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FREE RADICALS, SIGNAL TRANSDUCTION, AND HUMAN DISEASE

KLAUDIA JOMOVA[1] AND MARIAN VALKO[1,2]

[1]*Department of Chemistry, Faculty of Natural Sciences, Constantine The Philosopher University, Nitra, Slovakia*
[2]*Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, Slovakia*

2.1 INTRODUCTION

Free radicals are reactive species containing one or more unpaired electrons most often derived from oxygen (reactive oxygen species, ROS) or from nitrogen (reactive nitrogen species, RNS) [1]. ROS and RNS in living systems are generated by various enzymes, for example, by NAD(P)H oxidase or NO synthase (NOS). Beneficial effects of free radicals occur under low to moderate physiological concentration of radicals and maintain important physiological processes such as defense against infection and activation of various signaling pathways. Overproduction of free radicals as a consequence of, for example, the mitochondrial transport chain or overstimulated NAD(P)H results in oxidation stress that can be a mediator of damage to cell structures including lipids, proteins, and DNA [2].

Molecular oxygen has unique electronic properties; it contains two unpaired electrons on antibonding π^* orbitals possessing parallel spins and is itself a radical species. The addition of one electron originating, for example, from the mitochondrial electron transport system to the oxygen molecule leads to formation of superoxide anion radical $O_2^{\bullet-}$ ($O_2 + e^- \rightarrow O_2^{\bullet-}$) [3]. Superoxide radical is considered the "primary" upstream radical of the radical chain reactions in living systems, which can further react with other substrates to form "secondary" radicals. Superoxide radical is removed from the site of its action by a dismutation reaction [4].

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \qquad (1)$$

The dismutation reaction is catalyzed by the SOD enzyme working in conjunction with hydrogen peroxide-depleting enzymes such as catalases and glutathione peroxidases.

Under physiological conditions, redox-active metals are sequestered and cells contain only very limited amounts of free metal ions. Disruption of metal ion homeostasis leads to a state in which the concentration of free or unbound metals is elevated. Free metals can take part in catalytic decomposition reactions such as iron-catalyzed decomposition of hydrogen peroxide according to reaction [5]

$$Fe^2 + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad (2)$$

The hydroxyl radical ($^{\bullet}OH$) formed by this reaction has a half-life in aqueous environment of less than 1 ns and is one of the most reactive radicals occurring in biological systems. When produced in vivo it has a great ability to react at the site of its formation with neighboring biomolecules.

The reaction of superoxide radical with hydrogen peroxide can be described as the Haber–Weiss reaction, which is an overall reaction of the Fenton reaction (2) and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen [6]

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$$Fe^{2} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \quad \text{(Fenton reaction)} \tag{2}$$

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \tag{3}$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^{-} \quad \text{(Harber} - \text{Weiss reaction)} \tag{4}$$

Probably the most important RNS acting in biological systems is nitric oxide ($NO^{\bullet}$) generated by NOSs [7]. $NO^{\bullet}$ is an important signaling molecule participating in various important physiological processes involving regulation of blood pressure, smooth muscle relaxation, regulation of immune system, neurotransmission, and other processes [8]. $NO^{\bullet}$ contains one unpaired electron on an antibonding $2\pi_y^*$ orbital and therefore is a radical. $NO^{\bullet}$ and $O_2^{\bullet -}$ can react together to form an oxidant molecule, peroxynitrite [9]

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-} \tag{5}$$

Under in vivo conditions peroxynitrite can react with carbon dioxide, forming an nitrosoperoxycarbonate ($ONOOCO_2^-$). Peroxynitrite is oxidizing molecule causing oxidation of lipids and DNA fragmentation.

Under the conditions of imbalance between production and elimination of ROS and RNS termed oxidative stress, the organism uses various lines of defense. The first line of defense against deleterious action of free radicals is represented by antioxidant enzymes involving superoxide dismutase and catalase [10]. The second line of defense is represented by the small-molecular-weight antioxidants-vitamins, including vitamin C, vitamin E, carotenoids, lipoic acid, and others. Their structural properties allow them to donate an electron to a free radical and neutralize it.

2.2 REDUCTION, OXIDATION, AND THE THERMODYNAMICS OF FREE RADICAL REACTIONS

Oxidation and reduction reactions are called redox reactions and represent the basis for numerous biochemical mechanisms. When discussing redox reactions in biological systems, instead of the terms "reductant" and "oxidant," it is more appropriate to use the terms "antioxidant" and "prooxidant," respectively.

Free radical reactions are governed by the thermodynamic principles [11]. Thermodynamic properties of free radicals vary significantly, ranging from those capable of strong oxidation (e.g., reactive and damaging hydroxyl radical) to those capable of strong reduction (antioxidants such as vitamin C, glutathione, and others). It is convenient to use thermodynamic properties to predict a hierarchy for free radical reactions. The most important thermodynamic quantity to characterize the course of a free radical reaction is the half-cell reduction potential [11]. For example, the one-electron reduction of a compound "A" is related to the half-cell reduction potential of the couple:

$$A(OX) + e^{-} \rightarrow A^{\bullet -}(red)\ E[A/A^{\bullet -}] \tag{6}$$

The overall chemical reaction for an oxidation-reduction couple can be described by the following reaction equation

$$A(ox) + B(red) \rightarrow A(red) + B(ox) \tag{7}$$

TABLE 2.1 Half-Cell reduction potentials of selected couples (pH = 7)

Couple	E^0/mV
$^{\bullet}OH, H^+/H_2O$	+2310
$^{\bullet}OOH, H^+/H_2O_2$	+1060
$^{\bullet}OOR, H^+/ROOH$	+770–1440
$O_2^{\bullet -}, 2H^+/H_2O_2$	+940
α-TO$^{\bullet}$, H^+(Vit. E radical)/α-TOH (Vit. E)	+500
$H_2O_2, H^+/H_2O, {}^{\bullet}OH$	+320
$Asc^{\bullet -}$, H^+ (Ascorbyl rad.)/$AscH^-$ (Ascorbate)	+282
GSSG/2GSH	−248

Table 2.1 summarizes half-cell reduction potentials of selected couples. The values are listed from highly oxidizing (at the top of Table 2.1) to highly reducing (at the bottom of Table 2.1). Any oxidized species is capable of taking an electron (hydrogen) from any reduced species occurring below it in Table 2.1, or, conversely, each reduced species is able to donate an electron (hydrogen) to any oxidized species above it in Table 2.1 [11].

As an example of these equations we refer here to a very important reaction that takes place in biological systems, namely, the regeneration of vitamin E by vitamin C. Based on the half-cell reduction potential values of the α-tocopherol radical (α-T-O$^{\bullet}$)/α-tocopherol (α-T-OH, vitamin E) couple and an ascorbate radical anion ($Asc^{\bullet -}$)/ascorbate monoanion ($AscH^-$, vitamin C) couple it is clear that the ascorbate monoanion can react with the tocopherol radical to regenerate vitamin E:

$$AscH^{-} + \alpha\text{-T-O}^{\bullet} \rightarrow Asc^{\bullet -} + \alpha\text{-T-OH}$$

2.3 OXIDATIVE STRESS AND REDOX ENVIRONMENT OF A CELL

Similar to the process of regulation of pH, biological systems tightly regulate the redox state of a cell [9, 11].

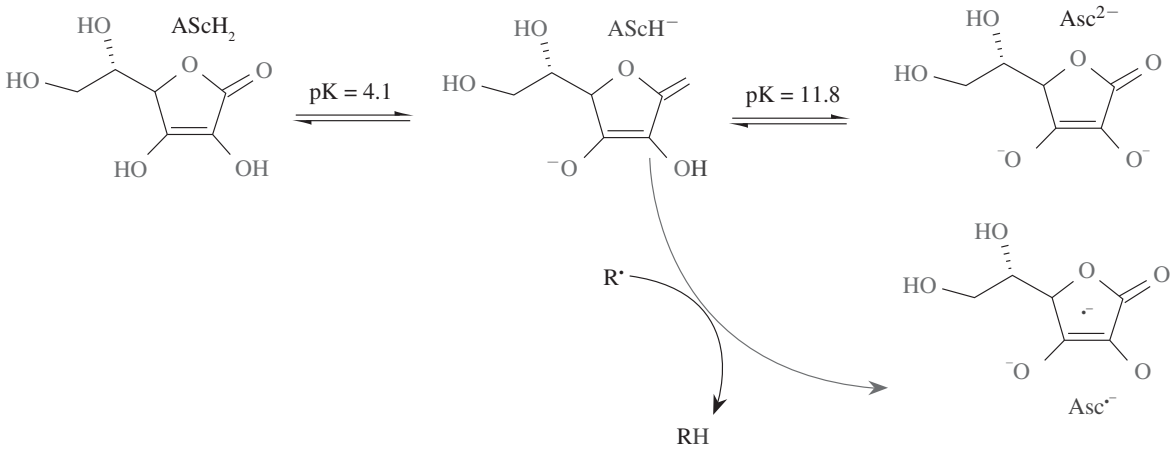

Fig. 2.1 Forms of vitamin C at various pH and its reaction with free radicals.

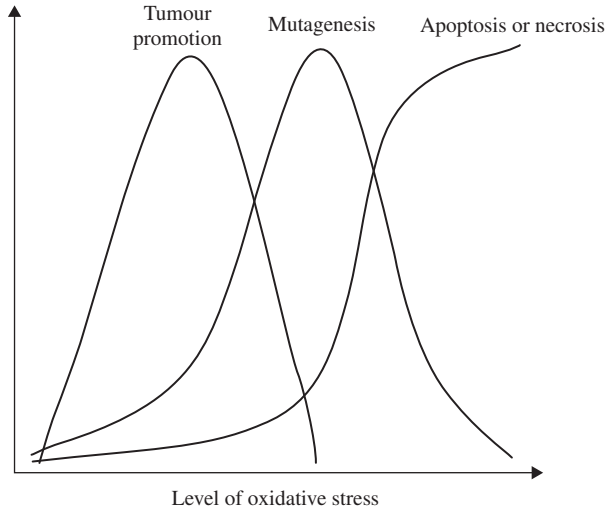

Fig. 2.2 Dose-dependent effect of relationship between level of oxidative stress and the tumor promotion process, the process of mutagenesis, and the process of apoptosis/necrosis.

The redox state of cells is kept in a narrow range and is given by the total "number of electrons" contained in various cellular constituents. The redox state of cells is maintained by the equilibrium of redox pairs, for example, $Asc^{\bullet -}/AscH^{-}$ (Asc = ascorbate), GSSG/GSH, and other couples. The tripeptide glutathione couple is one of the major cellular redox buffers [12]. Thioredoxin is an oxidoreductase enzyme and also participates in the maintenance of redox homeostasis [13]. In the course of the reduction of disulfide bonds by glutathione, this tripeptide is converted to its oxidized form, glutathione disulfide (GSSG). Conditions such as increased oxidative stress lead to increased pools of GSSG, which in turn lead to increased content of protein mixed disulfides. Proteins containing critical thiols that function as receptors in cell signaling pathways can thus possess altered properties [14]. This points to the fact that GSSG appears as a nonspecific signaling molecule. From this it follows that maintenance of high ratios of reduced to oxidized forms of glutathione and thioredoxin substantiated by the action of GSH reductase and flavoenzyme thioredoxin reductase, respectively, is of key importance in the mechanism of redox homeostasis.

In addition to the above-described redox buffering systems, there are low-molecular-weight antioxidants equilibrating redox homeostasis, of which one of the most important is ascorbic acid [15]. Ascorbate is a diacid containing two hydroxyl groups that can undergo ionization. Antioxidant activity of ascorbate is realized through the ascorbate anion form ($AscH^{-}$), the most abundant form of ascorbate under physiological conditions. $AscH^{-}$ is a donor antioxidant that reacts with free radicals forming the semidehydro-ascorbate radical anion ($Asc^{\bullet -}$) (Fig. 2.1). Thus exchange of electrons (and hydrogen atoms) between molecules of antioxidants and radicals determines the overall redox capacity of biological systems.

Changes in the redox environment of a cell are tightly linked with the cell cycle. A reducing environment is typical for cell proliferation [16]. Minor shifts in redox state toward a slightly oxidizing state are typical for cell differentiation. A more oxidizing environment of a cell is typical for apoptosis and necrosis (Fig. 2.2). This conclusion has been achieved using hydrogen peroxide, another oxidizing molecule significantly affecting the redox state of a cell. Experiments using cell lines showed that while low concentrations of hydrogen peroxide (up to 35 μM) induce apoptosis, necrosis is induced by high concentrations of oxidizing substances (more than 100 μM). A molecule of hydrogen peroxide is an uncharged species and can enter the cells by crossing the

biological membranes, a process that allows fluctuation of the redox environment of a cell.

2.4 ROS, SIGNAL TRANSDUCTION, AND HUMAN DISEASE

Signal transduction mechanisms enable induction of various biological processes, including cell growth, gene expression, muscle contraction, and others [17]. Proper functioning of such processes requires the presence of ROS and RNS acting as signaling molecules at various levels of the signal transduction process. Cytokines, growth factors, and hormones including, for example, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α stimulate formation of low levels of ROS/RNS. ROS production is a basis for constitution of the "physiological oxidative burst," necessary for the activation of various signaling pathways important for the maintenance of various physiological processes [9]. Redox-responsive signaling pathways control many physiological processes, for example, cell adhesion, immune response, NO production, sensing of oxygen concentration, regulation of vascular tone, and others.

Redox dysregulation substantiated by enhanced oxidative stress is a common denominator of various pathological conditions including cancer, neurological disorders, cardiovascular disease, metabolic disease, and aging [18]. Disease can be divided into two categories on the basis of origin: (i) The first group is represented by "mitochondrial disease" characterized by dysfunctional mitochondria demonstrated by the enhanced level of oxidative stress. The most typical disorders of this origin are cancer and diabetes mellitus. (ii) The second group of diseases are typical "inflammatory and oxidative" conditions. In addition, an enhanced activity of NAD(P)H oxidase leading to excess of ROS has been noted. This group of diseases is characterized by the enhanced levels of lipid peroxidation process, protein oxidation, and DNA damage caused by free radicals. The most typical diseases of this group are ischemic injury and atherosclerosis.

2.4.1 Cancer

As discussed above, low and transient concentrations of oxygen species participate in the process of cell proliferation [19]. On the other hand, high concentrations of oxygen species cause cell death and necrosis. ROS, redox-active, and redox-inactive metals interact with sulfhydryl groups of cysteine residues exposed on protein surfaces. The structural changes that occur at the active site of proteins trigger activation of several signaling cascades [19]. These include MAPK- and PI3-kinase-dependent signaling pathways and growth factor kinases—all of which in turn lead to activation of redox-dependent transcription factors AP-1, p53, HIF-1, NF-κB, and others (Fig. 2.3).

2.4.1.1 Dysregulation of Cellular Signaling in Cancer

Protein tyrosine phosphorylation plays a major role in various cellular processes such as proliferation, differentiation, and survival/apoptosis. The overall process of phosphorylation is driven by two antagonistic chemical reactions catalyzed by the protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) [20].

Ligands that trigger receptor tyrosine kinases involve insulin, insulin-like growth factor, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor, and others. Growth factors/growth factor receptors play a significant role in development, wound healing, and growth [9, 19].

Many cancers are tightly linked with the disruption of proper functioning of growth factors. Several lines of evidence have documented the influence of carcinogenic metals such as nickel, arsenic, and beryllium on growth factor receptors. These involve EGF receptor (EGFR), PDGF receptor (PDGFR), and VEGF receptors (VEGFR) [21].

Nickel compounds have been found to induce malignant tumors after intramuscular administration [22]. Overexpressed EGFRs have been detected in cancers of the urinary tract and lung cancers after exposure to increased concentration of nickel. The most profound carcinogenic effect of nickel has been reported for insoluble nickel compounds. Inhalation of nickel dust has also been associated with the development of cancers. The carcinogenic action of nickel is accomplished either by indirect damage though inflammation or directly by oxidative DNA damage via catalytic decomposition of hydrogen peroxide (Fenton reaction) forming reactive hydroxyl radicals [5].

Disruption of VEGF/VEGFR and EGF/EGFR pathways has been observed after arsenic exposure [23]. EGFR pathway activation has been shown to be activated by arsenic with the possible consequence of lung cancer [24]. Despite the various responses in patients suffering from non-small-cell lung cancers, a therapeutic approach based on the application of oral EGFR tyrosine kinase inhibitors (TKIs) appears to be a certain hope.

Overexpressed PDGF after exposure to arsenic has been described in lung, prostate, and ovarian cancers [25]. With the discovery of the new PDGF family members PDGF-C and PDGF-D, it has been shown that they play a role in renal disease, brain tumors (glioblastoma multiforme), and organ fibrosis.

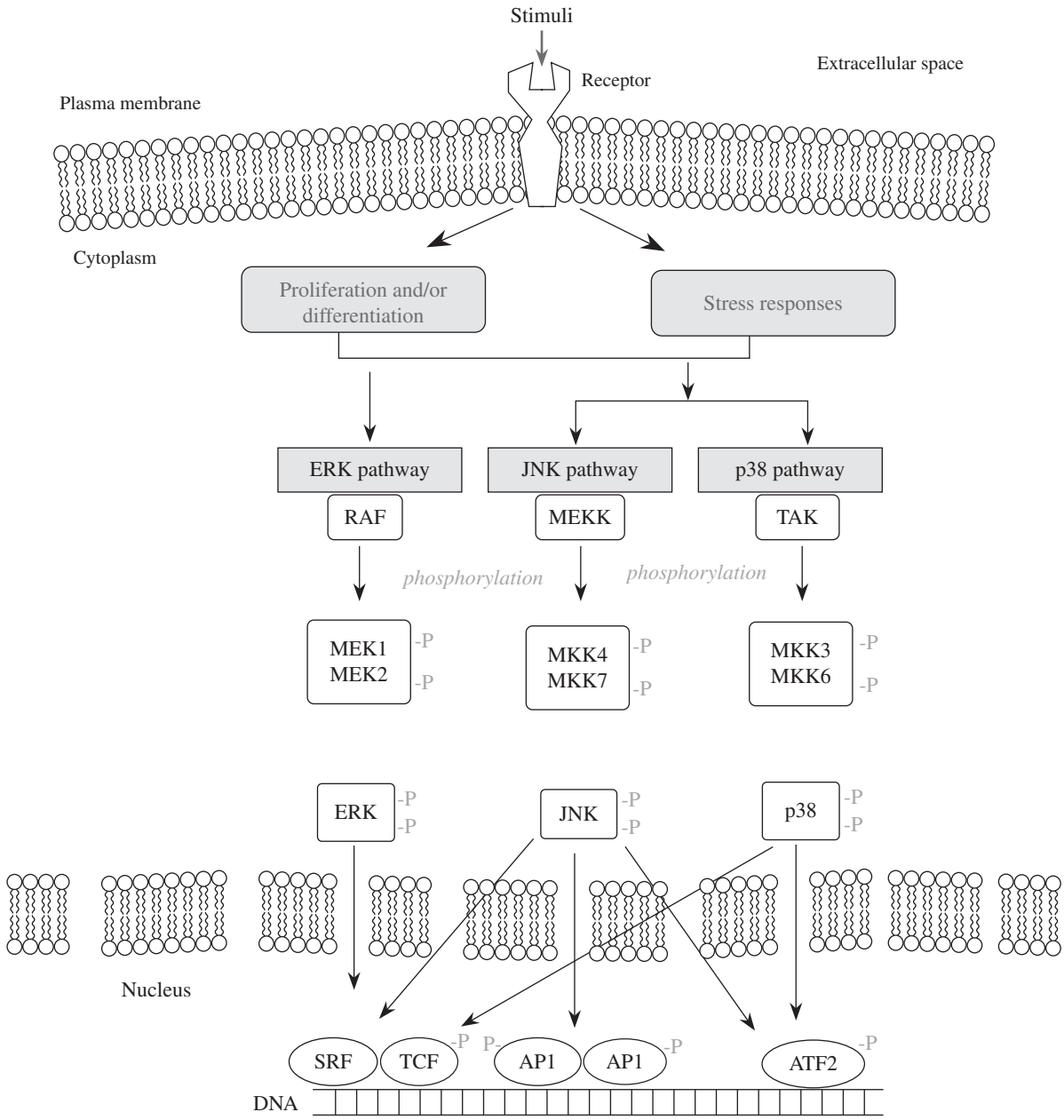

Fig. 2.3 Extracellular stimuli activate the MAPK pathways. Once MAPK kinase kinases [RAF, MEKK (MAPK/ERK kinase kinase) and TAK (TGFβ-activated kinase)] are activated, they phosphorylate MAPKKs on two serine residues. MAPKKs in turn phosphorylate the MAPKs ERK (extracellular-signal-regulated kinase), JNK (JUN N-terminal kinase), and p38 on both threonine and tyrosine residues. Activated MAPKs can translocate to the nucleus to phosphorylate a number of transcription factors such as activator protein 1 (AP1) and activating transcription factor 2 (ATF2), thereby altering gene transcription. SRF (serum response factor); TCF (ternary complex factor).

Nonreceptor protein kinases (PTKs) that belong to the Src family and Janus kinase (JAK) are also a target of ROS and RNS [26]. Hydrogen peroxide and superoxide radical anions have been documented to induce phosphorylation of several non-receptor protein kinases (PTKs) in various cell types, lymphocytes (T and B), and myeloid cells. PTKs belong to the Src family (Src kinases) and Janus kinase (JAK "just another kinase"). Src activated by arsenic(III), UV radiation, and chromium (III) triggers MAPK signaling pathways. Overexpressed Src has been documented in cancers of colon, breast, pancreas, and bladder.

ROS interact with protein tyrosine phosphatases (PTP) and regulate phosphorylation of many important

signaling molecules, for example, of the MAP kinase family [27]. PTPs are a direct target of ROS and have been involved in regulation of oncogenic transformation, cell growth, and differentiation.

There are four main groups of MAPK family in mammalian cells, and they are serine/threonine kinases [28]. These kinases are known to phosphorylate hydroxy groups of serine or threonine. They are regulated by the various redox-active/-inactive metals, including cobalt, chromium, and nickel (redox active) and cadmium and arsenic (redox inactive).

The most significant effect of metals on signaling pathways has been observed in the mitogen-activated protein (MAP) kinase/AP-1 and NF-κB signaling pathways, which are significantly affected by the effect of various stressors involving redox and nonredox metals and free radicals [5, 29].

Radiation, alcohol, benzpyrene, tetracyclic diterpenoids, asbestos, and other carcinogens represent external stress factors that activate NF-κB [30]. The exact mechanism of such activation is not yet clear; however, its role in cell growth, differentiation, and inflammation has been thoroughly described. ROS can be considered as second messengers involved in activation of NF-κB through IL-1 and TNF. Activated NF-κB has been reported for colon, breast, and pancreas cells [29]. The involvement of ROS and metals in the activation of NF-κB has been confirmed by studies utilizing antioxidants [31]. These studies suggested that thiols, polyphenols, carotenoids, vitamin E, L-cysteine, and other antioxidants can block activation of NF-κB by various stressors.

p53 is considered as one of the transcription factors sensitive to oxidative stress that has the ability to halt the cell cycle or initiate apoptosis and thus protect cells from tumorigenesis [32]. p53 is known to induce the expression of p85 (a regulator of PI3K), which may function as a signaling molecule during p53-triggered apoptosis. p53 is activated by various stress factors, including hypoxia, gamma radiation, UV radiation, and others.

The effect of metals on p53 has been reported; however, the mechanisms are not fully understood [33]. In the case of the effect of arsenic on p53 several controversial reports have appeared in the literature spanning from no effect of arsenic on p53 to an induced p53 phosphorylation. In patients with arsenic-related skin disease an overexpression of p53 gene has been reported. Zinc is very important in the binding of p53 to DNA; thus isostructural metals capable of replacing zinc in its binding sites may affect p53 functioning [34].

Mutations in p53 have been noted on exposure to nitric oxide [35]. A close association between iNOS expression and mutations in p53 has been detected in stomach, brain, and breast cancers. Nitric oxide and derivatives of nitric oxide may cause mutations in cancer-related genes and thus act as initiators/promotors of human carcinogenesis.

Metals can increase the intracellular levels of calcium, which activates the Ca^{2+}/calmodulin-dependent serine phosphatase calcineurin that in turn activates nuclear transcription factor NFAT [36]. In total there are five NFAT proteins evolutionarily related to Rel/NF-κB, of which four are calcium-dependent. Redox-active metals iron, nickel, and vanadium exhibit the ability to activate NFAT. Vanadium has been shown to activate NFAT not only by a calcium-dependent pathway but also via formation of hydrogen peroxide.

HIF-1 regulates the expression of many cancer-related genes including VEGF [37]. VEGF plays an important role in tumor progression as well as angiogenesis and has been found to be expressed in many types of cancer. Similar to the previous factors, HIF-1 is activated mainly by hydrogen peroxide and carcinogenic metals, most profoundly by nickel [38]. In addition, HIF-1 very sensitively reflects oxygen homeostasis and hypoxia. A possible replacement of iron by nickel in the oxygen carrier hybrid hemoglobin leads to a steady-state hypoxia, thus activating HIF-1 via an oxygen-sensitive pathway.

HIF-1 has been described to participate in the glycolysis pathway and glucose transport [5]. In this connection studies have been done employing organic vanadium complexes, which have been documented to emulate actions of insulin via expression of HIF-1.

2.4.1.2 Redox Environment of a Cell and Mechanism of Carcinogenesis There exist several theories explaining the mechanism of carcinogenesis [39]. One of the key theories is based on the disruption of an equilibrium between cell proliferation and cell death. Apoptosis is a normal physiological process that consists of the programmed mechanism of cell suicide [40]. A pivotal role in this process is played by the protein p53, and it is documented that more than half of cancer cases show defects in up- and downregulation of p53 expression. Uncontrolled apoptosis can destroy healthy cells; thus the delicate equilibrium between proapoptotic and antiapoptotic regulation must be maintained. In view of this, cancer can be considered as a disturbed equilibrium between cell proliferation and cell death shifted toward cell proliferation.

A three-stage "initiation-promotion-progression" model of carcinogenesis involves changes in the redox environment of a cell reflecting the concerted action of ROS, RNS, and antioxidants [29, 39]. Initiation involves DNA mutations (e.g., 8-OH-Gua being the most studied) caused by oxidative DNA damage that occurs through the attack of free radicals and redox metals [41]. The process of initiation further passes through the release of calcium from intracellular calcium

stores. The stage of promotion is still a reversible and dose-dependent process that depends on the intensity of tumor promotors. Tumor promotors have strong inhibitory effects on the cellular pool of antioxidants. Progression is irreversible, and the final stage of the process of cancer development is characterized by the accumulation of genetic damage and genetic instability. During this stage the process of cell transformation from benign to malignant occurs.

The redox environment of a cell is tightly linked with all three stages of carcinogenesis [42]. Low to moderate levels of oxidative stress can stimulate cell division in the stage of tumor promotion. On the other hand, moderate to high levels of oxidative stress are cytotoxic for the cell, halting the proliferation by triggering apoptosis. Very high levels of oxidative stress induce necrosis in cells. Thus fine-tuning of the redox environment of a cell by redox-active compounds appears to be a way of affecting the cell cycle with the intent that the oncogenic process may be suppressed.

2.4.1.3 Cancer and Antioxidants Antioxidant defense represents one of the mechanisms of maintaining redox homeostasis [43]. The most effective antioxidants acting in biological systems involve antioxidant enzymes, of which the most important are SOD, catalase, and glutathione peroxidase. The low-molecular-weight antioxidants are represented by vitamins C and E, glutathione, carotenoids, flavonoids, and others.

Chronic gastritis and gastric metaplasia are both precancerous lesions, and they have been found in individuals with decreased serum levels of ascorbic acid [44]. This is in line with the epidemiological studies exploring the positive effect of ascorbic acid in reducing the incidence of stomach cancer. Similar findings have also been found in cancers of the lung and colon and rectum.

Several concerns have been raised over a possible prooxidant effect of ascorbic acid in the presence of redox metals such as iron. It has been claimed that vitamin C and iron can react to form damaging hydroxyl radicals [45]. However, these studies were performed under nonphysiological in vitro conditions. Under physiological conditions vitamin C acts as an antioxidant that is also capable of regenerating vitamin E from its radical form, α-tocopherol radical [46]. Vitamin C has been reported to regulate AP-1 complex. Ascorbate significantly (over 50%) inhibits JNK/AP-1 signaling pathways in UV-B irradiated cells.

Vitamin E in combination with vitamin C reduces the incidence of colorectal cancer by triggered apoptosis of cancer cells by inducing the powerful p21/WAFI/CIP1 belonging to the class of protein kinase inhibitors [47].

Glutathione is a very powerful cellular antioxidant regulating redox signaling by alterations in the level of GSH and the ratio of GSSG (oxidized GSH) and GSH [48]. GSH activates various transcription factors involving NF-κB and AP-1. GSH protects cells from apoptosis; thus the effectiveness of various anticancer drugs must be maintained or even enhanced by coadministered GSH-depleting agents. GSH-1-depleting agents are often used in the form of transition metal complexes.

Lycopene possesses antiproliferative effects on various cancer lines by inhibiting the cell cycle [49]. Regulated transcription factors, including inhibition of AP-1 and reduced induction of insulin-like growth factor I have been reported for prostate, lung, and breast cancers [3]. Beta-carotene has been shown to enhance proapoptotic effect in colon cell lines via a redox-driven mechanism of increased formation of ROS and GSSG-to-GSH ratio interconnected with enhanced NF-κB activity. However, carotenoids, similar to other antioxidants, may behave under certain conditions as prooxidants [50]. To ascertain the effect of carotenoid supplements on human health, a long-term beta-carotene prevention trial was conducted by the National Institute of Health in Finland (ATBC trial) [51]. In this clinical trial supplemental beta-carotene (20 mg/day) was administered to 29,133 50- to 69-year-old male smokers in Finland for 5 to 8 years. The results were intriguing. It has been reported that men who took beta-carotene had an 18% increased incidence of lung cancers, which contributed to an 8% increased overall mortality! These findings suggest that carotenoids may elicit a prooxidant effect. Thus the antioxidant behavior of antioxidants depends not only on concentration of the antioxidant but also on the site of its action. In the case of beta-carotene, the oxygen-rich environment in lungs triggered the formation of car-OO$^{\bullet}$ radicals, which exhibited a significant harmful prooxidant effect.

Polyphenols represent another important class of compounds with antioxidant and chelating properties [52]. Their antioxidant capacity has a beneficial effect on human health. Flavonoids can prevent cancer, cardiovascular disease, and other pathological disorders. Increased flavonoid intake, mainly quercetin, has been associated with reduced incidence of lung, stomach, and pancreatic cancer.

2.4.2 Cardiovascular Disease

Oxidative stress contributes to the development of cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiac hypertrophy, and congestive heart failure [53].

Oxidative stress associated with enhanced formation of ROS and RNS has been linked to various cardiovascular disorders such as ischemic heart disease, hypertension, atherosclerosis, and heart failure [54]. Oxidative

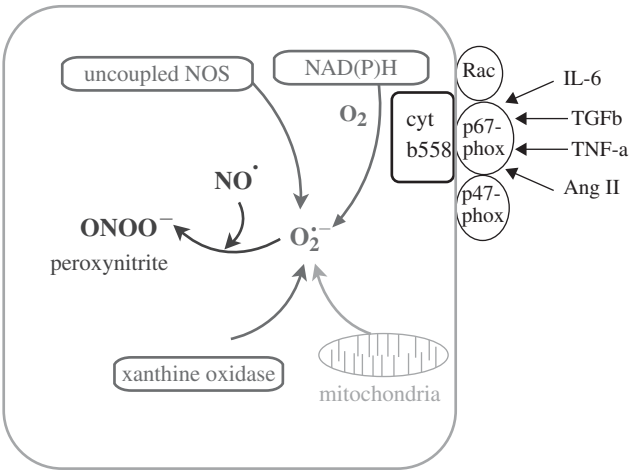

Fig. 2.4 Pathways of ROS and RNS generation in cardiovascular system.

stress in cardiac and cardiovascular systems has common denominators such as disruption of mitochondrial oxidative phosphorylation, activation of the xanthine dehydrogenase/xanthine oxidase system, uncoupled synthesis of nitric oxide, and activation of NAD(P)H by various activators (Fig. 2.4). The common feature for all these events is uncontrolled formation of superoxide anion radicals, which is the start of various deleterious radical reactions causing damage to biological tissues including the heart.

The most profound damage involves peroxidation and oxidation of thiol groups of biomolecules leading to changes in membrane fluidity and permeability, disruption of membrane lipids, and severe modification of various cellular proteins [55].

The studies dealing with the role of calcium in cardiovascular disease states are of key importance [56]. Heart mitochondria under conditions of oxidative stress show decreased membrane transport of calcium. Superoxide radical-incubated sarcolemma exhibited decrease in sarcolemmal ATP-dependent Ca^{2+} accumulation and calcium-stimulated ATPase activities. Radicals involving superoxide radicals, hydroxyl radicals, and nitric oxide all interact with sulfhydryl groups of ryanodine receptors, which in turn promote the release of calcium from the sarcoplasmic reticulum. Release of calcium results in the activation of kinases, such as protein kinase C (PKC), a member of the serine/threonine kinases. ROS-induced PKC activation has important functional consequences on downstream signaling pathways, namely, activation of MAPKs [57].

Several MAPK subfamilies have been identified in the mammalian cardiovascular system [30, 58]. The main MAPKs found in cardiac tissue are the extracellular signal-regulated kinases (ERKs), p38-MAPK, the stress-activated/c-Jun NH_2-terminal kinases (SAPK/JNKs), and ERK5/big MAPK 1 (BMK1). The ERKs are activated by physical stress; SAPK/JNKs and p38-MAPK are activated by various cell stresses, such as metabolic stress, UV radiation, heat shock, cytokines, and ischemia (Fig. 2.3). Activation of MAPKs represents pathogenesis of various processes occurring in the heart, for example, heart failure and ischemic and reperfusion injury [59]. In this respect, pharmacological modulations of MAPK activity and their impact on gene targeting or expression is of key importance.

2.4.2.1 Mitochondrial Dysfunction and Cardiovascular Disease Mitochondrial oxidative phosphorylation occurs within mitochondrial inner membranes and generates mitochondrial ATP used predominantly in the cytosol [56]. To transport ATP to the outside of the mitochondrial matrix, the organelle uses the ADP/ATP transmembrane protein carrier adenine nucleotide translocase, which governs the exchange of newly synthesized ATP in the mitochondrial matrix for ADP in the transmembrane space. In addition to soluble enzymes and small organic molecules, the matrix contains ribosomes and mitochondrial DNA (mtDNA). Mutations to mtDNA are responsible for many human diseases [60]. Apart from neurological, endocrine, and renal diseases, cardiac and cardiological disorders represent one of the major hallmarks of mtDNA mutations. Numerous experiments have confirmed that mtDNA is more prone to oxidative damage than nuclear DNA.

Mitochondrial production of superoxide radicals represents an evolutionary process by which cells regulate the concentration of various oxidants important in cell signaling pathways. However, mitochondrial formation of superoxide must be tightly controlled. Disruption of the mitochondrial balanced formation of superoxide radical leads to various diseases including the cardiovascular diseases. mtDNA mutations may lead to the enhanced formation of ROS and RNS, causing damage to mtDNA, which in turn triggers cardiovascular disorders [61]. To date more than several hundred mtDNA mutations have been reported, which can be divided into two major groups, point mutations and rearrangement mutations [56]. In fact, many mitochondrial diseases exhibit accumulation of mtDNA mutations and the progress of age-related decline in oxidative phosphorylation.

Production of mitochondrial ROS and RNS, mitochondrial antioxidants, and uncoupling protein activities, are all regulated by various physiological functions. Superoxide radicals can react with nitrogen oxide, forming peroxynitrite ($ONOO^-$). Peroxynitrite is a molecule with a damaging effect on various cellular components including DNA, proteins, and lipids. Mitochondrially generated superoxide radicals can be converted to

hydrogen peroxide by Mn-SOD [29]. Hydrogen peroxide is a signaling molecule that can be removed by catalase, forming water and oxygen. In the course of its production, hydrogen peroxide can be decomposed to damaging hydroxyl radicals by traces of transition metals such as iron(II) and copper(I).

Local concentrations of nitric oxide in mitochondria influence various process, such as superoxide radical formation and mitochondrial respiration. Relative concentrations of O_2 and NO thus influence the regulation of mitochondrial respiratory functions as well as concentrations of downstream reactive molecules, hydrogen peroxide and peroxynitrite [62]. An uncoupling protein (UCP) is a mitochondrial carrier catalyzing regulated electrophoretic proton transport across the inner mitochondrial membrane. Proton transport serves to reduce formation of oxidant molecules. The low expression levels of UCP are linked to suppressed proton transport and increased membrane potentials and increased formation of superoxide radicals [63]. The activation of UCPs is carried out by reactive alkenals, such as aldehydes (4-HNE) and other products of lipid peroxidation process.

The antioxidant pool is also an important factor in maintaining a physiological balance of mitochondrial oxidant molecules. Cytokines are a class of protein molecules that can directly or indirectly modulate the mitochondrial and cellular redox state. For example, PDGF mediates via mitogen-activated protein kinase 1 (MEK1) and ERK1/2 SOD2 transcription [64].

Mitochondria are exceptionally prone to damage by ROS and RNS. The most aggressive ROS and RNS causing damage to mitochondrial components are hydrogen peroxide and peroxynitrite, resulting in impaired mitochondrial protein synthesis and lowered redox state in vascular cells. This may lead, for example, to altered energy generation and redox signaling. It has been shown that prolonged ischemic injury in cardiac cells results in increased sensitivity of mitochondria to fluctuations in concentration of nitric oxide [65]. Protein synthesis inhibition in mitochondria has been documented by increased sensitivity to nitric oxide and induced apoptosis.

There is clear evidence pointing to an association between cardiovascular disorder incidence and mitochondrial impairments. Patients suffering from cardiovascular disease exhibited significantly increased abnormalities and damage to mtDNA and aorta compared with healthy subjects [66]. There are various molecular factors contributing to the increase of cardiovascular disease states. Among the most important is an elevated level of oxidative stress causing mitochondrial damage to the heart. The enhanced formation of free radicals participates in the increased mtDNA deletions and peroxidation of lipids in mitochondria [67]. Protection against these deleterious effects and enhanced tolerance to ischemia are provided by the mitochondrial antioxidants preventing these harmful mechanisms. There is a direct correlation between suppressed activities of SOD2 and increased susceptibility to cardiovascular risk.

Mitochondrial antioxidants and UCP protect the cardiovascular system against oxidative stress and the effects of ischemia and reperfusion. Therefore a deficiency in antioxidants and UCP in the heart has been linked with the triggering of cardiovascular disease under in vivo conditions.

2.4.2.2 Atherosclerosis and Hypercholesterolemia

There is a clear correlation between DNA damage and atherosclerosis [68]. Various DNA adducts have been linked with the prerequisite of the development of atherosclerosis. As shown by ^{32}P-postlabeling experiments, atherosclerotic patients exhibited significantly increased levels of aromatic DNA adducts in the thoracic aorta compared with control subjects [69]. The most frequently observed DNA adduct is 8-OH-Gua, which was also detected in plaques of the human carotid arteries. In addition, signs of enhanced DNA repair mechanisms have been observed in the atherosclerotic tissues. In line with all these results is the observation of significantly damaged mtDNA in cardiac tissues. However, the question of whether this damage is the cause or consequence of the cardiovascular disease state remains open.

The increased iron pool observed in atherosclerotic plaques is a good indicator of the iron-catalyzed formation of hydroxyl radicals (e.g., via the Fenton reaction), which may contribute to the development of atherosclerosis [70]. Increased cholesterol level and the uptake of oxidized low-density cholesterol (oxLDL) have been found to participate in the development of atherosclerosis. OxLDL mediates the formation of the superoxide anion radical, triggering apoptosis of vascular wall and plaque formation. Formation of superoxide radicals, and their ability to oxidize nitric oxide by forming peroxynitrite, is known to initiate peroxidation of lipids and oxidation of lipoproteins. These processes play a key role in the development of atherosclerosis.

Cholesterol administration in laboratory animals resulted in impaired mitochondrial energetic functions [71]. The activity of mitochondrial dehydrogenases was also impaired. Hypercholesterolemia leads to increased DNA damage. This has been substantiated by the observed 8-OH-Gua immunoreactivity and DNA strand breaks in atherosclerotic plaques in rabbits fed a cholesterol-rich diet for 6 months. The level of DNA strand breaks returned back to normal within a

month. However, reduction of 8-OH-Gua required between 3 and 6 months. While a high-fat diet reduced expression of genes involved in synthesis of antioxidant enzymes such as SOD and GPX, expression of genes responsible for the synthesis of stress proteins (Hsp 70) increased [72]. Intake of antioxidant supplements simultaneously with a high-fat diet reduced the deteriorating effect of cholesterol.

2.5 DIABETES

The majority of diabetes patients are non-insulin-dependent (type 2 diabetes), and about 10 of all patients are insulin-dependent (type 1 diabetes). The development of diabetes has been linked with the presence of oxidative stress substantiated by the formation of superoxide radicals and peroxidation of lipids, which in turn lead to the formation of isoprostanes, malondialdehyde, 3-nitrotyrosine levels, and DNA damage. DNA damage has been documented by the increased presence of oxidized DNA bases (one of the most abundant being 8-OH-Gua) in urine samples from diabetic individuals [73].

Under physiological conditions, complex I and ubiquinone-complex III in the mitochondrial membrane are major sources of electrons for the formation of ROS. However, under pathological conditions of diabetes mellitus, the primary site of superoxide radical formation becomes complex II [74]. This finding was revealed after application of the complex II inhibitor 2-thenoyltrifluoroacetone, which led to a decrease in ROS formation after treatment of various cell lines with high concentrations of glucose [75]. Formation of superoxide radicals in diabetic patients in mitochondria further increases the proton gradient across the inner mitochondrial membrane due to the overproduction of electron donors, for example, NADH. Overexpression of mitochondrial SOD2 counteracts the effect of superoxide radical, which in turn suppresses the activation of IL-1β/TNF-α/IFN-γ of NF-κB and induction of iNOS in insulin-producing cells. Conversely, suppression of SOD2 results in greater activation of NF-κB. These experimental findings suggest that mitochondrially derived ROS play a key role in the activation of the cytokine-sensitive transcription factor NF-κB.

NADPH oxidases are another major source of glucose-induced formation of free radicals and are considered as major mediators of diabetic complications [76]. Glucose-induced formation of free radicals by NADPH can be suppressed by the application of PKC inhibitors, pointing to the importance of this family of kinases in the regulation of hyperglycemia.

In addition to ROS, RNS has been implicated in the etiology of diabetes. $NO^{\bullet}$ forms with $O_2^{\bullet -}$ harmful peroxynitrite ($ONOO^-$), which reacts with the zinc cluster of NOS, leading to its uncoupling. Thus $ONOO^-$ not only depletes $NO^{\bullet}$ but, more importantly, causes damage to NOS and thus suppresses the formation of $NO^{\bullet}$.

Xanthine oxidase (XO) is another source of free radicals under diabetic conditions [77]. Allopurinol is an effective XO inhibitor and has been shown to reduce the concentration of oxidized lipids in plasma and to positively influence the blood pressure in type 2 diabetes patients.

Formation of ROS and RNS by the above-described sources depletes the antioxidant enzymes and low-molecular-weight antioxidants. Vitamin E supplementation in diabetic patients had a protective effect, mainly with respect to the level of lipid peroxidation [78]. The role of vitamin C, mainly at the plasma level in diabetic complications, has been studied. However, the results are not conclusive. Glutathione peroxidase (GPX) protects the organism from oxidative damage, and its relevance to diabetic complications has been investigated [79]. Hyperglycemia has been shown to affect the expression of GPX; however, the extent of the inhibition of expression and how it affects the cells is unclear.

The consequences of increased oxidative stress under diabetic conditions can sensitively be monitored with the use of appropriately selected biomarkers [80]. The most relevant include urinary and plasma levels of malondialdehyde and isoprostanes (nonenzymatic products of the oxidation of arachidonic acid). The role of 4-hydroxynonenal has not been proven. Modifications of side chain protein groups are referred as advanced glycation end-products (AGEs). AGEs were found in many tissues of various origin in rats and non-insulin-dependent humans [81].

2.6 NEUROLOGICAL DISORDERS

Alzheimer disease (AD) is a neurological disorder characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain [82]. The main constituent of amyloid plaques is a 39- to 42-residue peptide, amyloid-β protein (Aβ). Besides this, the major pathological feature of AD is the presence of an aberrant form of tau protein accumulated in the neurons in the form of neurofibrillary tangles.

The major neuropathological hallmark of Parkinson disease (PD) is the occurrence of intracellular inclusions called Lewy bodies that are located within the cytoplasm of neurons and consist of granular materials and filaments [83]. Lewy bodies show dense protein central cores with a rim of radiating filaments (7–20 nm in diameter).

2.6.1 Alzheimer Disease

Recent advanced scattering spectrometry experiments have shown significantly increased contents of metals within the amyloid plaques in AD brain with respect to surrounding tissue [84, 85]. The amyloid plaques have been found to contain significant amounts of Aβ organized into amyloid fibrils. Two major peptide fragments are formed, Aβ(1-42) and Aβ(1-40), displaying a different neurotoxicity that correlates with a marked difference in aggregation behavior [85]. While synthetic Aβ(1-40)s primarily exist as a monomer/dimer mixture, Aβ(1-42) contains a transient low-order potentially toxic oligomeric species. An increasing body of evidence suggests that various oligomeric Aβs of different sizes are involved in the development of AD.

While the majority of papers dealing with the origin of AD have considered the amyloid cascade hypothesis as the "null hypothesis," there is an alternative view proposing that amyloid-β is not a primary cause, but more probably a secondary event of the disease [86].

Without regard to such views, there is clear evidence that accumulated amyloid-β is able, under pathological conditions, to generate free radicals. Thus the pathology of AD is directly linked with the enhanced occurrence of oxidative stress of the brain [87]. The underlying factor of oxidative stress in the brain is the disrupted homeostasis of redox metals iron and copper and redox-inert metal zinc.

Copper(II) is abnormally elevated in amyloid plaques of AD brain and binds to Aβ through His13, His14, His6, and Tyr10 amino acid residues [88]. Besides copper (II), Aβ also binds iron(III) and zinc(II). Both metals are elevated in the amyloid plaques of subjects with AD. The neurotoxicity of Aβ is linked with its ability to reduce Cu(II) → Cu(I) and form hydrogen peroxide. The neurotoxicity of Aβ can be attenuated by the administration of free radical scavengers and various antioxidants including vitamin E.

The redox-inert metal zinc has a special role in AD. While the molecular mechanism of the action of zinc in AD is largely unknown, its preventive effects against Aβ toxicity in micromolar concentrations have been well documented. On the other hand, enhanced copper and iron-induced oxidative and nitrosative stress causes the release of zinc from vesicular pools, which may have serious neurotoxic consequences. Thus, under normal physiological conditions, there is a sensitive balance among zinc, copper, iron, and Aβ metabolism. Deposition of redox-active copper and iron induces an increase in oxidative stress that in turn may perturb the subtle metal ion balance substantiated by the uncontrolled zinc elevation from vesicular pools and, possibly, amyloid deposition.

Methionine35 (Met35) is significantly abundant in AD brain, which is in agreement with the high susceptibility of the sulfur atom of methionine to oxidation [89]. A recently presented model involves an oxidation reaction between C-terminal methionine (Met35) with N-terminally complexed Cu(II) according to the reaction

$$\mathrm{Met(35)S} + \mathrm{A\beta\text{-}Cu(II)} \leftrightarrow \mathrm{Met(35)S^{\bullet+}} + \mathrm{A\beta\text{-}Cu(I)} \quad (9)$$

forming the sulfide radical of methionine35 [$\mathrm{Met(35)S^{\bullet+}}$] and cuprous ions. The process of reduction of copper(II) is achieved via one-electron oxidation of methionine(35) forming the methionine radical cation $\mathrm{MetS^{\bullet+}}$, which plays a role in free radical formation and the neurotoxicity of Aβ. Met35 is the most vulnerable residue in Aβ to oxidation and may react with various radicals including superoxide radical anion [89, 90].

$$\mathrm{MetS^{\bullet+}} + \mathrm{O_2^{\bullet-}} \xrightarrow{\text{Met}} 2\mathrm{MetSO} \quad (10)$$

thus forming the Met-sulfoxide product (MetSO), which has been detected in AD senile plaques. Oxidation by the $\mathrm{Cu^{2+}}$-Aβ complex involves cell components such as fatty acids and cholesterol, leading to the formation of various markers of peroxidation process, the most neurotoxic being malondialdehyde (MDA), peroxynitrite, heme oxygenase (HO-1), and AGEs. Reduced copper (cuprous ions) can participate (via the Fenton reaction) in the catalytic decomposition of hydrogen peroxide, thus forming the reactive hydroxyl radical, which in turn is involved in the lipid peroxidation process. AGEs further activate proinflammatory cytokines, for example, IL-6. In addition, tyrosine residues can be a target of free radical attack, as documented by the observed accumulation of dityrosine and 2-nitrotyrosine in AD brain.

In vitro experiments employing neurotoxic forms of Aβ, Aβ(1-42) and Aβ(1-40), have been shown to stimulate copper-mediated oxidation of ascorbate ($\mathrm{AscH^-}$) and formation of hydroxyl radicals. The following mechanisms have been proposed [87]

$$\begin{gathered}\mathrm{A\beta\text{-}Cu(II)} + \mathrm{AscH^-} \leftrightarrow \mathrm{A\beta\text{-}Cu(I)} + \mathrm{Asc^{\bullet-}} + \mathrm{H^+} \\ \mathrm{A\beta\text{-}Cu(I)} + \mathrm{H_2O_2} \rightarrow \mathrm{A\beta\text{-}Cu(II)} \\ + \mathrm{^{\bullet}OH} + \mathrm{OH^-}\ \text{(Fenton)} \\ \mathrm{A\beta\text{-}Cu(I)} + \mathrm{O_2} \leftrightarrow \mathrm{A\beta\text{-}Cu(II)} + \mathrm{O_2^{\bullet-}}\end{gathered} \quad (11)$$

From the proposed mechanism it follows that copper (II) and ascorbate in the presence of hydrogen peroxide and oxygen (both of which are present in relatively high content in brain cells) leads to the formation of free radicals with damaging effects [5 and references therein].

We note that the above-described model is based on in vitro experiments and many other clinical trials employing high doses of vitamin C have disproved such conclusions. Based on the findings under in vivo conditions, it has been concluded that even high doses of vitamin C are not harmful to the organism (if not beneficial) and vitamin C does not act as a prooxidant [46]. In addition, vitamin C is able to regenerate vitamin E from its radical form (α-tocopheroxyl radical) back to α-tocopherol. Such combinations of preventive and chain-breaking antioxidants such as vitamin C and vitamin E could protect brain lipoproteins against oxidative stress.

An epidemiological trial of the use of vitamin E (2000 IU/day, 2 years) in patients with moderate AD has shown slowed functional deterioration (–53%) [91]. In addition, a combined supplemental intake of vitamins E and C was found to reduce prevalence (–78%) and incidence (–64%) of AD in elderly people [91]. In light of these results, vitamin E appears to act in concerted action with other antioxidants, predominantly with vitamin C, and thus provide protection of Aβ against oxidative damage.

2.6.2 Parkinson Disease

Parkinson disease (PD) is a progressive degenerative disorder of the central nervous system that affects motor skills and functions [92]. The majority of PD cases are sporadic (90–95%). Familial cases account for 5–10% of PD. Some studies have concluded that familial and sporadic PD patients display similar clinical features.

The main feature of PD is the degeneration of dopaminergic neurons containing and synthesizing the neurotransmitter and neurohormone dopamine in the substantia nigra pars compacta (SNc).

The most typical pathological feature of PD is the presence of intracellular inclusions called Lewy bodies that consist of aggregates of the presynaptic soluble protein called α-synuclein [93]. α-Synuclein is mainly a neuronal protein localized in neuronal mitochondria; however, it is also present in glial cells.

A relatively high level of oxidative stress in the SNc with respect to other brain regions has been found in postmortem studies of the brain of PD patients. The earliest signs of PD development are characterized by the rapid depletion of the antioxidant glutathione in the substantia nigra [94]. The loss of glutathione may have an effect on energy production in the mitochondria, most probably linked with the decline in the activity of mitochondrial complex I. α-Synuclein is localized in the inner membrane of mitochondria, and its inhibitory effect on mitochondrial complex I activity is dose dependent. Apart from increased markers of oxidative stress in the SNc, other factors, involving inflammation and the toxic action of $NO^{\bullet}$, may play a role in the development of PD [95, 96]. The role of trace metals has also been studied, showing increased iron levels in the PD midbrain, catalyzing the process of neurodegeneration [94]. The harmful effect of iron can be suppressed by the application of the iron chelator clioquinol [97]. Chelated iron in the form of the Fe-clioquinol complex does not participate in the formation of free radicals and prevents degeneration of dopaminergic midbrain neurons. The major features indicating the presence of oxidative stress in PD are lipid peroxidation markers, including 4-hydroxy-trans-2-nonenal (HNE), 4-oxo-trans-2-nonenal (4-ONE), 4-oxo-trans-2-hexenal, acrolein, and malondialdehyde. In addition, defects in protein clearance and toxic action of nitric oxide are all factors contributing to the development of PD.

In addition to α-synuclein, four other genes have conclusively been linked to dominant (LRRK2) and autosomal recessive (parkin, PINK-1, DJ-1) parkinsonism [98, 99]. Mutations in the LLRK2 gene are the most common cause of genetic PD mutations in PINK1. The PINK1 gene produces a protein called PTEN induced putative kinase 1, and mutations in PINK1 are the second most frequently cause of autosomal recessive parkinsonism. Mutations in the parkin gene induce a loss of parkin function, leading to the hypothesis that the accumulation of parkin substrates causes neurotoxicity and results in the death of dopaminergic neurons. Recent experiments revealed that the mutated protein Parkin is transported from the cytoplasm to damaged mitochondria and that this causes the breakdown of the mitochondria by processes acting within the cell.

DJ-1 is present mainly in the cytoplasm and less in the mitochondria and nucleus of dopaminergic cells [96]. Recently presented results suggest that various environmental toxins that may induce oxidative stress are linked with the role of DJ-1 [100]. Loss of DJ-1 leads to noteworthy susceptibility to the herbicide paraquat and the insecticide rotenone. This points to a possible role of DJ-1 in the protective action against environmental toxins inducing oxidative stress.

2.7 CONCLUSION

ROS and RNS are species possessing dual characters, acting under normal conditions as signaling molecules involved in many physiological functions of living systems and, conversely, under pathological conditions possibly inducing oxidative stress. Ironically, various ROS-mediated actions in fact protect cells against ROS-induced oxidative stress and reestablish or maintain "redox balance," also termed "redox homeostasis." The

dual character of ROS as pro- and antitumorigenic species is documented by the fact that on one hand they maintain the oncogenic phenotype of cancer cells but on the other hand they can also induce cellular senescence and apoptosis.

Overproduction of ROS resulting in oxidative stress is frequently achieved by excessive stimulation of NAD(P)H by cytokines, or by the mitochondrial electron transport chain and xanthine oxidase. The current problem in the quantification of the level of oxidative stress with respect to human disease and ageing is to determine the most reliable oxidative stress markers. Following this, the monitoring of healthy subjects over a few decades will be necessary to obtain reliable results.

Further effort is required to design effective redox-active agents interfering with the mechanism of ROS-induced apoptotic pathways. Detailed description of NO-driven redox-mediated signaling is anticipated to develop novel therapeuticals for heart failure.

Antioxidant enzymes and low-molecular-weight antioxidants provide protection against deleterious effects of oxidative stress. Since redox-active and redox-inert metals directly or indirectly participate in the formation of damaging ROS and RNS, the design of dual-functioning antioxidants, possessing both metal-chelating and ROS/RNS-scavenging properties is anticipated.

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