Novel Role of Red Wine-Derived Polyphenols in the Prevention of Alzheimer’s Disease Dementia and Brain Pathology: Experimental Approaches and Clinical Implications

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Abstract

Recent studies suggest that by the middle of this century, as many as 14 million Americans will have Alzheimer’s disease, creating an enormous strain on families, the health care system and the federal budget. There are still widespread misconceptions about issues related to the prevention and/or treatment of disease pathogenesis, leaving us unprepared to deal with the disease. To address these challenges, several therapeutic approaches are currently under investigation, mainly in an attempt to delay disease onset and eventually slow down its progression. Recent epidemiological evidence has implicated the protective role of dietary polyphenols from grape products against Alzheimer’s disease. Furthermore, experimental evidence supports the hypothesis that certain bioactive grape-derived polyphenols may protect against Alzheimer’s disease-type cognitive deterioration, in part by interfering with the generation and assembly of β-amyloid peptides into neurotoxic oligomeric aggregated species. Brain-targeting polyphenols have been shown to significantly reduce the generation of β-amyloid peptides in primary cortico-hippocampal neuron cultures, and preliminary results indicate that they may influence neuronal synaptic plasticity. Recent evidence has also implicated the role of certain grape-derived preparations in beneficially modulating tau neuropathology, including reducing tau aggregation. Studies suggest that dietary polyphenolics may benefit Alzheimer’s disease by modulating multiple disease-modifying modalities, both β-amyloid-dependent and independent mechanisms, and provide impetus for the development of polyphenolic compounds for Alzheimer’s disease prevention and/or therapy.

Introduction

Alzheimer’s 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, deregarded perception of space, inability to do calculations, depression, delusions, and other cognitive deficits. AD is ultimately fatal within 5 to 10 years of its onset. 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. The estimated cost of dealing with AD over the next 40 years is twenty trillion dollars. Up to 14 million people in the United States are projected to be affected by AD by the middle of this century [1]. To date, there is no cure for AD. The few agents that are approved by the FDA for the treatment of AD have only modest efficacy in terms of modifying clinical symptoms, and none appear to affect disease progression or prevention [2]. Scientists are continually exploring novel avenues for preventing or treating this condition.

Alzheimer’s Disease Neuropathological Features: Implications for Therapeutic Developments

While the classification and diagnosis of AD is based on the cognitive behavior of an individual, the roots of the disease lie in the neurological pathology of its victims [3]. The two defining neuropathological features of AD are abnormal aggregation and deposition of certain toxic peptide fragments known as β-amyloid (Aβ) peptides or tau protein in the brain as, respectively, extracellular neuritic plaques (NP) and intracellular neurofibrillary tangles (NFT) [4]. Aβ peptides are derived from the ubiquitous amyloid precursor protein
(APP) through amyloidogenic processing by β- and γ-secretases, rather than through non-amyloidogenic cleavage by α-secretase. In humans, genetic mutations leading to Aβ neuropathology in at least one of three genes, namely APP, presenilin 1 and presenilin 2, are causally linked to early onset AD cases and are associated with AD dementia [5,6]. In experimental animal models, these same mutations also accelerate Aβ deposition and cognitive deterioration [7]. Based on this, major efforts are focused on developing pharmacological strategies that delay the initiation and/or slow the progression of Aβ-mediated neuropathological responses. Recent evidence from experimental AD mouse models indicates that the 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 [8–12].

Despite strong genetic data arguing that Aβ neuropathology is sufficient to cause AD [13], progressive cognitive decline and neuron and synapse loss in AD are best correlated with tau neuropathology [14]. In the AD brain, tau proteins, particularly hyperphosphorylated tau, are found aggregated into progressively larger polymeric species that are ultimately deposited as insoluble NFTs [15]. NFTs themselves are not necessarily the tau species inducing neurotoxicity [16,17]. 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 alterations of microtubule-mediated processes, including abnormalities in axon transport, among others [18].

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 their depositions, may be a more productive approach to AD therapy. As discussed in more detail below, we recently demonstrated for the first time that dietary supplementation with red wines equivalent to moderate wine consumption in humans effectively attenuates the development of Aβ-mediated neuropathology and cognitive dysfunction in a mouse model of AD. Moreover, our evidence demonstrates that the grape-derived polyphenolics commonly found in red wines may also modulate tau-mediated neuropathology responses (© Fig 1).

Fig. 1 Grape-derived polyphenols from dietary grape products protect against AD-type cognitive dysfunction by attenuating Aβ-mediated neuropathological mechanisms. A. Schematic diagram illustrating the generation of monomeric Aβ peptides from the amyloid precursor protein and assembly of monomeric Aβ peptides into soluble high-molecular weight Aβ aggregates, which are key contributory factors of AD dementia. Abbreviation: APP, amyloid precursor protein. B. PICUP assay exploring in vitro protein–protein interaction of synthetic Aβ1–42 peptide in the absence or presence of a total polyphenolic extract from a red Muscadine wine. Lane 1: unaggregated monomeric Aβ1–42; Lane 2: aggregation of Aβ1–42 into dimeric, trimeric and oligomers following incubation in 37 °C; Lane 3: addition of a Muscadine polyphenol extract prevented Aβ1–42 aggregation. C. Generation of Aβ1–40 (left panel) and Aβ1–42 (right panel) peptides from primary cortico-hippocampal neurons generated from Tg2576 AD mice. * T-test, p < 0.05 vs. control, vehicle-treated neuron cultures. D. Dietary supplementation with a red Muscadine wine protected against the development of cognitive deterioration in Tg2576 AD mice. Dietary supplementation with the red wine was initiated at 4 months of age prior to development of cognitive dysfunction in Tg2576 mice. The dose provided daily was equivalent to moderate wine consumption in humans. Animals’ behavioral cognitive function was assessed using the Morris water maze paradigm at 14 months of age when Tg2576 mice are typically characterized by severe cognitive dysfunction. Results showed vehicle (water)-treated mice having difficulty in learning how to execute the task (locating the escape platform) whereas wine-treated mice showed significant improvements (reduced lag-time) in executing the task.
Red Wine-Derived Polyphenols and Alzheimer's Disease

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 and 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 [19]. Little is known about the beneficial role of red wine in AD dementia onset. The neuroprotective efficacy of red wine is typically attributed to the antioxidant activities of polyphenols in the wine. To explore how red wines might benefit AD, we tested whether dietary supplementation with red wines may beneficially modulate AD phenotypes in the Tg2576 AD mouse model [20]. Recapitulating select features of AD, Tg2576 mice are characterized by progressive development of Aβ neuropathology and cognitive decline with increasing age.

Potential Benefits of Moderate Consumption of Red Wine and Other Dietary Grape Polyphenol Products in Alzheimer's Disease

Polyphenols are members of a very large family of plant-derived compounds containing one or more phenolic group. Thousands of polyphenols have been identified to date, including bioflavonooids (anthocyanins, flavanols, flavonones, iso-favones and proanthocyanins), coumestans, lignans and stilbenoids. To illustrate, Fig. 2 presents examples of polyphenol structures by class. The content and composition of polyphenols among dietary grape products (and other plant products) vary tremendously depending on the type/source of plants used in the product, conditions under which these plants were grown, harvested and processed into specific dietary products, and how these products are stored and used [21–29].

Another key consideration for developing dietary polyphenols as novel dietary/supplemental approaches for preventing and/or treating AD dementia and brain pathology is that almost all of the bioactive polyphenols found in the brain are not directly available through our food supply, but are derived from Phase II xenobiotic metabolism of precursor, dietary polyphenols [30]. The bioavailability of polyphenols is a complex process known to be influenced by several factors including the food composition, dietary patterns, the dose and dose regime as well as the nutritional and pathophysiological status of an individual [30–38]. Many publications have discussed the potential role of dietary grape polyphenols in treating AD. Unfortunately, almost all of these are based on in vitro studies using the aglycone form of polyphenols, which are generally commercially available but are typically not physiologically relevant. Studies from our group [34] and from others [39] have shown that almost all dietary grape-derived polyphenols in circulating blood and, more importantly, in the brain [34], which is the key target tissue for AD interventions, are not in the aglycone form but rather in metabolically derivatized forms. Thus, follow-up bioactivity studies ex-
ploring the potential beneficial role of grape-derived polyphenols in AD should be conducted using specific metabolites that are accumulated in the brain. For example, we have recently identified selected epicatechin glucuronide derivatives from a grape seed polyphenolic extract that are capable of penetrating the brain [34] and potentially attenuating AD, in part by promoting neuroplasticity processes [40]. The structure of this bioactive epicatechin glucuronide as well as the structure of epicatechin, its parent compound, is illustrated in Fig. 3.

Consistent with epidemiological evidence implicating the protective role of dietary polyphenols from grape products against AD [19], our preclinical studies have demonstrated that dietary supplementation with a grape seed polyphenol extract [41], or moderate consumption of red wines [42,43] containing high contents of grape polyphenols, is effective in attenuating the onset and progression of Aβ-mediated AD-type neuropathology and cognitive deterioration in transgenic mouse models of AD. As schematically shown in Fig. 1A, Aβ peptides are derived from the ubiquitous protein APP through amyloidogenic processing by β- and γ-secretases rather than through non-amyloidogenic cleavage by α-secretase. Monomeric Aβ peptides (e.g., Aβ1–40 or Aβ1–42 peptides) tend to assemble into soluble, high-molecular weight neurotoxic Aβ aggregates that are key contributory factors to AD dementia. Our evidence has demonstrated that polyphenolic components from grape products may protect against AD dementia, in part by reducing Aβ-mediated neurotoxic mechanisms. Mechanistically, we found that grape-derived polyphenols may modulate Aβ toxicity by either reducing the generation of Aβ peptides from the amyloid precursor protein and/or by interfering with the assembly of Aβ peptides into high-molecular weight neurotoxic Aβ aggregated species (Fig. 1A).

For example, using a PICUP assay [44] we demonstrated that polyphenolic components from a red Muscadine wine, made from Vitis rotundifolia (Vitaceae), significantly interfere with Aβ protein-to-protein interactions critical for the initial assembly of monomeric Aβ peptides into increasingly large aggregated species (Fig. 1B). In another example, we showed that treatment with a polyphenolic extract from another red wine (Cabernet Sauvignon) significantly reduced the generation of Aβ1–40 (Fig. 1C, left panel) and Aβ1–42 (Fig. 1C, right panel) in a dose-dependent manner in primary cortico-hippocampal neuron cultures from Tg2576 mice in the presence of vehicle (control) or presence of total polyphenols from the wine or in the presence of the anthocyanin polyphenol subfraction.

Collectively, we have studied two unrelated red wines, a Cabernet Sauvignon and a Muscadine wine, for their efficacy in modulating AD phenotypes in the Tg2576 transgenic AD mouse model [42,43] and found that both wines are effective in attenuating Aβ-mediated neuropathology and cognitive dysfunction. Moreover, our evidence suggests that polyphenols from the two wines benefit Aβ-mediated phenotypes through different mechanisms – polyphenols from Cabernet Sauvignon wine are effective in reducing the generation of Aβ peptides [42] while polyphenols from the Muscadine wine attenuate Aβ aggregation, but have no impact on Aβ generation [43]. The two wines are characterized by a distinct composition of polyphenolic compounds. Thus, our studies revealed that distinct and varied polyphenolic compounds from red wines and other dietary sources may be bioavailable at the organism level and may beneficially modulate AD phenotypes through multiple Aβ-related mechanisms.

Ongoing studies aimed at identifying which of the polyphenols in the Cabernet Sauvignon and in the Muscadine wine might be responsible for Aβ-lowering and anti-Aβ aggregation activities, respectively. Outcomes will provide critical information for the...
identification of specific wines and other dietary products that might prove to be effective in AD prevention and therapy. Accumu-
larating evidence suggests that resveratrol, a naturally occur-
ing polyphenolic compound that is associated with beneficial ef-
effects on aging, metabolic disorders, inflammation and cancer in
animal models [45] and that is found in varying concentrations in
red wine and many food products, may enhance Aβ clearance by
promoting intracellular proteosome activity in vitro [46].
However, the role of resveratrol in our study on Cabernet Sau-
vignon treatment in Tg2576 mice [42] is not clear since the Ca-
ernet Sauvignon used had only 0.2 mg/L resveratrol, a concentra-
tion 10-fold lower than the minimal effective concentration
shown to promote Aβ clearance in vitro [46]. To gather insights
into the specific dietary grape-derived polyphenol(s) that might
be relevant to AD, we subfractionated polyphenols from bioactive
grape products (e.g., red wine and grape seed extract) containing
increasingly less complicated polyphenol compositions and con-
ducted in vitro and in vivo studies for exploiting potential benefi-
cial AD-modifying activities. We fractionated total polyphenols
from the red Cabernet Sauvignon wine and found that the Aβ-
lowering activity of Cabernet Sauvignon can be attributed to its
anthocyanin polyphenolic components (Fig. 4).

Grape Polyphenols Beneficially Modulate
Tau-Mediated Neuropathological Responses

In recent studies, we found that grape seed polyphenolic extracts
(GSPE) are capable of interfering with tau-mediated toxicity by
interfering with the abnormal aggregation of tau [47–49]. We used
both the TMHT [48] and JNPL3 [49] mutant tau mouse models of
AD, which overexpress the human TAU441 gene bearing missense
mutations V337M and R406W and express human tau protein
containing the P301L mutation, to test the efficacy of GSPE in
interfering with dementia resulting from abnormal tau functions.
We found that dietary supplementation with GSPE in these tau
mouse models effectively reduced the severity of abnormal tau
aggregation and neuropathology in the brain [47–49]. While on-
going studies are evaluating the efficacy of grape-derived prepa-
a tions in preserving cognitive function in these tau mouse models,
our data suggest that GSPE might also protect against AD and
other dementias in which tau neuropathology is a major contribu-
tory factor in the development of cognitive impairment.

Dietary Grape-Derived Bioactive Polyphenolic
Components in Alzheimer’s Disease Dementia

Evidence from our studies strongly supports the hypothesis that
moderate red wine consumption might provide preventive and/
or therapeutic value in AD. Our experimental evidence suggests
that in addition to providing antioxidant activities, polyphenols
from red wines and other grape products may also benefit AD
by directly modulating Aβ mechanisms as well as tau-related pathological
mechanisms in the brain (Fig. 5). Based on our observation that
multiple dietary grape products with distinct polyphenolic com-
ponent compositions effectively protect against the onset and
progression of AD phenotypes, we hypothesize that additional
dietary products containing similar polyphenol forms as grapes,
including other red wines, cocoa, tea, apple and berries, might also
provide beneficial disease-modifying activities in AD.

There is an urgent need for additional studies in order to identify
specific bioactive polyphenolics and corresponding polyphenolic
metabolic derivatives from red wines or other grape-derived die-
tary products that are physiologically bioavailable in target tis-


e s and in order to characterize the mechanisms of action of
these bioactive polyphenols. Such information will provide the
rational basis for developing selective bioactive dietary polypheno-
l(s) as lead compounds for clinical testing in AD. Moreover, this
information will facilitate the selection of food sources enriched
in targeted bioactive polyphenols that ultimately could be incor-
porated as key components in the development of dietary guide-
lines for AD prevention and/or management.

Acknowledgement

This material is the result of work supported in part with re-
ources and the use of facilities at the James J. Peters Veterans Af-
fairs Medical Center, Bronx, NY. In addition, Dr. Pasinetti holds a
Career Scientist Award in the Research and Development unit
and is the Director of the Basic and Biomedical Research and
Training Program, GRECC, James J. Peters Veterans Affairs Medical
Center. We also acknowledge that the contents of this manuscript
do not represent the views of the U.S. Department of Veterans Af-
fairs or the United States Government.

Conflict of Interest

There are no relevant conflicts of interest to be reported.
implications for Huntington’s disease and other neurodegenerative disorders. Exp Neurol 2011; 232: 1–6


Please note: This article was changed according to the following erratum on 5 Dec. 2012: Fig. 3 has been corrected, Panel B is now an epicatechin, as it is stated in the legend.

Please note: This article was changed according to the following corrigendum on 11 March, 2015: Affiliation #2 and Acknowledgements were added.