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THE PROTECTIVE ROLE OF GRAPE SEED POLYPHENOLS AGAINST OXIDATIVE STRESS IN TREATING NEURODEGENERATIVE DISEASES

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16.1 INTRODUCTION

Alzheimer disease (AD) is the most common type of dementia in the United States. Victims of AD commonly display a loss of memory, inability to learn new things, loss of language function, deranged perception of space, inability to do calculations, depression, delusions, and other cognitive deficiencies. AD is ultimately fatal within 5–10 years of its onset. It is estimated that approximately 5 million people in the United States currently have AD [1], with an estimated cost to society of more than $100 billion per year. Up to 14 million people in the United States are projected to be affected by AD by the middle of this century if effective therapies are not developed [1]. To date, there is no cure for AD; thus researchers are continually exploring novel avenues for the prevention and treatment of this condition. It is important to note that even delaying AD onset by a few years would lead to significant reductions in disease prevalence and, consequently, its burden on health care systems. The few agents that are approved by the FDA for the treatment of AD have only modest efficacy in modifying clinical symptoms, and none appears to affect disease progression or prevention [2]. Historically, the majority of candidate therapeutics have been directed toward cholinergic neurons, which are especially vulnerable in AD and cognitive alterations [2, 3]. More recently, agents are being developed to interfere with the β-amyloid (Aβ) protein precursor pathway, which is thought to be responsible for Aβ-mediated neuronal dysfunction and death [4, 5].

16.2 ALZHEIMER DISEASE NEUROPATHOLOGY FEATURES: IMPLICATIONS FOR THERAPEUTIC DEVELOPMENTS

While the classification and diagnosis of AD are based on the cognitive behavior of an individual, the roots of the disease lie in the neurological pathology of its victims [6]. The two defining neuropathology features of AD are abnormal aggregation and deposition of Aβ peptides and tau protein in the brain as, respectively, extracellular neuritic plaque (NP) and intracellular neurofibrillary tangles (NFT) [7].

Aβ species with different amino and carboxyl termini are generated from the ubiquitously expressed amyloid precursor protein (APP) through sequential proteolysis by β- and "proamyloidogenic" γ-secretases [8, 4, 5]. A 40-amino acid form of Aβ (Aβ1-40) is the major secreted species. However, a 42-amino acid form of Aβ (Aβ1-42), which contains two additional residues at its carboxyl terminus, is thought to initiate AD pathogenesis [9]. Aβ1-42 aggregates much more readily than Aβ1-40 in vitro and is also deposited earlier and more consistently in AD brain. In humans, AD-causing mutations elevate plasma Aβ1-42 levels by approximately 30–100% [10].

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Even mutations showing small increases in Aβ1-42 levels are associated with the onset of dementia in the fourth or fifth decade of life. In the Tg2576 transgenic mouse model of AD, the same mutations produce increases in Aβ1-42 levels and markedly accelerate Aβ deposition [11]. In light of this evidence, a major effort from both academia and industry is presently focused on developing pharmacological strategies that would delay the initiation and/or slow the progression of AD-type Aβ neuropathology in the brain. Recent evidence from experimental AD mouse models indicates that accumulation of soluble high-molecular-weight oligomeric Aβ species in the brain, rather than deposition of NP per se, may be specifically related to spatial memory reference deficits. In particular, experimental evidence demonstrated that high-molecular-weight oligomeric Aβ species may disrupt hippocampal long-term potentiation and synaptic plasticity and induce synaptic deficits [12–15]. Consistent with the hypothesis that soluble high-molecular-weight oligomeric Aβ species play a key role in AD memory dysfunction, experimental therapeutic evidence demonstrated that neutralization of soluble high-molecular-weight Aβ oligomeric species from the brain causally improves spatial memory functions in a mouse model of AD [16].

Despite strong genetic data arguing that Aβ neuropathology is sufficient to cause AD [17], progressive cognitive decline and neuron and synapse loss in AD are best correlated with tau neuropathology [18]. In the AD brain, tau proteins, particularly hyperphosphorylated tau, are found aggregated into progressively larger polymeric species that are ultimately deposited as insoluble NFTs [19]. NFTs themselves are not necessarily the tau species inducing neurotoxicity, as evidence in experimental mouse models suggests that neuronal loss and memory impairment may occur before NFT formation in the brain [20, 21]. Instead, evidence indicates that accumulation of multimeric tau aggregates may play a more important role in AD memory dysfunction [21] Consistent with this hypothesis, in transgenic mouse models levels of tau multimers are significantly correlated with memory index [21]. Moreover, neuronal loss and memory impairment in a mouse model of tauopathy can be mitigated by reducing tau expression without affecting the number of NFTs [20]. Aggregation of tau is a seed-nucleation process. Formations of hyperphosphorylated oligomers serve as nucleation sites that sequester additional hyperphosphorylated tau as well as normal nonphosphorylated tau [22]. Thus a predominant theory of tau-mediated neurodegeneration is based on a "toxic gain of function" model, in which abnormally phosphorylated tau promotes sequestration of both hyperphosphorylated and normal tau from microtubules, leading to microtubule instability and alteration of microtubule-mediated processes, including abnormalities in axon transport among others [22].

These considerations strongly suggest that reducing the accumulation of soluble oligomeric Aβ peptides and tau species in the brain, as opposed to dissociating or preventing NP and/or NFT formation or deposition, may be a more productive approach to AD therapy. Conceptually, it might be possible to reduce brain accumulation of oligomeric tau species by reducing tau aggregation and/or by promoting tau clearance. Similarly, it might be possible to reduce oligomeric Aβ peptides in the brain by reducing Aβ generation, reducing Aβ aggregation, and/or promoting Aβ clearance. As discussed in more detail below, we recently demonstrated for the first time that dietary supplementation with red wines that is equivalent to moderate wine consumption in humans effectively attenuated the development of Aβ-mediated AD-type neuropathology and cognitive dysfunction in a mouse model of AD. Moreover, our evidence demonstrated the polyphenolics in the red wines as principle bioactive components that may modulate neuropathology Aβ phenotypes by reducing Aβ generation as well as Aβ aggregation.

16.3 POTENTIAL ROLES OF RED WINES AND WINE-DERIVED POLYPHENOLS IN ALZHEIMER DISEASE PREVENTION AND/OR THERAPY

While genetic factors are highly relevant in early-onset AD cases, their significance diminishes in late-onset sporadic AD cases, which is the most common form of AD [2]. Nongenetic factors, including modifiable lifestyle dietary regimens such as moderate consumption of alcoholic beverages, are receiving increasing attention in AD research, especially in light of the recent epidemiological studies indicating that moderate wine consumption may influence the relative risk for AD clinical dementia [23]. For example, while little is known about the beneficial role of red wine in AD dementia onset, recent studies suggested that the neuroprotective efficacy of red wine may be mediated, in part, by the antioxidant activities of polyphenols in the wine. Wine-derived polyphenols are known to have strong inhibitory effects on lipid peroxidation. Moreover, evidence indicates that several types of natural polyphenols may have neuroprotective effects both in vivo and in vitro, possibly through their abilities to scavenge reactive oxygen species, which may have profound implications for the overall protective effects of red wine in neurodegenerative disorders and stroke outcomes [24].

Presently, little is known about the potential role of red wine in AD. Recent prospective studies showed that moderate intake of red wine may decrease the relative

risk for AD [23]. We therefore explored whether red wines may beneficially modulate AD phenotypes in the Tg2576 AD mouse model.

16.4 THE Tg2576 AD MOUSE MODEL

Tg2576 mice are transgenic mice engineered to express a mutant form of the human APP harboring the Swedish [Lys670 → Asn, Met671 → Leu] mutation that is found in a subset of familial AD patients. Recapitulating select features of AD, the Tg2576 mouse model is characterized by progressive development of Aβ neuropathology and cognitive decline. Originally generated by Dr. Karen Hsiao [25], Tg2576 is the most commonly used animal model for studying the mechanisms underlying Aβ-mediated AD neuropathology and cognitive deterioration. This animal model is also commonly used for testing novel AD therapeutic strategies.

16.5 EXPLORING THE POTENTIAL BENEFITS OF MODERATE RED WINE CONSUMPTION IN Tg2576 MICE

In our initial studies, we treated Tg2576 mice with a dietary supplement of a red Cabernet Sauvignon wine to explore the impact of wine consumption on Aβ neuropathology and cognitive functions [26]. This was generated from *Vitis vinifera* by our collaborators at the University of Florida as previously described [27]. Our Cabernet Sauvignon contained ~12% alcohol, as determined by ebulliometry, and had a titratable acidity (as tartaric acid) of 6 g/l and a pH of 3.6 [26]. The wine was delivered to the mice by dilution into the drinking water to a final ethanol concentration of 6% [26]. In a parallel control study, Tg2576 mice were provided with drinking water containing 6% ethanol [26]. Wine (or ethanol control) treatment was initiated at 3 months of age before animals developed Aβ neuropathology or cognitive impairment, and continued to about 10.5 months of age when Tg2576 mice are typically characterized by moderate Aβ neuropathology and cognitive dysfunction.

In our Cabernet Sauvignon study, we observed that each mouse consumed ~4 ml of wine-adulterated water per day. We calculated that ~7% of the total daily energy consumption in Cabernet Sauvignon-treated Tg2576 mice was derived from wine. This is equivalent to wine-derived energy intake in the human following moderate wine consumption of one 5-oz glass of red wine for a woman (accounting for 6.2% of energy intake) and two 5-oz glasses of red wine for a man (accounting for 10% energy intake) [26]. We also used an FDA-recommended criterion that incorporates body surface area for calculating equivalent drug doses across species [human equivalent dose in mg/kg = animal dose in mg/kg × (animal wt in kg/human wt in kg)] [27] as an independent means of estimating human wine consumption that is equivalent to what mice received in our study. We calculated that Cabernet Sauvignon-treated mice consumed ~8 g of alcohol/kg body wt/day, which is equivalent to a human daily intake of 39.5 g of alcohol per day, or daily consumption of 2.3 5-oz glasses (329.2 ml) of Cabernet Sauvignon. Thus, on the basis of two independent calculations, we determined that mice in our study consumed an amount of Cabernet Sauvignon considered moderate by the U.S. Departments of Agriculture and Health and Human Services [26].

16.6 MODERATE CONSUMPTION OF A RED CABERNET SAUVIGNON WINE ATTENUATES AD-TYPE NEUROPATHOLOGY AND COGNITIVE DETERIORATION IN Tg2576 MICE

At the end of the Cabernet Sauvignon treatment, we assessed cognitive functions in Tg2576 mice with the Barnes maze protocol. We found that moderate consumption of Cabernet Sauvignon attenuated the onset of cognitive deterioration in the Tg2576 transgenic AD mouse model (Fig. 16.1A). In parallel control studies we found that moderate Cabernet Sauvignon consumption had no detectable impact on cognitive behavioral performance in age-, sex-, and strain-matched wild-type (WT) mice [26]. We found that cognitive benefits of wine treatment were associated with significant reduction of AD amyloid neuropathology in the brains of Tg2576 mice (Fig. 16.1B). Moreover, we found that Cabernet Sauvignon treatment might modulate Aβ neuropathology, in part, by promoting brain activity of α-secretase that interferes with generation by cleaving the APP within the Aβ sequence [26].

In light of evidence that natural polyphenols may exert neuroprotective effects, we explored whether polyphenolic components in Cabernet Sauvignon may be responsible, in part, for the efficacy of this red wine to modulate Aβ-mediated neuropathology responses. We extracted total polyphenolic components from the Cabernet Sauvignon with acetonitrile-butanol (1:1, v/v), followed by vacuum centrifugation, to concentrate the extracted polyphenol compounds and remove volatile organic components, including ethanol from the wine as well as organic solvents used in the extraction [26]. HPLC analysis confirmed that the contents of polyphenol components in the extract were comparable to Cabernet Sauvignon (data not shown).

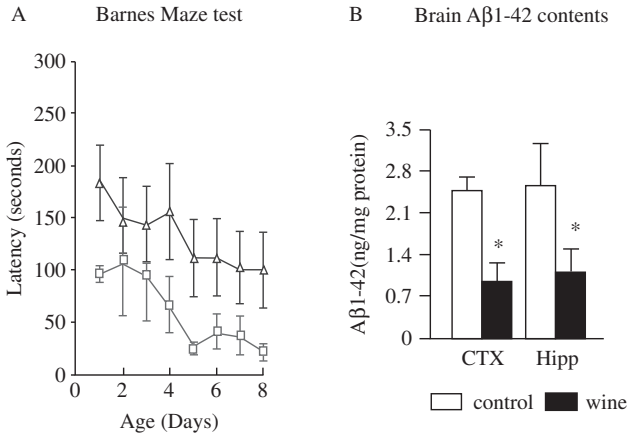


Fig. 16.1 Moderate consumption of Cabernet Sauvignon prevented cognitive impairment and attenuated Alzheimer disease (AD)-type neuropathology in the brains of Tg2576 mice. Tg2576 mice were treated with Cabernet Sauvignon by dilution of the wine into the drinking water. Control, nontreated mice were provided with unadulterated drinking water. (A) Spatial memory function assessment of Tg2576 mice treated with Cabernet Sauvignon at 10.5 months of age with the Barnes maze. Latency represents time (in seconds) to escape the platform, and points on the graph represent means (±SE). Statistical analysis by 2-way ANOVA: Cabernet Sauvignon group vs. control, 2-way ANOVA, $P < 0.0001$ for wine treatment, $P < 0.4379$ for escape latency over learning trials. (B) Assessment of Aβ1-42 peptide concentrations in neocortex (CTX) and hippocampus (Hipp) of Cabernet Sauvignon-treated or control Tg2576 mice. Bar graphs represent group means ± SE, $n = 6$–9 animals per group; $^*P < 0.05$, 2-tailed Student's *t*-test.

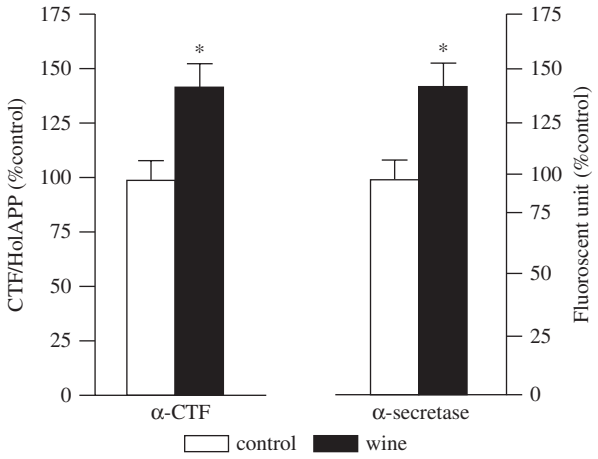


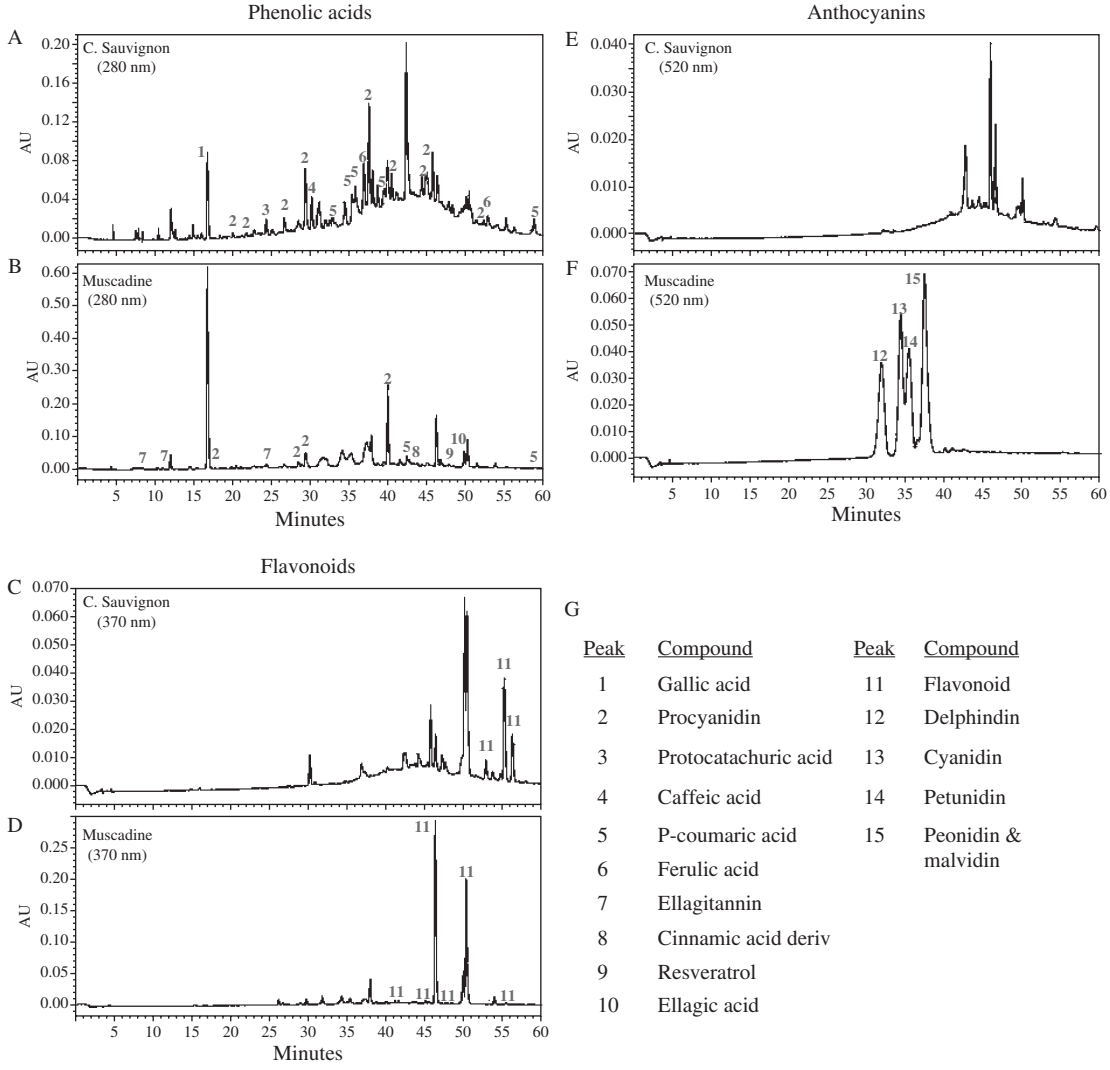
Fig. 16.2 Cabernet Sauvignon polyphenol extract promotes neuronal α-secretase activity. Primary corticohippocampal neuron cultures derived from Tg2576 mice were treated with 100 μg/ml Cabernet Sauvignon extract followed by assessments of neuronal α-secretase activity using independent methodologies. (Left) Assessment of the contents of amyloid precursor protein α-carboxyl terminal fragment (α-CTF). Cleavage of amyloid precursor protein by α-secretase generates α-CTF. Therefore, neuronal α-CTF content directly reflects α-secretase activity. (Right) Fluorometric assessment of α-secretase enzymatic activities in primary neuron cultures treated with 100 μg/ml Cabernet Sauvignon polyphenol extracts. Bar graphs represent group means ± SE, $n = 3$ per group; $^*P < 0.05$, 2-tailed Student's *t*-test.

We tested the efficacy of the Cabernet Sauvignon polyphenolic preparation to modulate the generation of Aβ peptides. For these studies, we prepared primary corticohippocampal neuron cultures derived from Tg2576 mice; these neuron cultures are known to generate Aβ peptides. We then treated primary corticohippocampal neuron cultures with the Cabernet Sauvignon polyphenolic preparation and measured Aβ generation by these neurons. Consistent with our observation in Cabernet Sauvignon-treated Tg2576 mice, we found that polyphenolics extracted from Cabernet Sauvignon significantly decrease generation of Aβ peptides by Tg2576 neuron cultures in a dose-dependent manner (Fig. 16.2). Similar to what we found in the brain of Cabernet Sauvignon treated Tg2576 mice, we also found that treatment with the Cabernet Sauvignon polyphenolic extract significantly reduced α-secretase activity in Tg2576 neuron cultures [26].

Collectively, observations from our in vivo studies on moderate consumption of the red Cabernet Sauvignon wine in Tg2576 AD mice and our in vitro mechanistic studies using Tg2576 corticohippocampal neuron cultures provide the first comprehensive experimental evidence supporting the hypothesis that polyphenolic components from red wines might provide benefit disease-modifying activities in AD.

16.7 EXPLORING FOR POTENTIAL BENEFICIAL AD DISEASE-MODIFYING ACTIVITY IN A RED MUSCADINE WINE WITH DIFFERENT POLYPHENOLIC COMPOSITIONS COMPARED TO CABERNET SAUVIGNON

To gather insights on the specific polyphenolic component(s) in red wines that might exert beneficial AD-modifying activities in vivo, we continued to assess potential AD-modifying activity in another red wine, namely, a red muscadine wine, whose polyphenolic content we found is distinctly different from the polyphenolic contents of the Cabernet Sauvignon, which we demonstrated to benefit AD phenotypes (see below). This red muscadine wine was also generated by our collaborator at the University of Florida from *Vitis rotundifolia* grapes [27]. The muscadine wine contains approximately 12% alcohol (determined by ebulliometry) and is characterized by a titratable acidity (as tartaric acid) of 6.9 g/l and a pH of 3.00. The phenolic



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Peak	Compound	Peak	Compound
1	Gallic acid	11	Flavonoid
2	Procyanidin	12	Delphindin
3	Protocatachuric acid	13	Cyanidin
4	Caffeic acid	14	Petunidin
5	P-coumaric acid	15	Peonidin & malvidin
6	Ferulic acid		
7	Ellagitannin		
8	Cinnamic acid deriv		
9	Resveratrol		
10	Ellagic acid		

Fig. 16.3 Chemical analysis of Cabernet Sauvignon and muscadine wines. Constituent polyphenolic components in Cabernet Sauvignon (A,C,E) and muscadine (B,D,F) wines were analyzed by reverse phase HPLC using a C18 column. (A,B) Detection of phenolic acid compounds at 280 nm. (C,D) Detection of flavonoids at 370 nm. (E,F) Detection of anthocyanins at 520 nm. (G) Identification of polyphenols corresponding to peaks detected in panel (A-F) based on spectroscopic interpretations.

composition (as gallic acid and measured by the Folin–Coicalteau method) was 1.731 mg/l [27].

We analyzed chemical compositions of muscadine and Cabernet Sauvignon by reverse-phase chromatography using HPC and an octadecyl silane column [29]. Different classes of polyphenolic compounds from the wines were detected at 280 nm (phenolic acids) (Fig. 16.3A,B), 370 nm (flavonoids) (Fig. 16.3C,D), and 520 nm (anthocyanins) (Fig. 16.3E,F), with select compounds identified based on spectroscopic interpretations from 200-600 nm (Fig. 16,3G) [29]. Our analysis demonstrated that the two wines are characterized by distinct component compositions of phenolic acid (Fig. 16.3A, B), flavonoid (Fig. 16.3C,D), and anthocyanin polyenenolic compounds (Fig. 16.3E,F).

16.8 MODERATE CONSUMPTION OF RED MUSCADINE WINE ATTENUATES AD-TYPE NEUROPATHOLOGY AND COGNITIVE DETERIORATION IN Tg2576 MICE

We treated Tg2576 mice with the red muscadine wine at a dosage equivalent to moderate wine consumption, using a procedure comparable to that we used with our Cabernet Sauvignon studies [29]. Similar to our observation with Cabernet Sauvignon, we found that moderate consumption of the muscadine wine significantly attenuated the development of spatial memory decline (Fig. 16.4A,B) and AD-type Aβ neuropathology (Fig. 16.4C) in the Tg2576 AD mouse model. However, in contrast to our previous observation with Cabernet

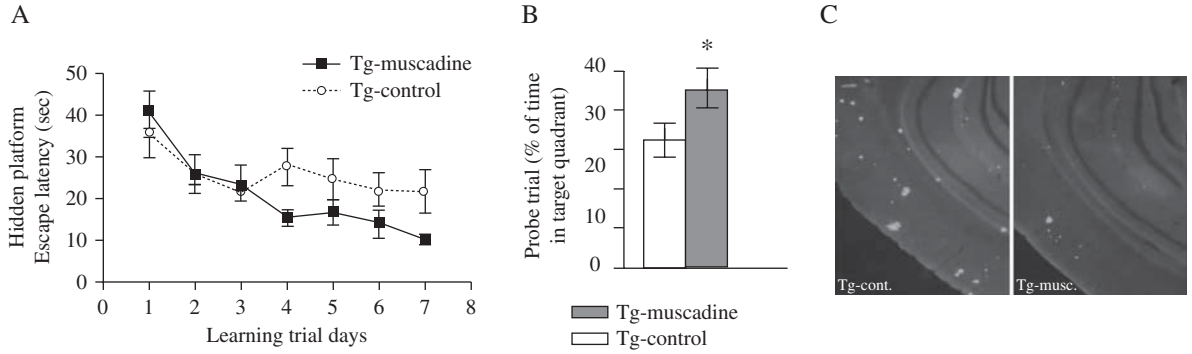

Fig. 16.4 Muscadine treatment improves spatial memory function and Aβ neuropathology in Tg2576 mice. (A,B) Assessments of spatial memory behavioral functions of 14 month old muscadine-treated (Tg-muscadine) and control, gender- and age-matched non-treated (Tg-control) Tg2576 mice using the Morris water maze protocol. (A) Learning trial hidden-platform acquisition curves. Tg-muscadine group performed significantly better than the control, non-treated group (Tg-control) [2-way ANOVA analysis of Tg-muscadine vs. Tg-control groups for muscadine treatment ($p < 0.05$, $F = 4.24$, DFn = 1, DFd = 84) and for training days ($p < 0.05$, $F = 6.43$, DFn = 6, Dfd = 84)]. (B) Probe trial conducted 24 hours after completion of hidden-platform training. Muscadine-treated Tg2576 mice exhibited a significantly higher preference for the target platform compared to control, non-treated Tg2576 mice ($p < 0.05$, 2-tailed Student t test). In (A,B) Values represent group mean (+SEM); $n = 7$–9 mice per group. (C) Assessments of Aβ neuropathology reflected by amyloid neuritic plaque density in cerebral cortex and in the hippocampal formation of brain specimens from muscadine-treated and control, non-treated Tg2576 mice. Representative micrograph of brain specimen stained for amyloid neuritic plaques in muscadine-treated (Tg-musc.) or in control, non-treated (Tg-cont.) Tg2576 mice. *(See color insert.)*

Sauvignon [26], we found that muscadine treatment did not modulate α-secretase activity or activities of other enzymes known to be involved in generation of Aβ peptides in the brain from APP [29]. Instead, we found that muscadine treatment reduced the accumulation of soluble high-molecular-weight oligomeric Aβ species in the brain, suggesting that muscadine treatment interferes with Aβ oligomerization (Fig. 16.5A). In parallel studies, we confirmed the efficacy of muscadine polyphenolics to interfere with the assembly of Aβ peptides into neurotoxic, high-molecular-weight oligomeric species (Fig. 16.5B). As illustrated by using a gel electrophoresis to assess steady-state Aβ aggregates, we demonstrated that polyphenolic components from the muscadine wine potently interfere with the formation of a synthetic Aβ peptide into high-molecular-weight aggregates (Fig. 16.5B). Collectively, our in vivo and in vitro evidence suggests that muscadine polyphenol may have benefited AD phenotypes in the Tg2576 mouse model of the disease by inhibiting the assembly of Aβ peptides into neurotoxic high-molecular-weight aggregates.

16.9 DIETARY GRAPE-DERIVED BIOACTIVE POLYPHENOLIC COMPONENTS: IMPLICATIONS IN AD THERAPY AND PREVENTION

The evidence from our studies with two independent red wines summarized above strongly supports the hypothesis that moderate red wine consumption might provide preventive and/or therapeutic value in AD. The potential health benefits of wine consumption are generally ascribed to the polyphenol compounds that are present in high abundance, particularly in red wines [30, 31]. Since many of the wine-derived polyphenols are strong antioxidants, it is thought that red wine polyphenols may benefit AD (and other neurodegenerative disorders) by reducing the content of reactive oxygen species in the brain [32]. Aside from potential antioxidant activities, our accumulating preclinical evidence suggests that red wine polyphenols may also benefit AD by directly modulating Aβ-related mechanisms in the brain. Results from our studies demonstrated that polyphenolic components from the Cabernet Sauvignon wine may protect against the onset of AD-type Aβ-neuropathology and cognitive deterioration by promoting α-secretase activity in the brain. In contrast, our studies suggest that polyphenolic compounds from a red muscadine wine may modulate AD phenotypes by interfering with aggregation of Aβ peptides into neurotoxic high-molecular-weight oligomeric Aβ species in the brain. Because the two red wines are characterized by distinct polyphenolic component compositions, our evidence suggests that selective bioactive polyphenolic compounds may be responsible for Aβ-lowering and anti-Aβ aggregation activities. More studies will be necessary to identify and characterize specific polyphenolic components from red wines capable of exerting Aβ-lowering and/or anti-Aβ aggregation activity in the brain. Collectively, our evidence suggests the possibility of developing a "combination" of dietary polyphenolic compounds for AD prevention and/or therapy by modulating multiple Aβ-related mechanisms (Fig. 16.6).

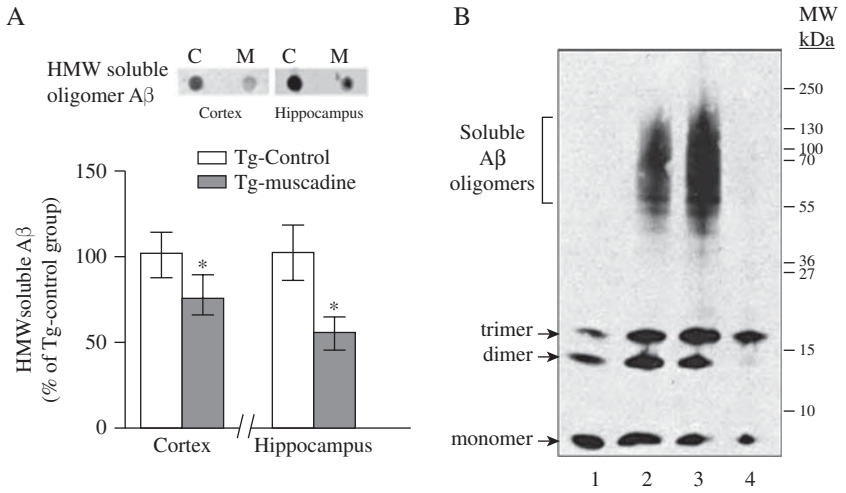

Fig. 16.5 Muscadine treatment reduces aggregation of Aβ peptides into neurotoxic high molecular weight aggregates. (A) Muscadine treatment significantly attenuated the accumulation soluble high molecular weight Aβ species in the brain of Tg2576 mice. The contents of soluble high molecular weight Aβ oligomeric species in the cerebral cortex and hippocampal formation of muscadine-treated or control, non-treated 14 month old Tg2576 mice were assessed by an immunological dot-blot (A) Immunodetection of high molecular weight Aβ oligomeric species in the cerebral using the oligomer-specific antibody A11 antibody (A, inset) Representative A11-immunoreactive dot-blot analysis of cortical and hippocampal formation brain specimens. Bar graphs represent means ± SEM., $n = 6-8$ per group; $^*P < 0.05$ vs. non-treated control Tg2576 group (2-tailed Student's t test). (B) Muscadine wine interferes with aggregation of synthetic Aβ1–42 peptides into high molecular weight oligomer Aβ species, *in vitro*. Synthetic Aβ 1–42 peptides were aggregated in the absence or in the presence of muscadine wine. Aβ species were then resolved by molecular size, transblotted onto a nitrocellulose membrane, followed by immunodetection of Aβ peptides using and the 6E10 antibody. Lane 1 represents non-aggregated Aβ1–42 peptides; Lane 2, aggregated Aβ1–42 peptides; Lane 3, Aβ 1–42 peptides aggregated in the presence of 1.2% ethanol (the same amount of ethanol presented in the aggregation assay in the presence of muscadine in Lane 4); Lane 4, Aβ1–42 peptides aggregated in the presence of 1 μl muscadine wine. In this assay, aggregation of Aβ without in the absence or presence of ethanol in lanes 2 and 3, respectively, lead to generation of high molecular weight Aβ species that migrated slowly in the assay. In contract, addition of muscadine completely eliminate the generation of high molecular weight Aβ aggregates.

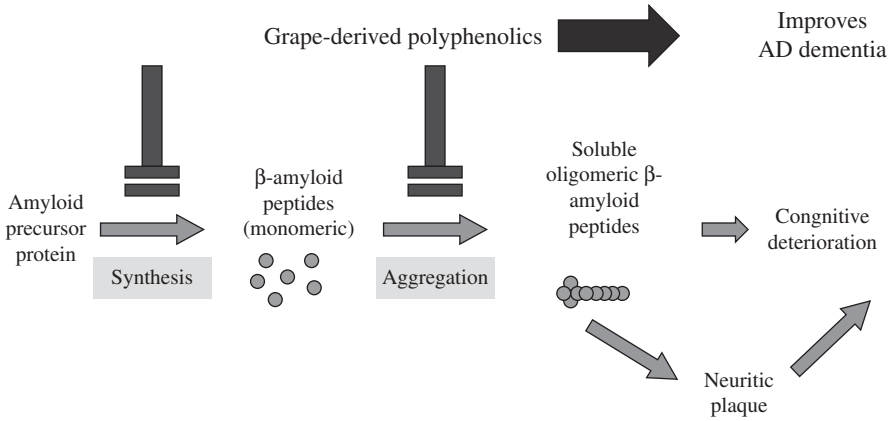

Fig. 16.6 Schematic of how grape-derived polyphenolics may benefit AD by modulating Aβ-mediated mechanisms. Aβ peptides are generated from amyloid precursor protein. Thereafter, monomeric Aβ peptides can be assembled into neurotoxic, soluble high-molecular-weight aggregates that may directly induce neuronal dysfunction that leads to cognitive deterioration. Further aggregation of soluble high-molecular-weight Aβ aggregates leads to deposition of insoluble Aβ aggregates as neurotic plaque in the brain that also induces neuronal dysfunction, in part, by promoting brain inflammatory responses. Bioactive polyphenols from grape-derived polyphenolics from red wine or other dietary products may improve interfere with Aβ-mediated pathological mechanisms by interfering with the generation (synthesis) of Aβ peptides from amyloid precursor protein and/or by interfering with assembly (aggregation) of Aβ peptides into neurotoxic, soluble high-molecular-weight species. This raises the possibility of improving AD dementia with a "combination" of dietary bioactive polyphenolic compounds.

In additional to the Cabernet Sauvignon and the muscadine wine, we recently demonstrated that other grape-derived products, namely, a grape seed polyphenolic extract [33] and a purple grape juice (Ho et al., unpublished observation), also exert bioactivity at the organism level and significantly interfere with the development of Aβ-related phenotypes in AD mouse models. In light of our observation that multiple dietary grape products with distinct polyphenolic component composition effectively protect against the onset and progression of AD phenotypes, we hypothesize that additional grape-derived products, including other red wines, might also provide beneficial disease-modifying activities in AD.

There is an urgent need for additional studies to identify specific bioactive polyphenolics from red wines or other grape-derived dietary products and to characterize the mechanisms of action of these bioactive polyphenolics. Information generated will provide the rational basis for developing selective bioactive dietary polyphenol(s) as lead compounds for clinical testing in AD. Moreover, this information will promote selection of food sources enriched in targeted bioactive polyphenols that ultimately could be incorporated as key components in the development of potential dietary guidelines for AD prevention and/or management.

REFERENCES

1. Alzheimer's Association. 2008 Alzheimer's disease facts and figures. *Alzheimer's Dementia* 2008; 4: 110–133.
2. Cummings JL. Treatment of Alzheimer's disease: current and future therapeutic approaches. *Rev Neurol Dis* 2004; 1(2): 60–69.
3. Sano M. Current concepts in the prevention of Alzheimer's disease. *CNS Spectr* 2003; 8(11): 846–853.
4. Religa D and Winbald B. Therapeutic strategies for Alzheimer's disease based on new molecular mechanisms. *Acta Neurobiol Exp* 2003; 63(4): 393–396.
5. Pangalos MN, Jacobsen SJ, and Reinhart PH. Disease modifying strategies for the treatment of Alzheimer's disease targeted at modulating levels of beta-amyloid peptide. *Biochem Soc Trans* 2005; 33(4): 553–558.
6. Duyckaerts C, Delatour B, and Potier MC. Classification and basic pathology of Alzheimer's disease. *Acta Neuropathol* 2009; 118(1): 5–36.
7. Davies P and Koppel J. Mechanism-based treatment for Alzheimer's disease. *Dialogues Clin Neurosci* 2009; 11(2): 159–169.
8. Kukar T, Murphy MP, Eriksen JL, Sagi SA, Weggen S, Smith TE, Ladd T, Khan MA, Kache R, Beard J, Dodson M, Merit S, Ozols VV, Anastasiadis PZ, Das P, Faug A, Koo EH, Golde TE. Diverse compounds mimic Alzheimer's disease-causing mutations by augmenting Aβ42 production *Nat Med* 2005; 11: 545–550.
9. Golde TE, Eckman CB, Younkin SG. Biochemical detection of Aβ isoforms: implications for pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Biochim Biophys Acta* 2000; 1502: 172–187.
10. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996; 2: 864–870.
11. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Hari gaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature* 1996; 383: 710–713.
12. Cleary JP, Walh DM, Hofmeister JJ, Shankar M, Kuskowski MA, Selkoe DJ, Ashe KH. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* 2005; 8(1): 79–84.
13. Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 2666; 440(7082): 352–357.
14. Jacobsen JS, Wu CC, Redwine JM, Comery TA, Arias R. Bowlby M, Martone R, Morrison JH, Pangalos MN, Reinhart PH, Bloom FE. Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2006; 103(13): 5161–5166.
15. Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J., Selkoe, D. J. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002; 416: 535–539.
16. Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM Betts V, Spooner ET, Jiang L, Anwyl R, Selkoe DJ, Rowan MJ. Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. *Nat Med* 2005; 11(5): 556–561.
17. Hardy J, Selkoe DJ The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002; 297(5580): 353–356.
18. Goedert M. Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Ann NY Acad Sci* 1996; 77: 121–31.
19. Sahara N, Murayama M, Lee B, Park JM, Lagalwar S, Binder LI, Takashima A. Active c-jun N-terminal kinase induces caspase cleavage of tau and additional phosphorylation by GSK-3beta is required for tau aggregation. *Eur J Neurosci* 2008; 27(11): 2897–906.
20. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash

A, Kuskowski M, Hyman B, Hutton M, Ashe KH. Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 2005; 309(5733): 476–481.

21. Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, Wszolek Z, Ashe K, Knight J, Dickson D, Andorfer C, Rosenberry TL, Lewis J, Hutton M, Janus C. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J Neurosci* 2007; 27(14): 3650–3652.
22. Sorrentino G, Bonavita V. Neurodegeneration and Alzheimer's disease: the lesson from tauopathies. *Neurol Sci* 2007; 28: 63–71.
23. Luchsinger JA, Mayeux R. Cardiovascular risk factors and Alzheimer's disease. *Curr Atheroscler Rep* 2004; 6: 261–6.
24. Mukamal KJ, Tolstrup JS, Friberg J, Jensen G, Grønbaek M. Alcohol consumption and risk of atrial fibrillation in men and women: the Copenhagen City Heart Study. *Circulation* 2005; 112: 1736–42.
25. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits. Aβ elevation, and amyloid plaques in transgenic mice. *Science* 1996; 274: 99–102.
26. Wang J, Ho L, Zhao Z, Seror I, Humala N, Dickstein DL, Thiyagarajan M, Percival SS, Talcott ST, Pasinetti GM. Moderate consumption of cabernet sauvignon attenuated beta-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J* 2006; 20: 2313–2320.
27. Percival SS, Sims CA. Wine modifies the effects of alcohol on immune cells of mice. *J Nutr* 2000; 130(5): 1091–4.
28. U.S. Food and Drug Administration. http://www.fda.gov/cber/gdlns/dose.htm).
29. Ho L, Chen LH, Wang J, Zhao W, Talcott ST, Ono K, Teplow D, Humala N, Cheng A, Percival SS, Ferruzzi M, Janle E, Dickstein DL, Pasinetti GM. Heterogeneity in red wine polyphenolic contents differentially influences Alzheimer's disease-type neuropathology and cognitive deterioration. *J Alz Dis* 2008; 16: 59–72.
30. Urquiaga I, Leighton F. Plant polyphenol antioxidants and oxidative stress. *Biol Res* 2000; 33: 55–64.
31. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases, *Crit Rev Food Sci Nutr* 2005; 45: 287–306.
32. Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. *Eur J Pharmacol* 2006; 545: 51–64.
33. Wang J, Ho L, Zhao W, Ono K, Rosesweig C, Chen L, Humala N, Teplow DB, Pasinetti GM. Grape-derived polyphenolics prevent Abeta oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease. *J Neurosci* 2008; 28(25): 6388–6392.