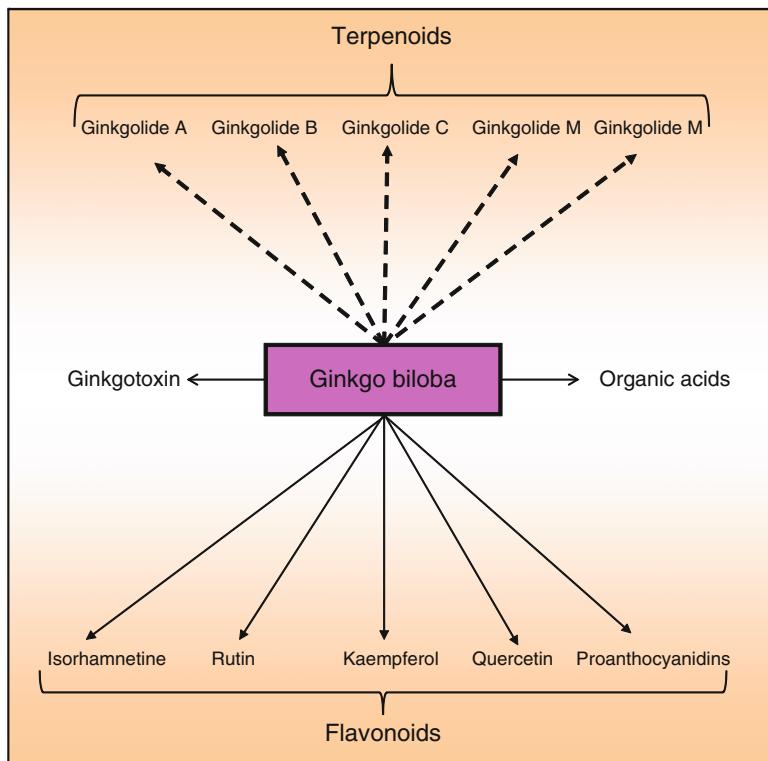


Fig. 8.1 Chemical constituents of EGb 761

on receptor desensitization (Bolanos-Jimenez et al. 1995). Other effects of EGb 761 include inhibition of monoamine oxidase A and B (White et al. 1996), decreasing retinal neovascularization following injury (Baudouin et al. 1992), altering the immune system (Braquet 1988), and promoting compensation from vestibular deafferentation (Lacour et al. 1991). Collective evidence suggests that active constituents of *G. biloba* extract scavenge free radicals, facilitate blood circulation, block clot formation, stabilize the membranes of neural cells, and protect nerve cells from harmful effects of lipid peroxidation.

8.2 Bioavailability of *Ginkgo Biloba* in the Brain

In general, the bioavailability of flavonoids of Egb 761 is relatively low due to limited absorption and rapid elimination (Goh and Barlow 2004). Unabsorbed flavonoids that reach the colon are metabolized by colon bacterial enzymes and then absorbed (DeFeudis and Drieu 2000). Once absorbed, flavonoids reach the

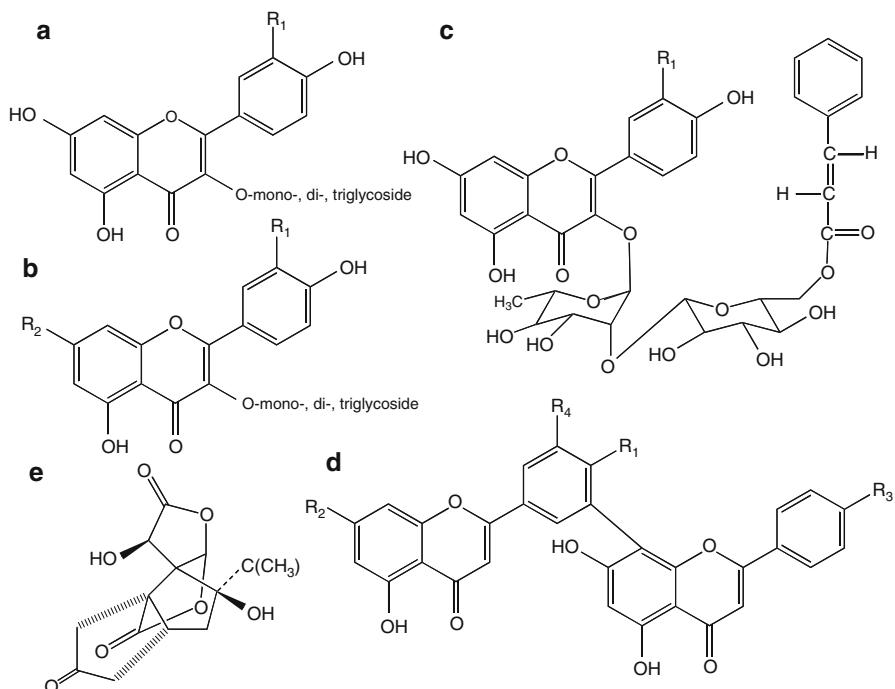


Fig. 8.2 Chemical structures of flavonoids glycosides and bilabiolide found in *Ginkgo biloba*. Kaempferol derivative, R₁ = (H) (a); apigenin derivative; R₁ = (H) and R₂ (O-glc) (b); quercetin derivative R₁ (OH) (c); ginigetin, R₁ (OCH₃), R₂ (OCH₃), R₃ (OH), R₄ (H) (d); and bilobalide A (e)

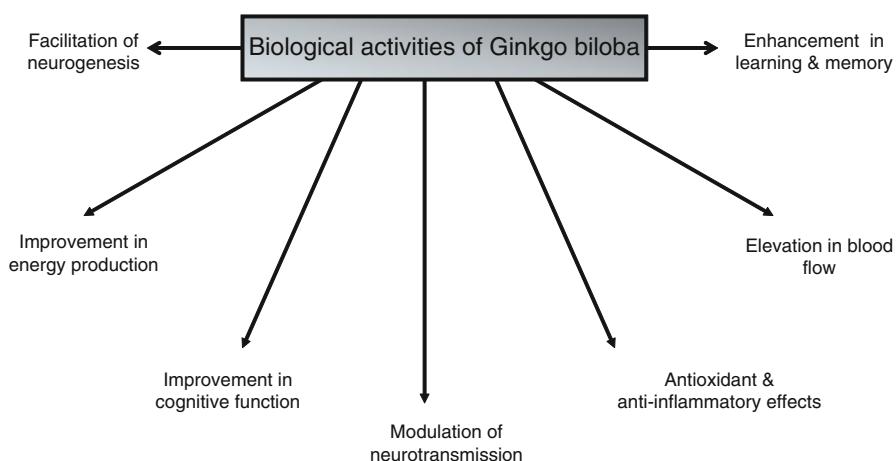


Fig. 8.3 Biological activities of *Ginkgo biloba*

liver where they are metabolized to conjugated derivatives (DeFeudis and Drieu 2000). The bioavailability of EGb 761 has been studied in rats and humans. Oral administration or injections of acute and subacute doses of EGb 761 in rats result in distribution of Ginkgo components in various tissues, and plasma follows linear pharmacokinetics in rat and human (Biber and Koch 1999; Woelkart et al. 2010). In human volunteers, oral intake of EGb 761 (120 mg) results in bilobalide plasma levels of 0.05–0.15 μM (Biber 2003; Woelkart et al. 2010). In rats, oral administration of EGb 761/or pure bilobalide produces dose dependent increases of bilobalide plasma levels from 0.5 to 7.5 μM (Biber 2003; Mauri et al. 2003). Although very little information is available on concentrations of Ginkgo constituents in the brain, it is becoming increasingly evident that components of EGb 761 can cross blood-brain barrier and reach at low micromolar concentrations in the brain. This allows efficient interaction with target molecules such as neurotransmitter receptors. Intraperitoneal injection of bilobalide to healthy mice results in appearance of bilobalide in the striatal dialysate in 10 min. Concentration of bilobalide reached 19 ng/mL (0.92 μM) after 40 min (Lang et al. 2010). The cerebral bioavailability of *G. biloba* extract has been confirmed by electroencephalography (Loew 2002). The oral bioavailability of EGb 761 components can be increased by the administration of phospholipidic complexes (Rossi et al. 2009), which are better absorbed than free EGb 761. In addition to better absorption, phospholipidic complexes provide better results than the conventional EGb 761 extracts (Murray 2003). In brain, EGb 761 exhibits a complex mode of action. It not only inhibits cPLA₂ (Zhao et al. 2011), produces antioxidant and anti-inflammatory effects (Naik et al. 2006; Chu et al. 2011; Lu et al. 2011), modulates gene expression (Sasaki et al. 2002; Smith and Luo 2004; Mahdy et al. 2011), and promotes memory (Mahdy et al. 2011), but also produces multiple effects on mitochondrial function and on the apoptotic pathway (Eckert et al. 2003; Rhein et al. 2010) including the stabilization of mitochondrial membrane potential, improvement of energy metabolism, upregulation of antiapoptotic Bcl-2 protein and downregulation of pro-apoptotic Bax protein, inhibition of cytochrome *c* release, reduction of caspase 9 and caspase 3 activity after oxidative stress, and reduction of apoptotic cell death (Rhein et al. 2010). Thus, EGb 761 has direct effects against apoptotic cell death of neurons and improves neural plasticity as evidenced in vestibular compensation. At the molecular and cellular levels, it is becoming increasingly evident that EGb 761 not only acts as free radical-scavenger and inhibits lipid peroxidation and maintains ATP content by protecting the mitochondrial respiration and preserving oxidative phosphorylation, but also exerts arterial and venous vasoregulator effects involving the release of endothelial factors and the catecholaminergic system (Clostre 1999). In addition, EGb 761 modulates ionic balance in damaged cells and exerts a specific and potent platelet activating factor antagonist activity (Clostre 1999). In visual system, flavonoid glycosides, terpene lactone (ginkgolides), and other organic acids found in EGb 761 promote visual activity by facilitating blood circulation. These constituents dilate blood vessels by increasing the release of endothelium-derived relaxing factor and prostacyclin (PGI₂) from vascular endothelial cells, and decrease blood viscosity by antagonizing platelet activating factor (Park et al. 2011).

8.3 Neurochemical Effects of EGb 761

As mentioned earlier, active compounds of EGb 761 (flavonol glycosides, ginkgolides, bilobalide, and proanthocyanidins) possess potent antioxidant, memory enhancing, anti-inflammatory, and blood flow promoting properties (Fig. 8.3), which play important roles in modulating brain activities, such as cognition, concentration, mental alertness, and decrease in the mental fatigue. Many of these brain activities are mediated by interactions between constituents of EGb 761 (bilobalide) and GABAergic (Huang et al. 2003; Kiewert et al. 2007) and glycinergic (Ivic et al. 2003; Kiewert et al. 2008) receptors that are located on neural cell surface. These receptors play an important role in memory formation, consolidation, and cognition (Ahlemeyer and Kriegstein 2003; Nathan 2000). In addition, EGb 761 enhances cholinergic processes in various cortical regions (Ramassamy et al. 2007; Mahadevan and Park 2008). In vitro, EGb 761 increases acetyl-choline (ACh) release in hippocampal synaptosomes (Kristofikova 1997). EGb 761 also improves performance on behavioral measures of spatial working memory (Rendeiro et al. 2009), attenuates the amnesia induced by scopolamine (Chopin and Briley 1992), and increases the density of hippocampal muscarinic receptors (Taylor 1986).

EGb 761 exerts its beneficial effects on endothelial dysfunction by enhancing the production of endothelial nitric oxide. The putative mechanism for this effect is suggested to be increased endothelial nitric oxide synthase (eNOS) promoter activity and eNOS expression in vitro (Koltermann et al. 2007). Collectively, these studies support the view that psychological and physiological benefits of EGb 761 are based on its primary action of regulating neurotransmitters, neurotransmitter receptors, and retardation of apoptosis. EGb 761 also benefits vascular microcirculation by improving blood flow in small vessels. Although there have been conflicting evidence about the benefits of Ginkgo, the number of studies supporting its beneficial role in brain tissue is steadily increasing (Birks and Grimley Evan 2009).

8.3.1 EGb 761 and Lipid Peroxidation

Reactive oxygen species (ROS), such as superoxide anions (O_2^-), hydroxyl ($\cdot OH$), alkoxyl ($RO\cdot$), and peroxy radicals ($ROO\cdot$), and hydrogen peroxide (H_2O_2) are constantly generated in neural cells as unwanted by-products of aerobic metabolism (Farooqui 2012). Although low physiological levels of ROS are needed for signal transduction processes and scavenged efficiently by the cellular antioxidant defense system, cell is unable to detoxify high ROS levels. This leads to an imbalance between ROS generation and the cellular antioxidant capacity producing the “oxidative stress.” Endogenous antioxidants (glutathione and lipoic acid) are the first line of defense followed by antioxidant enzymes. The antioxidant enzymes are not only involved in deactivation of ROS, but also detoxification of electrophiles (Liu et al. 2008a; Farooqui 2012). Because of their above-mentioned dual roles, antioxidant enzymes are not unequivocally distinguishable from phase-2 detoxification

enzymes, and vice versa (Liu et al. 2008a). Although antioxidant or detoxifying enzymes are rapidly induced in response to oxidative or electrophilic stress, such adaptive survival response is normally transient and prone to be overwhelmed by excess amount of ROS or electrophiles.

The antioxidant effect of the flavonoid fraction of EGb 761 may be either mediated through direct attenuation of O_2^- , $\cdot OH$, RO^\bullet , and ROO^\bullet , H_2O_2 , and nitric oxide radical (NO^\bullet) (Smith and Luo 2003, 2004) and chelation of prooxidant transitional metal ions (Gohil and Packer 2002) or caused by the expression of antioxidant proteins such as superoxide dismutase (SOD) and increase in antioxidant metabolites such as glutathione (Gohil and Packer 2002; Oken et al. 1998). Additionally, the phenolic hydroxyl groups of EGb 761 flavonoids and organic acids may chelate prooxidant transition heavy metal ions, such as Fe^{2+} and Cu^{2+} (Gohil and Packer 2002), and consequently preventing the formation of hydroxyl radicals (Ni et al. 1996; Zimmermann et al. 2002). The flavonoid fraction of EGb 761 extract appears to be more effective against hydroxyl radicals than the terpene fraction (Bastianetto et al. 2000; Zimmermann et al. 2002). Real time PCR studies indicate that EGb 761 elevates glutathione levels by inducing active heavy subunit of glutamate cysteine ligase (GCLC) in Hepa1c1c7 cells (Liu et al. 2008b). Collective evidence suggests that EGb 761 modulates neurotransmission, induces active heavy subunit of glutamate cysteine ligase (GCLC), elevates reduced glutathione levels, inhibits apoptotic cell death, stimulates energy metabolism of neurons, and stimulates cognitive processes in the brain (Liu et al. 2008b).

8.3.2 EGb 761 and Transcription Factors

Transcription factors interact with specific DNA sequences and thereby control the transfer of genetic information from DNA to mRNA. Transcription factors perform their function alone or with other proteins in a complex, by activating (activator) or inhibiting (repressor) the recruitment of RNA polymerase to specific genes. Examples of major transcription factors are NF- κ B, TLR, and CREB. NF- κ B is an important nuclear transcription factor, which initiates transcription of genes associated with inflammation in neurotraumatic and neurodegenerative diseases (Farooqui 2010). It controls the expression of numerous genes that modulate the immune and stress responses, onset and the resolution of inflammation, cell adhesion, calcium homeostasis, maintenance of intercellular communications, regulation of cellular proliferation, and protection against apoptosis (Mattson and Meffert 2006). Activation of NF- κ B occurs within minutes and persists for at least a week in response to neurotraumatic injuries. Its activation is associated with the expression of selected target genes such as TNF- α , IL-1 β , and IL-6 (Zhao et al. 2007). Inhibition of NF- κ B activity has been shown to have a therapeutic effect in neurotraumatic injuries, such as stroke, spinal cord injury, and traumatic brain injury (Farooqui 2010). TLRs are another family of signal transduction molecules and play a critical role in the induction of innate and adaptive immunity (Aderem and Ulevitch 2000). TLR-mediated signaling pathways mainly stimulate the activation of NF- κ B

(Hoshino et al. 2002). TLR4 contribute to the pathogenesis of stroke-mediated brain injury (Teng et al. 2009). Activation of TLR4 stimulates inhibitor of kappa B phosphorylation and degradation, resulting in nuclear translocation of NF-κB, which initiates transcription of genes associated with innate immune responses and inflammation (Anderson 2000).

cAMP Responsive Element Binding Protein (CREB) and its family members—the Activating Transcription Factor (ATF) and cAMP Response Element Modulator (CREM)—belong to basic/leucine zipper (bZIP) class of transcription factors. CREB is phosphorylated by several different protein kinases, including (Protein Kinase-A), PKC (Protein Kinase-C), CSNK (Casein Kinases), CaMKs (Calmodulin Kinases), and GSK3 (Glycogen Synthase Kinase-3). Phosphorylation by these kinases can either increase or decrease the activity of CREB. Ser133 phosphorylation of CREB is modulated by growth factors, neurotransmitter or hormone action on G-protein-coupled receptors (GPCR), or by Neurotrophin effects on Receptor Tyrosine Kinases (RTKs) (Wu et al. 2001). This activation of transcription factor contributes to neural cell survival, synaptic plasticity, and memory formation. Deletion of CREB and CREM in neurons of the developing brain is associated with apoptosis and postnatal ablation of these genes resulting in neuronal degeneration in adulthood. Adult striatal and hippocampal neurons are particularly vulnerable to CREB/CREM deficiency (Johannessen et al. 2004).

G. biloba extracts such as EGb-761 produce beneficial effects on the brain function, from enhancing cognitive function in dementia to facilitate recovery from acute forms of neural damage (Clostre 1999; Cho et al. 2009; Ahlemeyer and Kriegstein 2003). It is proposed that *G. biloba* extracts inhibit TLR4/NF-κB-dependent inflammatory responses as well as prevent neuronal cell apoptosis after neurotraumatic injury and in neurodegenerative diseases.

8.3.3 EGb 761 and Gene Expression

EGb 761 has been shown to modulate many genes associated with glucose and fatty acid metabolism (Bidon et al. 2009). Thus, it downregulates the transcripts for the fatty acid synthase (*Fasn*), an enzyme responsible for fatty acid synthesis, the acetyl-Coenzyme A acyltransferase2 (*Acaa2*), an enzyme involved in cholesterol and lipoprotein synthesis and fatty acid elongation, and hexokinase 1 (*Hk1*) and the phosphofructokinase 1 (*Pfk1*), enzymes associated with glucose metabolism. In contrast, EGb 761 treatment upregulates the transcripts for the pyruvate dehydrogenase kinase 4 (*Pdk4*) and fatty acid translocase (*Cd36*), enzymes involved in lipid metabolism and transport, respectively, along with the upregulation of genes for uncoupling proteins 1 and 3 (*Ucp1* and *Ucp3*), relevant for energy production (Bidon et al. 2009). Studies on the oral administration of EGb 761 for 5 days in developing fetal brain indicate that EGb 761 alters the expression of 187 genes in the hippocampi of male fetuses and 160 genes in those of female fetuses (Li et al. 2003). Among these clusters, 35 genes share a common expression pattern in male and female hippocampal development. These genes include insulin growth factor II,

insulin growth factor binding protein 2, testosterone repressed prostate message-2, glutathione-dependent dehydroascorbate reductase, lipoprotein lipase, guanylate cyclase, and DNA binding protein Brn-2 (Li et al. 2003). EGb 761 also increases expression of two transcription factors, the purinergic region binding protein α , (Pur- α ; fivefold), and nuclear I/X or NfiX protein (sevenfold). Although very little is known about the roles of these proteins in the brain, it is reported that Pur- α not only binds both single-stranded DNA and RNA, but also regulates the transcription by RNA polymerases of Pol II class and some Pol III class enzymes. These proteins have been shown to attenuate transcription of a variety of mRNAs expressed in neural cells. For example, Fe65, an adaptor protein that interacts with Alzheimer β -amyloid precursor protein (APP), is also transcriptionally regulated by Pur- α (Zambrano et al. 1997; Watanabe et al. 2001). Alterations in these genes have been confirmed by real-time quantitative polymerase chain reaction. In addition, EGb 761 also modulates growth-associated protein-43 (GAP-43), cyclic AMP response element-binding protein-1 (CREB-1), and glial fibrillary acidic protein (GFAP) expression in the prefrontal cortex, amygdala, and hippocampus (Oliveira et al. 2009). Thus, treatment with acute concentrations of EGb 761 results in underexpression of GAP-43 in several structures of brain and overexpression of GFAP in the amygdala and hippocampus compared to that of the control group. Treatment with subacute concentrations of EGb 761 produces decrease in GAP-43 expression in prefrontal cortex and hippocampus. Subacute treatment with EGb 761 also leads to a decrease in CREB-1 in the medial prefrontal cortex and increase in the hippocampus. CREB is a prosurvival transcription factor responsible for expressions of a large number of genes (Soule et al. 2006; Johannessen et al. 2004), such as brain-derived neurotrophic factor (BDNF) that is essential for neuronal activities, such as neural development, neural cell survival, morphogenesis, plasticity, and learning and memory (Numakawa et al. 2010). Although the molecular mechanism associated with EGb 761-mediated processes is not fully understood, wild-type neuroblastoma cells in the presence of amyloid beta (A β) show reduction in levels of phosphorylated CREB (pCREB) (Xu et al. 2007). Treatment with EGb 761 leads to increase in pCREB level and enhanced production of BDNF (Fig. 8.4). This increase in EGb 761-mediated CREB phosphorylation can be blocked by inhibitors of several upstream signaling pathways of CREB, including protein kinase C, ERK, ribosomal S6 kinase (RSK), and nitric oxide pathway (Xu et al. 2007) (Fig. 8.5). Moreover, these inhibitors differentially block the effects of individual components of EGb 761, ginkgolide C (BN-52022), quercetin, and bilobalide, which suggest diverse effects of the EGb 761 individual components. These studies support the view that EGb 761 support memory formation and consolidation at the molecular level (Oliveira et al. 2009). EGb 761 increases expression of mitochondrial enzymes like NADH dehydrogenases, which can modulate the ROS generation in the mitochondria. This leads to protection against uncoupling of oxidative phosphorylation and increase in ATP levels regulating energy metabolism (Tendi et al. 2002). EGb 761 inhibits cytokine-mediated endothelial adhesiveness by inducing HO-1 expression via the activation of p38 and Nrf-2 pathways, a mechanism in which oxidative stress is not directly involved. However, these processes produce antioxidant effects (Chen et al. 2011). Ginkgo also increases cytochrome *c* oxidase

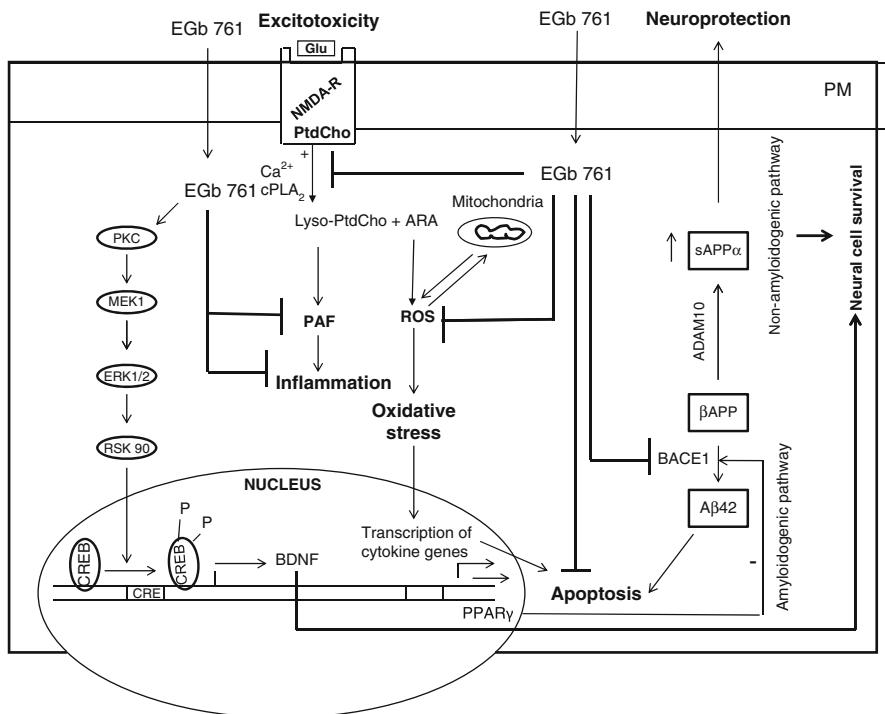


Fig. 8.4 Hypothetical diagram showing effect of EGb 761 on signal transduction processes in the brain. *N*-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); arachidonic acid (ARA); platelet activating factor (PAF); Protein kinase C (PKC); extracellular signal regulated protein kinase (ERK); Ras/Raf/mitogen-activated protein kinase (MEK); protein kinase A (PKA); ribosomal S6 kinase (RSK); AMP response element-binding protein (CREB); phosphorylated CREB (p-CREB); cAMP response element (CRE); brain-derived neurotrophic factor (BDNF); β -amyloid precursor protein (β APP); soluble amyloid precursor protein (sAPP); alpha-secretase (ADAM10); and β -secretase (BACE1 or beta-site APP cleaving enzyme). The symbols (+) indicate stimulation; and (−) (blocked arrow) represents inhibition

subunit I via upregulation of the gene and protein expression of its mitochondrial DNA-coded subunits, and modulation of cytochrome c oxidase subunit I activity by Egb 761 may be associated with its protective effects on mitochondrial function. Collectively, these studies are consistent with the suggestion that the EGb 761 modifies the mitochondrial functions in visceral and neuronal tissues by coordinating the expression of genes that enhance mitochondrial energy production by increasing lipid β oxidation and inhibiting glucose oxidation (Bidon et al. 2009).

8.3.4 EGb 761 and Mitochondrial Dysfunction

Mitochondrial dysfunction plays an important role in neurodegeneration in neurotraumatic and neurodegenerative diseases (Farooqui 2010). A decrease in

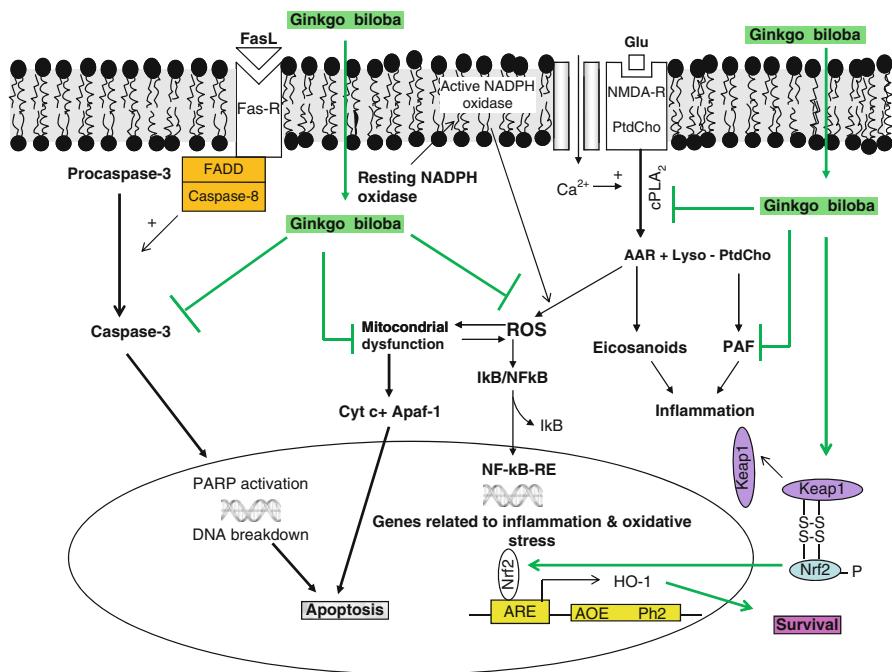


Fig. 8.5 Hypothetical diagram showing effect of EGb 761 on cPLA₂, mitochondrial dysfunction, Nrf2, and caspase-3 in the brain. N-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor- κ B (NF- κ B); nuclear factor- κ B response element (NF- κ B-RE); cytochrome c (Cyt c); apoptosome complex with apoptosis activating factor-1 (Apaf-1); poly(ADP)ribose polymerase (PARP); Kelch-like ECH-associated protein 1 (Keap1); and nuclear factor-erythroid-2-related factor 2 (Nrf2); and \neg (blocked arrow) represents inhibition

mitochondrial energy charge and redox state, loss of transmembrane potential (depolarization), mitochondrial respiratory chain impairment, and release of substances such as calcium and cytochrome *c* are closely associated with apoptotic cell death (Fig. 8.5). In addition, NADPH oxidase, which is localized in cytoplasm migrates to the plasma membrane and facilitates the production of more ROS leading to mitochondrial abnormalities that contribute to the pathogenesis of neurotraumatic and neurodegenerative diseases (Farooqui 2010). Mitochondria are not only involved in the production of ATP as the central source of cellular energy, but also are critical regulators of programmed cell death during aging (Sastre et al. 2000; Balaban et al. 2005). Mitochondrial function becomes less efficient during brain aging due to decrease in activities of respiratory chain components, such as complex I and to a lesser content of complex IV, which in turn causes enhancement in ROS formation, reduction in Ca²⁺ buffering capacity, and accumulation of mitochondrial DNA (mtDNA) mutations (Mattson 2007; Mattson et al. 2008). *Ginkgo biloba* extract (EGb 761) not only inhibits cPLA₂, the enzyme that releases arachidonic

acid from neural membrane phospholipid (Zhao et al. 2011), but also protects against ROS production and also retards age-associated oxidative damage to mtDNA and the oxidation of mitochondrial glutathione (Mattson 2007). In addition, EGb 761 extract also prevents changes in mitochondrial morphology, and function associated with aging of the brain and liver, and inhibits the activation of caspase-3 (Sastre et al. 2000).

8.3.5 EGb 761 and Memory Formation and Consolidation

Hippocampus plays an important role in the formation and consolidation of episodic and declarative memories (Scoville and Milner 2000; Squire and Zola-Morgan 1991). Changes in the efficacy and structural plasticity of synaptic connections in the hippocampus represent neurobiological mechanism underlying crucial aspect of cognition (memory encoding and storage) (Martin et al. 2000). EGb761 alters neuronal excitability, synaptic efficacy, and plasticity in the hippocampus of aged mice, which may be an appropriate explanation for its age-related effects on memory enhancement (Williams et al. 2004). Serotonergic system and 5-HT neurons in the hippocampus and amygdala play an important role in regulating emotional behavior, cognition, anxiety, memory formation, and circadian rhythm. Reduction in 5-HT turnover is consistently associated with impaired long-term memory functioning and cognitive flexibility (Mössner et al. 2000; Nishimura et al. 1995; Huguet et al. 1994). Treatment with EGb 761 significantly modifies 5-HT concentration and 5-HT turnover ratio in the prefrontal cortex and hippocampus, structures that are critically involved in spatial memory and behavioral flexibility (Blecharz-Klin et al. 2009). The effect of EGb 761 on spatial navigation in rats may be a consequence of an enhanced 5-HT neurotransmission in brain structures. This suggestion is supported by studies in which EGb 761-induced stimulation of serotonergic neurotransmission is significantly antagonized by the selective 5-HT1A antagonist WAY-100635, but is unaffected by the 5-HT2 antagonist pirenperone (Winter and Timineri 1999). This explanation provides a possible basis for the direct effect on memory and learning (Winter and Timineri 1999). Choline uptake is also upregulated in EGb761-treated hippocampal synaptosomes (Kristofíková and Klaschka 1997). Choline is a precursor for biosynthesis of the neurotransmitter acetylcholine, which plays a crucial role in memory and learning processes. Loss of basal forebrain cholinergic neurons has been directly related to Alzheimer disease (AD) (Bartus et al. 1982), suggesting that EGb 761-mediated stimulation of cholinergic neurons may support and contribute to the memory formation.

The ability of EGb761 to modulate several neurotransmitter systems (serotonergic, noradrenergic, cholinergic, dopaminergic, glutamatergic) and to enhance the acquisition and retention of memory has also been observed in several animal studies (Clostre 1999). Collectively, these studies indicate that the potential cognitive/nootropic activity enhancing and antioxidant properties of EGb 761 may not only be attributed to their elevating effect on the activity of antioxidant enzymes in the

hippocampus region, but also on modulation of synaptic efficacy and plasticity. Thus, accumulating evidence suggests that EGb 761 contains potent antioxidants, which are capable of scavenging free radicals, inhibiting nitric oxide synthesis, reducing lipid peroxidation, and protecting neural cells against apoptotic cell death (Clostre 1999).

8.3.6 EGb 761 and Antiapoptotic Effects

Apoptotic cell death is closely associated with the pathogenesis of neurodegenerative diseases (Farooqui 2009). It is reported that multiple mechanisms may contribute to the antiapoptotic effects of EGb761. Thus, EGb 761 facilitates the maintenance of the integrity of the mitochondrial membrane, prevents cytochrome *c* release from the mitochondria, and prevents the formation of the apoptosome and the apoptotic caspase cascade. EGb 761 also enhances the transcription of antiapoptotic Bcl-2-like protein, attenuates the transcription of pro-apoptotic caspase-12, and inactivates pro-apoptotic *c-Jun* N-terminal kinase (JNK). These processes not only lead to the “turning off” downstream target *c-Jun*, but also retarding the cleavage of caspase-3, resulting in the prevention of nuclear DNA fragmentation (Shi et al. 2009, 2010; Smith and Luo 2004). It is suggested that polyphenols present in EGb 761 may partly contribute to antiapoptotic properties. It is also reported that antiapoptotic effects of EGb 761 polyphenols may be associated with the modulation of specific proteins central to intracellular apoptotic signaling cascades such as the mitogen-activated protein kinase (MAPK) cascade. Thus, quercetin, one of the major phenolic constituents of EGb761 has no effect on JNK activity and apoptosis mediated by hydrogen peroxide and 4-hydroxy-2-nonenal (Uchida et al. 1999; Spencer et al. 2003; Shi et al. 2010). It is suggested that quercetin exerts its antiapoptotic effects not only by inactivating the peroxide-mediated JNK-*c-Jun*/AP-1 pathway and extracellular signal-regulated kinase (ERK)-*c-Fos*/AP-1 pathway [53,54], but also by promoting cellular survival through the activation of the MAPK pathway (ERK2, JNK1, and p38), leading to expression of downstream survival genes (*c-Fos*, *c-Jun*) and defensive genes (phase II detoxifying enzymes; GSH S-transferase, quinone reductase) (Kong et al. 2000). In addition, antiapoptotic effect of Egb 761 can also be produced by Bilobalide, ginkgolide B, and ginkgolide J. Although the molecular mechanisms associated with antiapoptotic effects of Bilobalide, ginkgolide B, and ginkgolide J are not fully understood, it is suggested that bilobalide can block neuronal apoptosis in the early stages by attenuating the elevations of c-myc, p53, and Bax and suppressing the activation of caspase-3 (Defeudis 2002; Shi et al. 2010).

8.3.7 EGb 761 and Thickening of Aorta

Elevated levels of homocysteine (Hcy) have been recognized as an independent risk factor for atherosclerosis leading to cardiovascular diseases. Plasma Hcy levels not

only have been reported to contribute to the thickening of intimal-media carotid artery wall (Malinow et al. 1993), but also are the risk of restenosis after percutaneous angioplasty (Morita et al. 2000). In animals hyperhomocysteinemia (Hhcy) exacerbates neointima formation after balloon injury (Morita et al. 2001). *Ginkgo biloba* extracts (EGb 761) have been reported to attenuate Hcy-induced endothelial dysfunction (Zhou et al. 2006) and downregulate the expression of cell adhesion molecules by Hhcy (Li and Peng 2004). Based on these findings it is hypothesized that EGb 761 supplementation may inhibit the intimal thickening of abdominal aorta in rabbits with high Hhcy after balloon injury through the regulation of migration and proliferation of VSMCs. Detailed investigations have revealed that EGb 761 decreases the neointima area and the ratio of the neointima area to the media area, downregulates the mRNA expression of matrix metalloproteinase-9 (MMP-9), and upregulates the protein expression of p21 WAF1/CIP1 (p21), supporting the view that EGb 761 can reverse the Hhcy-induced neointima formation in rabbits following balloon injury, and the suppressive effect of EGb 761 on the migration and proliferation of vascular smooth muscle cells (VSMCs) may be involved in these actions (Liu et al. 2008c).

8.4 EGb 761 and Neurological Disorders

Neurological disorders include neurotraumatic (stroke, traumatic brain injury, and spinal cord injury), neurodegenerative (Alzheimer disease, Parkinson disease, and Huntington disease), and neuropsychiatric (anxiety and depression) diseases. Most of above-mentioned neurological disorders are accompanied by marked increase in degradation of phospholipids, sphingolipids, and cholesterol along with the activation of phospholipases A₂, sphingomyelinases, and cholesterol hydroxylases; elevation in generation of phospholipid-, sphingolipid-, and cholesterol-derived lipid mediators; and increased production of eicosanoids, ROS, and lipid hydroperoxides. These metabolites induce oxidative stress and neuroinflammation (Farooqui and Horrocks 2007; Farooqui 2009, 2010). Accumulating evidence suggests that the above-mentioned neurological disorders are multifactorial dominant medical problems of aging populations, and development of effective strategies for their prevention or retardation is a critical issue for neuroscience research (Farooqui 2010). The lifelong cumulative effects of oxidative stress, toxicities of electrophiles, and inflammation are largely responsible for the pathogenesis of age-related neurological disorders. Brain tissue, which is low in antioxidant activity, has evolved an elaborate and functionally overlapping, highly inducible network of “phase 2” genes for its protection (Holtzman et al. 2004). The upregulation of phase 2 genes and their translated proteins (hemeoxygenase and NAD(P)H:quinone oxidoreductase) protects against damaging effects of oxygen- and nitrogen-based oxidants and other electrophiles (Dinkova-Kostova et al. 2005). As stated earlier, regulation of phase 2 genes involves the cytoplasmic repressor Keap1, which

Table 8.1 Effect of EGb 761 on neurological disorders

Disorder	Effect	References
Hypoxia	Beneficial	Karcher et al. (1984)
Ischemia	Beneficial	Pierre et al. (1999)
TBI	Beneficial	Attella et al. (1989), Menkü et al. (2003)
SCI	Beneficial	Ao et al. (2006)
AD (animal model)	Beneficial	Augustin et al. (2009)
PD (animal model)	Beneficial	Rojas et al. (2008)
HD (animal model)	Beneficial	Mahdy et al. (2011)
Anxiety	Beneficial	Sarris et al. (2011)
Depression	Beneficial	Hou et al. (2010)
Headaches	Beneficial	Usai et al. (2011)

Traumatic brain injury (TBI); spinal cord injury (SCI); Alzheimer disease (AD); Parkinson disease (PD); Huntington disease (HD)

contains cysteine residues that are the sensors of inducers. Under basal conditions, Keap1 binds and retains Nrf2 in the cytoplasm and targets it for ubiquitination and proteasomal degradation. Interactions with ROS modify specific cysteine residues of Keap1, which loses the ability to repress Nrf2 and migrates to the nucleus. In the nucleus Nrf2 binds to the antioxidant response elements (ARE) of phase 2 genes and activates their transcription (Dinkova-Kostova et al. 2005).

EGb contains flavonoids, ginkgolides, and bilobalide. It protects the brain against oxidative damage by blocking the formation of ROS in cerebellar neurons. Although, the detailed molecular mechanism by which EGb 761 provides neuroprotection and improves memory is not fully understood, but it is becoming increasingly evident that EGb 761 acts through multiple mechanisms. Thus, EGb 761 enhances cerebral glucose utilization as well as respiratory control ratio of mitochondria by protecting against uncoupling of oxidative phosphorylation. This increases ATP levels in the brain. Acute administration of EGb 761 reduces stress-mediated increases in whole brain levels of catecholamines and serotonin (5-HT) in rats (Shah et al. 2003). Similarly, chronic administration of EGb 761 enhances copulatory behavior and reduces serum prolactin levels in male rats, suggesting involvement of the dopaminergic system in the effects of EGb 761 (Yeh et al. 2008). These studies support the view that there may be a causal link between the central protective effects of Ginkgo extract and monoaminergic neurotransmission. The active constituents of EGb 761 are primarily flavonol glycosides and terpene compounds such as ginkgolides and bilobalide with smaller amounts of proanthocyanidins (DeFeudis and Drieu 2000). These constituents have been shown to possess potent free radical scavenging (antioxidant) and anti-inflammatory properties that may play an important role in memory boosting and neuroprotective effects of EGb 761 (Table 8.1) (Ahlemeyer and Kriegstein 2003) after neurotraumatic, neurodegenerative, and neuropsychiatric injuries. EGb 761 also influences a number of neurotransmitter systems that are considered critical in cognition and memory formation (Nathan 2000). In particular, EGb 761 enhances serotonergic, glutamatergic, and cholinergic receptor-mediated processes in various cortical and hippocampal regions (Mahadevan and Park 2008).

8.4.1 *Egb 761 and Stroke*

Stroke is the second leading cause of death in the world. It not only places a large burden on social service resources, but also is a leading cause of long-term disability in adults, which has a mortality rate of around 30 %. Stroke is accompanied by deprivation of oxygen, release of free fatty acids from neural membrane phospholipids, activation of phospholipases A₂, massive influx of calcium, excitotoxic glutamate efflux (neuronal component), and mitochondrial dysfunction along with depletion in ATP levels and accumulation of ROS in the brain (Farooqui 2010). Stroke injury also involves downstream endothelial responses, and blood–brain barrier (BBB) disruption, resulting in increased vascular-derived substances into the brain. These processes eventually lead to neural cell death through necrosis and apoptosis. EGb 761 treatment produces beneficial effects against ischemia/reperfusion injury. Thus, administration of EGb 761 and its constituents (bilobalides) 60 min before ischemic injury dose-dependently reduces the infarct area in mouse brain and the infarct volume in rat brain 2 days after the onset of the injury. Furthermore, 30 min of pretreatment with ginkgolide A and ginkgolide B also reduces the infarct area in the mouse model of focal ischemia. In addition, Ginkgolide B increases BBB permeability after ischemic injury both *in vivo* and *in vitro* (Fang et al. 2010). Concentration of Ginkgolide B in brain is also improved after middle cerebral artery occlusion. Ginkgolide B and bilobalide prevent glutamate-mediated neuronal damage, neuroinflammation, and apoptotic cell death hippocampal neuronal and astrocytic cultures (Urfková et al. 2006; Fang et al. 2010), supporting the view that constituents of EGb 761 induce their effects by inhibiting excitotoxicity, oxidative stress, and inflammation-mediated processes.

Although the mechanism of action of EGb761 in ischemia-induced brain injury is not known, it is reported that EGb 761 upregulates the expression of HO-1 in a dose- and time-dependent manner leading to neuroprotection and neuronal survival (Fig. 8.5). Additionally, EGb761 induces phase 2 genes through the Nrf2-antioxidant/electrophilic response element (ARE) signaling pathway and of known proteins, such as HO-1 that has the most ARE elements within its promoter region, making it a unique target for this EGb761-stimulated endogenous pathway (Zhuang et al. 2002; Liu et al. 2007). Some reports suggest that EGb 761 prevents ischemic injury-mediated neural cell death not only by preventing the injury-induced decrease of Akt phosphorylation (Cho et al. 2009), but also through the induction of HO-1 in ischemia/reperfusion brain injury (Zhuang et al. 2002; Saleem et al. 2008). In addition, EGb 761 promotes increase in cerebral blood flow, decreases blood pressure, mediates strong antithrombotic effects (Kriegstein et al. 1986; Ahlemeyer and Kriegstein 2003; Sasaki et al. 2002), improves memory impaired by ischemia (Tadano et al. 1998; Ahlemeyer and Kriegstein 2003), and protects cerebral function (Ma et al. 1999). EGb 761 not only retards the coagulation of platelets to inhibit thrombosis by antagonizing the platelet activating factor (Peng et al. 2003), but is capable of inhibiting the adhesion of monocytes and neutrophils to cultured cerebral microvascular endothelial cells (Xu et al. 1999). Collectively, these studies support

the view that EGb 761 can be used for the treatment of postischemic brain injury in animals. Although short-term human studies have provided some positive results, long-term and double blind studies on large human population have not been performed. So the use of EGb 761 for the treatment of stroke remains controversial.

8.4.2 EGb 761 and Alzheimer Disease

Alzheimer disease (AD) is an age-related progressive neurodegenerative disease that impairs the memory and intellectual abilities of the patient. The number of AD patients may reach 16 million by 2050 with the economic cost of its treatment reaching as high as 80–100 billion dollars.

Loss of synapse and memory impairment are an early and principal features of this disease. AD largely affects the basal forebrain cholinergic neuronal population and at advance stage produces massive neuronal loss in the cortex and hippocampus (Farooqui 2010). Extracellular as well as intracellular deposition of beta-amyloid (A β) peptide, intracellular formation of neurofibrillary tangles that are enriched in hyperphosphorylated tau protein, and neuronal loss are the neuropathological features of AD. AD is also accompanied by the activation of phospholipases A₂, sphingomyelinase, increased degradation of neural membrane phospholipid and sphingolipid, mitochondrial dysfunction, and deposition of A β along with depletion in ATP levels and accumulation of ROS in the brain (Farooqui 2010). Amyloid peptide accumulation interferes with the phosphorylation of cAMP-response element-binding protein (CREB) (Tong et al. 2001). High levels of intracellular amyloid peptide lead to persistent CREB hyperphosphorylation and block its translocation to the nucleus resulting in inhibition of cyclic AMP-response (CRE) directed gene expression (Arvanitis et al. 2007). Based on these results, it is suggested that inhibition of CREB translocation causes early synaptic dysfunction prior to the extracellular accumulation of amyloid peptide (Arvanitis et al. 2007).

In animal models of AD and in some human clinical trials of AD patients, EGb 761 produces beneficial effects by reducing cognitive dysfunction and age-associated memory impairment and dementia. Although the molecular mechanism associated with beneficial effects of EGb 761 is illusive, recent studies indicate that EGb 761 enhances the activity of α -secretase, which attacks APP inside the A β sequence, and therefore prevents formation of neurotoxic A β . After cleavage by α -secretase, the soluble N-terminal domain of APP (sAPP α), which possesses neurotrophic and neuroprotective properties, is released (Figs. 8.4 and 8.6) (Colciaghi et al. 2004; Postina 2008). In addition, EGb 761 component (bilobalide) may induce a decrease in A β levels as a downstream target of the activated PtdIns 3K pathway (Shi et al. 2009, 2011). Bilobalide has no significant effects on β -site of APP cleaving enzyme 1 (BACE-1) or γ -secretase but inhibits the β -secretase activity of cathepsin B, suggesting that bilobalide-induced A β reduction is probably mediated through modulation of cathepsin B rather than BACE-1. Similarly, inhibition of GSK3 β does not produce BACE-1 activity but decreases cathepsin B activity, supporting the view

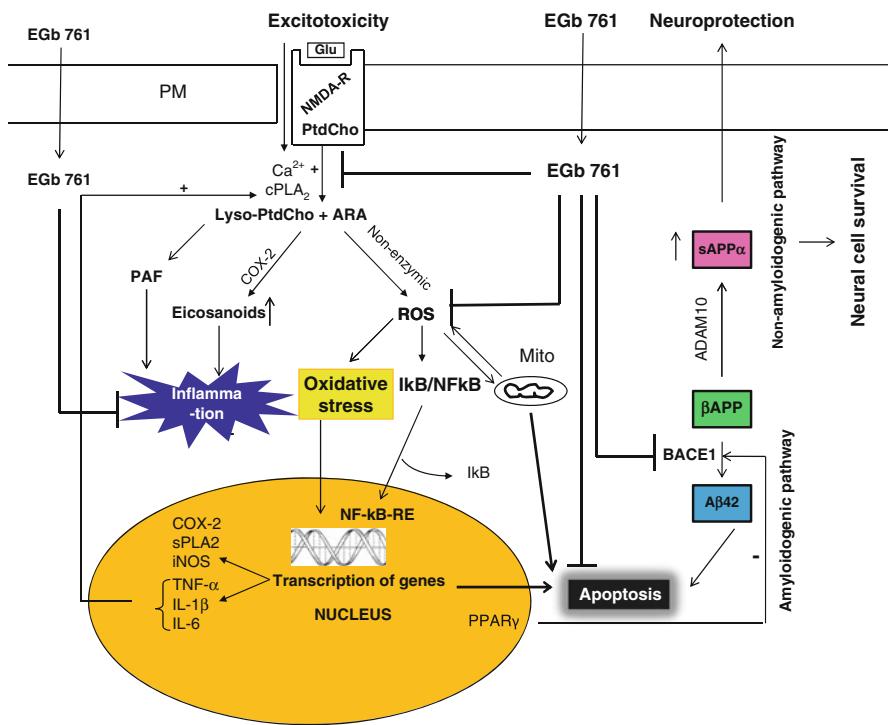


Fig. 8.6 Hypothetical diagram showing effect of EGb 761 on cPLA₂, ROS, inflammation, and APP processing in the brain. Plasma membrane (PM); *N*-Methyl-d-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (Lyso-PtdCho); arachidonic acid (ARA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor-κB (NF-κB); nuclear factor-κB response element (NF-κB-RE); β-amyloid precursor protein (βAPP); soluble amyloid precursor protein (sAPP); alpha-secretase (ADAM10); and β-secretase (BACE1 or beta-site APP cleaving enzyme). The symbols (+) indicate stimulation; and (−) (blocked arrow) represents inhibition

that the PtdIns 3K-GSK3 β pathway is probably associated with bilobalide-induced A β reduction (Shi et al. 2009, 2010, 2011). In contrast, stimulation of α -secretase activities can occur through several signaling cascades including phospholipase C, phosphatidylinositol 3-kinase, serine/threonine-specific kinases such as protein kinases C, and mitogen-activated protein kinases. Direct activation of protein kinase C and stimulation of distinct G protein-coupled receptors are known to increase α -secretase processing of APP (Postina 2008; Shi et al. 2011). Thus, long-term treatment (16 months) with EGb761 significantly lowers human APP protein levels by approximately 50 % as compared to that of controls in the cortex but not in the hippocampus. EGb 761 treatment in young mice has no effect on APP levels, indicating that APP may be an important molecular target of EGb761 in relation to the duration of the EGb 761 treatment and/or the age of the animals (Augustin et al. 2009).

A β -induced neurotoxicity in PC12 cells results in the generation of free radicals and treatment with EGb 761 prevents A β -peptide-induced free radical production, increases glucose uptake, apoptosis, and cell death, in a dose-dependent manner. It is proposed that EGb acts as a protective agent against amyloid fibril formation and this process may involve MAP-kinase cascade, SIRT1, and NF- κ B (Longpre et al. 2006).

Supplementation of EGb 761 also upregulates (>3-fold) the expression of transthyretin in the hippocampus. This protein is associated with the transport of thyroxine and retinol-binding protein in cerebrospinal fluid and serum (Kuchler-Bopp et al. 2000). Thyroid hormones regulate neuronal proliferation and differentiation in discrete regions of the brain during development and are necessary for normal cytoskeletal assembly and stability as well as for neuronal proliferation and outgrowth (Porterfield 2000). In vitro studies have indicated that transthyretin sequesters A β protein and prevent A β aggregation from arising in amyloid formation (Tsuzuki et al. 2000). Levels of transthyretin in cerebrospinal fluid are significantly decreased in AD patients. This may be another mechanism by which EGb 761 may exert its effects on the brain tissue. Collective evidence suggests that EGb761 inhibits the generation of A β from APP and A β aggregation, which are crucial processes related with pathophysiology of AD pathogenesis (Bastianetto et al. 2000).

Energy deficiency and mitochondrial failure are also early events associated with pathogenesis of AD. Chronic exposure of human neuroblastoma cells overexpressing human wild-type APP to A β results not only in activity changes of complexes III and IV of the oxidative phosphorylation system, but also in a reduction in ATP levels which may lead to loss of synapses and neuronal cell death in AD. Treatment of neuroblastoma cells with standardized *G. biloba* extract LI 1370 results in optimal activity of complexes III and IV of the oxidative phosphorylation system as well as restoration of A β -induced mitochondrial dysfunction (Rhein et al. 2010).

8.4.3 EGb 761 and Parkinson Disease

Parkinson disease (PD) is a common neurodegenerative disease caused by the progressive loss of dopaminergic neurons of the substantia nigra in the brain stem and subsequent depletion of dopamine (DA) in the striatum leads to movement impairment (Olanow and Tatton 1999). The major symptoms of PD are rigidity, tremor, and bradykinesia. Although the pathogenesis of PD is not fully understood, several mechanisms responsible for the neurodegeneration in PD have been proposed, including abnormal protein handling, oxidative stress, mitochondrial dysfunction, excitotoxicity, neuroinflammation, and apoptosis (Hirsch and Hunot 2009).

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been used to produce animal model of PD. This neurotoxin produces neurodegeneration in nigral cells with clinical symptoms similar to PD. Its active metabolite MPP $^+$ (1-methyl-4-phenylpyridinium ion) is taken up in dopaminergic terminals by the plasma membrane

Dopamine transporter (Przedborski et al. 2000). MPTP and MPP⁺-induced toxicity is accompanied by oxidative stress through the production of ROS (Przedborski et al. 2000). EGb761 has been shown to retard MPTP-mediated dopaminergic neurotoxicity in rodents. Although the molecular mechanism of EGb 761-mediated neuroprotective effect is not fully understood, it is becoming apparent that EGb 761 acts through multiple mechanisms including antioxidant and anti-inflammatory effects. MPTP administration produces a significant decrease in striatal dopamine levels and tyrosine hydroxylase immunostaining in the striatum and substantia nigra pars compacta (Rojas et al. 2008). Administration of EGb761 in mice significantly attenuates MPTP-induced loss of striatal dopamine levels and tyrosine hydroxylase immunostaining in the striatum and substantia nigra pars compacta. In addition, the neuroprotective effect of EGb761 against MPTP neurotoxicity is accompanied by decrease in lipid peroxidation and reduction of superoxide radical production (Rojas et al. 2008). EGb761 also improves MPTP-mediated impairment of locomotion in a manner that correlates with enhancement of striatal dopamine levels. In addition, *G. biloba* extract inhibit the activity of both forms of monoaminoxidase-A (MAO-A) and monoaminoxidase-B (MAO-B) in rodents (White et al. 1996). The activity of MAO is closely associated with mitochondrial function and production of hydrogen peroxide as a by-product of oxidation of monoamines. It is proposed that neuroprotective effect of *G. biloba* in PD is due to the ability of Ginkgo extracts to stabilize mitochondrial function (Abdel-Kader et al. 2007). The generation of hydroxyl radicals by Fenton reaction induces apoptosis in cerebellar granule cells. EGb 761 produces neuroprotective effects by decreasing Bcl-2, decreasing mRNA level, and increasing the protein levels. This supports the view that total flavonoid components of EGb761 and terpenes protect cerebellar granule cell from oxidative damage and apoptosis induced by hydroxyl radicals. Total terpenes of EGb761 do not protect against apoptosis and flavonoids and terpenes do produce a synergistic effect in this regard (Chen et al. 1999; Xin et al. 2000). Collective evidence suggests that, in mice, EGb761 attenuates MPTP-induced neurodegeneration of the nigrostriatal pathway and that an inhibitory effect against oxidative stress may be partly responsible for its observed neuroprotective effects. Long-term and double blind studies are needed on the use of EGb 761 in large human population to learn about therapeutic efficacy of this agent in PD.

8.4.4 EGb 761 and Huntington Disease

Huntington disease (HD), an autosomal dominant hereditary disease, is caused by a trinucleotide repeat mutation in the huntingtin gene that results in an increased number of glutamine residues in the huntingtin N terminus, which causes abnormal protein aggregation and ultimately neuronal death (Farooqui 2010). The disease is characterized by severe cognitive and personality alterations, which are accompanied by coordination decline and motor deficits that eventually lead to immobility. Major features of HD include reduction of prepulse inhibition (PPI)

of acoustic startle response, locomotor hypoactivity, bilateral striatal lesions, and CNS oxidative stress. At present, there is no cure for HD, and treatments are mainly focused on alleviating cognitive and psychological symptoms.

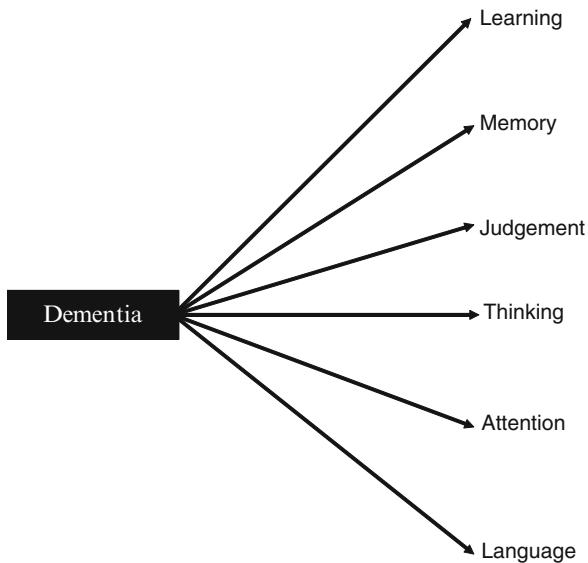
Administration of 3-nitropropionic acid (3-NP) has been used to develop animal models of HD. This toxin irreversibly inhibits succinate dehydrogenase and produces neurodegeneration similar to HD. Administration of EGb 761 not only reduces 3-NP-induced neurobehavioral deficits (Mahdy et al. 2011), but also inhibits 3-NP-mediated increase in striatal malondialdehyde. In addition, 3-NP-mediated increase in expression levels of striatal Bax and Bcl-xL genes and upregulation of striatal glyceraldehyde-3-phosphate dehydrogenase are also retarded by EGb 761 administration (Mahdy et al. 2011). Collectively, these studies suggest that EGb 761 has a neuroprotective role in 3-NP induced model of HD. Double blind and long-term studies in large human population are needed to learn about the therapeutic efficacy of EGb 761.

8.4.5 EGb 761 and Prion Diseases

Prion diseases are fatal neurodegenerative disorders characterized by the accumulation of abnormal isoforms of a host protein known as cellular prion protein (PrP^C), motor dysfunctions, dementia, and neuropathological changes such as spongiosis, astrocytosis, and neuronal loss (Prusiner 2001; Farooqui 2010). The cellular prion protein (PrP^C) is a membrane-bound glycoprotein, which is abundantly expressed in neurons and glial cells within the brain tissue. The role of PrP^C remains elusive. PrP^C binds copper (Viles et al. 2008; Singh et al. 2009) and retards the harmful effect of Cu^{2+} ions through the quenching of free radicals. In addition, PrP^C displays a superoxide dismutase-like activity, which may contribute to protective function against oxidative stress (Rachidi et al. 2005). PrP^C interacts with $\text{A}\beta$ oligomers (Lauren et al. 2009). Anti- PrP^C antibodies inhibit $\text{A}\beta$ oligomer binding to PrP^C and rescue synaptic plasticity in hippocampal slices from oligomeric $\text{A}\beta$ toxicity. Based on this observation, it is proposed that PrP^C mediates $\text{A}\beta$ -oligomer-induced synaptic dysfunction. These results also support the view that there is mechanistic similarities between AD and prion disease (CJD) (Lauren et al. 2009; Nygaard and Strittmatter 2009; Gunther and Strittmatter 2010). The scrapie prion protein (PrP^{Sc}) is a misfolded and altered β -sheet rich isoform of PrP^C formed by posttranslational modification of the PrP^C . Molecular mechanisms involved in conversion of PrP^C into PrP^{Sc} remains unknown. However, recent studies indicate that heparan sulfate stimulates conversion of purified PrP^C into PrP^{Sc} in vitro (Supattapone 2004).

Treatment of neuronal cultures with sPrP106 (a synthetic miniprion) at the nanomolar concentrations induces caspase-3, a biomarker for apoptotic cell death. The presence of ginkgolides, components of EGb 761, not only prevents the activation of apoptotic pathways in neurons, but also reduces microglial killing of neurons damaged by sPrP106 (Bate et al. 2004). These observations support the view that ginkgolides protect neurons from the toxic effects of sPrP106 (Bate et al. 2004).

Fig. 8.7 Effect of dementia on various processes



The ginkgolides also retard PAF-mediated neurotoxicity and reduces the production of prostaglandin E₂ in response to PAF or sPrP106 suggesting that PAF antagonists (the ginkgolides) can be useful in the treatment of prion diseases (Bate et al. 2004).

8.4.6 *EGb 761 and Dementia*

Dementia is a loss of brain function that occurs in certain neurological diseases. It not only includes learning and memory deficits, thinking, language, judgment, and behavior abnormalities, but also involves disturbances in other higher cortical functions preceded by deterioration in emotional control, intellect, and social behavior (Fig. 8.7). The prevalence of dementia increases from 1 % in people age 65–69 years to 30 % in people aged over 90 years, whereas the incidence of dementia increases from 10 cases to 200 cases per 1,000 person years (Lobo et al. 2000). Dementia is accompanied by many neuropsychiatric symptoms. In a large population-based longitudinal study, prevalence of neuropsychiatric symptoms in dementia amounted to up to 87 %, with depression (up to 77 %), apathy (up to 71 %), and anxiety (up to 62 %) being the most frequent single symptoms (Steinberg et al. 2008; Ihl et al. 2010). Similar rates of neuropsychiatric symptoms have been observed in AD and VaD patients (Lyketsos et al. 2000). These symptoms are also present in mild cognitive impairment patients than in healthy elderly subjects (Geda et al. 2008) and may predict future progression to dementia (Edwards et al. 2009).

EGb 761 has been effectively used for the symptomatic treatment of dementia. Daily oral treatment with EGb 761 reduces cognitive dysfunction in an animal

model of vascular dementia in gerbils (Rocher et al. 2011). The molecular basis of EGb 761 effect is not yet fully understood, but there is evidence of neuroprotective properties may involve the inhibition of A β oligomer production and toxicity (Hoyer et al. 1999; Ramassamy et al. 2007). In addition, as mentioned earlier, EGb 761 is an antioxidant that improves mitochondrial dysfunction (Eckert et al. 2003) and induces anti-inflammatory effects. EGb 761 decreases blood viscosity and enhances microperfusion (Költringer et al. 1995). It not only modulates serotonin neurotransmission (Ramassamy et al. 1992), increases dopamine levels in prefrontal cortical areas (Yoshitake et al. 2010), and attenuates activity of hyperactivated hypothalamus–pituitary–adrenal (HPA) axis (Rapin et al. 1993; Jezova et al. 2002), but also improves neuronal insulin sensitivity. These processes may play important roles in pathomechanisms that are common to dementia and behavioral disorders. Although double blind, long-term, and large population studies on the effect of EGb 761 have not been performed, above-mentioned studies support the view that EGb 761 slows the development of dementia in animal models of various neurological disorders.

8.4.7 EGb 761 and Depression

Stress is caused by numerous unexpected environmental, social, or pathological stimuli occurring during the life of animals, including humans, which determine changes in all of their systems. Although acute stress is essential for survival, chronic, long lasting stress can produce detrimental effects. Chronic stress mediates many effects in the brain, such as changes in neuroplasticity of brain structures that become functionally abnormal in major depression. In addition, stress-mediated dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis also contribute to pathophysiology of depression (Pittenger and Duman 2008). Clinical data suggest that a stressful lifestyle can be a risk factor for major depression and AD. Stress also triggers the release of high levels of cortisol and noradrenalin within minutes. Long-term exposure to elevated cortisol and noradrenalin levels affects the hippocampus which plays a central role in the regulation of HPA. The immune system responds not only by enhancing the levels of proinflammatory cytokines, which modulate behavioral changes associated with depression through their effects on neurotransmitters and neuropeptides function, but also by inducing changes in synaptic plasticity and neuroendocrine function (Pittenger and Duman 2008; Farooqui et al. 2012). Oxidative stress plays an important role in depression, animal models for depression-like behavior (Rojas et al. 2011), animal models of AD and, animal models of dementia. Pretreatment of male BALB/c mice with EGb761 daily for 17 days shows that EGb761 significantly decreases the immobility time (39 %) in the forced swimming test. This antidepressant-like effect of EGb761 is associated with a reduction in lipid peroxidation and superoxide radical production. The neuroprotective effect of EGb761 also involves the modulation of serotonergic and dopaminergic neurotransmission at the molecular level (Rojas et al. 2011). It is recently reported that quercetin and kaempferol stimulate depression-related signaling

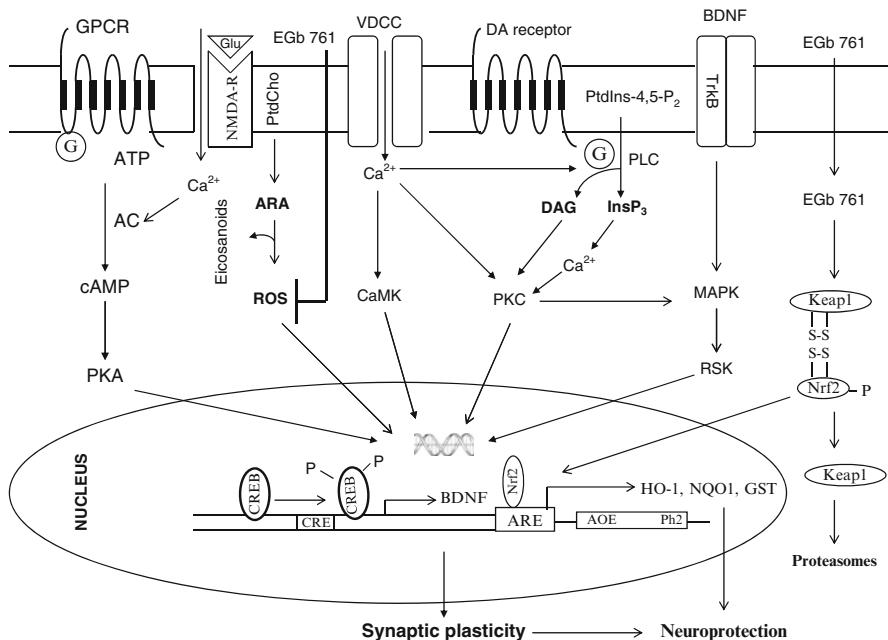


Fig. 8.8 Hypothetical diagram showing effect of EGb 761 on ROS, Nrf2, and APP processing in the brain. Plasma membrane (PM); *N*-Methyl-d-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A₂ (cPLA₂); lysophosphatidylcholine (lyso-PtdCho); arachidonic acid (ARA); platelet activating factor (PAF); phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P₂)phospholipase C (PLC); diacylglycerol (DAG); inositol 1,4,5-trisphosphate(InsP₃); reactive oxygen species (ROS); Kelch-like ECH-associated protein 1 (Keap1); nuclear factor-erythroid-2-related factor 2 (Nrf2); voltage-dependent calcium channels (VDCC); G protein-coupled receptors (GPCR); heme oxygenase (HO-1); NAD(P)H quinone oxidoreductase 1(NQO 1); and glutathione S-transferase (GST); and \perp (blocked arrow) represents inhibition

pathways involving brain-derived neurotrophic factor BDNF/phosphorylation of cyclic AMP response element binding protein CREB/postsynaptic density proteins PSD95 and reduce amyloid-beta peptide (A β) in neurons isolated from double transgenic AD mouse (TgAPPswe/PS1e9) (Fig. 8.8) (Pittenger and Duman 2008; Hou et al. 2010). In addition, enhanced BDNF expression and reduction of A β oligomers have been confirmed in hippocampus of the double transgenic mice administered with flavonol, which correlates with cognitive improvement behaviors in these mice supporting the view that stimulation of CREB and BDNF expression and reduction in A β toxicity by natural flavonols may be the molecular mechanisms involved in the adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of AD (Tchantchou et al. 2007; Xu et al. 2007; Pittenger and Duman 2008; Hou et al. 2010). These studies suggest that flavonols, the major active constituents of EGb 761, may upregulate CREB-BDNF pathway that contributes to its antidepressant activity.

8.4.8 EGb 761 and Attention-Deficit Disorder/Attention-Deficit/Hyperactivity Disorder

Attention-deficit disorder (ADD)/Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood disorder characterized not only by a persistent pattern of impulsiveness, a short attention span, and hyperactivity, but also by restlessness and irritability. ADD interferes especially with academic, occupational, and social performance (Rapport et al. 1994). In approximately half the cases the problem continues into adolescence and adulthood, evident by persisting disorganization and diminishing productivity. Although the molecular mechanism of ADD/ADHD is not fully understood, recent studies indicate that three genes may increase the susceptibility to ADHD. These genes include the D4 dopamine receptor gene, the dopamine transporter gene, and the D₂ dopamine receptor gene (Faraone and Biederman 1998). Studies of environmental adversity implicate the pregnancy and delivery complications, marital distress, family dysfunction, and low social class. The pattern of neuropsychological deficits associated with ADHD children indicate the involvement of working memory. Neuroimaging studies implicate abnormalities in frontosubcortical pathways involving catecholamine in ADHD (Faraone and Biederman 1998). Studies on behavioral measurement using Wender Utah ratings in ADD subjects indicate that *Ginkgo biloba* treatment improves mean scores by decreasing hyperactivity, inattention, and immaturity factors in ADD subjects with minimal side effects (Niederhofer 2010).

8.4.9 EGb 761 and Autism

Autism, a brain developmental disorder, is characterized by abnormal brain development resulting not only in lack of social and verbal and nonverbal communication skills, but also in restricted, repetitive, stereotyped patterns of behavior and impairment in imaginative activity with a markedly restricted repertoire of activities and interests (Amaral et al. 2008). Autism is the fastest growing serious disability in the USA among all the 3–12 year-old children in the country, 1 % children have an autism spectrum disorder. In all, autism now affects 1 in 110 children and 1 in 70 boys. Although the etiology of autism is not fully understood, growing evidence based on sleep functional MRI, diffusion tensor imaging, and proton magnetic resonance spectroscopy imaging suggests that diversity in morphological, functional, genetic, and neurotransmitter systems' alterations may contribute to the etiology of autism (Palmen et al. 2004; Polšek et al. 2011). At the molecular level, it is proposed that autism is associated with glutamate- and GABA-related abnormalities. It is hypothesized that a complex lack of local inhibition and long-distance excitation during brain development and later in life may contribute to the pathogenesis of autism (Polšek et al. 2011). Studies on the treatment of three autistic patients with

2×100 mg EGb 761 for 4 weeks indicate that there is some improvement in the aberrant behavior and symptom of autism (Niederhofer 2009). This is tempting to speculate that more studies are required on the efficacy of EGb 761 for the treatment of autism.

8.4.10 EGb 761 and Migraine Headache

A migraine headache is an intense, throbbing pain caused by pressing of nerves by the swollen blood vessels around the brain. Migraine headache is accompanied by nausea and increased sensitive to light and sound, numbness or tingling in their lips, face, or hands, weakness in their arms or legs, and difficulty focusing. Migraine headache is caused by abnormal brain activity mediated by stress, certain foods, and environmental factors (Wilson 2007). Gingkolide B, a component of EGb 761, is a natural antagonist of PAF. PAF is a strong proinflammatory and nociceptive agent released during inflammatory process in the brain. Gingkolide B inhibits nociception by interacting with PAF. In addition, Gingkolide B modulates the action of glutamate, the main excitatory neurotransmitter of the brain. Migraine headache is also caused by abnormal levels of glutamate in susceptible individuals along with PAF released from platelets and leukocytes during the first phase of migraine. Gingkolide B may inhibit pain by inhibiting cPLA₂ activity (Zhao et al. 2011).

8.5 Side Effects of EGb 761

EGb 761 is generally well tolerated by human (DeFeudis 1991). Sometimes it produces mild side effects, including gastrointestinal complaints, headache, allergic skin reactions, nausea, dizziness, restlessness, heart palpitation, and weakness (Mahadevan and Park 2008; Pittler and Ernst 2000). In addition to these mild effects, some studies indicate Ginkgo may increase the risk of bleeding when coadministered with other antiplatelet and anticoagulant agents (Koch 2005; Ryu et al. 2009). It is suggested that biologically plausible mechanism for an increased risk of bleeding with *G. biloba* may be through interactions with PAF and collagen that lead to decreased platelet aggregation. Although a clear cut cause of *G. biloba* intake and bleeding has not been established, it is proposed that the PAF antagonistic action of ginkgolides may be involved in this process. (Fong and Kinnear 2003; Bent et al. 2005; Yagmur et al. 2005). These side effects have been observed at range 80–160 mg/day in 2 weeks to 2 years time period. Above-mentioned studies indicate that in some individuals *Ginkgo biloba* may produce excessive bleeding and mild to strong seizures. Other side effects are related to headaches, allergic skin reactions, weakness of muscles, and digestive problems.

8.6 Conclusion

Ginkgo biloba extract (EGb761) is a complex mixture of ingredients with broad pharmacological effects on the brain tissue. Its mechanism of action include antagonism of PAF, enhanced constitutive nitric oxide bioavailability leading to increase in the peripheral and cerebral blood flow through nitric oxide-induced vasodilation, modulation of neurotransmitters, scavenging and inhibition of free radical production, along with inhibition of amyloid- β neurotoxicity, protection against oxidative stress and neuroinflammation, and cognitive enhancement in young and old humans. EGb 761 also enhances utilization of glucose and elevates respiratory control ratio of mitochondria by protecting against uncoupling of oxidative phosphorylation. EGb 761 restores the impaired phosphorylation of CREB and prevents the activation of NF- κ B and ERK1/2 pathways. EGb 761 also acts as a membrane stabilizer. These observations indicate that ginkgo extract can produce beneficial effects in neurological disorders.

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Chapter 9

Beneficial Effects of Garlic Components on Neurological Disorders

9.1 Introduction

Garlic is a dietary supplement derived from the bulb of *Allium sativum*, which belongs to the family Liliaceae. It is widely used as a flavoring agent for food and a medicinal agent for the treatment of a variety of diseases (Essman 1984). The chemical composition of garlic is complex and the most important and unique feature of garlic chemical composition is its high content of organosulfur compounds. Two classes of organosulfur compounds, such as (a) γ -glutamylcysteines, and (b) cysteine sulfoxides are found in whole garlic cloves. The γ -glutamylcysteine is hydrolyzed and oxidized to alliin (+S-allyl-L-cysteine sulfoxide). This compound is then converted to allicin (thio-2-propene-1-sulfinic acid S-allyl ester) by alliinase, which is released upon cutting, crushing, or chewing the garlic (Fig. 9.1). Alliinase is a very stable enzyme. Although the exact molecular mechanism of alliinase-catalyzed reaction is not fully understood, but it is suggested that alliinase catalyzes the formation of sulfenic acids from cysteine sulfoxides. Sulfenic acids spontaneously react with each other to form unstable compounds called thiosulfinate. In the case of alliin, the resulting sulfenic acids react with each other to form a thiosulfinate known as allicin (half-life in crushed garlic at 23 °C is 2.5 days). The formation of thiosulfinate is completed within 10–60 s of crushing garlic. The storage of garlic powder preparation up to 5 years shows little loss in ability to generate allicin. Allicin not only interacts with thiol-containing proteins, but also decomposes into 2-propenesulfenic acid (Rabinkov et al. 1998). This compound has ability to bind the free radicals and may be responsible for antioxidant effects of garlic (Vaidya et al. 2009). Whole garlic typically has ~1 % alliin, together with (+)-S-methyl-L-cysteine sulfoxide (methiin). The other components of garlic include (+)-S-(trans-1-propenyl)-L-cysteine sulfoxide, S-(2-Carboxypropyl)-glutathione, γ -glutamyl-S-allyl-L-cysteine, γ -glutamyl-S-(trans-1-propenyl)-L-cysteine, and γ -glutamyl-S-allyl-mercaptop-L-cysteine (Fenwick and Hanley 1985). Storage of garlic bulbs at cool temperatures induces alliin to accumulate naturally. On average, a garlic bulb contains up to 0.9 % γ -glutamylcysteines and up to 1.8 % alliin.

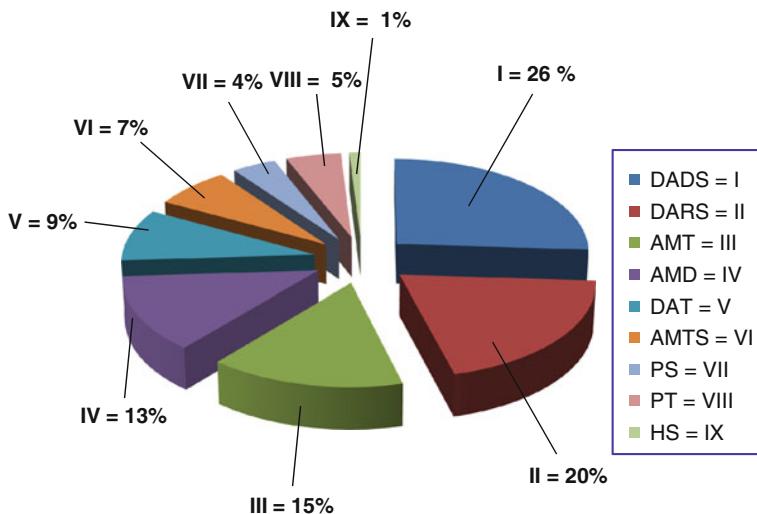


Fig. 9.1 Chemical composition of a typical commercial preparation of garlic oil. Diallyl disulfides (DADS) (I); diallyl trisulfides (DARTS) (II); allyl methyl trisulfides (AMT) (III); allyl methyl disulfide (AMD) (IV); diallyl tetrasulfide (DAT) (V); allyl methyl tetrasulfide (AMTS) (VI); dimethyl trisulfide (DTS) (VII); pentasulfide (PT) (VIII); and hexasulfide (HT) (IX). Adapted from Verma et al. (2008)

In addition to these main sulfur compounds, intact garlic bulbs also contain a small amount of *S*-allylcysteine (SAC), but no allicin. SAC is formed from γ -glutamyl cysteine catabolism. Allicin and related thiosulfinate are highly unstable and instantly decompose to yield various sulfur compounds including diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DARTS), dithiins, ajoene, methyl allyl disulfide, methyl allyl trisulfide, 2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin (Figs. 9.1 and 9.2) (Block 1985; Fenwick and Hanley 1985; Lawson 1998; Rybak et al. 2004). These compounds provide garlic its characteristic odor and flavor as well as most of its biological properties. It is estimated that 1 g of fresh garlic contains up to 2.5 mg of allicin and 500 mg of DADS or DATS. The amount of organosulfurs required for biological responses and beneficial effects is generated through dietary intake of garlic (Lawson 1998; Rybak et al. 2004). In addition to organosulfur compounds, garlic contains carbohydrates and proteins, which are the major components of garlic powder accounting for more than 80 %. Garlic also contains antioxidants vitamins A, C, and E as well as selenium, a key element for the synthesis of the antioxidant enzyme glutathione peroxidase (GPx) (Gorinstein Leontowicz et al. 2006).

Garlic preparations are available in several forms, such as aqueous garlic extract, heat-treated garlic extract, aged garlic extract, garlic powder, and garlic oil (Banerjee et al. 2003). Garlic has been used as a drug to prevent and treat a variety of diseases, including atherosclerosis, thrombosis, hypertension, dementia, cancer, cataract, and type 2 diabetes (Amagase et al. 2001; Verma et al. 2008). Beneficial effects of garlic

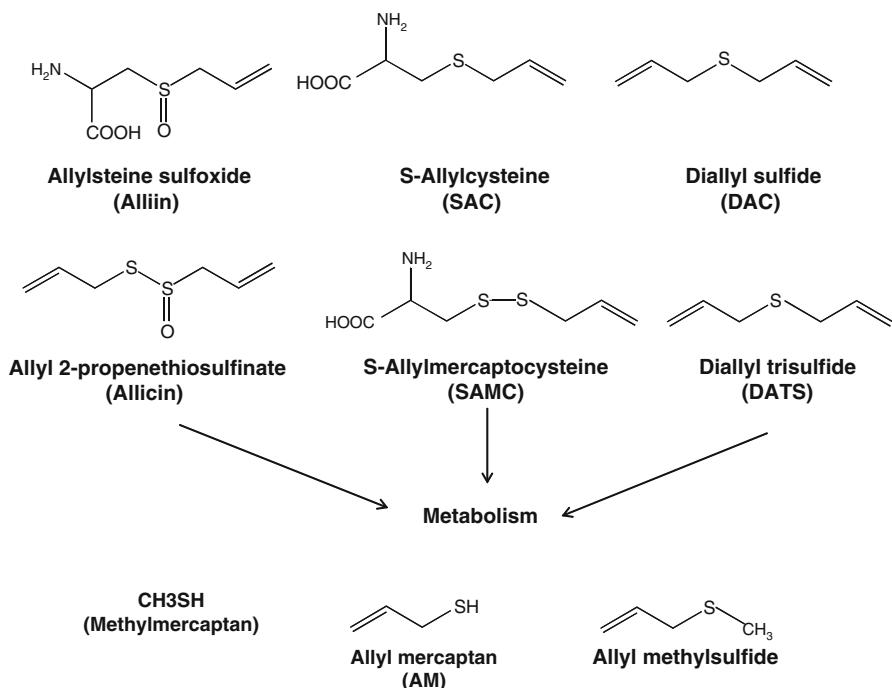


Fig. 9.2 Chemical structures of common organosulfur compounds found in garlic

are not only due to its antioxidant, anti-inflammatory, bacteriostatic, antiapoptotic effects, but also produced by immune system enhancing properties (increase in interleukin-1 levels in T lymphocytes and macrophages) and facilitating healthy blood circulation. Active components of garlic also induce some beneficial effects for livestock and produce hypocholesterolemic effects and growth-promoting and antioxidant activities in animals (Lewis et al. 2003). In addition, organosulfur compounds from garlic also induce carcinogen detoxification, inhibit tumor cell proliferation, produce antimicrobial effects, mediate free radical scavenging effects, inhibit DNA adduct formation, mediate cell cycle arrest, and induce apoptosis (Le Bon and Siess 2000; Moriarty et al. 2007).

9.2 Bioavailability of Garlic Constituents in the Brain

As stated earlier, factors that influence bioavailability of nutrients include gastric acid secretion, gastric emptying time, gastrointestinal blood flow, surface area, and absorption (Martinez and Amidon 2002), along with the effects of presystemic hepatic and gut metabolism and transport. Garlic contains many constituents. Some are easily absorbed in the gastrointestinal tract, while others are not. Studies on

bioavailability of SAC, a water-soluble organosulfur component indicates that its oral intake results in its rapid absorption in the gastrointestinal tract and distribution in plasma, liver, and kidney of rats, mice, and dogs (Nagae et al. 1994). The bioavailability of SAC is about 103.0 % in mice, 98.2 % in rats, and 87.2 % in dogs (Nagae et al. 1994). *N*-acetyltransferases transform SAC into *N*-Acetyl-SAC, which can be detected in the urine of dogs and humans (Steiner and Li 2001). Other oil-soluble organosulfur compounds of garlic, such as allicin, sulfides, ajoene, and vinylidithiins, are not found in blood or urine, even after consumption of a large amount of garlic (Lawson and Hughes 1992). Incubation of allicin with liver homogenate results in its disappearance very rapidly (Egen-Schwind et al. 1992). No allicin is detected in either serum or urine from 1 to 24 h after ingestion of 25 g of raw garlic (~90 mg allicin) (Lawson and Hughes 1992). The ingestion of allicin causes instability and no metabolite appears in the blood. It is reported that allicin quickly disappears from whole blood within a few minutes while its decomposition products (DAS and allylmercaptan) are formed and have been found in the blood (Freeman and Kodera 1995). This is because of the fact that allicin binds to the protein of red blood cells and oxidizes them immediately. Based on these findings, it is suggested that allicin may not contribute to the *in vivo* effects of garlic.

Little information is available on how many organosulfur compounds from garlic cross blood–brain barrier (BBB). What are their half lives? Studies on antioxidant effects of garlic extract in rat brain synaptosomes obtained from perfused young (3-month-old) and aged (14-month-old) male Wistar rats indicate that in young rat brain synaptosomes, the garlic extract inhibits the generation of 8-iso-PGF_{2 α} , both basally and after hydrogen peroxide-induced oxidative stimulation (Brunetti et al. 2009). In aged rats, 8-iso-PGF_{2 α} production is not affected by the garlic extract in the basal state, whereas, after hydrogen peroxide-induced oxidative stimulus, an antioxidant effect of the garlic extract takes place only at the higher concentration, supporting the view that garlic supplementation can be effective in preventing brain oxidative damage in young animals, whereas the aging brain seems to be resistant to the antioxidant effects of garlic, *in vitro* (Brunetti et al. 2009).

9.3 Biological Effects of Garlic Components

As stated before, beneficial effects of garlic on human health are due to its antioxidant, anti-inflammatory, anticancer, antifungal, and immunomodulatory properties. In addition, garlic extract also inhibits production of prostaglandin (Fig. 9.3). This suggests that organosulfur compounds in garlic act not only by targeting multiple signal transduction pathways, but also by modulating expression of many genes and enzymes, such as arylamine *N*-acetyltransferase, superoxide dismutase (SOD)-like activity, hydrogen peroxide (H₂O₂) scavenging activity, glutathione redox cycle enzymes, cytochrome P-450 reductase, and lactate dehydrogenase in the brain, liver, and other visceral tissues (Singh et al. 1997; Oelkers et al. 1992; Ali et al. 1991; Rahman 2003; Chung 2006). Collective evidence suggests that garlic constituents

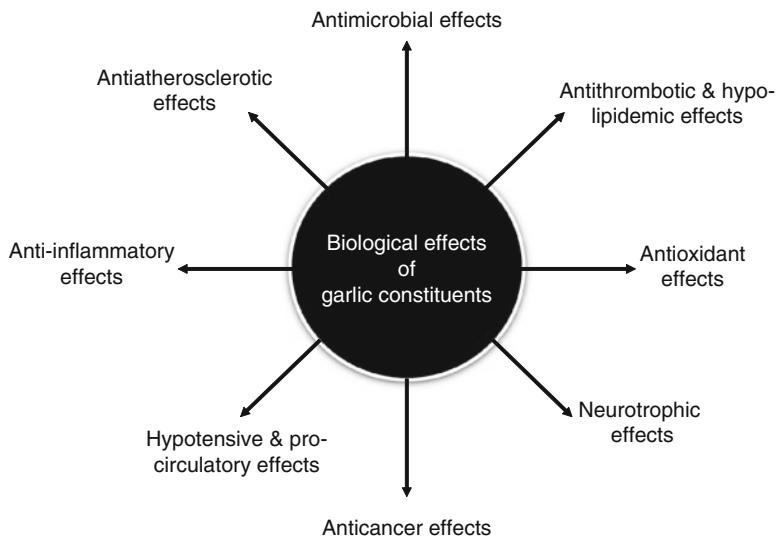


Fig. 9.3 Biological effects of garlic constituents

act through multiple mechanisms and produce antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic, and antitumor effects. Recent reports on cardioprotective effects of garlic show that freshly crushed garlic possesses superior and diverse cardioprotective abilities compared to processed garlic (Mukherjee et al. 2009). This suggestion is based on ability of freshly crushed garlic: (a) to mediate greater postischemic ventricular recovery, lower myocardial infarction, and decrease in cardiomyocyte apoptotic cell death compared to processed garlic; (b) to induce greater degree of survival signal by boosting antiapoptotic ERK1/2 and Bcl-2/Bax ratio and by suppressing the death signal by decreasing the phosphorylation of proapoptotic JNK and p38MAPK; (c) to stimulate Akt-FoxO survival network signaling; (d) to support redox signaling by activating Nrf2 and p65 subunit of NF- κ B; and (e) to reduce cardiovascular risk factors associated with diabetes and obesity through the upregulation of GLUT-4, PPAR α , and PPAR δ . In addition, superiority of freshly crushed garlic over processed garlic may also be due to the generation of H₂S, which is absent or present in considerably lower amounts in the processed garlic (Mukherjee et al. 2009).

9.3.1 Antioxidant Effects of Garlic Constituents

In vivo and in vitro studies on the effect of crude garlic and its organosulfur compounds not only act as potent antioxidants, but also stimulate the antioxidants enzymes, such as GPx, glutathione transferase (GST), catalase (CAT), and SOD

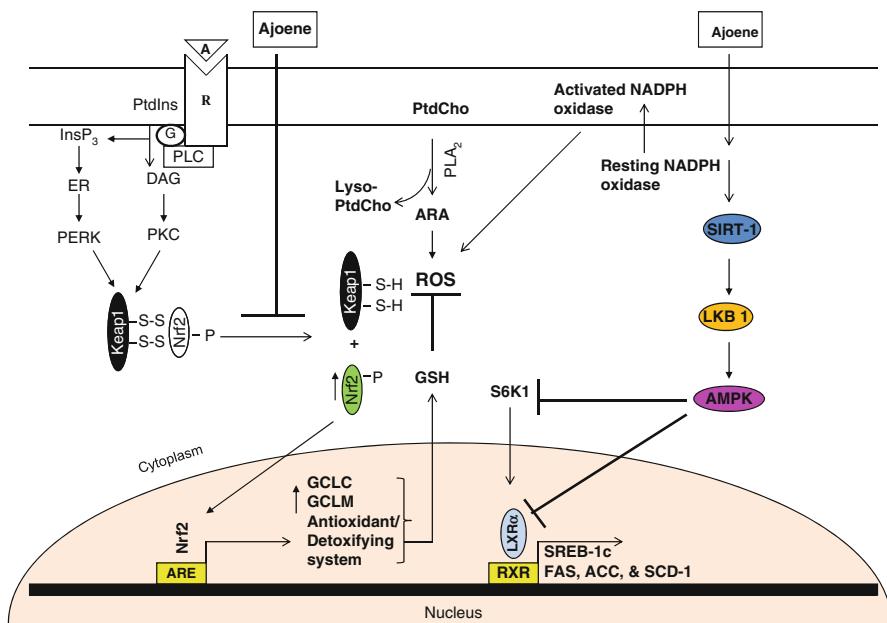


Fig. 9.4 Modulation of signal transduction processes by ajoene, an important garlic constituent. Phosphatidylinositol (PtdIns); diacylglycerol (DAG); inositol 1,4,5-trisphosphate (InsP₃); phospholipase (PLC); protein kinase (PKC); nuclear factor erythroid-2-related factor 2 (Nrf2); Kelch-like ECH-associated protein-1 (Keap1); genes encoding for the catalytic (GCLC); glutamate cysteine ligase modifier (GCLM); antioxidant response element (ARE); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); arachidonic acid (ARA); reactive oxygen species (ROS); glutathione (GSH); activate AMP-activated protein kinase (AMPK); p70 ribosomal S6 kinase-1 (S6K1); liver X receptor α (LXR α); and Sterol Regulatory Element-Binding Protein-1c (SREBP-1c); Stearoyl-CoA desaturase-1 (SCD1); and fatty acid synthesis (FAS)

in concentration and time-dependent manner. DAS, DADS, and DAT play differential modulatory roles on glutathione (GSH)-related antioxidant system in liver and red blood cells along with increased expression of GST (Chung 2006; Butt et al. 2009).

It is well known that GSH is the most abundant nonprotein thiol in mammalian cells. It is involved in defense against oxidative stress as a scavenger of reactive oxygen species (ROS) and electrophiles. Another antioxidant mechanism of neural and nonneuronal cells involves a transcription factor called nuclear factor erythroid-2-related factor 2 (Nrf2). The transcription factor protects neural and nonneuronal cells from oxidative stress by regulating activation of the antioxidant response element (ARE) in response to oxidative stress. Under normal conditions, Nrf2 is complexed with Kelch-like ECH-associated protein-1 (Keap1) and is localized in the cytoplasm (Fig. 9.4). Following oxidative stress or treatment with garlic constituents, Nrf2 translocates into the nucleus, where it interacts with ARE and modulates the induction of target genes (Itoh et al. 1999). These genes include glutamate-cysteine

ligase (GCL), heme oxygenase-1 (HO-1), thioredoxin reductase, NAD(P)H:quinone oxidoreductase-1 (NQO1), and GSH S-transferase (Nguyen et al. 2003). It is proposed that garlic constituents may protect from oxidative stress by modulating NrF2 activity.

In addition, garlic constituents modulate oxidative stress by scavenging ROS. Thus, alliin has been reported to scavenge superoxide, while allyl cysteine and allyl disulfide do not interact with superoxide. Allicin suppresses the formation of superoxide by the xanthine/xanthine oxidase system, probably by modulating a thiol exchange mechanism. Although alliin, allyl cysteine, and allyl disulfide have been shown to scavenge hydroxyl radicals, alliin, allicin, and allyl cysteine do not prevent microsomal lipid peroxidation. Alliin and allyl cysteine retard lipid peroxidation by scavenging hydroxyl radicals. In contrast, allyl disulfide inhibits lipid peroxidation by acting as terminator of lipid peroxidation. It is also suggested that organosulfur compounds of garlic inhibit the activation of the oxidant-induced transcription factor, nuclear factor (NF)- κ B, which has clinical significance in neurotraumatic (stroke) and neurodegenerative diseases (Alzheimer disease and Parkinson disease). Garlic components protect cellular DNA against free radical-mediated damage. This process may have a role in protecting against loss of brain function in aging and possesses other antiaging effects, as suggested by its ability to increase cognitive functions, memory, and longevity in a senescence-accelerated mouse model (Borek 2006). Collective evidence suggests that the degree of antioxidative efficacy of various garlic compounds or preparations differs according to variations in chemical structures and standardization procedures (Amagase 2006; Borek 2006; Chung 2006).

9.3.2 Antiinflammatory Effects of Garlic Constituents

Arachidonic acid is a major component of glycerophospholipid. It is released from neural membrane phospholipids through the action of phospholipases A₂ (PLA₂). It is oxidized by cyclooxygenases (COXs) and lipoxygenases (LOXs) into eicosanoids, which play an important role in the induction of neuroinflammation (Farooqui et al. 2007). Among the garlic constituents, DATS is the most potent compound that suppresses constitutive expression of COX-2 in neural and non-neuronal cells. It is proposed that in nonneuronal cells anti-inflammatory effects of DATS are probably mediated through blockade of AP-1 activation via downregulation of Akt and JNK signaling pathways (Shrotriya et al. 2010). In addition, DATs may also act through NF- κ B, a family of transcription factors that includes RelA (p65), NF- κ B1 (p50 and p105), NF- κ B2 (p52 and p100), c-Rel, and RelB. These transcription factors are sequestered in the cytoplasm by inhibitory I- κ Bs, which prevent NF- κ B activation, and inhibit its nuclear translocation. Following the degradation of inhibitor I- κ B as a result of phosphorylation of I- κ B by the activated I- κ B kinase (IKK) in the presence of DAT and other organosulfur compounds, NF- κ B migrates into the nucleus, where it typically forms homodimers or

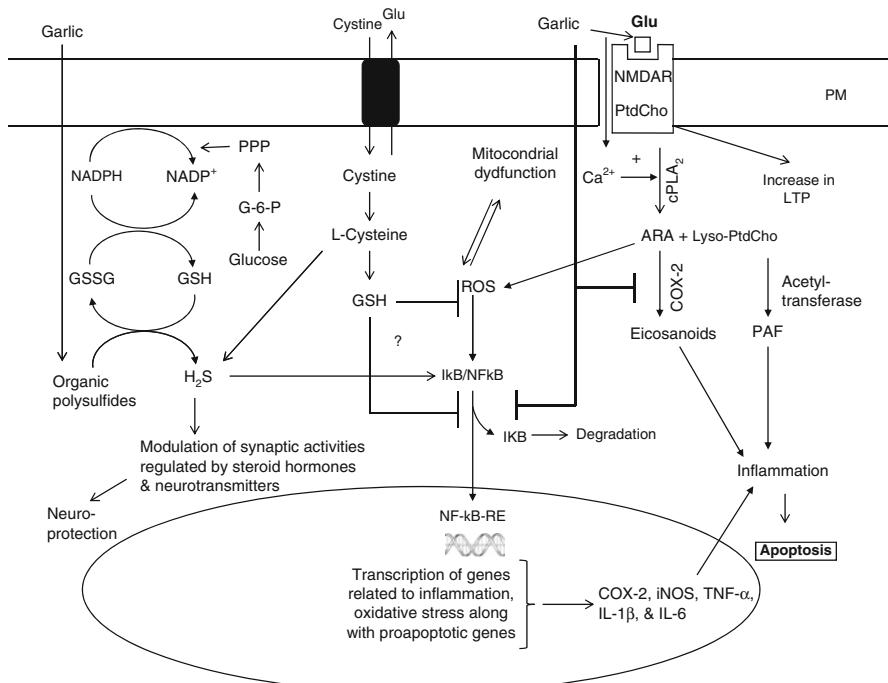


Fig. 9.5 Synthesis of hydrogen sulfide from organic polysulfide and inhibition of COX-2 and NF- κ B activities by garlic. Plasma membrane (PM); glutamate (Glu); *N*-methyl-D-aspartate receptor (NMDA-R); phosphatidylcholine (PtdCho); cytosolic phospholipase A_2 (cPLA $_2$); arachidonic acid (ARA); lyso-phosphatidylcholine (lyso-PtdCho); platelet activating factor (PAF); inducible nitric oxide synthase (iNOS); cyclooxygenase-2 (COX-2); nuclear factor-kappa B (NF- κ B); inhibitory form of nuclear factor kappa B (I κ B/NF- κ B); nuclear factor κ B-response element (NF- κ B-RE); inhibitory subunit of NF- κ B (I κ B); tumor necrosis factor- α (TNF- α); interleukin-1 β (IL-1 β); interleukin-6 (IL-6); glutathione (GSH); reactive oxygen species (ROS); pentose phosphate pathway (PPP); and long-term potentiation (LTP); and \dashv (blocked arrow) represents inhibition

heterodimers that bind to the promoters of many inflammatory response genes including genes for inflammatory cytokines (TNF- α and IL-1 β) as well as genes encoding cyclooxygenase-2 (COX-2) and iNOS. Thus, organosulfur compounds-mediated inhibition of NF- κ B may decrease the intensity of neuroinflammation by inhibiting the induction of TNF- α , IL-1 β , COX-2, and iNOS (Ban et al. 2007) (Fig. 9.5). Recently a novel sulfur compound has been isolated from garlic. It is called as thiacremonone (Ban et al. 2009). Thiacremonone not only inhibits LPS-mediated iNOS and COX-2 expression, but also decreases NF- κ B activity. These observations support the view that organosulfur compounds from garlic may act as anti-inflammatory agents.

9.3.3 Antitumor Effects of Garlic Constituents

It is well known that NF- κ B plays an important role in the promotion of tumor growth, angiogenesis, metastasis, and chemotherapeutic resistance through the expression of genes participating in malignant conversion and tumor promotion (Brown et al. 2008). Within this context, NF- κ B stimulates immune cell function and acts in a proinflammatory manner by inducing the expression of cytokines, chemokines, and their receptors (Bonizzi and Karin 2004). Moreover, when activated in these settings, NF- κ B inhibits programmed cell death by stimulating the transcription of antiapoptotic genes (Kucharczak et al. 2003). These aspects of NF- κ B function are undoubtedly not only central to the understanding of the overall behavior of NF- κ B, but also provide a foundation for therapeutic intervention in inflammatory diseases and cancer based on NF- κ B inhibition (Karin et al. 2004). The pro-oncogenic effects of NF- κ B arise from the overproduction of its normal target genes as a consequence of its chronic activation and nuclear localization in tumor cells. By modulating gene expression, NF- κ B facilitates other oncogenic processes, including tumor cell proliferation through its ability to induce proto-oncogenes such as cyclin D1 and c-Myc. In addition, NF- κ B also modulates metastasis through its ability to induce the expression of cellular adhesion molecules and matrix metalloproteinases, angiogenesis through regulation of vascular endothelial growth factor, and cell immortality through regulating telomerase (Perkins 2004). Finally, NF- κ B also provides the critical link between tumor development and chronic inflammation, a process that may be involved in 20 % of human cancers (Luo et al. 2005). As mentioned before, organosulfur compounds from garlic inhibit NF- κ B translocation from cytoplasm to the nucleus. This process may be considered as an adjuvant approach in combination with chemotherapeutics for the treatment of various cancers.

9.3.4 Antifungal Effects of Garlic Constituents

Garlic-derived organosulfur compounds, such as DADS act as a pro-oxidant to several fungal species and hence act as a potent antifungal in the management of fungal diseases. For example, treatment of candida cells with sublethal concentrations of DADS produces a decrease in the activity of all antioxidant enzymes (SOD, glutathione-S-transferase, and glutathione peroxidase) except catalase (Yousuf et al. 2011). Detailed investigations using two-photon fluorescence microscopy indicate that DADS treatment reduces intracellular GSH and increases intracellular ROS levels (Lemar et al. 2007). Additionally, DADS induces a marked decrease of $\Delta\Psi_m$ and reduction in respiration of cell suspensions and isolated mitochondria. In vitro kinetic studies with cell-free extracts indicate that glutathione-S-transferase (GST) is one of the intracellular targets of DADS (Lemar et al. 2007). Additional DADS targets include inhibition of a site or sites between complexes II and IV in the electron transport chain, as well as the mitochondrial ATP synthase. In addition, activity

of glucose-6-phosphate dehydrogenase decreases significantly following DADS treatment and can be correlated with a decrease in glutathione concentration in Candida species. Collective evidence suggests that DADS act as a pro-oxidant to Candida species and hence may act as a potent antifungal agent in the management of candidiasis (Lemar et al. 2007; Yousuf et al. 2011).

9.3.5 Immune System Enhancing Effects of Garlic Constituents

It is well known that IL-12 is the principal immunoregulatory cytokine, which induces the development of Th1 cells (Macatonia et al. 1993). This cytokine also has therapeutic role in infectious diseases and neuroinflammation associated with neurodegenerative diseases (Farooqui 2010) through the activation of p38 MAPK or ERK, which, in turn, may induce the production of IFN- γ mRNA and its protein in mouse spleen cells. T-helper-type 1 (Th1) responses are associated with immune system enhancement against malignant cells, viruses, and microbial infections (Pober and Cotran 1990). These observations are supported by earlier studies, which indicate that intracellular T-cell IFN γ and IL-2 production is impaired in children with acute lymphoblastic leukemia (ALL) (Nash et al. 1993). In addition, impairment in T-cell TNF α production is also observed. This impairment in TNF α not only inhibits tumor cell growth in a variety of hematological malignancies (Reed and Pellecchia 2005), but also stimulate immune response to tumors (Vassalli 1992). Organosulfur compounds from garlic compounds block the impairment of T-cell proliferation or Th1 responses by T cells from normal healthy control children. Furthermore, garlic contains lectin. This lectin induces IFN- γ production and promotes the formation of Th1 type T cells. This process enhances the immune system to protect cells from viral infections (Dong et al. 2011).

9.3.6 Cholesterol Lowering Properties of Garlic Constituents

Although it is controversial, many studies have indicated that garlic produces its cardioprotective effects not only by its antioxidant and anti-inflammatory effects, but also by its cholesterol lowering and anti-hypertensive properties (Borek 2001). These properties are due to the presence of DADS and dipropyl disulfide. The organosulfur compounds mediate antioxidant and antiatherosclerotic effects of garlic by increasing resistance of LDL to oxidation (Borek 2001). In addition, aged garlic extract inhibits the in vivo oxidation of LDL by chelating Cu $^{2+}$, scavenging superoxide ions, thus inhibiting the oxidation of protein and lipid moiety of human LDL-cholesterol (Dillon et al. 2003). It is also suggested that some constituents of garlic may not only act as inhibitors for hydroxyl methyl glutaryl CoA reductase, the rate-limiting step for the synthesis of cholesterol (Durak et al. 2004), but also by improving blood lipid profile and increasing the plasma antioxidant capacity and oxidation resistance by increasing antioxidant enzymes activities (SOD and GSH-Px).

9.3.7 *Memory Retention and Garlic Constituents*

Chronic garlic administration enhances memory function (Haider et al. 2008). This may be due to the modulation of neurotransmitters. Thus, garlic administration in rats increases brain serotonin (5-hydroxytryptamine [5-HT]) levels. 5-HT, is a neurotransmitter, which is not only involved in a number of physiological functions, but also enhances cognitive performance (Haider et al. 2008). Memory assessment by a step-through passive avoidance paradigm (electric shock avoidance) test indicates that the levels of plasma free total tryptophan (TRP) are significantly increased and plasma total TRP levels are significantly decreased in garlic-treated rats. In addition, brain TRP, 5-HT, and 5-hydroxyindole acetic acid (5-HIAA) levels are also significantly increased following garlic administration. A significant improvement in memory function is observed in garlic-treated rats in the passive avoidance test. Based on these results it is proposed that the memory-enhancing effect of garlic may be associated with increased brain 5-HT metabolism in rats (Haider et al. 2008).

9.4 Hydrogen Sulfide and Garlic

Hydrogen sulfide (H_2S) is a gas endogenously produced from cysteine in a reaction catalyzed by two pyridoxal-5-phosphate-dependent enzymes, namely cystathione β -synthase (CBS) and cystathionine γ -lyase (CSE), each of which utilizes L-cysteine as substrate (Stipanuk and Beck 1982; Hosoki et al. 1997). In addition, 3-mercaptopropruvate sulfurtransferase (3-MST) also catalyzes the formation of H_2S from L-cysteine and α -ketoglutarate. Acute intoxication with H_2S results in inhibition of monoamine oxidase and induces changes in levels of neurotransmitters in the brain (Warenycia et al. 1989a; Kimura 2010). Electrophysiological studies on toxic effects of H_2S on neurons also indicate that sulfide activates Ca^{2+} channels as well as Ca^{2+} -dependent K^+ channels and suppresses the delayed rectifier (Kombian et al. 1993). Recent studies indicate that like other endogenous gaseous molecules, such as nitric oxide (NO) and carbon monoxide (CO) (Wang et al. 1997a, b), H_2S also fulfills many physiological roles of gaseous messengers. It plays important roles in regulating cardiovascular, cerebrovascular, and neuroprotective functions (Abe and Kimura 1996; Kimura 2010) through the modulation of cellular calcium, regulation of vascular tone, modulation of apoptosis, modulation of neurotransmitter release, blood pressure, and stimulation of ATP-sensitive K^+ channels (Fig. 9.6). H_2S is present at relatively high levels in the brain. It is shown that CBS is highly expressed in the hippocampus and the cerebellum; CBS inhibitors, hydroxylamine and amino-oxyacetate, suppress the production of brain H_2S ; and CBS activator, S-adenosyl-L-methionine, enhances H_2S production, indicating that CBS contributes to the production of endogenous H_2S . Physiological concentrations of H_2S selectively enhance NMDA receptor-mediated responses and facilitate the induction of hippocampal long-term potentiation

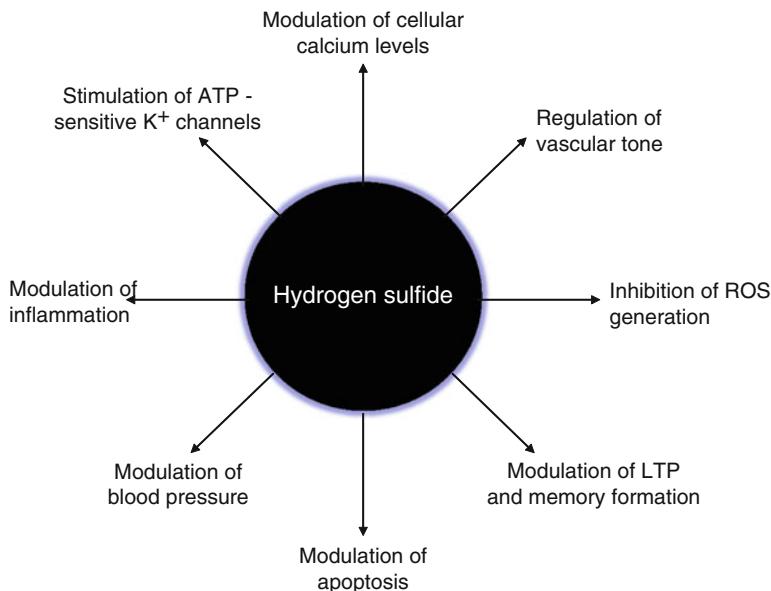


Fig. 9.6 Biological activities of hydrogen sulfide

(LTP), a process closely associated with memory formation (Kimura 2010) (Fig. 9.6). Similar, LTP induction has also been reported with NO and CO. However, there are differences in mechanism of LTP induction. In addition to its actions upon neurons, H₂S also appears to influence astrocytes (Kimura and Kimura 2004). It is shown that H₂S increases glutathione levels by modulating glutamate transporters. The uptake of glutamate into astrocytes is an energy-dependent process driven by electrochemical gradients of intracellular and extracellular Na⁺ and K⁺ along with ATP consumption. It is well known that the disruption of ATP synthesis in astrocytes after exposure to H₂O₂ is a predominant cause for the impairment of glutamate uptake and H₂S reverses H₂O₂-mediated suppression of ATP generation and therefore enhances glutamate uptake function (Sung et al. 2003). Thus, H₂S increases GSH production in rat cortical astrocytes by improving the activity of glutamate transporters.

NO and CO are retrograde neurotransmitters and they do not require NMDA receptor activation (Zhuo et al. 1993), while H₂S requires NMDA receptor activation. The blockade of NMDA receptor by NMDA antagonists does not facilitate the LTP induction with H₂S. Another critical difference in LTP induction is that NO and CO activate soluble guanylyl cyclases leading to increase in intracellular cGMP, while H₂S does not (Abe and Kimura 1996; Kimura 2010). In addition, there occurs a cross talk (interplay) between H₂S and NO. Thus, H₂S not only decreases the sensitivity of the cGMP pathway to NO (Warenciety et al. 1989b), but also reduces the expression level of NO synthase (NOS). Likewise, NO not only increases the

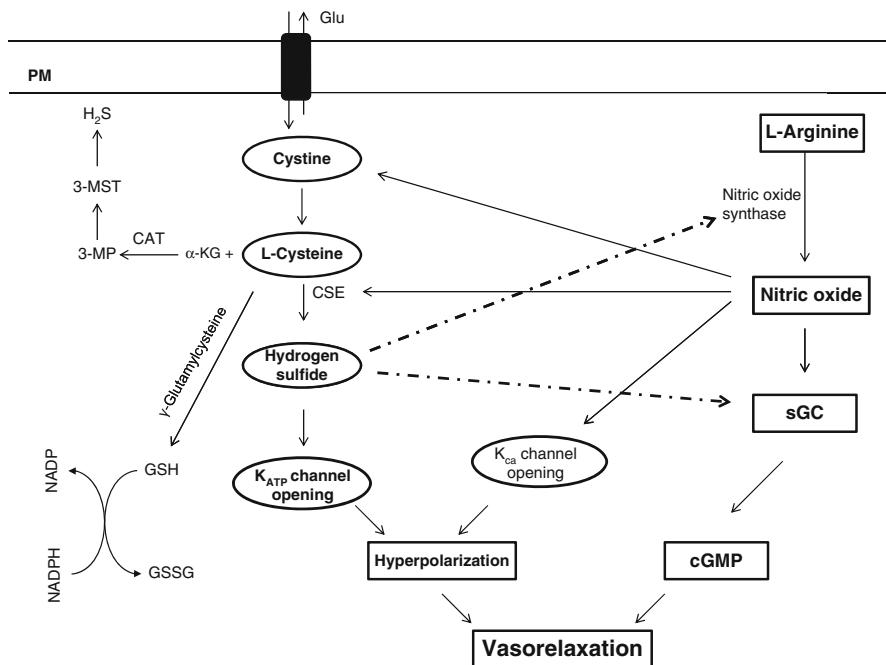


Fig. 9.7 Interplay between hydrogen sulfide and nitric oxide. Plasma membrane (PM); reduced glutathione (GSH); oxidized glutathione (GSSG); cystathionine γ -lyase (CSE); nitric oxide synthase (NOS); α -Ketoglutarate (α -KG); cysteine aminotransferase (CAT); mercaptopyruvate sulfur transferase (MST); and 3-mercaptopyruvate (3-MP)

expression of CSE, but also increases the cellular uptake of cystine. Furthermore, H₂S also modifies K_{Ca} channels to decrease their sensitivity to NO (Fig. 9.7) (Wang 2002). These observations suggest that endogenous H₂S functions as a neuromodulator and gasotransmitter in the brain (Kimura 2010). H₂S not only protects neurons from oxidative stress during excitotoxicity by increasing the levels of GSH through enhancing the activity of cystine transporters (Kimura and Kimura 2004), but also by modulating the cystine/glutamate antiporter coupled influx of cystine, an H₂S precursor, with efflux of glutamate from neurons and influx of glutamate by astrocytes (Lu et al. 2008). In addition, H₂S also inhibits the release of insulin (Ali et al. 2007; Kaneko et al. 2006, Yang et al. 2005). Although the molecular mechanism associated with inhibitory effect of H₂S on insulin release is not fully understood, it is becoming increasingly evident that H₂S-mediated inhibition of insulin secretion is closely associated with K_{ATP} channel-dependent pathway (Yang et al. 2005; Ali et al. 2007). Administration of the K_{ATP} channel antagonist glibenclamide reverses the inhibition of insulin secretion by NaHS in β -cells (Ali et al. 2007). It has also been demonstrated that H₂S can directly open K_{ATP} channels in β -cell line INS-1E cells (Yang et al. 2005). It is also reported that H₂S suppresses calcium oscillation,

thereby causing decreased insulin secretion in isolated mouse pancreatic β -cells (Kaneko et al. 2006). H_2S also acts as an endogenous modulator of neuroinflammation (Zanardo et al. 2007; Li et al. 2005). H_2S may exert anti-inflammatory actions not only through the inhibition of the proinflammatory factors, but also by enhancing the production of anti-inflammatory cytokines (Hu et al. 2011; Zhi et al. 2007). It is also suggested that H_2S may upregulate endogenous antioxidants through a nuclear-factor-E2-related factor-2 (Nrf2)-dependent signaling pathway (Calvert et al. 2009). Nrf2 regulates gene expression of a number of antioxidant proteins (heme oxygenase-1) and phase II detoxification enzymes (for example, glutathione S-transferase) (Lee et al. 2003).

Another target of H_2S in brain is the hypothalamic-pituitary-adrenal (HPA) axis. It is central to stress responses by eventually releasing glucocorticoids that coordinate adaptation responses to the stressors (Tsigos and Chrousos 2002). H_2S plays important roles in the regulation of the HPA axis and neuroendocrine responses to stress (Dello Russo et al. 2000). Thus, in cultured hypothalamic explants, NaHS (H_2S donor) inhibits KCl-stimulated corticotropin-releasing hormone (CRH) release (Dello Russo et al. 2000). Similarly, in vivo administration of *S*-adenosyl-L-methionine (SAMe) suppresses the plasma corticosterone level in rats under physical stress. These observations support the view that the inhibitory effect of NaHS and SAMe on glucocorticoid level in plasma may be associated with inhibition of CRH release in the hypothalamus (Oi et al. 2001).

It is well known that NO and CO mediate vasorelaxation by increasing the cellular cGMP activity and/or stimulating K_{Ca} channels in vascular smooth muscle cells (SMCs) (Kimura 2010). It is recently reported that beneficial effects of garlic and garlic-derived organic polysulfides (DATS and DADS) on cardiovascular diseases may be due to the production of H_2S in aortic and heart tissues. Garlic-derived organic polysulfides induce H_2S generation through their interactions with biological thiols including reduced glutathione (GSH) in a dose-dependent manner (Benavides et al. 2007). The utilization of glucose through pentose phosphate pathway (PPP) is necessary for maintaining GSH pool via the reduction of $NADP^+$ to $NADPH$. Based on electrophysiological studies, it is proposed that H_2S generation leads to vasorelaxation via vascular SMCs K_{ATP} -linked hyperpolarization (Zhao et al. 2001) (Fig. 9.6). Thus, generation of H_2S protects cardiac muscle from ischemia reperfusion injury not only by preserving mitochondrial function (Elrod et al. 2007), but also through the stimulation of K_{Ca} channels. In addition, H_2S also exerts antinociceptive effects in the gastrointestinal tract while in other peripheral tissues it produces nociceptive responses (Distrutti et al. 2006; Nishimura et al. 2009).

9.5 Adverse Effects of Garlic

Little is known about the effect of freshly crushed garlic and AGE on brain in vivo. Most information is based on the effect of garlic on neural cell cultures (Brenya et al. 1999; Kosuge et al. 2003; Peng et al. 2002). Significant information on the

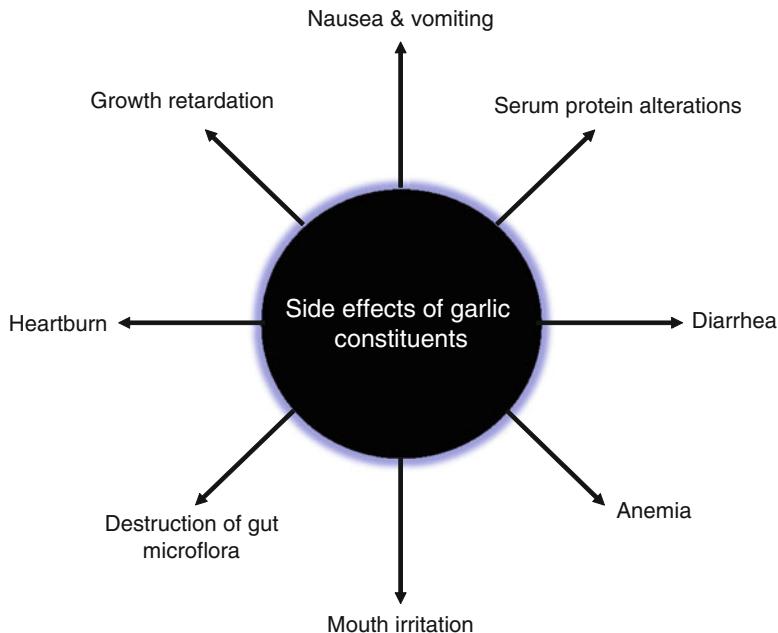


Fig. 9.8 Side effects of garlic constituents

effect of garlic constituents is available on cardiovascular system. Although earlier studies on the consumption of raw garlic have indicated that freshly crushed garlic induces many side effects including anemia, growth retardation, and destruction of gut microflora and alteration of serum protein levels (Nakagawa et al. 1984; Shashikanth et al. 1984) (Fig. 9.8). Clinical trials indicate the consumption of high amount of garlic results in “garlic breath” and body odor. In addition, garlic consumption may cause allergic reactions (allergic contact dermatitis, generalized urticaria, angioedema, pemphigus, anaphylaxis, and photoallergy), alteration of platelet function and coagulation (with a possible risk of bleeding), and burns (when fresh garlic is applied on the skin, particularly under occlusive dressings). Intake of high amounts of garlic by nursing mothers induces behavioral changes in infants during breast feeding. Finally, garlic has been reported to enhance the pharmacological effect of anticoagulants (e.g., warfarin, fluindione) and to reduce the efficacy of anti-AIDS drugs (i.e., saquinavir) (Borrelli et al. 2007). In addition, garlic consumption in some individuals has been reported to produce heartburn, abdominal pain, nausea, vomiting, flatulence, and diarrhea (Mulrow et al. 2000, 2004). Sulfurous nature of garlic constituents makes it a prime breeding ground for botulism (*Clostridium botulinum*). The worst danger from botulism comes if raw garlic is stored in oil at room temperature—or even for too long in the refrigerator. Another serious adverse effect of garlic consumption is related to

uncontrolled bleeding. Several studies have indicated that garlic intake may be associated with serious postoperative or spontaneous bleeding (Rose et al. 1990; Burnham 1995; Carden et al. 2002). Higher concentrations of garlic extract produce clastogenic effects (disruption or breakages of chromosomes) in visceral tissues in mice, which can be appreciably reduced at lower concentrations (Das et al. 1996). Feeding garlic homogenate (1,000 mg/day) for 30 days induces a significant loss of the normal cellular architecture of the heart, liver, and kidneys (Banerjee et al. 2001, 2002). Occupational exposure to garlic powder or dust in some individuals with asthma has been reported to trigger allergic responses (Anibarro et al. 1997). Collective evidence suggests that consumption of freshly crushed garlic or garlic powder may not only cause several undesirable side effects in certain groups of human population (Desai et al. 1990; Nakagawa et al. 1980; Nakagawa et al. 1984) but may induce allergic reactions (Lybarger et al. 1982) (Fig. 9.8). To overcome the adverse effects of fresh garlic preparations, aged garlic extract (AGE) has been developed by soaking sliced garlic in water–ethanol mixture for 20 months, which removes several irritant sulfur-containing compounds and also stabilizes some unstable compounds such as allicin (Borek 2006; Morihara et al. 2006).

9.6 Effects of Garlic Constituents and Neurological Disorders

In vitro studies on the effect of *S*-allyl-L-cysteine (SAC) and allixin indicate that garlic constituents protect PC12 cells against beta amyloid ($A\beta$) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicities in a concentration-dependent manner (Brenya et al. 1999; Kosuge et al. 2003; Peng et al. 2002; Rojas et al. 2011). SAC has no effect on $A\beta$ -induced cell death in cultured cerebellar granule neurons, which can be blocked by a caspase-3 inhibitor. Collective evidence suggests that SAC protects against the neuronal cell death that is triggered by the dysfunction of endoplasmic reticulum in the hippocampus. SAC has no effect on neuronal cell death that is dependent upon the caspase-3-mediated apoptotic cell death. Another constituent of garlic, DADS at low concentration induces neuroprotective effects in differentiated PC12 cells by activating phosphatidylinositol 3-kinase (PtdIns 3K/Akt) and by inhibiting glycogen synthase kinase-3 (GSK-3) activation, cytochrome c release, caspase-3 activation, and PARP cleavage, whereas at high concentration DADS (100 μ M) produces cytotoxicity by blocking PtdIns 3K/Akt and by promoting activation of GSK-3 and caspase-3, release of cytochrome c, and cleavage of PARP (Koh et al. 2005). Studies on the effects of DATS in a transgenic mouse model of amyotrophic lateral sclerosis (ALS) indicate that oral administration of DATS beginning at clinical onset stage significantly prolongs disease duration and extends life span for about one week. DATS treatment induces HO-1 and reduces GFAP expression in the lumbar spinal cord of SOD1-G93A transgenic mice. It is proposed that DATS has multifunctional neuroprotective effects in SOD1-G93A transgenic mice (Guo et al. 2011).

It is well known that mild hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular disease and atherothrombosis (Weiss 2005). Although mechanisms by which homocysteine (Hcy) exerts its proatherogenic effects are still not fully understood, it is suggested that Hcy-mediated production of free radicals may be one of the mechanisms causing cell damage in the vascular wall (Weiss 2005). At the molecular level, increase in oxidative stress due to the overproduction of ROS and/or activation of PKC-MAP kinase pathway through PPAR γ stimulation might be the mechanism by which Hcy contributes to the activation of CD36 (a class B scavenger receptor expressed by monocytes/macrophages) and formation of atherosclerotic lesions (Morihara et al. 2011). This process seems to be minimized by AGE through the enhancement of intracellular glutathione contents in endothelial cells (Ide et al. 2006). Therefore, it has been speculated that antioxidant effects of AGE may prevent Hcy-induced CD36 expression in human monocytes/macrophages by inhibiting activation of PPAR γ (Morihara et al. 2011). Thus, AGE can modulate the formation of early atherosclerotic lesions and may be useful for preventing atherosclerosis in cardiovascular and cerebrovascular diseases.

9.6.1 Garlic Constituents and Ischemic Injury

Deprivation of blood supply (stroke), with the subsequent deficiency of glucose and oxygen, triggers an important number of mechanisms (e.g., excitotoxicity, oxidative stress, and inflammation) leading to irreversible neuronal injury in the brain. According to data of the World Health Organization, stroke is the third highest cause of morbidity and mortality in the developed countries of the world, immediately following ischemic heart disease and malignant diseases.

Stroke is accompanied not only by severe long-term disability, but also sudden loss of motor, sensory, and cognitive function. Following ischemic episode levels of ROS are markedly increased during reperfusion (Halliwell 2006). The neurochemical mechanisms associated with brain damage during reperfusion subsequent to ischemia are attributed to energy failure, intracellular accumulation of calcium ions, increased excitatory amino acids, increased intracellular hydrogen ions, increased production of free radicals and free radical-mediated damage, and intracerebral synthesis of platelet-activating factor (Farooqui 2008). In vitro studies indicate that organosulfur compounds from garlic protect neuronal cell from oxidative stress.

Middle cerebral artery (MCA) occlusion (MCAO) in rats not only produces significant decrease in GSH and glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), but also causes significant elevation in MDA, glutamate, and aspartate. The activities of Na $^+$, K $^+$ -ATPase, SOD, and CAT are decreased significantly by MCAO. The neurobehavioral activities (grip strength, spontaneous motor activity, and motor coordination) are also decreased significantly in the MCAO group (Saleem et al. 2006). Above ischemia-mediated neurochemical events are significantly attenuated by pretreatment with aqueous garlic extract 30 min before the induction of MCAO and correlate well with histopathology by decreasing

the neuronal cell death after MCAO and reperfusion (Saleem et al. 2006). Similarly, treatment of animals, which have been subjected to 1 h of ischemia plus 24 h of reperfusion with aged garlic extract (AGE) (1.2 ml/kg weight, i.p.), at the onset of reperfusion indicates that AGE treatment diminishes the neurological alterations (61.6 %), the infarct area (54.8 %), and the histological damage (37.7 %) caused by cerebral ischemic injury (Colín-González et al. 2011). AGE administration attenuates the elevation in 8-OHdG levels (77.8 %), in TNF α levels (76.6 %), and in COX-2 protein levels (73.6 %) and activity (30.7 %) caused by 1 h of ischemia plus 24 h of reperfusion. Based on these results, it is suggested that the neuroprotective effect of AGE may not only due to its antioxidant properties, but also mediated by diminishing the increase in TNF α levels and inhibition of COX-2 protein expression and activity (Colín-González et al. 2011). Collective evidence suggests that AGE not only modulates neurobehavioral changes, but also lowers blood pressure (BP) and protects neural cells from the deleterious effects of ischemia/reperfusion injury. Although the molecular mechanism associated with BP lowering effects of garlic is not fully understood, it is suggested that garlic-derived organic polysulfides are converted by erythrocytes into hydrogen sulfide which relaxes vascular smooth muscle, induces vasodilation of blood vessels, and significantly reduces blood pressure. In addition, studies on ischemic injury in the central nervous system indicate that exogenous H₂S protects neurons from glutamate toxicity (Kimura and Kimura 2004), improves survival of HT22 cells (Kimura et al. 2006), attenuates cell death and radical formation (Tyagi et al. 2009), and reduces infarct size by 50 % (Florian et al. 2008). Moreover, H₂S also decreases expression of apoptotic proteins and activation of antiapoptotic proteins (Minamishima et al. 2009).

It is suggested that high levels of organosulfur compounds in AGE may help in preventing the oxidant-mediated damage that occurs during ischemia or reperfusion. The neuroprotective effects of AGE are observed in a preclinical study of ischemia. It is also shown that treatment with SAC not only attenuates damaging effects of ROS, but also prevents brain injury through the reduction of infarct volume (Chun et al. 2003). Collective evidence suggests that beneficial effects of garlic on ischemia/reperfusion injury are due to antioxidant properties of organosulfur compounds.

9.6.2 *Garlic Constituents and Alzheimer Disease*

Alzheimer disease (AD) is one of the most common forms of elderly dementia, which is characterized by cerebrovascular and neuronal dysfunctions leading to a progressive decline in cognitive functions. Pathological hallmarks of AD are neurofibrillary tangles consisting of hyper-phosphorylated microtubule-associated protein called tau and extracellular amyloid plaques (Farooqui 2010). The main component of amyloid plaques in AD brains is amyloid β (A β) peptide, whose deposition results in the production of ROS leading to the hyperphosphorylation of tau mediated by glycogen synthase kinase-3 (GSK-3). It is recently reported that

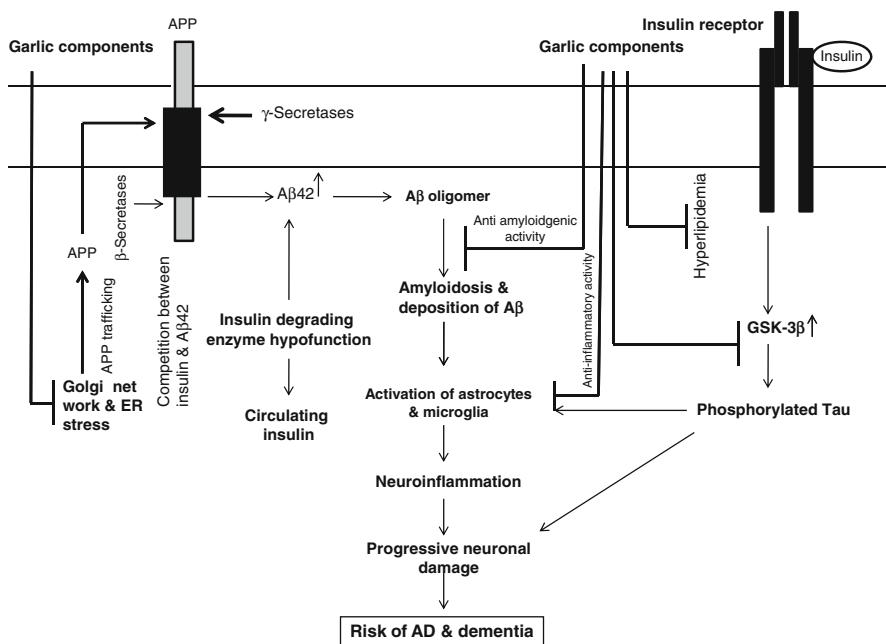


Fig. 9.9 Antiamyloidogenic effects of garlic constituents and inhibition of astrocytic and microglial activation by garlic in animal models of Alzheimer disease. Glycogen synthase kinase 3 (GSK-3); amyloid precursor protein (APP); beta amyloid (A β); upward arrow indicates increase; endoplasmic reticulum (ER); and Alzheimer disease (AD)

GSK-3 β phosphorylates preassembled tau filaments at a minimum of five sites promoting the filaments to combine into optically visible tangle-like structures similar to those formed by prephosphorylated tau and those isolated from AD patients (Rankin et al. 2008a, b). Hyperphosphorylation interferes with the binding of tau with tubulin inducing destabilization of axonal structures. This process may contribute to neuronal damage in AD (Fig. 9.9). In addition, in AD deposition of A β not only initiates a cascade of events causing synaptic failure, apoptotic neuronal death, cognitive decline and deficits in spatial memory, but also activation of microglia. The interactions between microglia and A β not only promote the generation of more ROS, but also facilitate the expression of cytokines (interleukins) (ILs) and tumor necrosis factor- α (TNF- α) and chemokines leading to severe neuronal damage (Coraci et al. 2002; Farooqui, 2010; Ray et al. 2011a).

Recent studies indicate that S-allyl-L-cysteine (SAC), the most active component of “aged garlic extract” (AGE) due to its antioxidant properties, can lower A β levels and toxicity (Ray et al. 2011b). Treatment of neuronal cultures with AGE and SAC results in protection against H₂O₂-mediated oxidative stress. Pretreatment with AGE alone also induces neuroprotection in 80 % neurons in cultures from ROS-mediated oxidative damage. In addition, AGE also preserves presynaptic protein

called synaptosomal-associated protein of 25 kDa (SNAP25) from ROS-mediated insult. Thus, treatment with AGE and SAC independently enhances SNAP25 levels (~70 %) and synaptophysin in Alzheimer amyloid precursor protein-transgenic mice, which are significantly decreased in AD (Ray et al. 2011a, b). Intrahippocampal injections of A β produce increase in lipid peroxidation. Pretreatment with S-allylcysteine (300 mg/kg, i.p.) 30 min before peptide injection decreases lipid peroxidation (Pérez-Severiano et al. 2004a). In addition, A β -induced abnormalities in learning and memory can also be prevented by SAC treatment. Observations on the effect of SAC on A β aggregation and levels are supported by studies on "Thioflavin-T, transmission electron microscopy," SDS-PAGE, size exclusion-HPLC. Under in vitro aggregating conditions, SAC not only dose dependently blocks A β fibrillation, but also destabilizes preformed A β fibrils (Gupta and Rao 2007). Further studies with circular dichroism and fluorescence quenching also support the ability of SAC to bind A β along with induction of a partially folded conformation in A β . SAC has been reported to protect neurons against the caspase-12-dependent neurotoxicity induced by A β (Kosuge et al. 2003). It is well known that hypercholesterolemia is a risk factor for AD. In Alzheimer transgenic model Tg2576, it is suggested that SAC may act as HMG CoA reductase inhibitor and mediate its beneficial effect by lowering cholesterol levels (Chauhan 2006). Garlic compounds are known to reduce A β -induced neuronal apoptosis, possibly by enhancing the endogenous antioxidant defenses (Peng et al. 2002). SAC has been shown to exhibit neurotrophic activity in cultured neurons (Moriguchi et al. 1997a, b). Thus, accumulating evidence suggests that SAC may prevent the progression of AD by multiple mechanisms *in vivo*.

Cerebroprotective and cardioprotective effects of dietary garlic may also be mediated through the generation of H₂S (Benavides et al. 2007). As mentioned earlier, garlic-derived organic polysulfides are not only converted by erythrocytes into H₂S, which relaxes vascular smooth muscle, induces vasodilation of blood vessels, but also decrease the risk of AD by lowering cholesterol levels, inhibiting neuroinflammation, reducing homocysteine, preventing oxidative brain injury, and protecting neurons against apoptosis triggered by oxidative stress. In addition, AGE prevents atrophy in the frontal brain of early senescence mice models, improves learning and memory retention and increases longevity (Moriguchi et al. 1997a). It is also shown that allixin, a component of garlic and AGE enhances the survival of neurons and increase the branching points in axons of hippocampus neurons (Moriguchi et al. 1997b).

Homocysteine is also a risk factor not only for cardiovascular diseases, but also for stroke, vascular dementia (arteriosclerotic dementia), and AD. Elevated homocysteine damages endothelial cells that line blood vessels and induces thrombosis that can lead to heart attacks and stroke. Homocysteine induces breaks in DNA and facilitates apoptosis, a major cause of neuronal death in dementia and AD (Seshadri et al. 2002; Kruman et al. 2002). The link between high levels of homocysteine and dementia including AD is based on the epidemiological studies and confirmed in case-control studies, where people with vascular dementia and AD have higher levels of homocysteine than healthy people. A direct link between increased plasma

homocysteine and loss of cognition is observed in recent studies. This observation supports the view that in adults with intact cognition, an elevation in plasma homocysteine, over time, is associated with an increased incidence of dementia, including AD. Consumption of AGE has been shown to reduce homocysteine levels (Yeh et al. 1999). This may be another mechanism by which garlic constituents protect from the risk of developing AD.

9.6.3 *Garlic Constituents and Parkinson Disease*

Parkinson disease (PD) is a chronic and progressive neurological disorder characterized by uncontrolled muscle tremor, rigidity, and bradykinesia. It affects over 1 % of people over the age of 65 years. PD is characterized by the degeneration of dopaminergic neurons in substantia nigra due to oxidative stress caused by monoamine oxidase-mediated abnormal dopamine metabolism and hydrogen peroxide generation (Farooqui 2010). Typical PD cases have intracellular proteinaceous inclusions called Lewy bodies and Lewy neurites in the brainstem and cortical areas. At the cellular level, the loss of dopaminergic neurons causes pathological changes in neurotransmission in the basal ganglia motor circuit (Farooqui 2010). Interactions among α -synuclein with microglia and astrocytes contribute to neuroinflammation and enhance expression of GFAP (Kim and Joh 2006).

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP $^{+}$)-treated mice have been widely used as animal models for PD. 1-Methyl-4-phenyl pyridium ion (MPP $^{+}$), the active metabolite of MPTP, selectively accumulates in the mitochondria of dopaminergic neurons via the dopamine transporter and confers toxicity and neuronal death through complex I inhibition (Vila and Przedborski 2003). Protection from MPP $^{+}$ neurotoxicity by various drugs has been used as index for neuroprotection.

As mentioned earlier, SAC, the most abundant organosulfur compound in AGE, produces beneficial effects of garlic through multiple mechanisms. It is well known that MPP $^{+}$ neurotoxicity involves oxidative stress via free radical production. Pretreatment of C57BL/6J mice with SAC for 17 days, followed by MPP $^{+}$ neurotoxicity not only attenuates MPP $^{+}$ -mediated neurotoxicity, but also results in significant decrease in dopamine levels in the striatum (Rojas et al. 2011). In addition, neuroprotective effect of SAC against MPP $^{+}$ neurotoxicity also blocks lipid peroxidation and reduces superoxide radical production as judged by the upregulation of Cu-Zn-SOD activity. Behavioral analyses indicate that SAC improves MPP $^{+}$ -mediated impairment in locomotion (35 %) (Rojas et al. 2011). Collectively, these studies suggest that in mice, SAC attenuates MPP $^{+}$ -induced neurotoxicity in the striatum and that an antioxidant effect against oxidative stress may be partly responsible for its observed neuroprotective effects (Rojas et al. 2011). In addition, it is recently shown that endogenous H₂S also protects PC12 cells from MPTP toxicity. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor protects PC12 cells against the MPP $^{+}$ -mediated neurotoxicity by upregulating endogenous H₂S generation. This upregulation of endogenous

H_2S generation decreases MPP⁺-mediated accumulation of intracellular ROS, which consequently upregulates Bcl-2 expression and prevents MPP⁺-stimulated mitochondrial membrane potential loss and Cyt-c release (Tang et al. 2011). These studies shed light on the mechanisms of H_2S -mediated neuroprotective effects on MPP⁺ toxicity.

9.6.4 Effect of Garlic Constituents in Animal Model of Huntington Disease

3-Nitropropionic acid is a neurotoxin that irreversibly inhibits succinate dehydrogenase, which is associated with the complex II of the respiratory chain during mitochondrial electron transport. 3-Nitropropionic acid has been used to produce an experimental model of Huntington disease (HD), which is characterized by increase in oxidative/nitrosative stress. Oxidative/nitrosative stress is closely associated with the pathogenesis of HD. 3-Nitropropionic acid (0.75–2.5 mM) induces enhancement in lipid peroxidation (Pérez-De La Cruz et al. 2006). Treatment with increasing concentrations of SAC (0.1–2 mM) reduces the peroxidative action of 3-nitropropionic acid (1 mM) in synaptosomal fractions in a dose-dependent manner. SAC at 0.75 mM blocks the 3-nitropropionic acid (1 mM)-mediated mitochondrial dysfunction supporting the view that 3-nitropropionic acid-mediated oxidative stress can be prevented by SAC (Pérez-De La Cruz et al. 2006).

Like 3-nitropropionic acid, infusion of quinolinic acid in striatum also produces neurotoxicity through the generation of ROS, elevation in lipid peroxidation, and induction of oxidative stress. SAC has been shown to retard quinolinic acid-mediated oxidative stress through the inhibition of ROS production (Pérez-Severiano et al. 2004b). Mounting evidence suggests that *in vivo* SAC ameliorates quinolinic acid striatal toxicity through several mechanisms, including: (a) scavenging free radicals; (b) decreasing oxidative stress; and (c) preserving the striatal activity of Cu, Zn-superoxide dismutase (Cu, Zn-SOD). These mechanisms may be responsible for the preservation of the morphological and functional integrity of the striatum (Pérez-Severiano et al. 2004b).

9.6.5 Effect of Garlic Constituents on Depression

Depression is a common psychiatric disorder that is universally experienced by virtually everyone at some point of time in life. Although multiple pathways are associated with the pathogenesis of depression, currently monoaminergic pathway has received considerable attention. According to monoamine hypothesis, depression is caused by a functional deficit in monoamines (norepinephrine, serotonin, and dopamine) and monoaminergic signaling at certain sites in brain (Gold et al. 1988).

Increase in monoamine oxidase results in reduction of monoamine metabolite levels in the cerebrospinal fluid of depressed individuals (Leonard 2000; Nutt 2002). In addition to reduction in monoamine signaling pathways, decrease in GABA levels has been reported to occur in the cerebrospinal fluid of depressed patients (Gold et al. 1980; Roy et al. 1991). An acute and chronic treatment with CGP56433A, a selective GABA_B receptor antagonist, decreases immobility in forced swim test, suggesting that GABA_B receptor antagonism may serve as a basis for the generation of novel antidepressants (Mombereau et al. 2004).

Administration of ethanolic garlic (25, 50 and 100 mg/kg p.o.) to mice, for 14 successive days shows significant antidepressant-like activity as judged by Forced Swim Test (FST) and Tail Suspension Test (TST). This efficacy of garlic extract is comparable to fluoxetine and imipramine (Dhingra and Kumar 2008). Garlic extract does not produce significant changes in the locomotor activity of mice, as compared to the control group, indicating that garlic extract does not produce any motor effects. The antidepressant-like effect of garlic extract can be significantly reversed by the treatment of animals with prazosin (α_1 -adrenoceptor antagonist), sulpiride (a selective dopamine D₂-receptor antagonist), p-CPA (serotonin synthesis inhibitor), and baclofen (GABA_B agonist) when tested in TST, suggesting that garlic extract mediates antidepressant-like effect through interaction with α_1 -adrenoceptors, dopamine D₂ receptors, and serotonergic and GABAergic receptors (Dhingra and Kumar 2008). These interactions elevate the levels of norepinephrine, dopamine, and serotonin and reduce GABA levels in the brain of mice. In addition, administration of garlic extract (100 mg/kg p.o.) to mice, for 14 successive days, significantly reduces brain MAO-A and MAO-B activities as compared to the control group. Collective evidence suggests that garlic extract produces antidepressant-like activity probably by inhibiting MAO-A and MAO-B levels, and through interaction with adrenergic, dopaminergic, serotonergic, and GABAergic systems (Dhingra and Kumar 2008).

9.7 Conclusion

Organosulfur compounds of garlic (allicin, SAC, diallyl sulfide, and diallyl trisulfide) not only produce antibacterial, antiviral, antifungal, and antiprotozoal effects, but also mediate beneficial effects on the cardiovascular, cerebrovascular, and immune systems resulting into cardioprotective and neuroprotective effects. Besides its antimicrobial effect, garlic constituents also produce effective antioxidant, anti-inflammatory, and antitumor effects. Although nothing is known about the half-lives of organosulfur compounds of garlic in the brain in general, and neuronal and glial cells in particular, organosulfur compounds of garlic have been reported to cross BBB and produce their antioxidant and anti-inflammatory effects by modulating signal transduction processes in animal models of stroke, Alzheimer disease, and Parkinson disease. Garlic extracts have been shown to have multiple biological activities and prevents cognitive decline by protecting neurons from A β

neurotoxicity and apoptosis. Garlic not only produces antiaging effects, but also improves learning and memory impairments and has neurotrophic effects. In addition, the generation of H₂S may also contribute to the beneficial physiological effects garlic exerts on the cardiovascular and cerebrovascular systems. Based on above information, it can be proposed that large, long-term, fully blinded, and well-controlled studies using a standardized preparation of garlic with known active components are necessary for realizing the beneficial effects of garlic in animal models of stroke, Alzheimer disease, and Parkinson disease. Based on results of animal model studies, one can design large, long-term, fully double-blinded controlled studies in normal humans and patients with above neurological disorders.

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Chapter 10

Beneficial Effects of Propolis on Neurological Disorders

10.1 Introduction

Propolis (bee glue) is a resinous natural substance gathered by worker honeybees from certain parts (buds and barks) of plants, and thus chemical composition of propolis depends on the phytogeographic characteristics of the collection site. In different habitats, bees choose different plant species as propolis sources and consequently the chemical composition of this bee product is highly variable. In spite of different chemical composition, propolis always demonstrates similar biological activities (Banskota et al. 2001). In temperate zones, propolis originates from the bud exudates of *Populus* species, and therefore has relatively constant qualitative composition (Greenaway et al. 1990). In tropical regions, there are no poplars, and the bees are known to find other sources of their glue (Park et al. 2002). Honeybees use propolis for the construction and repair of their hive as well as for the defense purposes. It not only contains sticky compounds coming from various plants, but also waxes and other honeybee excretions (Castaldo and Capasso 2002). Humans use propolis as a natural remedy because of its numerous health benefits including antioxidant, anti-inflammatory, vasodilatory, and immunostimulating properties (Fig. 10.1) (Banskota et al. 2001; Kujumgiev et al. 1999; Sforcin 2007; Seidel et al. 2008). In addition, propolis is used as a popular remedy and is sold in the form of capsules, as an extract, as a mouthwash, in throat lozenges, creams, and in powder form for gargling. Propolis is also claimed to be useful in cosmetics and as a constituent of health foods. Oral administration of propolis extract also results in suppression of overall weight gain in mice, the accumulation of visceral adipose tissue weight, and the increase in serum and liver triglycerides that normally result from feeding a high-fat diet to C57BL/6N mice (Koya-Miyata et al. 2009). Real-time PCR studies indicate that the antiobesity effects of propolis extract can be attributed to reduction in the expression of fatty acid synthesis genes in the liver (Koya-Miyata et al. 2009). In addition, propolis extract also inhibits body weight gain, lowers blood pressure, and liver triglycerides in obesity-induced by a high-fat diet. Since it is well known that accumulation of visceral adipose tissue and hyperlipidemia associated with

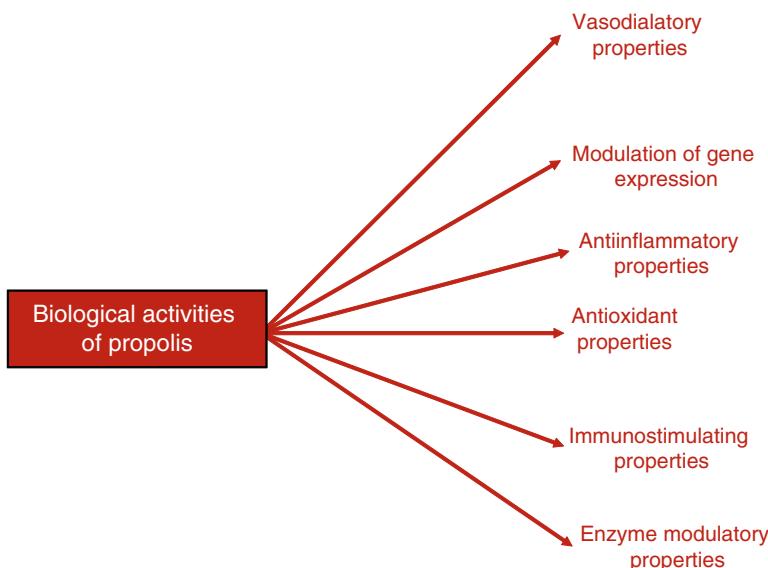


Fig. 10.1 Biological activities of propolis

metabolic syndrome. These studies indicate that propolis extract may prevent and mitigate metabolic syndrome caused by excessive intake of a high-fat diet, and this may involve downregulation of lipid metabolism-related gene expression (Koya-Miyata et al. 2009). Recently, propolis is being widely used in food, beverage, and pharmaceutical industries as a health supplement (Banskota et al. 2001).

10.2 Chemical Composition and Biological Activities of Propolis

Propolis is composed of 30 % wax, 50 % resins and vegetable balsams, 10 % essential oils, 5 % pollen and other substances (Burdock 1998). The color of propolis varies from green, red to dark brown. The chemical composition of propolis is very complex and represents great variability (Bankova 2005; Sforcin 2007). More than 300 compounds, such as amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, terpenes, caffeic acid, ferulic acid (Fig. 10.2), caffeic acid phenethyl ester (CAPE), sesquiterpenes quinines, coumarins, steroids, and inorganic compounds, have been identified in propolis samples. The contents of these compounds depend on the collecting location, time, and plant source (Lofty 2006). The presence of 12 different flavonoids, pinocembrin, acacetin, chrysanthemum, rutin, catechin, naringenin, galangin, luteolin, kaempferol, apigenin, myricetin, and quercentin, and two phenolic acids, cinnamic acid and caffeic acid, has been reported

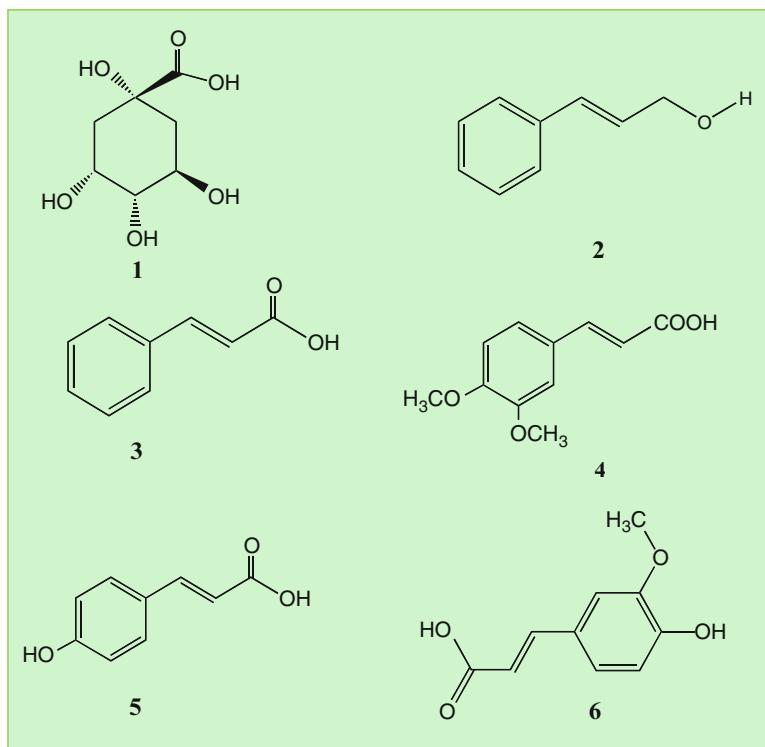


Fig. 10.2 Major phenolic compounds (flavonoids and phenolic acid derivatives) in propolis: (1) quinic acid, (2) cinnamic alcohol, (3) cinnamic acid, (4) 3,4-dimethoxy cinnamic acid, (5) *p*-coumaric acid, and (6) ferulic acid

to occur in propolis extracts (Fig. 10.3) (Lofty 2006). In addition, propolis also contains some minerals such as Mg^{2+} , Ca^{2+} , K^+ , Na^+ , Cu^{2+} , Zn^{2+} , Mn^{2+} , and Fe^{2+} as well as some vitamins like B1, B2, B6, C, and E, and a number of fatty acids. It also contains some enzymes such as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase, and acid phosphatase (Tikhonov and Mamontova 1987). Propolis contains copper 26.5 mg/kg, manganese 40 mg/kg, and the ash residue having iron, calcium, aluminum, vanadium, strontium, manganese, and silicon (Moreira 1986). Phenolic acid esters, flavonoids, cinnamic acid, terpenes, caffeic acid, ferulic acid, and CAPE account for strong radical-scavenging activity of propolis. Propolis flavonoids need a 2–3 carbon double bond, a carbonyl group at carbon 4 of the C-ring, and two hydroxyl groups at carbons 5 and 7 of the A-ring (Fig. 10.4) for anti-inflammatory activity (Farooqui and Farooqui 2010; Lotito and Frei 2006). Antioxidant activity of flavonoids is due to their ability to reduce free radical formation, scavenge free radicals, and chelate metal ions (Ahn et al. 2009). Flavonoids in propolis possess Fe^{2+} chelating properties (van Acker et al. 1996). Flavonoids require a hydroxyl group at carbon 3 of the C-ring and two hydroxyl

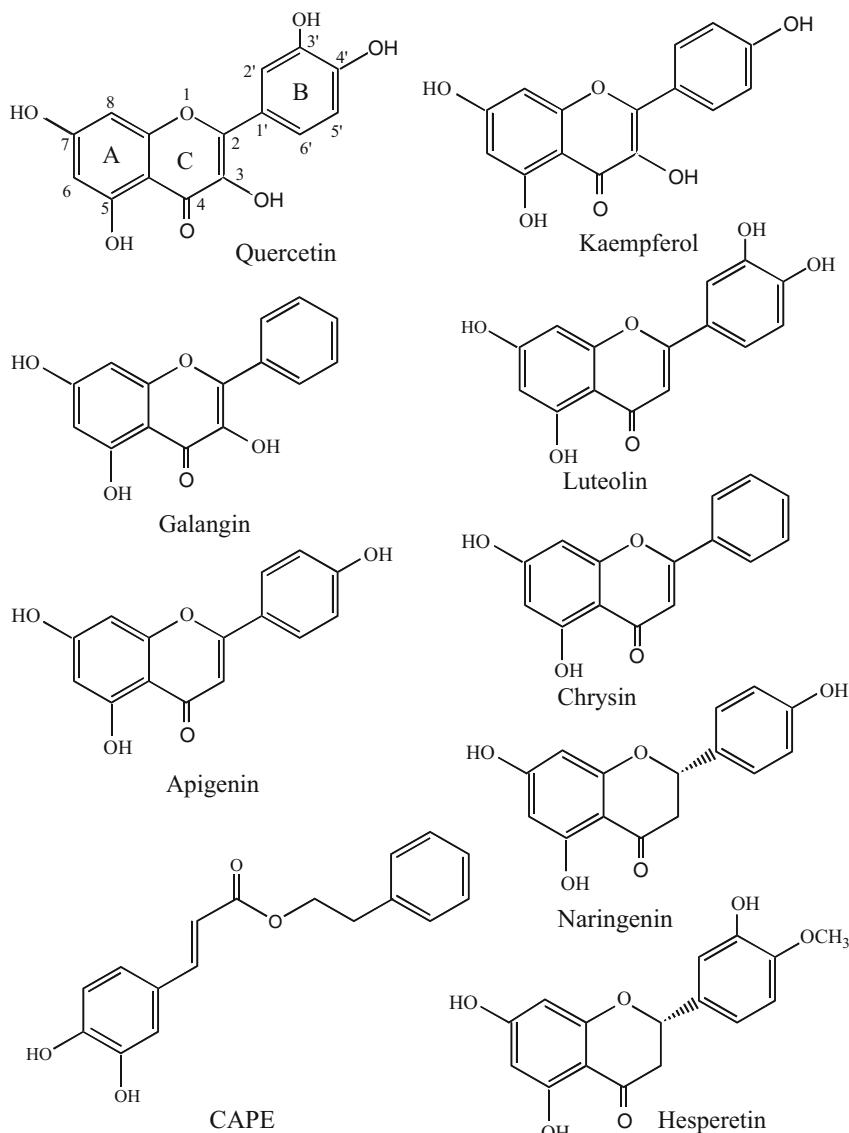
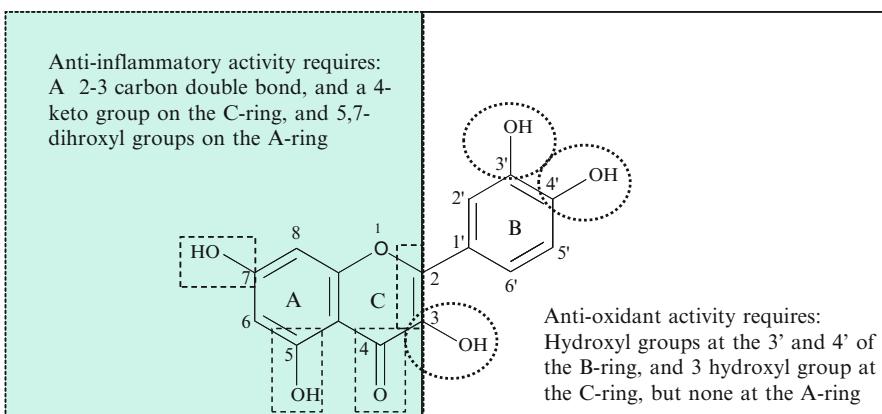


Fig. 10.3 Chemical structures of flavonoids found in propolis

groups at carbons 3' and 4' of the B-ring (Fig. 10.4) for their antioxidant activity (Farooqui and Farooqui 2010; Jovanovic et al. 1994; Amić et al. 2007).

The good scavenging activity requires the presence of a catechol moiety on ring B along with 3-OH moiety in combination with a C2 C3 double bond in chelators (Farooqui and Farooqui 2010). These structural requirements increase Fe^{2+} chelating and inhibiting or decreasing the rate of lipid peroxidation (van Acker et al. 1996).

Anti-oxidative activity



Anti-inflammatory activity

Fig. 10.4 Structural requirements of flavonoids for their anti-inflammatory and antioxidative activities

Propolis flavonoids have also been shown to exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, and vaso-dilatory activities. In addition, flavonoids inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the activity of enzyme systems like cyclooxygenase and lipoxygenase (Viuda-Martos et al. 2008).

Antiplatelet activity of flavonoids can be attributed to the increased production of prostacyclin by endothelial cells. Prostacyclin decreases aggregation via synthesis of cAMP and increases the cAMP concentration inhibiting the expression of platelet GPIIb/IIIa receptors (Dell’Agli et al. 2008). Among flavonoids, quercetin is an excellent free-radical scavenging antioxidant, even if such an activity strongly depends on the intracellular availability of reduced glutathione. In addition, propolis also contains baccharin, beturetol, kaempferide, isosakuranetin, and drupanin, which modulate HIF-1-dependent luciferase activity associated with Brazilian green propolis (Hattori et al. 2011) (Fig. 10.5). It is well known that hypoxic injury is accompanied by an uncontrolled growth and insufficient vascularization. Neural cells respond to hypoxic injury by stimulating the expression of hypoxia-inducible factor (HIF), which is critical for the survival of neural cells under hypoxic conditions (Fan et al. 2009). HIF is a heterodimer consisting of the O₂-regulated sub-unit, HIF-1 α , and the constitutively expressed aryl hydrocarbon receptor nuclear translocator, HIF-1 β . Under hypoxic conditions, oxidative stress-mediated injury promotes the migration of HIF-1 α to the nucleus where it binds with HIF-1 β to form the complex (HIF-1 α + HIF-1 β). The initiation of transcription is promoted by the binding of the complex (HIF-1 α + HIF-1 β) to hypoxia-responsive elements (HREs). This complex [(HIF-1 α + HIF-1 β) + HREs] stimulates the expression of genes involved in angiogenesis, vascular permeability, and neuroinflammation

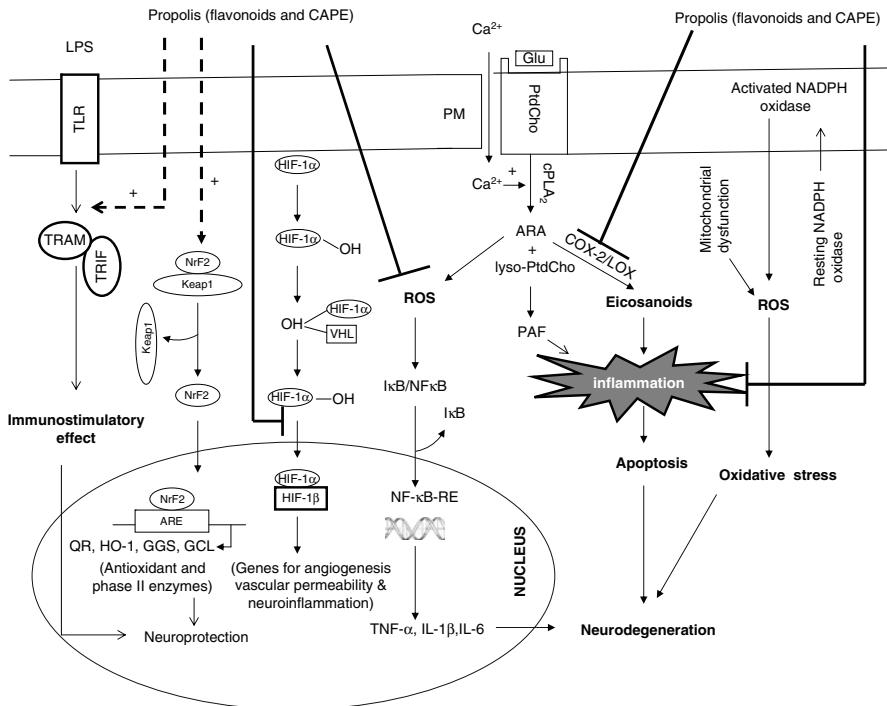


Fig. 10.5 Hypothetical molecular mechanism underlying beneficial effects of propolis. Cellular injury involves the generation of ROS by multiple mechanisms, activation of NF- κ B, alterations in redox status, and gene expression during oxidative stress. Cytosolic phospholipase A₂ (cPLA₂); cyclooxygenase-2 (COX-2), lipoxygenase (LOX); cytokines (TNF- α and IL-1 β); nuclear factor- κ B (NF- κ B); nuclear factor- κ B response element (NF- κ B-RE); nuclear factor (erythroid-derived 2)-like 2 (NrF2); and kelch-like erythroid Cap'n'Collar homologue-associated protein 1 (Keap1). Propolis components inhibit HIF prolyl hydroxylase (HIF-PH). Propolis components not only inhibit HIF, COX and LOX, ROS production, and neuroinflammation, but stimulate TLR and NrF2. Positive sign indicates stimulation; and \neg (blocked arrow) represents inhibition

(Nangaku et al. 2006; Fan et al. 2009; Chen et al. 2009). CAPE inhibits the migration of HIF-1 α to the nucleus and prevents the expression of genes responsible for the induction of neuroinflammation. In addition, cinnamic acid derivatives that occur in propolis not only significantly inhibit the expression of HIF-1 α protein, but they also retard downstream target genes such as glucose transporter 1, hexokinase 2, and vascular endothelial growth factor A (Hattori et al. 2011). Propolis not only inhibits the expression and enzyme activity of glucose 6-phosphatase (G6Pase), but also blocks the activity of glycogen synthases kinase 3 alpha and beta (GSK3 α and β) via the inhibition of serine and tyrosine phosphorylation. Although propolis shows antioxidant activity and antidiabetic effect, these activities are not influenced by hydrogen peroxide and N-acetylcysteine, supporting the view that propolis inhibits the expression of G6Pase by blocking the autophosphorylation of Y279 and Y216 of GSK3 α and β , respectively, which are involved in the activation of GSK3

(Bojić et al. 2011). Collective evidence suggests that due to the presence of phenolic acids and flavonoids, propolis exhibits antioxidant, anti-inflammatory, and vasodilatory activities (Kumazawa et al. 2004, 2010). Modulation of activities may exert positive preventive effects in neurotraumatic and neurodegenerative disorders not only because of modulation of antioxidant activity and their capacity to affect the expression of several detoxifying enzymes, but also their ability to modulate protein signaling cascades (Williams et al. 2004).

Studies on the effect of propolis on Th1/Th2 cytokines production by spleen cells of acutely stressed mice indicate that the effect of propolis is not antagonized by stress-induced inhibition on IFN- γ secretion (Pagliarone et al. 2009). However, propolis facilitates the production of IL-4 by spleen cells. This observation indicates its immunorestorative role, since stress may lead to a higher susceptibility to infectious diseases and humoral immunity is extremely important to host defense against infections by extracellular microorganisms. Although the molecular mechanisms involved in immunostimulatory effect of propolis is still not fully understood, recent studies on Toll-like receptors (TLRs) indicate that propolis modulates TLR-2 and TLR-4 expression (Orsatti et al. 2010) (Fig. 10.5). TLRs are expressed by various cell types of the immune system (Kawai and Akira 2006). They recognize conserved pathogen-associated molecular patterns shared by different microorganisms, such as bacterial lipopolysaccharide, bacterial or mycobacterial lipopeptides, viral RNA and DNA, and not only play an important role in the innate immune response, but also in the initiation of adaptive immune response (Hopkins and Sriskandan 2005). Basal levels of IL-1 β production and TLR-2 and TLR-4 expression are not only increased in peritoneal macrophages of propolis-treated mice, but also in the spleen cells of propolis-treated mice. These observations support the view that propolis activates the initial steps of the immune response by upregulating TLRs expression and the production of pro-inflammatory cytokines in mice (Orsatti et al. 2010).

10.3 Bioavailability of Propolis Components in the Brain

Phenolic compounds, like caffeic acid CAPE are small lipid soluble molecule with multiple biological effects, such as antioxidant and anti-inflammatory agent (Russo and Vanella 2002). It passes rapidly into blood after intraperitoneal injection and due to its lipophylic nature and small molecule size, it can cross blood–brain barrier (BBB) and enter easily into cells by crossing neural cell membranes. At low doses, it not only regulates NF- κ B (Natarajan et al. 1996), but retards the expression of proinflammatory cytokines, resulting in the downregulation of oxidative stress and neuroinflammation (Fig. 10.5). It is suggested that CAPE-mediated inhibition of NF- κ B may be due to the suppression of nuclear translocation and DNA binding of p65 or phosphorylation of p65, which is dependent or independent on I κ B α protein degradation in the cytosol (Ang et al. 2009).

In a dose-dependent manner, CAPE inhibits MCF-7 (hormone receptor positive, HR+) and MDA-231 (a model of triple negative BC (TNBC) tumor growth, both

in vitro and in vivo without much effect on normal mammary cells, and strongly influences gene and protein expression. It induces cell cycle arrest and apoptosis, and reduces the expression of growth. CAPE interacts with human estrogen receptor b (hERb) and reduces human estrogen receptor a (Era) expression in MCF-7 and MDA 231 cells (Jung et al. 2010). In the yeast estrogen receptor transcription assay, CAPE facilitates the transcriptional activity of estrogen-responsive element with EC₅₀ values of 3.72×10^{-6} M, but does not increase the growth of MCF-7 estrogen receptor-positive breast cancer cells in doses ranging from 10^{-7} to 10^{-5} M. Collective evidence suggests that CAPE is a selective agonist to hERb, but does not produce any estrogenic effect on estrogen receptor-positive breast cancer cells and in immature rat uterine tissue (Jung et al. 2010). CAPE also downregulates the GTPase Rac, a direct activator of the kinase PAK1 (an enzyme that is associated with pathogenesis of tumors in humans) (Xu et al. 2005). As a consequence, CAPE eventually inactivates PAK1. This anti-PAK1 action of CAPE may contribute to the usefulness of CAPE for the treatment of these PAK1-dependent cancers or tumors. Propolis preparations from New Zealand (NZ) have been reported to contain the highest CAPE content (6–7 % of extract) of a variety of propolis samples from around the world, whereas Brazilian green propolis contains another anticancer ingredient called ARC (artepillin C), instead of CAPE, which also inactivates PAK1 (Maruta and Ohta 2008).

Nuclear factor-erythroid 2 p45 (NF-E2)-related factor 2 (Nrf2) is another important cytoprotective transcription factor, which is located in the cytoplasm as an inactive complex with its cytosolic repressor Kelch-like ECH associated protein 1 (Keap1). This protein mediates proteasomal degradation of Nrf2 by acting as an adaptor protein of an E3 ubiquitin ligase complex (Tong et al. 2006). When challenged by oxidants, Nrf2 is released from Keap1 repression and migrates to the nucleus, where it interacts with antioxidant response element (ARE) and mediates the expression of several detoxifying enzymes. CAPE activates Nrf2 pathway and the inhibitory effect of CAPE on NFκB can be attenuated by knockdown of Nrf2 (Lee et al. 2010) (Fig. 10.5). It is demonstrated that removal or modification of the catechol moiety, Michael reaction acceptor, or phenethyl ester moiety in CAPE structure impairs its ability to inhibit NFκB (Lee et al. 2010). In addition, CAPE is a potent inducer of HO-1 in neural cells (astrocytes and neurons) (Scapagnini et al. 2002).

The bioavailability of propolis-derived flavonoids in various visceral tissues is very low due to their intestinal and hepatic metabolism (Williams et al. 2004; Spencer et al. 2004). Flavonoids are not only metabolized by several enzymes located in the small intestine and colon, but also in the liver. Molecular sites of metabolic modification include methylation on the B-ring catechol group, influencing both antioxidant and pro-oxidant properties, and glucuronidation/sulfation on the A ring (and C and B rings in certain structures), altering lipophilicity. Glutathionylation can occur on the C ring and may be an important metabolic step in cell detoxification and export, but can potentially influence the homeostatic control of cell redox and thiol-dependent signaling. Intramolecular cleavage sites are involved in colon metabolism. Ring fission via the C ring can lead to a variety of simple phenolics

depending on the position of ring cleavage. These various metabolites may also be absorbed from the large intestine and subsequently undergo further hepatic metabolism (Spencer et al. 2004).

The bioavailability of flavonoids in the brain is even lower than visceral tissues and information on molecular mechanisms of their action is still poorly understood. The degree of BBB penetration of propolis flavonoids depends on their lipophilicity (Youdim et al. 2003). Thus, less polar O-methylated metabolites are capable to greater brain uptake than the more polar flavonoid glucuronides. However, some studies indicate that certain glucuronides may cross the BBB by specific uptake mechanism for glucuronides *in vivo* (Aasmundstad et al. 1995). Their brain entry may be also modulated by their interactions with specific efflux transporters expressed in the BBB, such as P-glycoprotein (Lin and Yamazaki 2003) which appears to be responsible for the differences between naringenin and quercetin flux into the brain *in situ* (Youdim et al. 2004).

10.4 Beneficial Effects of Propolis Components on Neurological Disorders

Brain aging is accompanied by oxidative damage to nucleic acid, carbohydrate, protein, and lipids, which accumulates over the years. Oxidative damage is particularly detrimental to neurons, which are largely postmitotic. Therefore, damaged neurons cannot be replaced readily via mitosis (Farooqui 2010). During normal aging, the brain undergoes morphological and functional modifications resulting in behavioral declines such as decrements in motor and cognitive performance. These declines are intensified by neurodegeneration in neurotraumatic [stroke, spinal cord injury (SCI), and traumatic brain injury] and neurodegenerative diseases (Alzheimer disease and Parkinson disease). Neurodegeneration is a multifactorial process, which involves oxidative stress, neuroinflammation, reduction in the expression of trophic factors, and accumulation of protein aggregates (Farooqui 2010). Prominent neurochemical alterations in neurotraumatic and neurodegenerative diseases include increased breakdown of neuronal membrane phospholipids (phosphatidylcholine and plasmalogen), sphingolipids (sphingomyelin), and cholesterol due to the stimulation of PLA₂, sphingomyelinases (SMase), and cytochrome P450 hydroxylases (Farooqui 2010) along with increase in levels of lipid mediators (Farooqui 2011) and the abnormal accumulation of iron in the degenerating neurons as well as in the surrounding microglia and astrocytes. Ramified microglial cells constantly survey the environment with their long protrusions (Nimmerjahn et al. 2005). Microglial activation may result in neuroprotection not only through the release of neurotrophic factors, but also through the induction of phagocytosis of accumulated proteins (A β and α -synuclein), which are critical neurotoxic component of AD and PD brain, respectively. Concurrently, microglial activation causes elevated inflammatory responses that lead to paracrine damage to neurons.

Activated microglial cells also receive continuous inhibitory signals from neurons retarding microglial neurotoxicity (Cardona et al. 2006; Hamby and Sofroniew 2010). Breakdown of the microglia-neuron cross-talk (Dick et al. 2003), local danger signals, such as released ATP (Haynes et al. 2006), or neurotransmitter gradients (Ransohoff and Perry 2009) can lead to a functional transformation of resting microglia into activated microglia with a variety of effectors, including the generation of NO and expression of cytokines and chemokines. Consequently, alarmed microglia and activated astrocytes along with reactive microgliosis and astrogliosis have been identified in a variety of neurotraumatic and neurodegenerative diseases (Farooqui 2010).

Reactive microgliosis and astrogliosis are not merely a marker of neuropathology of neurotraumatic and neurodegenerative diseases, but play essential roles in orchestrating the injury response as well as in regulating inflammation and repair in a manner that markedly impacts functional and clinical outcomes (Sofroniew 2005).

During inflammatory response, activation of glial cells is accompanied by the generation of nitric oxide (NO) via induction of CD23 receptor-mediated stimulation of iNOS. This process is in turn activated by cytokines such as TNF- α and IL-1 β . NO diffuses to neighboring neurons where it inhibits mitochondrial respiration at cytochrome c oxidase. NO may also react with superoxide radical to generate peroxynitrite which can cause damage to proteins, inhibit mitochondrial respiration, and activate cell death genes and signaling pathways, leading ultimately to neurodegeneration in neurotraumatic and neurodegenerative diseases (Farooqui 2009). Furthermore, cytokines such as TNF- α and IL-1 β may directly trigger neurodegeneration by interacting with their receptors on neuronal surface. This process also results in the stimulation of apoptotic cell death (Farooqui 2009). It is proposed that flavonoids exert their neuroprotective effects (at low, physiological concentrations) through their interactions with critical neuronal/glial intracellular signaling pathways pivotal in controlling neuronal resistance to neurotoxins, including oxidants ("indirect" antioxidant nature) (Levites et al. 2001) and inflammatory mediators (Spencer 2009), or through their chelation of transition metal ions such as iron (Levites et al. 2002; Mandel et al. 2005; Spencer et al. 2012). It should be noted that flavonoids have close structural homology to specific inhibitors of cell signaling cascades, such as PD98059, a MAPK inhibitor and LY294002, a phosphatidylinositol-3 kinase (PtdIns 3K) inhibitor. PD98059 and LY294002 effectively inhibit iNOS expression and NO production in activated glial cells (Bhat et al. 1998). Like above mentioned inhibitors, flavonoids also retard the expression of iNOS and NO production, supporting the view that flavonoids may act as anti-inflammatory molecules in this signaling pathway (Spencer et al. 2012). Propolis-derived flavonoids, polyphenols, and phenolic acids not only exert anti-inflammatory effects, but are able to chelate iron and thereby inhibiting lipid peroxidation. Propolis-derived flavonoids may also inhibit PLA₂, PLC, COX, LOX, and protein kinase activities (Rezai-Zadeh et al. 2005; Kim et al. 2010). In addition, many propolis flavonoids induce adaptive cellular stress responses, by which neural cells achieve ability to counteract stressful situations (Calabrese et al. 2008). Adaptive cellular stress responses not only require the activation of pro-survival genes, but also induction of the expression of antioxidative and antiapoptotic molecules and their neurochemical activities (Calabrese et al. 2008).

The vitagene system has emerged as a neurohormetic potential target for novel cytoprotective interventions of polyphenolic phytochemicals (Calabrese et al. 2008). These genes encode for survival proteins such as HSP70 and HO-1 as well as thioredoxin/thioredoxin reductase (Calabrese et al. 2008, 2009). It is becoming increasingly evident that neurohormetic phytochemicals including flavonoids suppress disease processes in animal models of PD (Mattson et al. 2007; Kim et al. 2010).

10.4.1 Beneficial Effects of Propolis Components in Ischemic Injury

Stroke (ischemia) is a metabolic insult caused by severe reduction in cerebral blood flow due to a clot formation in cerebrovascular artery. This decrease in cerebral flow not only decreases oxygen and glucose delivery to brain tissue but also results in the breakdown of BBB and build-up of potentially toxic products in brain (Farooqui 2010). Two major types of strokes (ischemic and hemorrhagic) are known to occur in the mammalian brain. Ischemic strokes are brought about by critical decrease in blood flow to various brain regions causing neuronal cell death. Ischemic stroke is the most common type of stroke, constituting around 80 % of all strokes, of which 60 % are attributable to large-artery ischemia. Hemorrhagic strokes are caused by a break in the wall of the artery resulting in spillage of blood inside the brain or around the brain (Farooqui 2010). During ischemic injury, interruption in oxygen supply and depletion in ATP generation, as well as mitochondrial dysfunction result in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The initial response to ATP depletion in ischemic injury is depolarization, which causes Na^+ influx into axons. Prolonged depletion of ATP produces a massive Ca^{2+} influx and accumulation that facilitates neurodegeneration (Farooqui 2010).

Propolis not only contains high concentration of pinocembrin, a flavanone with antioxidant, antibacterial, anti-inflammatory, and antifungal properties, but also has phenolic acids and phenolic acid esters, which produce antioxidative and neuroprotective effects. Studies on neuroprotective effect of pinocembrin in a model of middle cerebral artery occlusion and reperfusion in rats indicate that pinocembrin increases rat viability, reduces infarct volumes and neurological deficit scores in rats (Liu et al. 2008). Similarly in a cell culture model of ischemia/reperfusion-like injury, treatment of primary cortical neuronal cultures with pinocembrin at the start of reoxygenation results not only in increase in neuronal survival rates and decrease in LDH release, but also alleviates both neurite length and apoptosis (Liu et al. 2008). These processes are accompanied by the reduction in ROS, nitric oxide, and neuronal nitric oxide synthase (nNOS) and inducible NOS (iNOS), and increase in glutathione. In addition, pinocembrin-treated group also shows a decrease in DNA laddering, downregulation in caspase-3 activity, and alleviation in PARP degradation (Liu et al. 2008).

Like pinocembrin, CAPE also mediates a protective effect on brain injury in a right focal permanent middle cerebral artery occlusion (pMCAO) model of ischemia in rabbits (Khan et al. 2007; Altuğ et al. 2008). Intraperitoneal administration

of CAPE for 7 days in rabbits indicates that CAPE treatment significantly reduces the percentage of infarction in the ipsilateral hemisphere compared with the ischemia group. CAPE treatment significantly attenuates the elevation of plasma malondialdehyde, catalase, and xanthine oxidase content, whereas it significantly increases the levels of plasma GSH and NO. Therefore, subacute CAPE administration plays a protective role in focal pMCAO due to attenuation of lipid peroxidation and its antioxidant activity (Altug et al. 2008). Treatment with CAPE also decreases lipid peroxidation and nitrotyrosine levels, and enhances cerebral blood flow (Khan et al. 2007). CAPE downregulates neuroinflammation by inhibiting nuclear factor kappa B activity. The affected mediators include adhesion molecules (intercellular adhesion molecule-1 and E-selectin), cytokines (tumor necrosis factor-alpha and interleukin-1 β), and iNOS. Antineuroinflammatory action of CAPE can be further documented by the reduction in ED1 (marker of activated macrophage/microglia) expression. In addition, CAPE treatment also retards apoptotic cell death by downregulating caspase 3 and upregulating antiapoptotic protein Bcl-xL. Collective evidence suggests that CAPE provides neuroprotection against cerebral ischemic injury through its antioxidant and anti-inflammatory actions (Altug et al. 2008; Tsai et al. 2006; Khan et al. 2007).

10.4.2 Beneficial Effects of Propolis Components in Spinal Cord Injury

SCI causes severe and often permanent neurological deficits due to the loss of descending motor and ascending sensory axonal pathways, and demyelination (Bunge et al. 1993). The pathophysiology of SCI involves the initial primary injury followed by secondary injury processes involving cascades of biochemical, molecular, and cellular changes, which can produce even more extensive damage. Although mechanical disruption of the nerve axons in the spinal cord cannot be treated because of instantaneous neuronal cell death, changes in secondary injury are susceptible to therapeutic intervention (Sekhon and Fehlings 2001; Klussmann and Martin-Villalba 2005). Neurochemical changes during secondary event include microvascular ischemia, edema, elevation in excitatory amino acids (excitotoxicity), dysregulation, neuroinflammation, and oxidative stress (generation of ROS). Events in secondary injury are primarily mediated by a robust cellular inflammatory response involving macrophage and microglial activation, and chemokine and cytokine production (Farooqui 2010). These processes not only effect neuronal activities, glial cell reaction, and demyelination involving oligodendrocytes, but also modulate leukocyte infiltration and activation of macrophages and vascular endothelial cells (Bramlett and Dietrich 2004) along with the failure of axonal regeneration due to the expression of axonal growth-inhibiting molecules (Fawcett and Asher 1999), the lack of neurotrophic factors (Widenfalk et al. 2001), and/or inflammatory reactions (Franzen et al. 1998). Studies on the effect of ethanol extract of Chinese propolis, which is enriched in pinocembrin, indicate that its intraperitoneal injections in rats suppress

iNOS gene expression, reduce NO generation, and increase the expression of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) mRNAs at the lesion site (Kasai et al. 2011). In addition, ethanol extract of Chinese propolis ameliorates the locomotor activity after SCI. This improvement is accompanied by a reduction in lesion size at the injury site (Kasai et al. 2011). Based on these results, it is suggested that injections of ethanol extract of Chinese propolis may be useful for the treatment of SCI in rats.

10.4.3 Beneficial Effects of Propolis Components in Epilepsy

Epilepsy is one of the most common serious neurological disorders in the world. Epileptic seizure is defined as the clinical manifestation resulting from an excessive and abnormal discharge of a population of neurons. Studies on the effect of fish oil and propolis in pilocarpine-mediated epilepsy in rats indicate that propolis produces neuroprotective effects in epilepsy (Manna et al. 2011).

10.4.4 Beneficial Effects of Propolis Components in Parkinson Disease

Parkinson disease (PD) is a neurodegenerative disease characterized by the death of pigmented dopaminergic neurons in the substantia nigra pars compacta of the mid-brain. This neurodegeneration produces a reduction of dopamine (DA) levels in the striatum (ST), which promotes a cascade of abnormal neural circuits that are manifested as distinct symptoms of PD (Farooqui 2010). Lewy bodies-cytoplasmic inclusions that contain aggregated proteins and the degeneration of substantia nigra dopamine neurons represent the pathological hallmarks of PD (Farooqui 2010). The disease is mainly characterized by tremor, bradykinesia, rigidity, and postural instability. Loss of the ability to synthesize dopamine is an important step in the development of PD.

Injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mouse have been used to produce mouse model for PD. Recent evidence shows that glial-related response plays a key role in the MPTP neurotoxic process. In addition, dopamine transporters (DAT) play an important role in MPTP neurotoxicity because an MPTP metabolite must first gain access to the dopaminergic neurons via DAT (Watanabe et al. 2005) to induce its toxic effects on dopaminergic neurons (Kurosaki et al. 2003). Injections of CAPE, an active component of propolis, attenuate dopaminergic neurodegeneration and dopamine loss in the MPTP injected mice. The neuroprotective effect of CAPE is associated with marked reductions in iNOS and caspase 1 expression. Additionally, CAPE inhibits MPP⁺-mediated neurotoxicity in vitro and directly inhibits MPP⁺-mediated release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria. Thus, CAPE may have beneficial effects in slowing or preventing the progression of PD (Fontanilla et al. 2011).

Similarly, 6-hydroxydopamine (6-OHDA), a neurotoxin specific to dopaminergic neurons, has also been used to produce rodent models to PD. It induces neuronal death either via uncoupling mitochondrial oxidative phosphorylation resulting in energy deprivation or alternatively, is associated with its ability to produce hydrogen peroxide, hydroxyl, and superoxide radicals (Wei et al. 2008). In cerebellar granule neurons, CAPE significantly blocks 6-OHDA-mediated cell death in a dose-dependent manner. Furthermore, CAPE also modulates the Ca^{2+} -mediated release of cytochrome c in isolated liver mitochondria (Noelker et al. 2005). Caspase-3 activation following 6-OHDA treatment is markedly inhibited in the presence of CAPE. These observations support the view that propolis component, CAPE produces neuroprotective effect by inhibiting apoptotic cell death (Noelker et al. 2005). PD symptoms in Dawley rats can be induced by a single intracisternal injection of 6-OHDA. Quercetin, a flavonoid found in propolis increases the striatal dopamine and antioxidant enzyme levels compared with the group treated with 6-OHDA alone. A significant decrease in protein carbonyl content in the striatum is observed in quercetin-treated rats compared with rats that did not receive quercetin. Furthermore, significant increase in neuronal survivability has been reported to occur in quercetin-treated rats after 6-OHDA administration (Haleagrahara et al. 2011). Collectively, these studies indicate that propolis components (CAPE and quercetin) protect neural cells from MPTP and 6-OHDA-mediated neurotoxicity in animal and cell cultures models of PD.

10.4.5 Beneficial Effects of Propolis Components in Alzheimer Disease

Alzheimer disease (AD) is one of the most prevalent neurodegenerative disorders in the United States. It is characterized by the accumulation of amyloid-beta ($\text{A}\beta$)-containing plaques, hyper-phosphorylated tau protein containing neurofibrillary tangles, as well as synapse and neuron loss. $\text{A}\beta$ and tau represent the main neuropathological hallmarks of AD. It is proposed that senile plaques and tangles play a pivotal role in the pathogenesis of AD. Within the $\text{A}\beta$ toxicity cascade, mitochondrial dysfunction and energy metabolism deficiencies have been recognized as earliest events (Leuner et al. 2007; Moreira et al. 2010). Alterations in mitochondrial processes lead to neuronal dysfunctions inducing to a progressive decline in cognitive functions, memory loss, and dementia (Farooqui 2010). The pathophysiology of AD is complex. It not only includes defective beta-amyloid ($\text{A}\beta$) protein metabolism, abnormalities of glutamatergic, adrenergic, serotonergic, and dopaminergic neurotransmission, but also increases in neuroinflammatory, oxidative stress, and hormonal pathways (Farooqui 2010). Studies on the effect of water-soluble derivative of propolis (WSDP) on scopolamine-mediated impairment in learning and memory with both hidden-platform acquisition training and probe mice trials in Morris water maze test indicate significant mitigation scopolamine-mediated amnesia in mice (Chen et al. 2008). Furthermore, WSDP also significantly inhibits

AChE activity in the hippocampus of scopolamine-treated mice (Chen et al. 2008), indicating that WSDP mitigates amnesia in vivo through inhibition of AChE activity in the hippocampus. In addition, the main components of WSDP such as apigenin, kaempferol, and luteolin induce neuroprotective effects due to their antioxidative capacity and free-radical scavenging activity (Kang et al. 2004). The Brazilian green propolis mediates neuroprotective effects in PC12 cell culture by acting as an antioxidant against lipid peroxidation and free-radical production in vitro (Shimazawa et al. 2005). Based on these observations, it is suggested that propolis may have potentials as neuroprotective agent in animal models of AD.

10.4.6 Beneficial Effects of Propolis Components on Experimental Allergic Encephalomyelitis

Multiple sclerosis (MS) is a chronic autoimmune neurological disorder of unknown etiology. It is characterized by disseminated focal immune-mediated demyelination. The inflammatory lesions in MS involve massive infiltration by a heterogeneous population of cellular and soluble mediators of the immune system, including T cells, B cells, macrophages, and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement, and other toxic substances.

Demyelination is accompanied by variable axonal damage and loss and reactive gliosis. ROS generated by activated macrophages and microglial cells are thought to play a major role in damaging myelin and myelin-producing cells oligodendrocytes in MS (Bruck and Stadelmann 2003). Experimental allergic encephalomyelitis (EAE) is an animal model for MS. Studies on the effect of CAPE in EAE indicate that CAPE not only significantly inhibits EAE-mediated ROS generation, but also ameliorates clinical symptoms of EAE in rats (Ilhan et al. 2004). It is suggested that CAPE exerts its anti-inflammatory effect by blocking ROS production at the transcriptional level through the suppression of nuclear factor kappaB activation, and by directly inhibiting the catalytic activity of inducible nitric oxide synthase (Ilhan et al. 2004).

10.4.7 Beneficial Effects of Propolis Components in Anxiety

Anxiety is a psychological and physiological state characterized by nervousness, fear, apprehension, and excessive worrying about everyday life events with no obvious reasons (Cortese and Phan 2005). Anxiety disorders include generalized anxiety disorder (GAD), specific and social phobias, posttraumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), and panic disorder. Anxiety disorders are thought to be caused by dysfunction of one or more neurotransmitters and their receptors. Most data, which are derived from study of the benzodiazepine- γ -aminobutyric acid receptor complex, indicate that alteration of the influx of chloride

ions within this receptor complex is associated with the development of anxiety disorders. Compounds that target γ -aminobutyric acid and the serotonergic systems have received great attention within the development of treatments for anxiety disorders (Salzman et al. 1993). Recently, the glutamatergic system, the major mediator of excitatory synaptic transmission in the mammalian brain, has been the focus of pathophysiological studies of human anxiety disorders (Cortese and Phan 2005).

Propolis essential oil (PEO) can significantly reverse the anxiety-like behavior of restraint-stressed mice, but has no effect on locomotor activity (Li et al. 2011). Furthermore, PEO significantly decreases the plasma levels of cortisol (CORT), adrenocorticotropic hormone (ACTH), and malondialdehyde (MDA), whereas it increases the activity of superoxide dismutase (SOD) in restraint-stressed mice. These results strongly suggest that PEO has therapeutic effects on anxiety through antagonizing the hyperfunction of hypothalamic-pituitary-adrenal (HPA) axis and improving the ability of antioxidation in brain tissue (Li et al. 2011).

10.5 Challenges and Side Effects of Propolis

In spite of above-mentioned beneficial effects of propolis, the most challenging problem is uncertainty about its correct dosage, bioavailability of its flavonoids, and safety. In some individuals, propolis induces allergic reactions including wheezing, headache, itchy throat, hives, or skin flushing. In other cases, application of propolis causes erythema, eczema, vasculitis, and pruritus. Discontinuation of propolis use results in complete recovery from above conditions. Although the complete information regarding its toxicity remains elusive, recent studies indicate that in male rats oral consumption of propolis for 45 days shows no significant behavioral and clinical toxicity in animals (Mohammadzadeh et al. 2007). Propolis is a potent sensitizer and should not be used in patients with an allergic predisposition, in particular an allergy to pollen. Therefore, healthcare practitioners and public should be aware of the risk of allergic reactions to propolis. Another important problem is the occurrence of genetic polymorphism in humans. This may be the reason of inconsistent findings from epidemiological studies on beneficial effects of propolis. Despite of increasing number of in vitro and in vivo studies trying to unravel the mechanisms of action of propolis, the research in this field is still developing and more studies are needed on the molecular mechanism of propolis action in neurological disorders.

10.6 Conclusion

Propolis is a naturally occurring resinous substance collected by bees from the leaf buds and bark of trees. Bees use the propolis along with beeswax to construct their hives. Propolis also plays an important role in the protection of bee colony against

the invasion and infection of bacteria, fungi, and viruses. The chemical composition of propolis is very complex. Propolis contains phenolic compounds like flavonoids (galangin, quercetin), cinnamic acid and its derivatives (chlorogenic acid, ferrulic acid, CAPE), various steroids, and amino acids. The fundamental properties of propolis flavonoids (galangin and quercetin) are not only their antioxidant and anti-inflammatory properties and their ability to interact with transition metals ions (Fe^{2+} , Cu^{2+} , or Zn^{2+}), but also include scavenging properties for superoxide anions, oxygen singlet, and lipidic peroxyradicals. In addition, flavonoids may also regulate signal transduction processes, which involve modulation of PtdIns 3-kinase/Akt, and MAP-kinases pathways and inhibition of PLA₂, COX, LOX, and NADPH oxidase-catalyzed reactions to regulate pro-survival gene expression and transcription factors. Emerging evidence suggests that propolis-derived flavonoids may exert neuroprotective effects by suppressing the activation of microglia and inhibiting neuroinflammation. At the molecular level, propolis-derived flavonoids and CAPE may act not only by blocking the release of proinflammatory cytokines (TNF- α and IL-1 β) from activated glia and inhibiting the induction of iNOS induction, but also by retarding the activation of NADPH oxidase and subsequent ROS generation in activated glia and downregulating the activity of NF- κ B. Collectively, above-mentioned studies suggest that CAPE and flavonoids in propolis may be responsible for antioxidant, anti-inflammatory, and neuroprotective activities. Clinical studies are now also in progress to verify the effects of propolis in the prevention and treatment of atherosclerosis and metabolic syndrome.

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Chapter 11

Perspective and Direction for Future Research on Phytochemicals in Neurological Disorders

11.1 Introduction

Phytochemicals are heterogeneous group of bioactive compounds produced by plants, which are extensively researched by scientists for their health-promoting potentials in humans. Unlike vitamins and minerals, phytochemicals are not required for sustaining cell viability, but they play an important role in protecting tissues and cells from the harmful effects of oxidative stress and neuroinflammation-mediated changes (Farooqui and Farooqui 2010). In addition, phytochemicals also stimulate detoxification enzymes, immune and hormonal response in humans who consume colored edible fruits, plants, vegetables, and herbs. Because plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods that contain those phytochemicals (Joseph et al. 1999, 2009; Williams and Spencer 2012). Examples of phytochemicals include flavonoids, catechins, resveratrol, ginkgo biloba, and sulfur compounds found in garlic. Plants produce phytochemicals and store them in vulnerable regions (the skin, seeds, and leaves) in order to discourage insects and other organisms from eating and killing the plant (Trewavas and Stewart 2003; Mattson et al. 2007). In addition, phytochemicals also function in chemical defense against environmental stress and contribute to repair wound healing process in the plant.

Phytochemicals exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals (Joseph et al. 2009; Williams and Spencer 2012). Although at high concentrations phytochemicals produce their carcinogenic, neuro-toxic, and/or cardiotoxic effect in mammals, at low concentrations (amounts normally consumed by humans), these phytochemicals promote beneficial effects on human health (Mattson et al. 2007; Joseph et al. 1999, 2009).

11.2 Problems Associated with the Use of Phytochemicals for Human Health

The use of phytochemicals for improving human health has gained considerable attention in recent years because it is believed that these natural products are safer and produce lower side effects than synthetic drugs (Raskin et al. 2002). It is also proposed that phytochemicals improve the quality of life, enhance physical or cognitive performance, and heighten self-esteem. Dietary supplements with phytochemicals as their constituents are produced, sold, and consumed without strict regulations. Due to the lack of clear identification of their active ingredients and their amount and combinations, it is difficult to standardize supplements produced by different companies. Today, approximately six in ten Americans regularly consume some phytochemicals, and approximately one in six Americans uses phytochemicals (herbal remedies) on a regular basis (Gershwin et al. 2010). Unlike drugs (pharmaceuticals), which undergo extensive clinical trials in animals and humans prior to FDA approval, dietary phytochemicals are not tested for their efficacy and safety. Plants products contain complicated mixtures of organic chemicals, the levels of which may vary substantially depending upon many factors related to the growth of plants, harvesting, production, and storage conditions. These factors may cause alterations in chemical compositions of dietary supplement resulting in batch variation (Bent and Ko 2004; Gershwin et al. 2010). Significant US population believes that economically the use of supplement is better than drugs because drugs are more expensive than supplements. Consumption of phytochemicals in the form of supplements is generally greater among women, people undergoing surgery, and elderly men than younger people (Ang-Lee et al. 2001; Morelli and Naquin 2002). Because of this reason, research activity on phytochemicals in form of supplements has increased not only in United States, but all over the world and new terms such as “functional food” and “nutraceutical” have been introduced. Functional foods and nutraceuticals contain biologically active ingredients and are intended to be consumed as part of the normal diet, which can not only enhance health, but also reduce risk of chronic diseases (Subirade 2007), for example, the addition of antioxidative polyphenols to virgin olive oil and supplementation of n-3 fatty acids to cheese and milk. Functional foods entered the market in the early 1990s and have shown promise in reducing the risk of some chronic diseases, such as reduction in risk of cardiovascular and cerebrovascular diseases and certain types of cancers. The precise molecular mechanisms through which specific phytochemicals exert their beneficial biological effects still remain the subject of intense investigations. Brain responds to the effect of phytochemicals through the stimulation of receptors located on neuronal membranes. For example, brain responds to capsaicin (the phytochemical responsible for the “hotness” of hot peppers) or allicin in garlic through the stimulation of sensory neurons in the mouth and associated structures (Mattson et al. 2007). Similarly, psychoactive phytochemicals such as those found in marijuana and peyote stimulate neurons containing cannabinoid receptors. The stimulation of these receptors has been reported to promote neuronal survival through the stimulation of Ca^{2+} influx and phosphatidylinositol-3-phosphate (PtdIns 3) kinase and mitogen-activated

protein (MAP) kinases (Howlett 2005). In addition, neurons are also sensitive to other phytochemicals (ALA, curcumin, sulforaphane, and resveratrol), which are absorbed through the gut and distributed through the blood stream to various organs including brain, which is protected by blood–brain barrier (BBB). The bioavailability of most phytochemicals to visceral organs is relatively higher than the brain. This is not only because BBB, but also due to rapid metabolism and elimination of phytochemicals in the urine (Mattson et al. 2007). To enter the brain, a phytochemical must be either highly lipid soluble or subjected to uptake transport processes through ABC transporter. Thus, there occurs a complex interplay between the physicochemical properties of phytochemicals and active ABC transporters (Vaidyanathan and Walle 2003; Youdim et al. 2004). The chemical structures of phytochemicals are often used as “parent structures” for generating their synthetic analogs with improved pharmacological activities along with optimal bioavailability and pharmacokinetic profiles. When phytochemicals reach in the brain, they play a vital role in protecting neural cells from oxidative stress and neuroinflammation associated with normal aging and chronic age-related diseases. Neurons are particularly sensitive to oxidative stress-mediated injury not only because of their large dependence on oxidative phosphorylation for energy as compared to glial cells, but also due to many major antioxidant defense mechanisms, such as GSH, Nrf-2, and metallothionein are localized in astrocytes. The demand for oxygen consumption by neurons is extremely high and 1–2 % of the oxygen is converted into superoxide anion radicals (O_2^-) and hydrogen peroxide, leading to oxidative stress (Farooqui 2012). Neurochemical effects of phytochemicals are mediated through their abilities to counteract, reduce, and also repair damage resulting from oxidative stress and neuroinflammation, processes that are modulated by transcription factor, NF- κ B. In addition, phytochemicals also modulate enzymes, various transcription and growth factors, inflammatory cytokines, and subcellular signaling pathways associated with neurodegeneration (Fig. 11.1). Phytochemicals also stimulate the synthesis of adaptive enzymes and proteins that favor resistance to cellular stress (detoxifying and antioxidant enzymes). They are effective modulators that act synergistically on membrane, cytoplasmic, and nuclear pathways to dampen cellular hyperproliferation, hyperactivity, and re-equilibrate metabolic activity, and promote apoptosis of neural cells that have injured from oxidative stress (Kops et al. 2002; Mattson et al. 2007; Joseph et al. 2009; Farooqui and Farooqui 2010, 2011). In activated microglia, phytochemicals inhibit NO, IL-1 β , and TNF- α production (Farooqui and Farooqui 2011, 2012). Flavonoids (quercetin, wogonin, baicalein, epigallocatechin gallate, and genistein) attenuate microglia and/or astrocyte-mediated neuroinflammation by blocking of (a) iNOS and cyclooxygenase (COX-2) expression, (b) NO production, (c) cytokine release, and (d) NADPH oxidase activation and subsequent reactive oxygen species generation in astrocytes and microglia (Lee et al. 2003; Chen et al. 2005; Li et al. 2004; Lau et al. 2007). Flavonoids may also exert their effects via direct modulation of protein and lipid kinase signaling pathways, for example via the inhibition of MAPK signaling cascades, such as p38 or ERK1/2 which regulate both iNOS and TNF- α expression in activated glial cells. The effects of flavonoids on these kinases may influence downstream pro-inflammatory transcription factor (NF- κ B) important in iNOS transcription, supporting the view that there is interplay between signaling

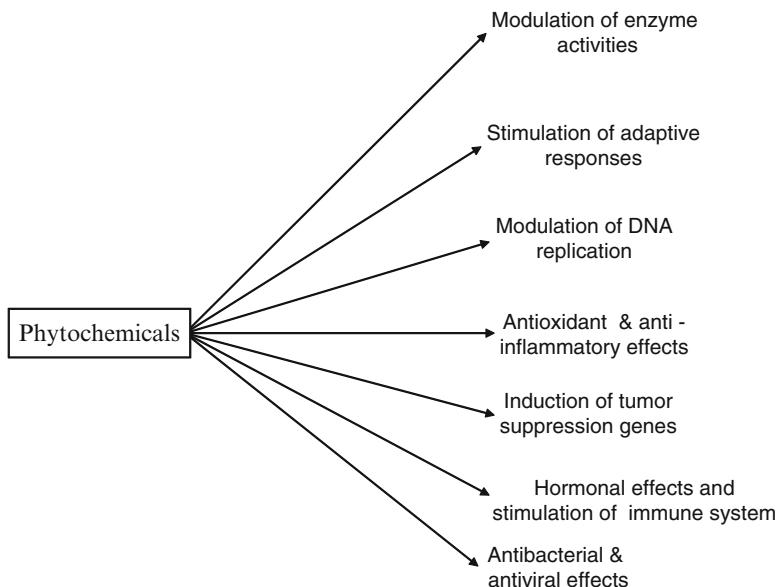
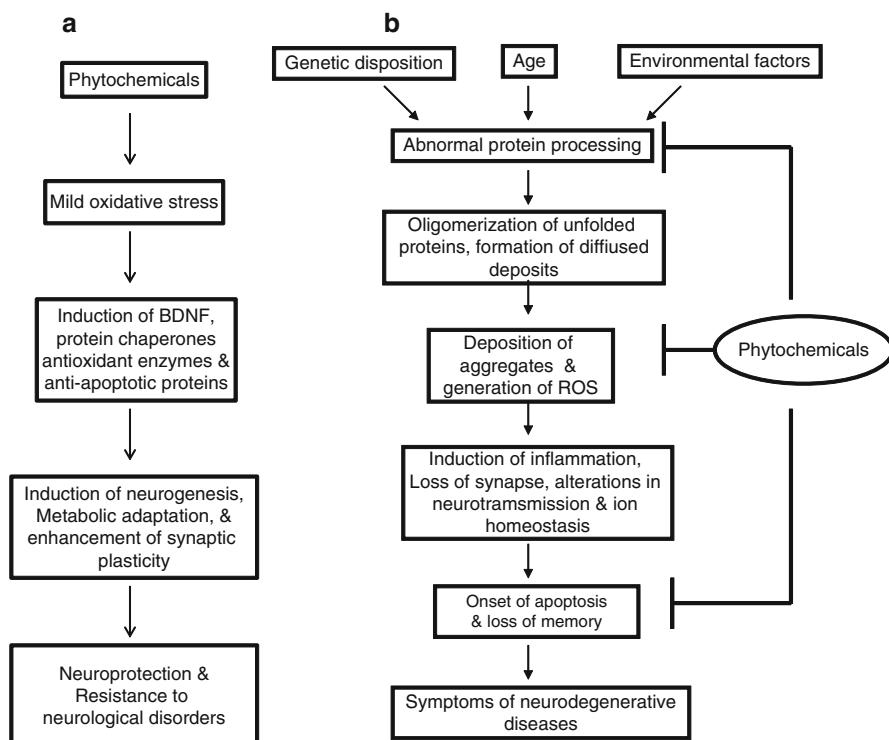


Fig. 11.1 Modulation of biochemical activities by phytochemicals

pathways, transcription factors, and cytokine production in determining the neuroinflammatory response in the brain (Bhat et al. 2002). Other effects of phytochemicals include alterations in immune function and changes in metabolism. At low doses, phytochemicals (flavonoids, catechins, resveratrol, curcumin, quercetin, ginkgo biloba, and sulfur compounds found in garlic) not only improve neuronal and cognitive functions, ocular health, and memory formation but also protect genomic DNA integrity, inducing neuronal regeneration, and help strengthen the immune system (Table 11.1 and Fig. 11.1) (Choi et al. 2001; Kim et al. 2001; Lim et al. 2001; Ono et al. 2003, 2004; Rezai-Zadeh et al. 2005; Mattson et al. 2007; Joseph et al. 2009; Zhang et al. 2009; Schiltkne et al. 2010; Farooqui and Farooqui 2010, 2011). Phytochemicals not only target oxidative stress and inflammation, but also modulate angiogenesis, ionic homeostasis, and redox imbalance through cross-talk signaling networks involved in controlling energy metabolism (Farooqui and Farooqui 2010). The biphasic dose-response relationship for many phytochemicals (low-dose beneficial effects and high-dose toxic effects) indicates that an exclusive antioxidant action may not occur *in vivo*. Aging, genetic disposition, and environmental factors induce oxidative stress, abnormal protein processing, induction of neuroinflammation, loss of synapse, abnormalities in neurotransmission, and ion homeostasis leading to symptoms of neurological disorders (Fig. 11.2a). Consumption of phytochemicals induces mild oxidative stress initiating neurohormetic response that results in the expression of adaptive stress-resistance genes responsible for encoding antioxidant enzymes, protein chaperones, and BDNF (Fig. 11.2b) (Mattson and Cheng 2006). Neurohormesis not only provides a framework for explaining the dose-response

Table 11.1 Phytochemical and their effects on human health

Phytochemical	Active component	Mechanism	References
Flax seeds	α -Linolenic acid	Anti-inflammatory, antioxidant effects	Eckert et al. (2010), Blondeau et al. (2009)
Olives	Oleic acid, tyrosol, and hydroxytyrosol	Anti-inflammatory, antioxidant effects	Alarcón de la Lastra et al. (2001), Cornwell and Ma (2008)
Flavonoids	Polyphenols	Anti-inflammatory, antioxidant effects	Middleton et al. (2000), Croft (1998)
Green tea	Catechins	Anti-inflammatory, antioxidant effects	Bastianetto et al. (2006), Chacko et al. (2010)
Tumeric	Curcumin	Anti-inflammatory, antioxidant effects	Aggarwal and Harikumar (2009), Ataie et al. (2010)
Red wine	Resveratrol	Anti-inflammatory, antioxidant effects	Bastianetto et al. (2009), Athar et al. (2009)
Garlic	Organosulfur compounds	Anti-inflammatory, antioxidant effects	Brunetti et al. (2009), Koh et al. (2005)
Propolis	Flavonoids and phenolic acids	Anti-inflammatory, antioxidant effects	Farooqui and Farooqui (2011, 2012)

**Fig. 11.2** Neurohormesis effects of phytochemicals on brain (a) and sites of action of phytochemicals in neurodegenerative diseases (b)

relationships between concentration of phytochemicals and beneficial effects, but also explains a key insight for improving the accuracy of the therapeutic dose of phytochemicals within the highly heterogeneous human population (Calabrese et al. 2011). Based on the stimulation of signal transduction network and adaptive stress-resistance genes, it is proposed that the use of phytochemicals from childhood to old age along with regular exercise and three to four time consumption of fish per week and low glycemic index fruits is an inexpensive strategy for maintaining normal aging and delaying onset of age-related neurological disorders (stroke, Alzheimer disease, AD, and Parkinson disease, PD) (Joseph et al. 2009; Farooqui and Farooqui 2010, 2011). It is well known that normal aging is accompanied by increase in oxidative stress and neuroinflammation, decrease in receptor sensitivity, reduction in antioxidant status, and alterations in Ca^{2+} homeostasis (Farooqui and Farooqui 2010). Risk factors for stroke, AD, and PD include old age, positive family history, unhealthy lifestyle, consumption of high fat diet, and exposure to toxic environment (Fig. 11.2a). These risk factors contribute to abnormal protein processing leading to oligomerization of unfolded proteins, generation of ROS, induction of neuroinflammation, and apoptotic cell death in neurological disorders (Farooqui 2010). Although phytochemicals have no effects on positive family history and gender, their long-term use may retard the effect of unhealthy lifestyle by delaying or slowing the onset of stroke, AD, and PD (Choi et al. 2001; Kim et al. 2001; Lim et al. 2001; Ono et al. 2004; Rezai-Zadeh et al. 2005; Mattson et al. 2007; Joseph et al. 2009; Schültke et al. 2010; Farooqui and Farooqui 2010, 2011). These processes may lead to improvement in health status and quality of life in older age.

11.3 Molecular Mechanisms Associated with Beneficial Effects of Phytochemicals

Phytochemicals may slowdown and partially reverse age-related decline in memory formation through their ability to interact with the cellular and molecular architecture of the brain responsible for memory formation. These interactions include their ability to up-regulate signaling pathways critical for controlling and maintaining synaptic plasticity through the modulation of growth factors in various regions in the brain (Spencer et al. 2009). The molecular mechanisms through which phytochemicals retard oxidative stress-mediated neurotraumatic, neurodegenerative, neuropsychiatric diseases are related to their ability to counteract toxic production of both ROS and RNS, along with the up-regulation of phase 2 enzymes and vitagenes, such as members of the heat shock protein (Hsp) family, heme oxygenase-1, thioredoxin, and sirtuin protein systems (Calabrese et al. 2010; Son et al. 2008; Tosetti et al. 2009) (Fig. 11.3). It is also shown that phytochemical-mediated phosphorylation of specific serine or threonine residues in Nrf2 by upstream kinases, such as MAP kinases, phosphatidylinositol-3-kinase/Akt, protein kinase C, and casein kinase-2, promotes Nrf2 signaling pathway and induces the expression of cytoprotective genes (Surh et al. 2008). At the molecular level, phytochemicals activate the extracellular signal-regulated kinase (ERK1/2) and the protein kinase B

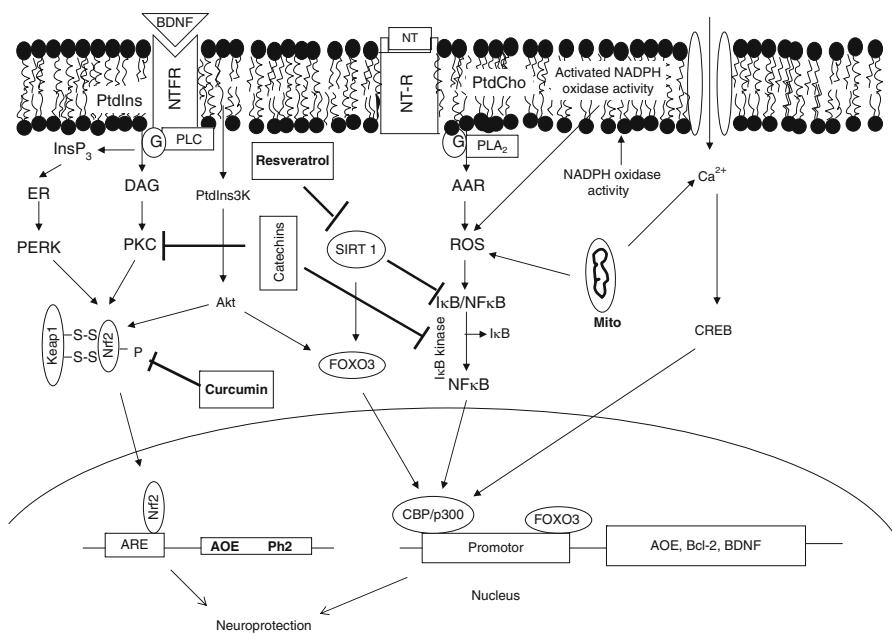


Fig. 11.3 Hypothetical scheme showing phytochemicals-mediated signal transduction pathways associated with neurohormetic responses. Neurotransmitter receptor (NT-R); neurotrophic factor-activated specific receptor (NTFR); phosphatidylinositol (PtdIns); phospholipase C (PLC); diacylglycerol (DAG); protein kinase C (PKC); phosphatidylinositol 3-kinase (PtdIns3K); inositol 1,4,5-trisphosphate (InsP_3); phospholipase A₂ (PLA₂); nuclear factor- κ B (NF- κ B); nuclear factor- κ B-response element (NF- κ B-RE); inhibitory subunit of NF- κ B (I κ B); I κ B kinase (I κ K); protein kinase B (Akt); PRK-like endoplasmic reticulum kinase (PERK); phosphatidylcholine (PtdCho); arachidonic acid (ARA); reactive oxygen species (ROS); nuclear factor (erythroid-derived 2)-like 2 (Nr2F); kelch-like erythroid Cap'n'Collar homologue-associated protein 1 (Keap1); antioxidant response-element (ARE); antioxidant enzymes (AOE); phoase-2-protein (Ph2); mitochondria (Mito); silent information regulator two protein1 (SIRT1); Foxhead box O (FOXO) transcription factor; brain-derived neurotrophic factor (BDNF); cAMP response element binding protein (CREB); CREB-binding protein (CBP); and sirtuins (SIRT 1). Adapted from Mattson et al. (2007)

(PKB/Akt) signaling pathways, leading to the activation of the cAMP response element-binding protein (CREB), a transcription factor responsible for increasing the expression of a number of neurotrophins that are important in mediating memory formation (Spencer et al. 2009; Spencer 2010). Furthermore, phytochemicals such as resveratrol modulate the activity of numerous other proteins, such as peroxisome proliferator-activated receptor coactivator-1 α (PGC-1 α) and the Forkhead boxO (FOXO) family. FOXO family members have been shown to control cellular function in cell processes such as survival via the regulation of cell cycle progression, cell longevity, and apoptotic cell death (Kops et al. 2002). FOXO proteins are downstream targets of phosphatidylinositol 3-kinase (PI3-K)/AKT signaling and are phosphorylated by protein kinase B (PKB) (Tang et al. 1999) (Fig. 11.3).

Aging or neurodegenerative disease-mediated oxidative stress in the brain leads to the stimulation of phospholipid metabolism and decrease in Nrf2 ubiquitination and degradation. As a result, Nrf2–Keap1 complex dissociates and free Nrf2 migrates into the nucleus, where in cooperation with sMaf or other transcription factors (ATF4 or JunD, or PMF-1) transactivates the antioxidant response elements (AREs). Nrf2/ARE pathway induces protein chaperones, phase-2 enzymes (heme oxygenase-1, glutamate cysteine ligase, glutathione S-transferase, glutathione peroxidase, thioredoxin, NAD(P)H:quinone oxidoreductase-1, and γ -glutamylcysteine synthase), neurotrophic factors, and other cytoprotective proteins (Son et al. 2008; Tosetti et al. 2009). Above-mentioned enzymes and growth factors are associated with neural cell survival. Upon recovery of cellular redox status, Keap1 translocates into the nucleus and facilitates the dissociation of Nrf2 from the ARE. Subsequently, the Nrf2–Keap1 complex is exported out of the nucleus by the nuclear export sequence (NES) in Keap1 (Wakabayashi et al. 2010). Although, studies on distribution of Nrf2 expression in AD indicate that Nrf2 is predominantly localized in cytoplasmic fraction of hippocampal neurons compared to age matched control brain indicating that Nrf2 does not migrate to the nucleus, but in brain from PD patients, Nrf2 predominately occurs in the nuclear fraction of substantia nigral neurons compared to age matched control, where Nrf2 is present in the cytoplasm (Ramsey et al. 2007). These observations support the view that Nrf2-mediated transcription is not induced in neurons in AD despite the presence of oxidative stress. In PD, nuclear localization of Nrf2 is strongly induced, but this response may be insufficient to protect neurons from degeneration (Ramsey et al. 2007). Thus, it is likely that there are differences in neuronal response to oxidative stress in AD as compared with PD and more studies are needed on the regulation of Nrf2 in subpopulations of neurons in neurodegenerative diseases.

Phytochemicals can also modulate signaling pathways associated with another redox-sensitive transcription factor, nuclear factor-kappa B (NF- κ B) pathways. Normally, NF- κ B exists in an inactive cytoplasmic form (a heterotrimer consisting of p50, p65, and the inhibitory subunit I κ B α), but enters the nucleus in response to various stimuli, such as oxidative stress and inflammation (Farooqui 2010). These stimuli initiate intracellular signaling cascades through phosphorylation of the inhibitory protein κ B (I κ B) by I κ B kinases (IKKs). The activation of NF- κ B is accompanied by the expression of almost 400 different genes, which include enzymes (e.g., COX-2 and iNOS), proinflammatory cytokines (such as TNF- α , IL-1 β , IL-6, IL-8, and chemokines), adhesion molecules, cell cycle regulatory molecules, and angiogenic factors (Ahn and Aggarwal 2005) (Fig. 11.4). Phytochemicals retard NF- κ B signaling and inhibit the expression of proinflammatory cytokines, adhesion molecules, and cell cycle regulatory molecules blunting the process of neurodegeneration (Farooqui and Farooqui 2011). Some phytochemicals (EGb 761) not only inhibit ROS production and neuroinflammation, but stimulate phosphorylation of CREB (Xu et al. 2007) and enhancement in production of BDNF. This increase in EGb 761-mediated CREB phosphorylation can be blocked by inhibitors of several upstream signaling pathways of CREB, including protein kinase C, ERK,

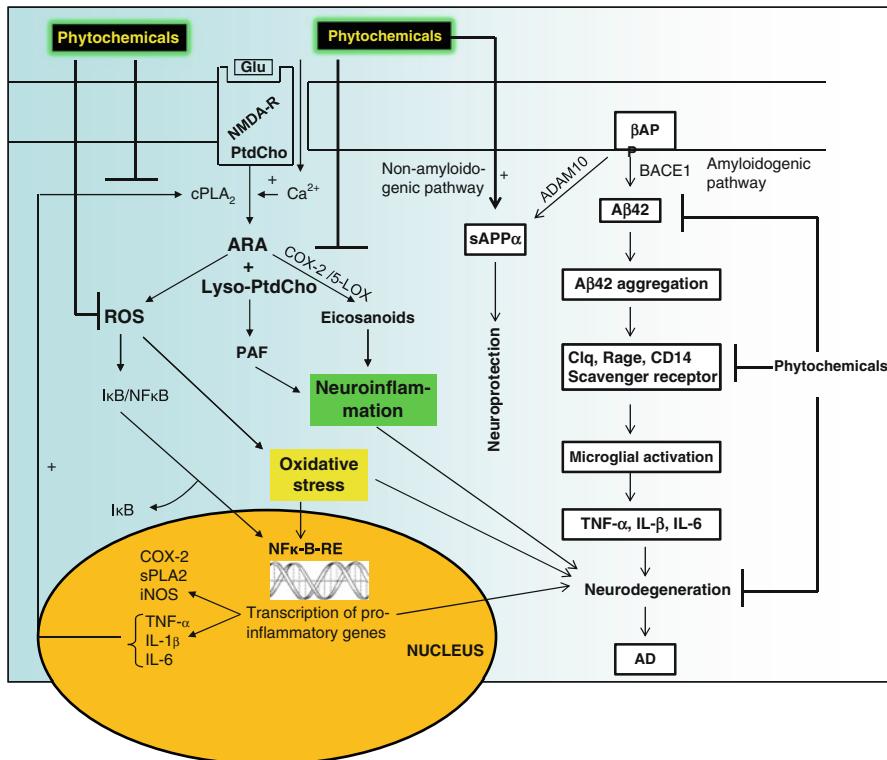


Fig. 11.4 Hypothetical diagram showing neuroprotective effects of phytochemicals in AD. *N*-methyl-D-aspartate receptor (NMDA-R); glutamate (Glu); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A₂ (cPLA₂); arachidonic acid (ARA); platelet activating factor (PAF); cyclooxygenase2 (COX-2); 5-lipoxygenase (5-LOX); 15-lipoxygenase (15-LOX); secretory phospholipase A₂ (sPLA₂); inducible nitric oxide synthase (iNOS); reactive oxygen species (ROS); tumor necrosis factor-alpha (TNF- α); interleukin 1beta (IL-1 β); interleukin-6 (IL-6); nuclear factor- κ B (NF- κ B); β -amyloid precursor protein (β APP); soluble amyloid precursor protein (sAPP); alpha-secretase (ADAM10); β -secretase (BACE1 or beta-site APP cleaving enzyme). The symbols (+) indicate stimulation

ribosomal S6 kinase (RSK), and nitric oxide pathway (Xu et al. 2007). In addition, EGb 761 enhances the activity of α -secretase, which attacks APP inside the A β sequence, therefore prevents the formation of neurotoxic A β . After the cleavage by α -secretase, the soluble N-terminal domain of APP (sAPP α) possessing neurotrophic and neuroprotective properties is released (Fig. 11.4) (Colciaghi et al. 2004).

AMP-activated protein kinase (AMPK) is an energy sensor that is activated by the increase of intracellular AMP:ATP ratio. AMPK is a crucial survival factor that is associated combating with several metabolic stresses, stimulating energy production via glucose and lipid metabolism and reducing energy consuming

Table 11.2 Neuroprotective effects of phytochemicals in animal and cell culture models of neurological disorders

Animal model of neurological disorder	Phytochemical	Effect	References
Ischemia	Polyphenol	Neuroprotection	Wang et al. (2005)
	Curcumin	Neuroprotection	Yang et al. (2004)
Epilepsy	Curcumin	Neuroprotection	Sharma et al. (2010)
TBI	Quercetin	Neuroprotection	Schültke et al. (2005)
SCI	Quercetin	Neuroprotection	Schültke et al. (2010)
AD	(–)EGCG	Beneficial	Lee et al. (2009), Feng (2006)
PD	(–)EGCG	Beneficial	Strömb erg et al. (2005), Mandel et al. (2008)
HD	(–)EGCG	Beneficial	Ehrnhoefer et al. (2006), Ho et al. (2010)
MS	Luteolin	Beneficial	Theoharides (2009)

TBI Traumatic brain injury, SCI spinal cord injury, AD Alzheimer disease, PD Parkinson disease, HD Huntington disease, MS multiple sclerosis

functions (Kohno et al. 2011). In hypothalamic neurons, AMPK signaling is involved in controlling the food intake and whole-body energy balance (Varela et al. 2011). Many phytochemicals (curcumin, quercetin, and ginsenoside Rh2) activate AMPK, either by modulating upstream kinases or increasing local AMP concentration (Hwang et al. 2009). These processes may lead to the repression of inflammatory factors, such as NF-κB and contribute to phytochemical-mediated neuroprotection in the brain.

Some phytochemicals (flavonoids) modulate the microglial transcriptome and inhibit the production of IL-1β, TNFα, NO, and prostaglandin E₂. Thus, many flavonoids (luteolin) produce anti-inflammatory, antioxidative, and neuroprotective effects (Dirscherl et al. 2010). Similarly, another flavonoid (quercetin) retards LPS-mediated NO generation and inducible nitric oxide synthase (iNOS) gene transcription by inhibiting activation of IκB kinase (IKK), NF-κB, and AP-1 (Chen et al. 2005). As mentioned in Chap. 4, nanomolar concentrations of flavanols and flavanones activate the ERK pathway via flavonoid-binding sites, such as ligated ion channels, G-protein-coupled receptors, tyrosine kinase receptors, and steroid receptors at the neural membrane surfaces (Williams and Spencer 2012). The flavone backbone (2-phenyl-1,4-benzopyrone) has close structural homology to specific pharmacological modulators of ERK signaling, such as PD98059 (2'-amino-3' methoxyflavone). The activation of ERK by flavanols may lead to downstream cAMP response element binding protein (CREB) activation. This process is closely associated not only with long-lasting changes in synaptic plasticity and memory (Finkbeiner et al. 1997), but also with up-regulation of neuroprotective pathways including induction of BDNF (Williams et al. 2008). Collectively, these studies suggest that some flavonoids may be promising candidates for developing immuno-modulatory and neuroprotective therapies for the treatment of neurodegenerative disorders (Dirscherl et al. 2010). Based on these results, it is suggested that

consumption of phytochemicals from childhood to old age may not only promote good health by retarding the motor and cognitive behavioral deficits that occur during normal aging, but also delays the onset of stroke, AD, PD, anxiety, and improving mood and cognitive performance (Farooqui and Farooqui 2012; Joseph et al. 2009; Pittler and Ernst 2003; Kennedy et al. 2006) (Table 11.2). Above suggestion is based on epidemiological as well as neurochemical studies.

11.4 Unresolved Issues Associated with Therapeutic Use of Phytochemicals

It is well known that consumption of fruits, vegetables, and berries is associated with a lower risk (30–35 %) of all-causes mortality (cardiovascular disease, stroke, AD, and various types of cancers) (Wilson et al. 2006; Letenneur et al. 2007; Farooqui and Farooqui 2010; Krikorian et al. 2010). Fruits not only contain little fat and salt and enriched in vitamins (vitamin C, folic acid), but also have potassium and fiber, which promote beneficial effects on human health. Although many studies have been published on the beneficial effects of phytochemicals, studies on the efficacy of phytochemicals in human beings are not supported by placebo-controlled, randomized, double-blind clinical trials in large human populations. Although cell and animal models offer an appealing and ethical alternative to human experimentation for initial efficacy and mechanistic studies, they can provide an indication of biological activity, bioavailability, pharmacokinetics, and molecular mechanism for the phytochemicals in question, which can be used to design human experiments. The main advantage of the use of *in vitro* cell and *in vivo* animal models is practical convenience, such as ease of culturing, their relatively low cost, and moderate throughput capabilities. Thus, most studies have been performed in cell cultures and animal models which provide mechanistic support for beneficial or harmful effects of phytochemicals. However, it is not possible to assume that results obtained in cell culture or animal models of chronic diseases can be valid for humans. So, the exact molecular mechanisms of phytochemical-mediated beneficial effect in humans remain unknown. Stability, purity, bioavailability (Ross and Kasum 2002), and information on pharmacokinetics of phytochemicals are other major problems (Wu et al. 2002), which may be effected by a number of factors, including the food matrix, structural differences among phytochemicals (whether they are oxygenated or conjugated), and interactions with other food components (Bidlack and Wang 1998; German and Dillard 1998; Farooqui and Farooqui 2011). Very little is known about half-lives and ability of phytochemicals to reach and accumulate at the specific site (subcellular organelle) within the brain in humans. Furthermore, information on optimal levels of phytochemicals at the specific site, which can delay or retard oxidative stress and neuroinflammation, remains unknown. It is very likely that bioavailability of phytochemicals may differ greatly among various human body tissues. Long-term supplementation of diet with high doses of

phytochemical may result in harmful effects such as development of cancers. Detailed investigations on metabolic fate of absorbed phytochemicals and their levels in human tissues in general and brain in particular have not been performed. Answers to above questions are essential for not only understanding the beneficial effects of phytochemicals on human brain, but also for determining the ability of phytochemicals to delay the onset and blocking the pathogenesis of neurotraumatic and neurodegenerative diseases.

11.5 Challenges for Studies on Therapeutic Use of Phytochemicals in Neurological Disorders

Most information on the beneficial effects of phytochemicals (curcumin, resveratrol, green tea catechins, garlic components, and *Ginkgo biloba*) is based on either in cell culture models or in animal models of neurological disorders, which partially mimic some aspects of stroke and neurodegenerative diseases (AD and PD) at the molecular level. Thus, animal models of stroke, AD, and PD are not homologous to pathological changes in stroke, AD, and PD in human subjects because there are substantial anatomical differences between the rodent and human brains, particularly that the rodent brain has a higher gray-to-white matter ratio than human brain (Feuerstein et al. 2008; Ford 2008). Furthermore, in animal model studies occlusion of blood vessel is performed by artificial methods, whereas during stroke *in vivo* occlusion occurs through the clot formation. Animal model studies ignore the effect of clot-derived substances (such as thrombin) that may be flushed into the ischemic region by residual flow, possibly confounding the ischemic insult (Feuerstein et al. 2008; Ford 2008). Similarly, it is not possible to produce all symptoms of AD and PD in any known animal model system (Farooqui 2010). Human clinical trials are performed for longer time in AD and PD patients with extensive neuronal loss and damage due to the onset of neurological disorder. Most cell culture or animal studies in animal models are performed on a short-term basis. More long-term studies are needed to determine their beneficial effects in slowly developing AD. In addition, it is not possible to measure pathological changes during phytochemical treatment-mediated recovery in living patients during the course of neurodegenerative diseases due to ethical reasons (Quinn et al. 2004). Other problems in human clinical trials include appropriate dosing, clearance rates, and tissue distribution of phytochemicals. It is not possible to extrapolate doses that have been used in animal models for human clinical trials. Although interspecies scaling methods (Mahmood 2002; Meibohm et al. 2005) have been used for predicting dosing, clearance rates, and volume of distribution in human subjects in the case of single drug, (phytochemical), this approach is limited in the case of complex phytochemical extracts, especially when active ingredients are unknown (Quinn et al. 2004). Thus, as stated above for the beneficial effect, stability of phytochemical in plasma is necessary for constant delivery to the brain.

11.6 Conclusion

The term phytochemicals refers to a variety of small-molecular weight compounds produced by plants not only for the protection against environmental stress, but also for supporting wound repair process in the plant. Diet supplemented with phytochemicals not only provides beneficial effects on normal aging process in humans, but also retards and delays the onset of neurotraumatic and neurodegenerative diseases. Phytochemicals produce modulatory actions on neural cells by interacting with a wide spectrum of molecular targets through cell-signaling machinery involving activation of MAP-kinase, protein kinase C, serine/threonine protein kinase Akt/PKB, and phase II antioxidant detoxifying enzymes; downregulation of proinflammatory enzymes (COX-2 and iNOS) through the activation of peroxisome proliferator activated receptor γ ; regulation of calcium homeostasis; inhibition of phosphoinositide 3-kinase, tyrosine kinases, NF- κ B, c-Jun, and antioxidant response element pathways; as well as modulation of several cell survival/cell-cycle genes. Many of the above-mentioned processes inhibit oxidative stress not only by scavenging free radicals and neuroinflammation, but also by stimulating immune responses and regulating gene expression. Phytochemicals disrupt the Nrf2–Keap1 association, thereby releasing Nrf2, which translocates to the nucleus and drives the gene expression of detoxifying phase II enzymes (heme oxygenase and GST) which not only helps in detoxifying and eliminating cancer-causing agents from the body, but also protect neural cells from neurotraumatic and neurodegenerative diseases. The sulfur compounds in garlic stimulate beneficial DNA repair. By modulating the activity of transcription factors (Nrf2 and FOXO3) and their upstream signaling molecules, naturally occurring dietary phytochemicals promote apoptosis in abnormal and diseased cells. At present, supplementation of diet with phytochemicals provides the most efficacious methods for increasing “health span” and delaying the onset of age-related chronic diseases. Most of this evidence is preclinical, so more clinical trials are needed to further strengthen this evidence. Because chronic neurodegenerative diseases incubate for 40–55 years before they manifest, therefore designing and structuring drugs for such clinical trials may be difficult. As stated earlier, it remains unknown what amounts of phytochemicals are needed and for how long and whether it is better to consume phytochemicals in food or if supplements will suffice. The evidence that phytochemicals are safe, multitargeted, efficacious, and affordable demands further investigation on the use of phytochemicals in chronic neurodegenerative diseases. Although the molecular mechanisms for the beneficial effects of many phytochemicals have yet to be discovered, it is clear from above discussion that phytochemicals induce health benefits by inhibiting oxidative/inflammatory stress signaling, increasing neuroprotective signaling, and inducing neurohormetic effects to protect against oxidative stress and neuroinflammation.

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