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Review Article

Curcuminoids and resveratrol as anti-Alzheimer agents

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Abstract

Alzheimer disease (AD) is by far the most common cause of dementia globally. This neurodegenerative disorder of the brain is chronic and progressive, characterized clinically by the deterioration in the key symptoms of behavioral and cognitive abilities. Treatment options for this disease currently are limited. Deposition of amyloid-β and tau hyperphosphorylation are cardinal pathologic features of AD that lead to the formation of neuronal plaques and neurofibrillary tangles, respectively. In addition to mounting research on herbal compounds for the treatment of AD, curcuminoids and resveratrol appear to be beneficial as anti-AD agents. Curcuminoids (curcumin and demethoxycurcumin) and resveratrol possess unique properties that make them especially worthy of further studies. This review article revisits and presents the current research done on the potential of the curcuminoids curcumin and demethoxycurcumin and the polyphenolic compound resveratrol as anti-AD compounds.

Keywords: Alzheimer's disease; amyloid-β; curcuminoids; resveratrol; tau hyperphosphorylation

Introduction

Demographic ageing is a global process that denotes the success of improved healthcare over the past years. Today, human life expectancy is significantly longer resulting to a much greater proportion of elderly people. Nevertheless, ageing does impose some challenges as well. It has been well documented that corollary to ageing there is also an increase in cases of Alzheimer's disease and other dementias in the senile but modern society. It is the aim of this review paper to present the benefits and potentials of curcumin, demethoxycurcumin and resveratrol as a therapeutic agent for Alzheimer's disease.

Alzheimer disease—an emerging burden on society

The globally prevalent illness Alzheimer disease (AD) is a genetically complex disorder that accounts for most cases of dementia experienced by older people. It is a neurodegenerative disorder affecting major brain areas including the cortex and limbic system, and is characterized by progressive decline

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in memory and impairment of at least one other cognitive function [1]. AD often begins with symptoms such as short-term memory loss and progresses with more widespread cognitive and emotional dysfunction. The so-called late-onset AD occurs after age 65 years. AD features ongoing deterioration of the patient's functioning, which results in substantial and long-lasting disability over the approximate 7–10 years from diagnosis to eventual death [2]. A diagnosis of AD is correct when the disorder is not limited to the period of delirium, is present for at least 6 months, and has no alternative explanation [3].

The health and social welfare of elderly people have long been underprioritized in the public health policy of most countries. The progressive mental deterioration in old age has been recognized and described throughout history. However, it was not until 1906 that a German physician, Alois Alzheimer, identified a collection of brain cell abnormalities as a disease [4]. He presented in his paper the results of postmortem studies on his patient, a 51-year-old woman named Auguste D. who had experienced a progressive presenile dementia [5], the first case of AD ever documented. In Dr. Alzheimer's paper, the patient showed strong feelings of jealousy toward her husband, followed by rapid memory impairment and disorientation in time and space. She then became bedridden and incontinent. She died 4.5 years after the onset of the illness. Her brain autopsy revealed an evenly affected atrophic brain without microscopic foci. The larger cerebral vessels showed arteriosclerotic changes [6]. Dr. Alzheimer also observed the presence of usual deposits in the cortex that were refractory to staining.

Dementia has contributed 11.2% of years lived with disability in elderly people age 60 years and older; more than stroke (9.5%), musculoskeletal disorders (8.9%), cardiovascular disease (5.0%), and all forms of cancer (2.4%) as reported in the global burden of diseases of the 2003 World Health Report. Based on the 2005 Lancet study by Ferri et al [7] on the global prevalence of dementia, 4.6 million new cases of dementia are projected to be recorded each year, with a rate of one case per 7 seconds, and the number of people affected will double every 20 years to as high as 81.1 million by 2040. It was predicted that most people with dementia will live in developing countries (60% in 2001, rising to 71% by 2040). However, the rates of increase are not uniform; thus, numbers in developed countries are forecasted to increase by 100% between 2001 and 2040, but more than 300% in India, China, and their South Asian and Western Pacific neighbors. In addition, there are currently an estimated 18 million people worldwide in whom AD has been diagnosed, according to the World Health Organization. In 2025, it is estimated that this figure will double to approximately 34 million [8]. Although people with dementia are heavy consumers of health services, direct costs in developed countries occur mostly from community and residential care. Despite technologic advancements in medicine, research revealed that most people currently living with dementia have not received a formal diagnosis. In countries with high income, only 20–50% of dementia cases are recognized and are documented during primary care. This treatment gap is without a doubt much greater in low- and middle-income countries, with one study in India suggesting 90% remain unidentified [9]. If the current statistics are extrapolated to other countries worldwide, it suggests that approximately 28 million of the 36 million people with dementia have not received a diagnosis and therefore do not have access to the treatment, care, and organized support that a formal diagnosis can provide. This situation clearly presents a major concern, given that the world's population is aging; new cases of dementia and AD are steadily increasing. Therefore, early diagnosis and intervention are important mechanisms by which the treatment gap can be closed.

Amyloid β—the culprit in AD pathology

In past years, important progress has been made in the understanding of the pathogenic mechanisms of AD, and new therapeutic drugs have become available that allowed the underlying disease process to be treated directly. In this respect, the "amyloid hypothesis" has become the dominant theory in this field. It is believed that amyloid beta (Aβ) accumulation in plaques or as partial soluble filaments initiates a pathologic cascade leading to tangle formation [10], neuronal dysfunction, and possibly inflammation and oxidative damage, with neurodegeneration and dementia as the final outcome. The Aβ is a 40–42 amino acid peptide that has a β-pleated sheet amyloidogenic nature, as detected by Congo red and thioflavin-S staining. The Aβ peptides, which are 3–4 kDa in length, are the building blocks of both the amyloid fibrils in neuritic plaques, in addition to the vascular deposits that accumulate in the brains of AD patients. The Aβ is derived from the proteolytic processing of one or more isoforms of the amyloid-β precursor protein (APP) [11]. Furthermore, the Aβ can exist in two primary forms: as a soluble peptide or in an aggregated state as insoluble amyloid deposits in a beta-sheet conformation; this aggregated state forms the basis of the senile plaques characteristic of AD. Previous studies have shown the neurotrophic characteristic of Aβ at low dosages (10^{-10} M to 10^{-8} M); nevertheless, at higher doses ($>10^{-7}$ M) the peptide becomes neurotoxic [12].

There are also some reports that the protofibrils of Aβ, which are metastable intermediates in amyloid fibril formation, may be toxic [13]. The size of Aβ oligomers is distributed over a wide molecular weight range (from <10 kDa to >100 kDa), with structural polymorphism in Aβ oligomers of similar sizes. The soluble Aβ oligomers are found to be more cytotoxic than fibrillar Aβ aggregates in general and inhibit many critical neuronal activities including long-term potentiation, a classic model for synaptic plasticity and memory loss *in vivo* and in culture [14,15]. Low-molecular-weight Aβ oligomers found in the soluble fractions of the human brain and amyloid plaque extracts [16] were suggested to be the building blocks of larger oligomers or insoluble amyloid fibrils [17,18]. The high toxicity of low-molecular-weight Aβ-oligomers is also supported by *in vitro* studies showing that Aβ dimers are threefold more toxic than monomers and that tetramers are 13-fold more

toxic [16]. Synthetic Aβs were also found to be toxic because these can form fibrillar aggregates that have properties similar to those found in AD plaques in the brain. Lambert et al [14] published the formation of small globular Aβ oligomers (approximately 5 nm in diameter) in Ham's-F12 medium. These globular oligomers were referred as Aβ-derived diffusib ligands. These molecules strongly bind with the dendritic arbors of cultured neurons to cause neuronal death and the blocking of long-term potentiations [14].

Upon reaching the critical concentration, with the Aβ aggregates forming insoluble amyloidogenic deposits, there is then an observed general age-related increase in Aβ generation by the neural cells [19]. Studies have shown that the 42-amino-acid form of the Aβ is the more amyloidogenic of the two forms of the peptide, and it manifests a greater tendency to create insoluble deposits commonly seen in brains affected by AD [20–22]. In certain familial AD, including cases of APP mutation [23] and presenilin mutations [24], there is an increase in the $A\beta_{42}$:$A\beta_{40}$, with the larger form of the peptide acting as a potential "seed" for the deposition of the amyloid peptide and plaque formation. The deposition of the Aβ appeared to be an early event in AD-associated neurodegeneration, with this peptide acting as a seed for the subsequent deposition of the $A\beta_{40}$ peptide.

Amyloid-β precursor protein—a multifunctional molecule in the central nervous system

Although there are many components contained within the neuritic plaques of the brain affected by AD, the APP has a distinct role toward the pathogenesis of AD. The APP isoforms are Type I transmembrane sialoglycoproteins encoded for by a single gene on chromosome 21 that contains 19 exons [25]. The three primary APP isoforms are as follows: APP_{695}, APP_{751}, and APP_{770}. The APP is a multifunctional protein that serves in both cell adhesion and neurite outgrowth. The APP protein has been demonstrated to interact with a number of elements of the extracellular matrix that include collagens I and IV [26], laminin [27], fibronectin [28], heparan sulfate [29], and some glycosaminoglycans [30]. APP has also been proposed to play a role in the modulation of synaptic plasticity [31]. This protein can also play a role in long-term potentiation [32]. The soluble form of APP may also elicit both neurotrophic and neuroprotective properties. The APP acts as a neuroprotective agent, stabilizing intracellular calcium levels wherein the treatment of cells with APP resulted in rapid and prolonged decrease in cellular Ca^{2+} [33]. The APP was able to attenuate the toxic actions of the Aβ peptide by overcoming the Aβ-associated elevation of free intracellular Ca^{2+} levels with the associated induction of free radical species [34]. As a neurotrophic agent, APP stimulates neuronal cell differentiation and neurite outgrowth. Transfection of an APP-null neural cell with APP complementary DNA (cDNA) resulted in an increased neurite outgrowth. The binding of a larger peptide (Ala_{319} to Met_{335}) to cell membrane preparation is both saturable and specific, suggesting that soluble APPs (APPs) mediates its effect by a receptor-mediated mechanism [35].

With regard to APP protein processing, two alternate pathways are known. The primary pathway produces APP after the cleavage at Lys^{16} within the Aβ region of the protein by the enzyme, alpha (α)-secretase. The alternative pathway can be through the cleavage of the APP protein by the β- and γ-secretase to generate the 40–42 amino acid Aβ peptide. The major species of the Aβ produced at normal circumstances is the 40 amino acid form ($A\beta_{40}$). However, $A\beta_{42}$ was found to be increased in AD and was observed to be more amyloidogenic and acts as a seed for amyloid deposition [36]. The pathway by which APP is processed can be influenced by a multitude of factors that may be extracellular or intracellular. In addition, the processing mechanisms could be cell-, tissue-, or species-specific. There is also an increasing number of evidence that the carboxy-terminal fragment of the APP may also exhibit neurotoxicity in addition to Aβ [37]. The toxic fragment was identified as a 100-amino-acid C-terminal fragment (C100 protein). This fragment inhibits the Mg^{2+}/Ca^{2+}-adenosine triphosphatase within the endoplasmic reticulum and also the Na^{+}/Ca^{2+} exchanger. This in turn compromises the ability of the cells to sequester Ca^{2+} within the endoplasmic reticulum, thereby raising the level of the intracellular Ca^{2+} [38,39]. The C100 appears to be a more potent neurotoxin than the Aβ [37]. It also exhibits neurotoxic actions *in vivo*, and transgenic mice overexpressing the peptide displays some neuropathologic features characteristic of the AD brain [40,41]. The processing of the APP is one critical event in the pathogenesis of AD; these processes can be modulated by different factors that include neurotransmitter receptors and activation of a multitude of second messenger systems. These can then provide therapeutic potentials for the action of anti-AD agents that may elicit certain pharmacologic action toward the increased secretion of soluble APPs and diminished production of the amyloidogenic Aβ peptide.

Tau protein—its role in AD progression

Tau (τ) is a major microtubule-associated protein that was almost simultaneously discovered in the United States and Europe [42–44]. It was first identified as a "factor essential for microtubule assembly" from purified tubulin. The τ protein is abundantly expressed in both the peripheral and the central nervous systems, [45] although low levels of τ protein are also present in the oligodendrocytes and astrocytes [46]. Tau undergoes a developmentally regulated phosphorylation having 38 phosphorylation sites [47]. The fetal brain as well as the pathologic brain has highly phosphorylated τ if compared with the normal adult brain [48]. The cDNA for τ was first isolated from the mouse brain expression library [49], then it was cloned from other species such as goat [50], chicken [51], bovine [52] and human [53,54].

The human τ gene is located on the human chromosome 17q21 and has at least 16 exons. To date, at least six different known major τ protein isoforms are brought about by messenger RNA (mRNA) alternative splicing [55] involving exons 2, 3, and 10, whereas exon 4A is only transcribed in the peripheral nervous system [56,57]. The expressed protein

is "the big τ" isoform with approximate molecular weight of 100 kDa. The six isoforms consist of 352–441 amino acids with apparent molecular weight between approximately 37–46 kDa and pI values of 6.85–9.46 [58]. In some articles, the apparent molecular weights of the τ isoforms ranges from 60 kDa to 74 kDa [59] The τ-isoform's structure becomes more complex due to various posttranslational modifications such as nitration, ubiquitination, glycosylation, phosphorylation, and truncation. Structurally, τ protein is hydrophilic and appears as a random coiled protein as analyzed by circular dichroism. The brain τ isoforms have two large domains: the projection domain, which comprises the amino acid terminal two-thirds of the molecule, and the microtubule-binding domain, which contains the carboxy-terminal third of the molecule. Furthermore, the projection domain can be divided into two regions: the amino terminal region with a high proportion of acidic residues and the proline-rich region. The microtubule binding domain can also be divided into the basic, true tubulin-binding region and the acidic carboxy-terminal.

AD is the best-known "tauopathy" because it is characterized by the aggregation of the abnormally phosphorylated τ protein. It is apparent that during normal development and as mentioned earlier, the τ protein undergoes various post-translational modifications. As a result, an increased amount of the modified τ has been associated with a large number of neurodegenerative disorders. In the process of aggregation to form paired helical filaments (PHFs), there should be a reduced affinity of the τ protein for the microtubules to accompany the release of τ into a soluble form. Dissociation of τ from the microtubule that may have been a result of the phosphorylation at some sites in the τ protein bring about microtubule destabilization. The resultant soluble τ protein becomes an ideal target for posttranslational modifications and may change directly or indirectly the conformation of the τ protein, thereby inducing τ dimerization in an antiparallel manner. Subsequently, the stable τ dimers then form τ oligomers and the aggregation process continues, therefore, constituting the subunits of filament called protomers. Coiling of two protomers with each other then forms PHFs. These PHFs then mature and assemble to form neurofibrillary tangles (NFTs). The NFTs are intraneuronal aggregates of the abnormally phosphorylated τ. NFTs correlate with the degree of dementia in AD. Currently, the molecular and cellular mechanisms that could explain the formation of τ lesions remain elusive. These lesions appeared to be controversial as to whether it could be considered a primary etiologic factor of the disease. Phosphorylation of τ is by far the most common and well-studied posttranslational modification associated with AD. It was observed that there is an obvious increased level of hyperphosphorylated τ in brains of AD patients that is three to four times higher than that found in normal adult brain [60,61] because normally a τ phosphoprotein contains one to three molecules of phosphate (PO_4^{3-}) per molecule of τ. Protein phosphorylation involves the addition of a phosphate group via esterification on the following amino acid residues: serine (S), threonine (T), and tyrosine (Y). There are 85 putative phosphorylation sites on τ: 45 (53%) are on serines; 35 (41%) are on threonines; and 5 (6%) are on tyrosines. Phosphorylation is also modulated by various kinases and phosphatases. Therefore, hyperphosphorylation is a consequence of a dysregulation of the kinases and phosphatases. Currently there are more than 10 serine/threonine protein kinases known to phosphorylate τ protein *in vitro*. These kinases are categorized into two according to the motif specificity: proline-directed and nonproline-directed protein kinases. The proline-directed protein kinases include extracellular signal-regulated kinase 1/2 (erk-1/2), cell division cycle 2 kinase (cdc 2), cyclin-dependent kinase 2 (cdk 2), and the cdk 5. Although proline is not needed strictly in the phosphorylation of τ protein by Glycogen synthase-3 (GSK-3), still the presence of proline improves the phosphorylation efficiency. The nonproline-directed kinases are calcium- and calmodulin-dependent protein kinase II, PKA protein kinase (PKC), casein kinase I, casein kinase II and $p^{110MARK}$.

The τ protein may also undergo prolyl isomerization, a reaction that allows the rearrangement of disulfide bonds in proteins. This involves the modification of the conformation of the protein of interest from *cis* to *trans* conformation and vice versa. The change in the conformation of the τ protein from *cis* to *trans* allows a shift of the peptide chain in space, allowing them to be more available for the action of phosphatases. Prolyl isomerization of τ can be achieved by the aid of the enzyme, peptidylprolyl isomerase Pin-1 (peptidylprolyl *cis/trans* isomerase NIMA interacting-1; NIMA: never in mitosis gene A) [62]. Pin-1 interacts with τ to prevent its pathologic accumulation [63]. Also, τ nitration proceeds by the addition of nitrogen dioxide on tyrosines of the molecule that occurs particularly at four sites Y18, [64] Y29, Y197, and Y394 [65]. This reaction was observed to be involved in the aggregation of the τ protein [66]. Polyamination is a reaction catalyzed by transglutaminases involving a glutamine residue as an acyl donor and a lysine as an acyl acceptor. The reaction results to the formation of γ-glutamyl-ε-lysine isopeptide bond and provokes protein cross-linking [67]. The primary donor site is Q424 and the principal acceptor is in the region between residues K163 and K240, [68] thereby linking τ polyamination to the NFT formation process. Oxidation can also occur on the τ protein that leads to the assembly of the paired helical filaments [69,70]; it occurs at amino acid residue C322 localized in the R3 domain of the microtubule-binding domain (MDB) region. The C322 is linked to the formation of tau lesions.

To date, it is still difficult to ascertain which among the previously described post-translational modifications is preferentially involved in τ pathology and AD. Most certainly, an orchestration among several τ modifications is required for τ aggregation into NFTs. Therefore, studying τ regulation at transcription and translation levels is of high significance in our understanding of the physiologic role of τ and its involvement in the development of AD. This is also particularly important in identifying compounds that are of therapeutic value for AD.

Curcuminoids and resveratrol on AD

Scientists around the globe have been trying for decades to treat and put an end to the pathologic symptoms of AD that deprive elderly persons of their intellect, but have had limited success. Despite these efforts, only four Food and Drug Administration-approved drugs are currently commercially available in the United States to treat AD pathology [71]. These drugs mainly target cholinergic functions associated with AD, leaving most other potential AD targets almost unaffected by the treatment. As a result, there is an urgent need for developing drugs based on multiple pathomechanisms of AD. The enormous diversity of functions in natural compounds extracted and isolated from either plant or marine sources may provide a new generation of drugs for AD therapy and management [72,73]. Plant-derived drugs are popular because the public believes that herbs are naturally safer than synthetic drugs. These beliefs may account for the sudden increase in the use of herbal treatment during the last decades [74]. This review also presents an update on the current researches undertaken on curcumin, demethoxycurcumin, and resveratrol regarding their potential for use in the treatment of AD.

Curcuminoids

Curcumin (Fig. 1) is a bright yellow powder that was first identified in 1910 [75]. This compound is also known as deferuloylmethane, natural yellow 3 and (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione. It has a formula weight equivalent to 368.38 g mole^{-1}. This substance was isolated from the plant *Curcuma longa* or turmeric that is commonly used for culinary purposes. This substance possesses several functional groups wherein the planar aromatic ring systems are attached to α,β-unsaturated carbonyl groups. The diketones generate stable enols that could be easily deprotonated to form enolates. The α,β-unsaturated group can undergo nucleophilic addition and Michael reaction. Curcumin has been shown to possess a wide range of pharmacologic activities, including anti-inflammatory [76,77], antioxidant [78], anticancer [79], wound healing [80], and antimicrobial effects. Curcumin has been shown to exhibit activity against various neurologic diseases, including AD [81], multiple sclerosis [82], Parkinson disease [83], epilepsy [84], cerebral injury [85], age-associated neurodegeneration [86], schizophrenia [87], spongiform encephalopathies (Creutzfeldt–Jakob disease) [88], neuropathic pain [89], and depression [90].

The oxidation process occurs mostly in the brain and it consumes 20% of the body's oxygen despite accounting for only 2% of the total body weight. With normal aging, the brain accumulates metals ions such as iron (Fe), zinc (Zn), and copper (Cu). Thus, the brain requires copious amounts of antioxidants that control and prevent the damaging effects of reactive oxygen species generated via the Fenton reaction concening redox-active metal-ion reduction and activation of molecular oxygen [91]. In AD, copper ions are also involved in the progression of the disease, and copper uptake is regulated by the APP. Copper has high affinity to both APP and Aβ; Huang et al [92] recently reported that curcumin may play a dual role in protecting the rat cortical neuron against the Cu(II)-induced oxidative damage. Low dosages of curcumin (i.e. 1, 5, and 10 μM) were able to depress oxidative stress levels exacerbated by Cu(II); however, high dosage (>10 μM) of curcumin failed to decrease the Cu(II)-induced oxidative stress [92]. It was found that curcumin and its structural analogs, demethoxycurcumin and bisdemethoxycurcumin, can protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from Aβ-induced oxidative stress, and that these compounds were better antioxidants than α-tocopherol [93]. Zhang et al [94] in 2010 investigated the effect of curcumin on the metabolism of Aβ and APP in various cell lines including rat neuroblastoma cells overexpressing human APP_{751}. Curcumin significantly increased the levels of matured APP at low concentrations (5 and 10 μM) and there was an observed decrease in the level of matured APP at 15 and 20 μM. As a result, curcumin treatment markedly increased the ratio of matured APP and immature APP at lower concentrations and again the ratio was decreased significantly at higher concentrations of curcumin [94]. In a study by Ishrat et al [95], supplementation of curcumin on intracerebroventricular-streptozotocin infused rats significantly alleviated cognitive deficiencies resulted from intracerebroventricular-streptozotocin infusion. Curcumin further prevented oxidative damage to the hippocampus and cerebral cortex as ascertained through the following biomarkers: (1) increased 4-hydroxynonenal, malondialdehyde, thiobarbituric reactive substances, hydrogen peroxide, protein carbonyl and oxidized glutathione; and (2) diminished amounts of reduced glutathione and enzymes glutathione peroxidase and glutathione reductase [95]. The potential reversal of the intracerebroventricular-streptozotocin-induced alterations in cognitive behavioral and biochemical parameters suggests that addition of curcumin as part of our diet may help reduce the incidence of age-related sporadic dementia of Alzheimer type.

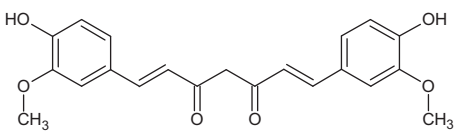

Fig. 1. Structural formula of curcumin.

In 2005, Yang et al [96] showed that curcumin could inhibit aggregated as well as disaggregated Aβ40. In this regard, curcumin was seen to be a better Aβ40 aggregation inhibitor than ibuprofen or naproxen. In this regard, studies by Yanasigawa et al [97] on derivatization of curcumin aiming to increase the anti-Aβ aggregation activity of curcumin revealed that the curcumin derivatives have high levels of binding to Aβ aggregates and not the Aβ monomers. In the same study, the enol form was more predominant among the curcumin derivatives that interacted with the Aβ aggregates. This may be because compounds may only be able to bind with Aβ aggregates. In addition, the compound needs to be coplanar

and have double-bond conformation with certain optimal carbon chains also known as the Goldilocks model [98]. The curcumin derivatives used in the study by Yanasigawa et al [97] conformed with the Goldilocks model for the compound, and maintained the coplanarity and the presence of the extended double bond conjugation between the two phenyl rings which gave the strong binding affinity of curcumin to the Aβ aggregates. The microtubule-associated protein 2 (MAP2) is a neuronal cytoskeletal component that plays an important role in the preservation of cellular architecture and internal organization, with certain involvement in the determination of cellular shape, in cell division, and cellular processes [99]. Changes in the architectural framework of the cytoskeleton bring forth malfunctions of the nerve cells in brain tissues of patients with AD. In a study by Xiao et al [100], it was reported that chemically synthesized curcumin and its derivatives can increase the viability of human neuroblastoma SK-N-SH cells after $A\beta_{1\text{-}42}$ insult. Treatment of SH-N-SK cells with curcumin and its derivatives resulted in an increase expression of the MAP2. The upregulation of the MAP2 protein may be associated with the reorganization of the cytoskeleton during the degeneration of neuronal cells in AD and this implied that curcumin may have played a pivotal role in improving abnormal neuronal cell morphology by being able to restore normal physiology within the cell. In addition, the molecular structure of curcumin and its derivatives may also be responsible for their neuroprotective function. The important role of β-diketone moiety, 4-hydroxyl group on the benzene ring might be a key group of resistance to Aβ neurotoxicity, whereas 3-methoxy could indirectly affect the beneficial effect of curcumin derivatives related to suppressing 4-hydroxyl [101]. It was also described that the hydrogen atom donation from the β-diketone part of the lipid alkyl or the lipid peroxyl radical is the potentially more important antioxidant action of curcumin [93]. Recently, solid-state nuclear magnetic resonance studies demonstrated that curcumin can interact with the 12th and 17th to 21st amino acid residues of the $A\beta_{42}$ fibrils. Furthermore, based on the clear cross-peaks between carbons adjacent to methoxy and/or hydroxy groups revealed by C^{13} to C^{13} 2D solid-state nuclear magnetic resonance experiments, the presence of the functional groups on the aromatic rings plays an important role in the anti-Aβ aggregative property of curcumin [102]. In a study conducted by Liu et al [103], curcumin exhibited the most potent activity in the restraint of the production of $A\beta_{42}$ in swap HEK293 cells followed by demethoxycurcumin and bisdemethoxycurcumin. Their group also found that curcumin but not other curcuminoids was able to decrease the APP protein expression, suggesting that the antiamyloidogenic property of curcumin can be attributed to its effect on the APP metabolism.

Demethoxycurcumin (Fig. 2) is a structural analog of curcumin less one methoxy group and is also isolated from the plant *C. longa*. It comes as orange powder with a formula weight equal to 338.35 g $mole^{-1}$. Its IUPAC name is (1*E*,6*E*)-1-(4-hydroxy3-methoxyphenyl)-7-(4-hydroxyphenyl) hepta-1,6-diene-3,5-dione and may commonly be referred to as desmethoxycurcumin, monodemethoxycurcumin, or

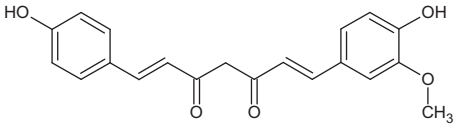

Fig. 2. Structural formula of demethoxycurcumin.

parahydroxycurcumin. Several articles have been published associating members of the curcuminoid family with AD. Listed herein are some of the results gathered by researchers implicating demethoxycurcumin with AD. β-Amyloid (Aβ) induced oxidative stress is a well-established pathway of neuronal cell death in AD. The curcuminoids curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were observed to protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from $A\beta_{42}$ insult, as measured by MTT assay. The ED_{50} values of curcumin, demethoxycurcumin, and bisdemethoxycurcumin toward PC12 and human umbilical vein endothelial cells were 7.1 ± 0.3, 4.7 ± 0.1, 3.5 ± 0.2 μg/mL and 6.8 ± 0.4, 4.2 ± 0.3, and 3.0 ± 0.3 μg/mL, respectively. These compounds were found to be better antioxidants than α-tocopherol as determined by DPPH radical trapping experiment. α-Tocopherol did not protect the cells from Aβ (1–42) insult even at >50 μg/mL concentration. The results suggested that these compounds protected the cells from $A\beta_{42}$ insult through antioxidant pathway [104]. Ahmed and Gilani [105] in 2009 reported on their conducted experiments on curcuminoids including demethoxycurcumin for anti-acetylcholinesterase (AChE) activity *in vitro* and *ex vivo*. It was observed that, *in vitro*, demethoxycurcumin has a marginal effect on AChE inhibition compared with bisdemethoxycurcumin and curcumin has the lowest activity. *Ex vivo*, both demethoxycurcumin and bisdemethoxycurcumin were able to show anti-AChE activity [105]. Investigation on the effects of curcuminoid mixture and individual constituents on spatial learning and memory in an Aβ peptide-infused rat model of AD and on the expression of post-synaptic density protein-95 (PSD-95), synaptophysin, and Ca^{+2}/calmodulin-dependent protein kinase IV (camkIV) were carried out. Curcuminoid mixture exhibited a memory-enhancing effect in rats displaying AD-like neuronal loss marginally at 30 mg/kg, whereas individual components were effective at 3–30 mg/kg dosages. A short period of treatment with test compounds showed that the curcuminoid mixture and bisdemethoxycurcumin increased PSD-95 expression in the hippocampus at 3–30 mg/kg. The maximum effect was observed at a low dose of 3 mg/kg with respective values of 470.5 and 587.9%. However, after a longer treatment time, both demethoxycurcumin and curcumin also increased PSD-95 level up to 331.7 and 226.2%, respectively, at 30 mg/kg. Meanwhile, their effect on synaptophysin in the hippocampus after the longer duration treatment was studied. The curcuminoid mixture and all three individual components increased synaptophysin expression. Among these, demethoxycurcumin was the most effective, showing a 350.1% increase ($p < 0.01$) at 30 mg/kg compared with the neurotoxin group. The compounds' effect

on camkIV expression in the hippocampus was also determined. Only demethoxycurcumin increased camkIV levels to 421.2% at a concentration of 30 mg/kg. These compounds salvaged PSD-95, synaptophysin, and camkIV at the expression levels in the hippocampus in the rat AD model, suggesting multiple target sites giving out the potential of curcuminoids in enhancing the spatial memory and disease progression in AD. Recently, the effect of a curcuminoid mixture and its individual components on inflammatory and apoptotic genes expression in AD using an Aβ + ibotenic acid-infused rat model was explored. Five days after treatment with demethoxycurcumin, the amounts of hippocampal IL-1β were decreased to 118.54 ± 47.48 and 136.67 ± 31.96%, respectively, at 30 mg/kg and 10 mg/kg, compared with the amyloid treated group (373.99 ± 15.28%). Also, 5 days treatment with the curcuminoid mixture and demethoxycurcumin effectively decreased the levels of glial fibrillary acidic protein, a specific marker for astrocytes hyperactivity, often elevated in inflammatory conditions and AD, in the hippocampus. The curcuminoid mixture and bisdemethoxycurcumin effectively decreased caspase-3 level in the hippocampus after 20 days of treatment, where bisdemethoxycurcumin showed a maximal rescuing effect that is 92.35 ± 3.07% at a concentration of 3 mg/kg. Furthermore, the curcuminoid mixture also at 30 mg/kg decreased hippocampal Fas ligand levels to 70.56 ± 3.36% after a 5-day treatment and 19.01 ± 2.03% after 20 days. Measurement of the amount Fas receptor, demethoxycurcumin decreased the levels after 5 days of treatment, with all three doses showing a maximum effect (189.76 ± 15.01%) at 10 mg/kg. Individual compound was efficient 20 days posttreatment in plummeting Fas receptor levels in the hippocampus. This study demonstrated the important outcomes of curcuminoids administration on gene expressions.

Resveratrol

Resveratrol (Fig. 3) was first reported in 1939 by a Japanese researcher, Dr. Michio Takaoka, after it was extracted, purified, and isolated from a poisonous but medicinal herb, *Veratrum album* var. *grandiflorum* [106]. Resveratrol is a stilbenoid, a derivative of stilbene, that is also known as 3,4,5′-trihydroxystilbene. It has a molecular weight equivalent to 228.24 g mole^{-1}. This compound appears as white powder with slight yellow tinge. It exists in nature as two geometric isomers, *cis*- and *trans*-resveratrol. The *trans*-resveratrol is the more biologically active isomer. However, exposure to ultraviolet light of the *trans*-resveratrol leads to its conversion to its inactive form, *cis*-resveratrol [107]. In grapes, *trans*-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as *Botrytis cinerea* [108]. Also, most of this compound is found in the skin and seed of the grape's fruit (*Vitis vinifera*) [109]. An epidemiologic association between the common red wine and a low occurrence of cardiovascular diseases led to the beginning of what was referred to as the "French Paradox", coined by Serge Renaud, a scientist from Bordeaux University, despite the saturated fat-rich diet consumed by the French people. Furthermore, resveratrol attracted greater attention when its presence in red wine was suggested as an explanation for the cardioprotective effects of wine. Wine and grape extract have been shown to reduce platelet aggregation, uphold vasorelaxation, prevent atherosclerosis, decrease lipid peroxidation, and ameliorate serum cholesterol and triglyceride concentrations [110].

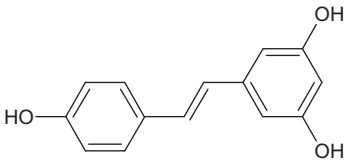

Fig. 3. Structural formula of resveratrol.

Because resveratrol has shown unprecedented promise for the development of new drugs, intense efforts are being made to understand its use in promoting healthy aging, longevity, and neuronal protection. Reports were made telling the significance of resveratrol as a neuroprotective agent. To date, it is still not clear how resveratrol may be essential in neuronal protection. Scientists currently still have to prove whether resveratrol takes its action through the traditionally known antioxidant properties that could be more effective than the cytoplasmic signal transduction pathways, or whether it may act through direct modulation of neuronal functions. Studies strongly implicate sirtuins in neuronal protection, and have also shown that calorie-restriction types and calorie-restriction mimetics can elevate sirtuin production in the human brain. As mentioned previously, resveratrol can act as a calorie-restriction mimetic, making it a good neuroprotective candidate compound acting via the sirtuin pathway [110]. Sirtuins, particularly SIRT-1, may play an important role in protecting neurons from the devastating effects of reactive oxygen species, peroxides, nitric oxide, β-amyloid peptides, and other intracellular and extracellular insults that could be present in the brain with AD. Resveratrol-induced SIRT-1 overexpression has been found to deacetylate and suppress the activity of p53 in neurons, thus preventing apoptotic death of neurons. In addition to this finding, the apoptotic activities of the FOXO proteins were also inhibited by resveratrol-induced SIRT-1 expression [111]. FOXOs has functional similarities and takes part in the cross-talk with p53. In another study, it was observed that resveratrol-induced SIRT-1 inhibited the signaling pathway of the nuclear factor kappa B (NF-κB) in microglia and astrocytes resulting in the protection against Aβ-induced toxicity [112]. Soluble forms of Aβ can accumulate in the endothelial cells of the blood vessels of the brain with AD that ultimately inhibits the endothelial nitric oxide synthase (eNOS) activity. This phenomenon later causes toxic effects on nitric oxide functions such as Ca^{2+} dyshomeostasis [113]. This study suggests that in brains with AD, resveratrol may increase eNOS, modulate the expression of SIRT-1, and affect other cellular metabolic functions. Resveratrol also protects both nerves and blood vessels against Aβ insults. Moreover, amyloid peptides trigger the activation by acting with a number of Toll-like receptors (TLRs) including TLR4.

It was shown by Capiralla et al [114] that resveratrol can prevent the activation of murine RAW 264.7 macrophages (mouse leukaemic monocyte macrophage cell line) and the microglial BV-2 cells treated with the TLR4 ligand, lipopolysaccharide. The activation may be due to the reason that resveratrol may have acted upstream during the activation cascades interfering in the TLR4 oligomerization after the stimulation of the receptors. In this same study, resveratrol was demonstrated to hinder the proinflammatory events as consequences of the presence of the fibrillar Aβ on macrophages by effectively inhibiting the effect of Aβ on the phosphorylation of IκB, activation of STAT1 and STAT3, and on the secretion of tumor necrosis factor-α and interleukin-6 [114].

In transgenic APP mice experiments, excessive and persistent reactive oxygen species production caused by the interaction of Aβ with Aβ-induced alcohol dehydrogenase may result in oxidative damage to mitochondrial and cellular biomolecules, resulting in a shut-down of mitochondrial energy production [115]. Finally, resveratrol had long been considered as a powerful antioxidant, and evidence has indicated the potential of resveratrol as a neuro-protecting agent. In PC12 cells, resveratrol was able to protect cells from $A\beta_{25-35}$-induced toxicity; it was also able to attenuate apoptotic cell death, reduce changes in mitochondrial potential and inhibited accumulation of reactive oxygen species [116]. Another important finding by Marambaud et al [117] regarding the neuroprotective role of resveratrol is its ability to interact with the ubiquitin proteosome system (UPS) by affecting the proteosome-mediated degradation of Aβ through a mechanism that does not directly increase the activity of the proteosome. The UPS is one of the main cellular, protein quality control mechanisms that enzymatically labels, transports, and eventually degrades the misprocessed and misfolded proteins [118]. It also controls all the metabolic reactions of the components of APP. Unfortunately it has been found that accumulation of Aβ causes the reduction of the UPS. Recently, it has been demonstrated that resveratrol was cytoprotective in human neuroblastoma cells by exerting its role not through the inhibition of β-amyloid aggregation but rather on its scavenging abilities from the toxic effect of the accumulation of Aβ and Aβ-metal complexes (Aβ-Fe, Aβ-Cu, Aβ-Zn, and Aβ-Al).

Resveratrol at a concentration of 15 μM acted as a reactive oxygen species scavenger against those generated by Aβ-Fe, Aβ-Cu, Aβ-Zn complexes, thereby reducing their toxicity; however, resveratrol is not sufficient to fully block the toxicity induced by metal complexes [119]. Furthermore, it was previously reported in an *in vivo* experiment that 2% resveratrol did not alter the holoAPP, αC-terminal (C83), and β-terminal fragments (C99) when it was given *ad libitum* to transgenic APP695 mice, Tg19959 [120].

The poor bioavailability of resveratrol limits its potential for use in the treatment of AD because (1) polyphenols such as resveratrol undergo extensive biochemical reactions including oxidation, reduction, hydrolyses, glucuronic acid, sulfate, and methyl conjugation immediately after ingestion, and (2) there is a considerable person-to-person variability in drug absorption and metabolic process [109]. However, it is presumed that the circulating form of resveratrol in the human body, thought to modulate numerous cellular and biochemical effects, is predominantly the modified metabolite and not the aglycone. This finding is true because the total recovery of glucuronic acid and sulfates conjugated with resveratrol in human urine and feces were about 71–98% through oral routes and 54–91% via the intravenous route [121].

Conclusion

AD is an incapacitating disease that deprives elderly individuals of their will and intellect. Apart from the diverse biologic qualities such as anti-inflammatory, antioxidant, anticancer, wound-healing enhancer, antimicrobial, and antiaging shown by curcumin, demethoxycurcumin, and resveratrol, these compounds had also found their niche in the anti-AD research. Researchers considered curcumin, demethoxycurcumin and resveratrol as possible anti-AD compounds due to their ability to prevent the aggregation of amyloid-β peptides. For many of the pharmacologic activities including the anti-AD activities that have been reported on curcumin, demethoxycurcumin, and resveratrol, clinical data are very limited. Clinical efficiency and potential toxicity of these compounds in larger trials requires further evaluation before recommendation regarding their use for AD treatment.

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