16 Actions of Bioactive Phytochemicals in Cell Function and Alzheimer's Disease Pathology

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16.1 INTRODUCTION

Phytochemicals are broadly defined as compounds produced by plants and include the phenols, terpenes, and organosulfurs. Many of these chemicals have pigment, odorant, and/or irritant properties that may help the plant's biochemical defenses against metabolic byproducts [e.g., reactive oxygen species (ROS), protein misfolding] and environmental insults (e.g., pathogens, insects, ultraviolet radiation). Consumption of plants or their phytochemicals can confer some of these beneficial

properties and modulate a number of biological pathways, including inflammatory, enzymatic, neurotransmitter systems, apoptosis, and neurogenesis.[1]

Several dietary, medicinal, and isolated phytochemicals have been shown to modulate various aspects of Alzheimer's disease (AD), the most common neurodegenerative disorder of aging and cause of dementia. This chapter presents an overview of AD etiology followed by a survey of phytochemicals that may affect its neurophysiological and/or neuropathological sequelae.

16.2   AMYLOID β–RELATED NEUROTOXICITY IN ALZHEIMER'S DISEASE BRAIN

AD currently affects approximately 10% of the population over the age of 65 years and 50% of the population over the age of 85 years, but the incidence is expected to rise as the population ages. The first, and most prominent, symptom is loss of memory for recent events, followed by a progressive decline in general cognition (e.g., memory, language, executive functions) and motor abilities. Neuropathological evidence of AD includes the accumulation of protein deposits ("plaques") between and surrounding the brain's neurons and neurofibrillary tangles inside the neurons. In addition to the characteristic plaques and tangles found during postmortem examination of AD brains, evidence of mitochondrial dysfunction, inflammation, astrogliosis, microglial activation, synaptic loss, neuronal damage, and apoptosis is also observed.

The plaques are primarily composed of amyloid-β (Aβ) peptides[2] that are enzymatically snipped from the much larger amyloid precursor protein (APP) by the γ-secretases and β-secretases. Other proteins (e.g., apolipoproteins) and non-proteins (e.g., ROS, hemes, metals) are also found within the plaques, which generally begin to accumulate in the medial temporal lobes years before the first behavioral symptoms emerge. Brain structures in this region include the entorhinal cortex and hippocampal formation, which play important roles in learning and memory. With age, the plaques gradually spread throughout the cortical and subcortical areas.[3]

Neurons within the plaques often have abnormally twisted axons and dendrites resulting from the neurofibrillary tangles. These tangled cytoskeletal microtubules destabilize the structure of long neuronal processes and disrupt intracellular transport mechanisms. The tau protein normally plays a role in stabilizing cytoskeletal microtubules within neurons, but the Aβ-related build-up of abnormal tau in the cell destabilizes the microtubules, which eventually leads to the cell's demise. Neurotransmitter systems, especially acetylcholine (ACh) and glutamate, become dysfunctional as the neurons that produce these chemicals atrophy and die.[4] Thus, the age-related accumulation of Aβ in the brain is associated with neuronal dysfunction that ultimately leads to the behavioral symptoms associated with AD.

Aβ's putative toxicity probably involves several inter-related mechanisms. Aβ causes intracellular tau disruption and neuronal death in hippocampal cell cultures.[5] It also induces hypersensitivity to excitotoxic damage by glutamate[6] and oxidative stress, which occurs when the build-up of potentially harmful ROS cannot be effectively controlled. For example, Aβ-heme peroxidase complexes form within plaques that can cause inflammation, release of ROS, and damage to muscarinic ACh receptors, and these effects are prevented by antioxidant compounds.[7–11] The plaques eventually disrupt synaptic structures, and synaptic loss within plaques provides a better predictor of cognitive dysfunction than the amount of plaque deposition.[12–15] This observation suggests that certain individuals may be more or less susceptible to the effects of Aβ deposition. The deleterious effect of Aβ on synaptic function is also demonstrated by experiments that assess long-term potentiation (LTP), which is a neuronal model of learning and memory.[16–20] Finally, numerous studies have demonstrated that the age-related accumulation of brain Aβ is associated with progressive cognitive impairments in transgenic mouse models of AD. In vivo imaging shows that Aβ plaques can form quite rapidly (over the course of 24 hours) in the brains of these mice and that signs of neurodegeneration around the plaques are seen shortly thereafter.[21]

16.2.1 Accelerating Amyloid-β Deposition Increases the Risk for Alzheimer's Disease

Thus, the gradual accumulation of potentially toxic Aβ in the brain is associated with progressive oxidative stress and various downstream events that cause structural damage to neurons. This process eventually leads to functional deficits, cognitive and behavioral impairments, and death. Pathophysiological conditions that accelerate Aβ accumulation in the brain increase the risk of developing AD. For example, Down syndrome is characterized by the overproduction of APP in the brain, which leads to elevated Aβ production and deposition and dementia by around 50 years of age.[22,23]

Furthermore, several inheritable mutations in the genes for APP or constituents of γ-secretase lead to elevated APP and Aβ production. These genes are associated with early onset of AD,[24,25] and their identification has spawned the development of several lines of APP transgenic mice. These transgenic mouse models of AD generally express relatively high brain levels of human APP and develop age-related neuropathology and cognitive deficits coincident with the accumulation of Aβ aggregates and deposits.[26–34]

Mounting evidence also suggests that brain inflammation and oxidative stress resulting from traumatic brain injury, stroke, or even chronic low-level insult (e.g., hypoxia due to breathing problems[35] or high-cholesterol diet[36–42]) can induce accumulation of APP and Aβ in the brain and elevate the risk of developing AD. Indeed, oxidative stress, a common component of all types of brain injury, is sufficient to induce Aβ accumulation,[43] initiating a vicious circle of progressive oxidative load in the brain.

16.2.2 Inhibiting Amyloid-β Deposition Decreases the Risk for Alzheimer's Disease

Further evidence for the support of Aβ accumulation as a causative factor in AD comes from experiments using APP transgenic mouse models of AD. Many studies have described systemic treatments that lower levels of Aβ in the brains of these mice (e.g., monoclonal anti-Aβ antibodies, dietary manipulations) and prevent or even reverse neuropathology and behavioral deficits.[16,34,44–51] Interestingly, reducing the brain's oxidative load can improve cognitive function in APP transgenic mice without reducing Aβ levels,[52,53] suggesting that Aβ contributes to oxidative overload in the brain that gradually impacts the function of cortical circuits involved in learning and memory.

16.2.3 Summary: Alzheimer's Disease Etiology

Thus, AD is associated with the abnormal build-up of Aβ in the brain, which induces events that lead to even greater Aβ accumulation. The idea that this process creates a vicious circle of neurodegenerative decline is known as the amyloid cascade hypothesis of AD.[54–56] Current pharmacological approaches for treating AD include stabilizing glutamatergic activity by blocking NMDA glutamate channels (e.g., memantine) and inhibiting acetylcholinesterase (AChE), an enzyme that breaks down ACh and has been shown to promote the aggregation of Aβ (e.g., galantamine, tacrine, donepezil, rivastigmine). However, mounting epidemiological and experimental evidence suggests that diet and other sources of bioactive phytochemicals can also decrease the risk of developing AD.[57–59]

16.3 BIOACTIVE PHYTOCHEMICALS AND ALZHEIMER'S DISEASE

There is a growing body of literature demonstrating that phytochemical compounds can affect various aspects of AD via antioxidant and other pathways. Antioxidant compounds found in plants include minerals (e.g., selenium, zinc), vitamins (e.g., ascorbic acid, α-tocopherol), and other organic compounds (e.g., phenols, terpenes, organosulfurs), all of which can neutralize ROS by giving up electrons to oxygen ions, peroxides, and free radicals. However, evidence suggests that these compounds may also work by a variety of other mechanisms. Indeed, many currently available pharmaceuticals have roots in traditional herbal medicines. For example, galantamine is a pharmaceutical

AChE inhibitor derived from daffodils, and the anti-inflammatory blood thinner aspirin is derived from salicylic acid, a polyphenol found in willow bark. Both of these drugs are currently used to control AD. The following subsection provides a survey of the epidemiological and experimental evidence for the effects of other plants and isolated phytochemicals on AD processes.

16.3.1 EPIDEMIOLOGICAL EVIDENCE OF PHYTOCHEMICAL EFFECTS ON ALZHEIMER'S DISEASE

Regular consumption of a variety of fruits and vegetables may decrease the risk for or slow the progression of AD. For example, a large study of elderly Japanese Americans found that drinking fruit and vegetable juices was associated with a lower risk for AD,[60] and a group of studies on elderly French subjects showed that daily consumption of phenol-rich fruits and vegetables significantly decreased the risk of developing AD with age.[61–63]

Epidemiological evidence that isolated dietary phytochemicals can affect AD remains elusive. For example, one study reported that dietary tocopherols (isoforms of vitamin E), vitamin C, β-carotene, and tea were not correlated with the risk of developing AD.[60] However, another study found a lower incidence of AD with high intake of food-based α-tocopherols and γ-tocopherols[64] and a large study from the Netherlands associated high intake of dietary (but not supplemental) vitamins E and C with a lower risk for AD. In that study, the effects were stronger for tobacco smokers, who also benefited from dietary β-carotene and polyphenols.[65] Finally, a recent study found that supplemental vitamin E and/or C did not reduce the risk of developing AD over 5 years of follow-up.[66] Thus, the evidence suggests that acquiring phytovitamins through a varied diet may provide more protection against AD than the use of supplemental vitamins.

Some epidemiological evidence suggests that dietary phospholipids, such as the omega-3 fatty acids [e.g., α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid (DHA)], which are found in flax, nuts, algae, and the oil of fish that eat algae, may protect against developing AD.[62,67–69] Other sources of bioactive phytochemicals include colorful, flavorful, and aromatic spices, which can contain high concentrations of phenols, terpenes, and organosulfurs. For example, a study of elderly Asians showed that those whose diets included curry performed significantly better on neuropsychological tests of cognitive performance.[70] Curry is a mixture of spices including the bright yellow turmeric and curcumin, its associated polyphenol.

Additionally, moderate wine consumption by the elderly is associated with reduced risk for AD,[71] but this effect could be due to grape polyphenols, such as resveratrol and ethanol (which is a derivative of phytosugars). Interestingly, smoking tobacco may also protect against Aβ deposition. In a postmortem examination of AD brains, there were significantly lower levels of Aβ in smokers' entorhinal cortex, which plays a role in learning and memory.[72] However, another study showed no protective effect of smoking on the risk of developing symptoms of dementia.[73]

Thus, epidemiological evidence suggests that eating a wide variety of fruits and vegetables may provide several bioactive phytochemical compounds that may work collectively to lower the risk for AD. Relatively few experimental clinical trials have been published assessing the effects of plants or phytochemical compounds on AD in humans. However, a number of experimental preclinical studies using APP transgenic and/or in vitro models have provided evidence that various aspects of AD can be manipulated by plants and their phytochemicals. The following subsection surveys the experimental literature describing the potential effects of bioactive phytochemicals on AD.

16.3.2 EXPERIMENTAL EVIDENCE OF PHYTOCHEMICAL EFFECTS ON ALZHEIMER'S DISEASE

16.3.2.1 Ginkgo Biloba and Its Phytochemicals

The leaves of the ginkgo biloba tree have been used to improve cognition for centuries. In part because of this history, ginkgo biloba is one of the most studied plants in terms of its effects on AD. Its biologically active compounds include polyphenols (e.g., kaempferol, quercetin) and terpenes

(ginkgolides and bilobalides). Ginkgo biloba is often studied experimentally using a commercially available extract (EGb761) that is standardized to contain 24% polyphenols and 6% terpenes.

Several clinical trials of EGb761 have suggested that daily treatment for 3–6 months may provide mild cognitive benefit over placebo in elderly demented patients.[74–78] Several other studies have compared the clinical effects of the extract with clinically used AChE inhibitors. One study found that ginkgo biloba extract was as effective as the pharmaceutical AChE inhibitor donepezil.[78] A meta-analysis of placebo-controlled studies of demented patients that continued for at least 6 months determined that the reported efficacy of EGb761 was similar to that of four pharmaceutical AChE inhibitors (tacrine, donepezil, rivastigmine, and metrifonate). Tacrine was the only one of the five treatments that was associated with a high dropout rate due to negative side effects.[79] Another study demonstrated that the extract produced electroencephalogram changes in elderly demented patients similar to those produced by tacrine.[80]

However, other studies have found no significant cognitive effect of ginkgo biloba in elderly demented[81–83] or non-demented[84] subjects. The difficulty of demonstrating a strong clinical effect in humans is not surprising, considering that the currently available pharmaceutical therapies provide only modest and short-term clinical benefits. However, a number of animal and in vitro studies have provided evidence that the phenols and terpenes in ginkgo biloba can modulate aspects of AD pathology.

For example, ginkgo biloba extract was shown to eliminate cognitive deficits in APP transgenic mice without reducing levels of brain Aβ.[85] Beneficial effects on synaptic function may underlie ginkgo biloba's putative cognitive effects, as an extract enriched with terpene ginkgolides and bilobalides (70% vs. 6% in EGb761) prevented Aβ-induced neurotoxicity and inhibition of LTP (a neuronal model of learning and memory) in rodent hippocampal neurons. Ginkgolide J, the major terpenoid component of the extract, provided similar protection as the whole extract.[86]

EGb761 has antioxidant properties and can modulate a variety of cellular signaling pathways.[87–91] For example, the extract protected cultured rat hippocampal neurons from Aβ- and protein kinase C-induced neurotoxicity and oxidative stress via effects on nitric oxide. The isolated polyphenolic components of the extract produced a similar but less potent effect, whereas the isolated terpene component was ineffective at preventing neurotoxicity.[88,89] This pattern of results suggests that the combined phytochemicals have a synergistic antioxidant effect. Similarly, ginkgo biloba extract significantly reduced ROS-induced apoptosis in mice[90] and prevented oxidative stress and reversed neuritic dystrophy associated with Aβ plaques in transgenic APP mice.[92] EGb761 and its polyphenolic components also attenuated the build-up of ROS in cultured APP-producing neurons and transgenic *Caenorhabditis elegans* nematodes that express human Aβ.[93,94]

Besides its antioxidant properties, ginkgo biloba extract inhibits Aβ aggregation, caspase-3 activity, and apoptosis in cultured APP transgenic neurons[95] and increases α-secretase processing of APP in rat hippocampal slices. Cleavage of APP by α-secretase (rather than γ- and β-secretases) not only prevents the production of Aβ but also yields the potentially neuroprotective peptide sAPPα.[96] Cholesterol may provide another pathway for the effects of ginkgo biloba on AD, as treating aged rats with EGb761 lowered circulating levels of cholesterol and brain levels of APP and Aβ. In vitro experiments suggested that the inhibition of Aβ production was associated with enhanced clearance of intracellular cholesterol.[97]

Thus, there is some evidence that ginkgo biloba may provide benefits to elderly demented individuals similar to those of the current clinically approved pharmaceutical AChE inhibitors. Because ginkgo biloba is generally not a dietary plant and because phytochemical concentrations in the whole leaf are probably too low to provide acute benefits at usable doses, any significant beneficial effects of ginkgo biloba will likely come from concentrated extracts and pharmaceutical derivatives rather than from incorporation of whole ginkgo biloba leaf into the diet.

16.3.2.2 Pomegranate and Its Phytochemicals

Pomegranates have been used as food and medicine for centuries. Chemical assays show that they contain very high concentrations of bioactive polyphenols, including phenolic acid tannins such as

the punicalagins that hydrolyze (break down in water) to smaller phenols such as ellagic acid and gallic acid.[85,98–100] Several human and animal studies of pomegranate juice have demonstrated a variety of biological effects, including antioxidation.[101–107] Additionally, one report suggests that it inhibits the activation of oxidation-sensitive genes in response to cellular stress, and another demonstrated modulation of endothelial nitric oxide synthase expression. In addition to the antioxidant properties of the whole fruit juice, other studies have reported anti-apoptotic, anticancer, antibacterial, and cardiovascular effects.[101–109]

Animal experiments in which mice were fed diluted pomegranate juice through their water demonstrate the neuroprotective effects of these phytochemicals. In these studies, dietary supplementation with pomegranate juice provided the mice with an average daily dose of polyphenols roughly equivalent in human terms to 250–500 ml of full-strength pomegranate juice. The juice improved maze performance and significantly reduced levels of Aβ in the hippocampus of APP transgenic mice.[16] Additionally, when fed to pregnant mice, it protected their neonatal offspring from hypoxic-ischemic brain injury.[110]

Ellagic acid extracted from pomegranate husks was shown to inhibit β-secretase activity,[111] suggesting that it may prevent the formation of Aβ from APP in vivo. Additionally, ellagic acid suppresses the pro-inflammatory nuclear transcription factor-κB (NF-κB) activation pathway[112] and modulates other cell-signaling pathways.[113] However, in vitro experiments assessing its antioxidant and anti-apoptotic properties suggest that the whole juice may provide synergistic benefit over isolated phytochemical components.[106] Interestingly, one study suggests that the sugar fraction of pomegranate juice, which consists of conjugated sucroses, fructoses, and glucoses, may have significant antioxidant properties independent from those of the phenolic compounds.[104] Thus, dietary pomegranate juice may provide significant behavioral and neuropathological protection against AD and a host of other age-related diseases.

16.3.2.3 Turmeric and Curcumin

A diet that includes high amounts of the spice mixture curry is associated with improved cognitive performance in the elderly.[70] Curcumin is a phenolic acid and yellow pigment found in the curry spice turmeric (a member of the ginger family), which has some interesting AD-related properties. Dietary curcumin prevented learning deficits, oxidative stress, synaptic damage, and cortical microgliosis (while increasing plaque-associated microgliosis) in a rat model of AD that uses intracerebroventricular infusion of Aβ.[114] Curcumin also lowered levels of oxidized proteins and plaque burden in APP transgenic mice.[115–117] Its antioxidant properties were further demonstrated by its reduction of heme-Aβ peroxidase damage to muscarinic ACh receptors.[7]

In addition to its effect on Aβ plaque burden in vivo, curcumin also prevented and reversed Aβ aggregation in vitro. Interestingly, dietary or injected curcumin binds to amyloid fibrils in the brain similarly to histological stains such as thioflavin S and Congo red and can be viewed under fluorescent light to visualize Aβ plaques. Other mechanisms by which curcumin could act on AD processes include inhibition of pro-inflammatory NF-κB activity[112] and modulation of other cell-signaling pathways.[113]

16.3.2.4 Garlic and Its Phytochemicals

Garlic has a number of aromatic sulfur-containing phytochemicals, including *S*-allyl cysteine (SAC) and di-allyl-disulfide, known collectively as organosulfurs. Adding aged garlic extract, SAC, or di-allyl-disulfide to the diets of APP transgenic mice prevented cognitive deficits and lowered brain levels of inflammation, Aβ plaques, and abnormal tau. The whole extract was more effective than the isolated components, suggesting a synergistic effect.[118,119] Isolated SAC also inhibited and reversed Aβ aggregation in vitro by binding to Aβ and altering its conformation,[120] suggesting another mechanism by which garlic may reduce Aβ deposition. Finally, garlic extract and its organosulfur components have inhibited Aβ-induced generation of ROS, pro-inflammatory NF-κB, caspase-3 activation, DNA fragmentation, and apoptosis.[112,121]

These observations suggest that garlic-based organosulfurs may act on AD processes by several pathways.

16.3.2.5   Omega-3 Fatty Acids

As mentioned in the epidemiology section, dietary phospholipids such as the omega-3 fatty acids (found mainly in flax, nuts, algae, and fish oil) may decrease the risk of developing AD. Phospholipids increase the fluidity of neuronal membranes and promote α-secretase processing of APP, which not only prevents the formation of the Aβ peptide but also yields sAPPα, a neuroprotective peptide.[122] Dietary omega-3 essential fatty acids have been shown to reduce learning deficits, Aβ plaques, and synaptic neuropathology, while increasing cerebral blood volume in APP transgenic mice.[123–126] One well-studied omega-3 fatty acid, DHA, comprises around 15% of the brain's total fatty acids and 30%–40% of its gray matter.[68] DHA protects against an Aβ-induced model of neurotoxicity in the rat brain,[127,128] and has anti-inflammatory and anticancer properties.[68] Therefore, diets high in flax and other seeds or nuts with high omega-3 content may provide benefit to the brain and reduce the risk for AD by several potential mechanisms.

16.3.2.6   Phytovitamins

Some epidemiological studies have found a protective effect of dietary antioxidant tocopherols (vitamin E isoforms) against the risk of developing AD, and studies of transgenic APP mice support those data. One study found that vitamin E prevented oxidative stress associated with Aβ plaques and reversed neuritic dystrophy associated with amyloid plaques in APP transgenic mice,[92] while another study found that chronic dietary administration of vitamin E to young, but not to old, APP transgenic mice reduced Aβ deposition.[129] Furthermore, dietary administration of vitamin E to APP transgenic mice before and after repetitive traumatic brain injury ameliorated behavioral impairments, oxidative stress, and injury-accelerated Aβ formation.[130] Therefore, human and animal data suggest that dietary tocopherols, which have well-known antioxidant properties, may serve to protect the brain from oxidative stress that could eventually lead to increased Aβ deposition and AD.

Another study looked at the effects of a diet deficient in folic acid (an isoform of vitamin B_9 found in many fruits and vegetables) on neuropathology in APP transgenic mice. Although Aβ levels were not affected, significant neurodegeneration within the hippocampus was noted in the brains of folic acid-deprived mice.[131] Another in vitro study found that folic acid deprivation increased expression of the genes involved in encoding the γ- and β-secretases along with increased levels of Aβ.[132] Together with other data showing the neuroprotective effects of folic acid on the developing nervous system and the data on dietary tocopherols, several pieces of evidence suggest that various phytovitamins may play a protective role against neurodegenerative processes such as AD.

16.3.2.7   Tea and Its Phytochemicals

Although one epidemiological study showed that tea consumption was not correlated with the risk for AD, other lines of evidence suggest that consumption of tea may protect against oxidative stress[133] and tea has been used as a medicinal tonic for centuries. Tea leaves contain several bioactive phytochemicals, including polyphenol catechins and tannins (up to 25% of the mass of a tea leaf), and psychoactive xanthines, such as caffeine. Several experimental studies have shown that some of its compounds may have protective effects against AD.

For example, an extract of tea catechins reduced cognitive deficits, inflammation, and oxidative stress in rats subjected to intermittent oxygen deprivation as a model of obstructive sleep apnea, which produces brain damage that is associated with an increased risk for AD.[35,134] Isolated compounds in tea have been studied in more depth. Epigallocatechin-3-gallate (EGCG), a polyphenol catechin found in tea, improved cognitive performance in APP transgenic mice,[135] reduced production and deposition of brain Aβ, and increased levels of α-secretase processing and the subsequent neuroprotective peptide sAPPα.[135,136] The effect was observed whether EGCG was injected or administered via the drinking water. EGCG has also been shown to prevent fibrillogenesis of both

Aβ and α-synuclein by directly binding to the peptides and preventing aggregation[137] and to prevent abnormal protein folding in Huntington's disease models.[138] Tannic acid, a phenolic tannin responsible for the astringency in tea's taste, has also been shown to inhibit Aβ aggregation in vitro.[139] Thus, the polyphenols isolated from tea may protect against AD by a variety of mechanisms.

16.3.2.8   Caffeine

This psychoactive xanthine probably explains the worldwide popularity of tea and coffee (another plant with relatively high concentrations of the polyphenol caffeic acid[140,141] and antioxidant properties[142–145]). Caffeine functions as an insecticide in the plant and as a psychostimulant in mammals, primarily via competitive inhibition of adenosine receptors. Because of its stimulant effect, which is generally devoid of the euphoric properties of other phytostimulants, such as cocaine and ephedrine, caffeine has been used for centuries as a general enhancer of cognition. Caffeine added to the drinking water of APP transgenic mice at a dose equivalent to roughly five cups of coffee per day prevented the learning deficits observed in the noncaffeinated transgenic mice. The cognitive benefit was associated with inhibition of β-secretase and decreased Aβ deposition, suggesting that drinking caffeine decreased the production of Aβ. Caffeine also decreased Aβ production in neuronal cultures[146,147] and prevented cholesterol-induced disruption of the blood-brain barrier in rabbits, which develop age-related brain Aβ deposition similar to that seen in human AD patients and APP transgenic mice.[148] Interestingly, "caffeinol," a mixture of caffeine and ethanol, has been shown to have a potent synergistic neuroprotective effect in rodent stroke models.[149] Therefore, caffeine may improve general cognition via its mild stimulant effects and can prevent AD neuropathology by inhibiting Aβ production and protecting the brain from insult.

16.3.2.9   Phytocannabinoids

Cannabis is another plant with a long history of medicinal and recreational use. It contains a wide variety of phytochemicals that bind with CB_1 and CB_2 cannabinoid receptors. Collectively known as the phytocannabinoids, these terpene compounds include tetrahydrocannabinol (THC), cannabidiol, and cannabinol. CB_1 receptors are expressed mainly in the brain and are thought to be responsible for the well-known psychoactive effects of cannabis, whereas CB_2 receptors are expressed mainly in the periphery and thought to play a role in inflammatory processes. Aging is associated with a gradual loss of cannabinoid receptor binding in the brain and may even be further reduced in the hippocampus and caudate of AD brains.[150] Other studies have found that Aβ plaques in AD and Down syndrome brains express high levels of CB_2 receptors but that CB_1 receptor expression in plaques is unchanged or even reduced.[151–153] The increased expression of CB_2 receptors in brain Aβ plaques suggests a role for inflammatory mediation by the endogenous cannabinoids (e.g., anandamide),[154] and this notion is supported by the observation that both THC and anandamide can inhibit production of the free radical nitric oxide.[155,156] Indeed, the cannabinoids are antioxidant, anti-inflammatory, and neuroprotective against excitotoxicity in vitro and against acute brain damage in vivo.[157,158]

THC strongly inhibits AChE activity and prevents AChE-induced Aβ fibrillogenesis by binding directly to a site on AChE that mediates Aβ aggregation.[159] Interestingly, the cannabinoids can also stimulate neurogenesis within the adult hippocampus.[160] Other experimental evidence for a role of the cannabinoids in AD comes from studies of several psychoactive and nonpsychoactive synthetic cannabinoids. Independent of their antioxidant and/or psychoactive properties, these have been shown to prevent cognitive impairment, neurodegeneration, and microglial activation in rats subjected to models of Aβ-induced neurotoxicity[152,161] and chronic brain inflammation[162] and to block Aβ-induced microglial activation and neurotoxicity in vitro.[152] Thus, several studies suggest a number of mechanisms by which the phytocannabinoids may affect AD processes, but there are currently no reported epidemiological data on the incidence of AD among long-term cannabis users.

16.3.2.10   Nicotine

Nicotine is a psychoactive alkaloid that functions as an insecticide in the tobacco plant and has stimulant properties in mammals, primarily due to its nicotinic ACh receptor agonism. Similar to caffeine, nicotine has a long history as a general cognitive enhancer due to its stimulant qualities. As noted earlier, tobacco smokers may have reduced levels of Aβ in the entorhinal cortex,[72] although another study found no protective effect of tobacco smoking on risk of developing AD.[73] Other lines of evidence are provided by experiments showing that nicotine decreased accumulation of Aβ in the cortex and hippocampus of APP transgenic mice.[163,164] Possible mechanisms may include activity at the nicotinic ACh receptor, which results in decreased levels of Aβ and nitric oxide, and inhibition of NF-κB and apoptosis.

In addition to the potential cognitive enhancing, anti-inflammatory, and anti-Aβ effects attributed to nicotine, nornicotine is a long-lived psychoactive nicotine metabolite and minor constituent of tobacco that can inhibit Aβ aggregation by forming permanent covalent bonds with Aβ and glucose via glycation.[165] This study provides another explanation as to the putative neuroprotective effects of nicotine.

16.3.2.11   Huperzine A

Huperzine A is a terpene alkaloid and AChE inhibitor derived from a traditional medicinal plant called Chinese club moss.[166] In some clinical studies, treatment with huperzine A for 8 weeks was associated with mild cognitive improvement over placebo.[167,168] As compared with tacrine (a pharmaceutical AChE inhibitor), huperzine A protected cultured neurons from Aβ-induced neurotoxicity and oxidative stress[169] and caspase-3-induced apoptosis [170] to an equal degree.

However, its neuroprotective properties may be independent of its AChE inhibitory properties, since its enantiomer[(molecular mirror image; (+)-huperzine A] is 50-fold less potent at inhibiting AChE but equally potent at protecting cultured neurons from Aβ-induced toxicity.[171] Evidence for other possible mechanisms of huperzine A includes protection of cultured cells exposed to oxygen glucose deprivation,[169] non-competitive antagonism of the NMDA glutamate receptor (similar to memantine),[172] and increased sAPPα release from cultured human neurons that overexpress APP. The elevated sAPPα was suppressed by muscarinic ACh receptor antagonists and protein kinase C inhibitors. Thus, huperzine may join galantamine in the world of plant-derived AChE inhibitory pharmaceuticals. Indeed, huprine X is a hybrid AChE inhibitor synthesized by combining parts of huperzine A with tacrine that inhibits AChE to a much larger extent than either huperzine A or tacrine alone.[173–177]

16.3.2.12   Other Phytochemicals

Experimental evidence suggests that a number of other foods and isolated phytochemicals have potential anti-AD properties. For example, the dark blue pigments in blueberries are polyphenols with potent antioxidant properties, and dietary blueberries improved cognitive performance in APP transgenic mice without decreasing Aβ plaque levels.[52] Furthermore, a blueberry-enriched diet fed to aged rats elevated levels of blueberry polyphenols in the brain and improved cognition.[178] Various raw fruit extracts have prevented in vitro Aβ-induced calcium flux deficits[179] and neurotoxicity induced by oxidative stress.[53]

Experiments with other isolated phytochemicals also suggest possible roles in AD-related processes. For example, the common dietary polyphenol luteolin, found in the leaves of many plants, was found to reduce levels of Aβ in the brains of APP transgenic mice. Luteolin also demonstrated in vitro inhibition of the enzyme glycogen synthase kinase 3, which plays a role in the cleavage of APP to Aβ.[180] Resveratrol, a polyphenol stilbenoid found in grapes and nuts, was shown to increase clearance and decrease levels of Aβ in vitro by intracellular proteasome-facilitated degradation of Aβ[181] and can modulate a number of other cell-signaling pathways.[113] Its biochemical effects may explain the epidemiological evidence for a decreased risk for AD among elderly subjects who

consume moderate amounts of wine. The phenolic acids rosmarinic acid (from rosemary) and nor-dihydroguaiaretic acid (from creosote) prevented and reversed Aβ aggregation in vitro,[117,182] and myricetin and quercetin (polyphenols that are found in a variety of fruits, vegetables, and spices) reduced heme-Aβ damage to muscarinic ACh receptors.[7–9]

Finally, phytochemicals may improve AD symptoms via effects on the cellular processes of learning and memory. For example, fisetin, an isolated polyphenol found in strawberries and other fruits and vegetables, enhanced long-term memory in normal mice and induced cAMP response element binding phosphorylation and enhanced LTP in rat hippocampal slices.[183] Fisetin's effect on cAMP response element binding (which plays an important role in learning and memory mechanisms) and its functional effects of enhanced LTP and cognition suggest that it may improve synaptic plasticity. Additionally, caffeine and nicotine have long histories of anecdotal and experimental use as general cognitive enhancers, most likely due to their psychostimulant effects. Finally, cannabis also has a history of use for cognitive and creative enhancement but has acute deleterious effects on short-term memory processes. Thus, the cellular effects of phytocannabinoids on learning and memory processes may suggest targets for cognitive enhancement via selective cannabinoid antagonists.

16.3.3 Summary

Several epidemiological and experimental studies show that dietary and isolated phytochemicals may have beneficial effects on cell function and AD-related pathology. In particular, phenols, terpenes, and organosulfurs all have well-characterized antioxidant properties and have demonstrated a variety of other mechanisms by which they may affect AD processes. Other phytochemically mediated pathways related to AD include modulation of the enzymatic processes that produce Aβ from APP, inhibition of Aβ aggregation via direct binding, increased intracellular clearance of Aβ and cholesterol, modulation of the glutamatergic and cholinergic neurotransmitter systems, anti-inflammatory effects via modulation of NF-κB and microglia, anti-apoptotic effects via inhibition of caspase-3, protection of the blood-brain barrier, and even stimulation of neurogenesis.

Low-level accumulation of Aβ in the brain occurs over the lifetime of individuals, and a variety of acute or chronic brain insults can accelerate this process and increase the risk of developing AD. Because age- and insult-related neuropathologies include the build-up of oxidative Aβ and because this gradually increases the oxidative load on the brain, the Aβ deposition process may self-propagate. Traumatic brain injury often occurs in young patients, planting early seeds for future AD neuropathology, and elderly individuals have an increased risk for stroke, which adds to the overall oxidative stress in the brain and subsequent Aβ cascade. Whereas accelerating brain Aβ accumulation can increase the risk of developing AD, reducing Aβ accumulation may decrease the risk. Currently, pharmacological strategies for controlling AD progression include modulation of the glutamatergic system by blocking NMDA receptor channels and preventing the degradation of ACh by inhibition of AChE. Unfortunately, these treatment strategies provide mild to moderate benefits at most.

However, a lifetime of consuming high levels of bioactive phytochemicals and low levels of cholesterol may help attenuate Aβ-related neuropathology associated with age and/or insult. Therefore, an AD-protective diet could reduce the susceptibility to the progression of AD neuropathology and symptoms. Because AD is a progressive disease of the elderly, delaying the onset by as little as a few years would significantly decrease its incidence. Given that Aβ accumulation seems to cause oxidative stress and inflammation, leading to the accumulation of even more Aβ, keeping oxidative stress in check with an AD-protective diet may reduce the slow but steady accumulation of Aβ in the brain with aging.[184,185] Interestingly, several studies suggest that the wide variety of phytochemicals and their isoforms found in whole plant preparations may provide synergistic benefit over isolated phytochemical compounds.[88,106,186,187]

In summary, epidemiological and experimental lines of evidence suggest that diets consisting of a wide variety of brightly colored and spicy foods should provide a broad degree of chronic

background protection against the deleterious effects of aging, including Aβ deposition and its associated consequences. In addition to the antioxidant effects of these compounds, a host of effects on other biochemical pathways that may provide additional lifetime benefits have been identified.

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