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Review

Anti-amyloidogenic therapies: strategies for prevention and treatment of Alzheimer's disease

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Abstract. Deposition of amyloid β-protein (Aβ) in the brain is an early and invariant neuropathological feature of Alzheimer's disease (AD). The current search for anti-AD drugs is mainly focused on modification of the process of accumulation of Aβ in the brain. Here, we review four anti-amyloidogenic strategies: (i) reduction of Aβ production, which has mainly been approached with secretase inhibition, (ii) promotion of the Aβ degrading catabolic pathway, including an Aβ degrading enzyme, neprilysin, (iii) immunotherapy for Aβ and (iv) inhibition of Aβ aggregation. We have reported that AD patients have a favorable molecular environment for Aβ aggregation and that various compounds, such as polyphenols, interfere with Aβ aggregation and destabilize preformed Aβ fibrils.

Keywords. Alzheimer's disease, amyloid β-protein, anti-amyloidognic therapy, secretase, neprilysin, polyphenols.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the deterioration of cognitive function and behavioral changes [1, 2]. Senile plaques, neurofibrillary tangles and extensive neuronal loss represent the main histological hallmarks observed in AD brains. Two main disease mechanism-based approaches are based on the involvement of two proteins, amyloid-β protein (Aβ) and tau. Aβ is the main constituent of senile plaques, and tau is the main component of neurofibrillary tangles.

High levels of fibrillary Aβ are deposited in the AD brain, which is associated with loss of synapses, impairment of neuronal functions and loss of neurons [3–6]. Aβ was sequenced from the meningeal vessels and senile plaques of AD patients and individuals with Down's syndrome [7–9]. The subsequent cloning of the gene encoding the β-amyloid precursor protein (APP) and its localization to chromosome 21 [10–13], coupled with the earlier recognition that trisomy 21 (Down's syndrome) invariably leads to the neuropathology of AD [14], set the stage for the proposal that Aβ accumulation is the primary event in AD pathogenesis. In addition, certain mutations associated with familial AD and hereditary cerebral hemorrhage with amyloidosis have been identified within or near the Aβ region of the coding sequence of the APP gene [15–19], and these mutations cluster at or very near the sites within APP that are normally cleaved by proteases called α-, β- and γ-secretases (Fig. 1) [20]. Furthermore, other genes implicated in familial AD include presenilin-1 (PS1) and presenilin-2 (PS2) [21–23], which alter APP metabolism through a direct effect on the γ-secretase [24, 25]. These facts support the notion that aberrant APP metabolism is a key feature of AD.

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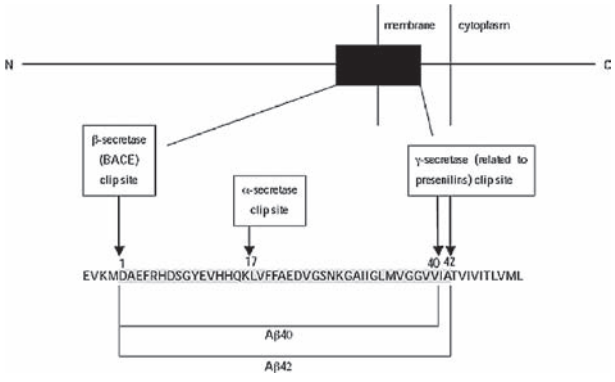

Figure 1. Diagram of amyloid precursor protein (APP) and its principal metabolic derivative, amyloid β(Aβ). Aβ is generated from APP by two proteases, β-secretase and γ-secretase, while a third protease, α-secretase, competes with β-secretase for the APP substrate.

Mutations in the gene encoding the tau protein cause frontotemporal dementia with parkinsonism, which is characterized by severe deposition of tau in neurofibrillary tangles in the brain, but no deposition of Aβ [26, 27]. Thus, genetic and pathological evidence strongly supports the notion that accumulation of Aβ in the brain is the first pathological event leading to AD (amyloid cascade hypothesis; Fig. 2), and neurofibrillary tangles seen in AD brains are likely to have been deposited after changes in Aβ metabolism and initial plaque formation [28].

Aβ deposited in the brain consists of two major species, Aβ40 and Aβ42, which differ according to the whether the C terminus of Aβ ends at the 40th or 42nd amino acid, respectively (Fig. 1) [29–31]. In the brains of AD patients, Aβ42 is the predominant species deposited in the brain parenchyma [32]. In contrast, Aβ40 appears to be the predominant species deposited in the cerebral vasculature (cerebral amyloid angiopathy) [31]. There is a strong correlation between Aβ40 and mature senile plaques [31], and in the brains of Down's syndrome patients, Aβ42 can form numerous diffuse plaques as early as age 12 years, whereas Aβ40 is first detected in the plaques almost 20 years later [33]. Further experimental studies indicate that Aβ42 aggregates more easily than Aβ40 [34], and Aβ42 is essential for amyloid deposition in the parenchyma and also in the vasculature [35].

Therefore, inhibition of the accumulation of Aβ, especially Aβ42, in the brain (anti-amyloidogenic therapy) is the primary strategy for the development of AD therapies, and currently the most active area of investigation. Here, we review four potent anti-amyloidogenic strategies: (i) reduction of Aβ production, (ii) promotion of the Aβ degrading catabolic pathway, (iii) immunotherapy for Aβ and (iv) inhibition of Aβ aggregation and destabilization of aggregated Aβ; among them, special attention is directed to the fourth strategy, i.e. anti-Aβ aggregation, on which we focus our research.

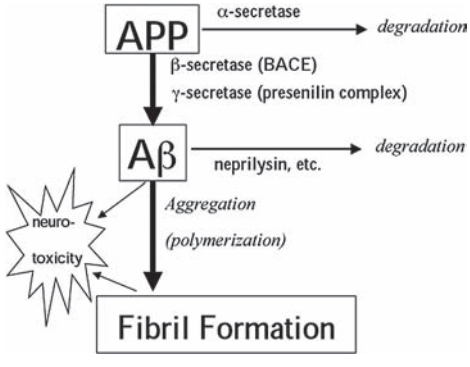

Figure 2. The amyloid cascade hypothesis. This hypothesis proposes a series of pathogenic events leading to Alzheimer's disease (AD). Cerebral amyloid β (Aβ) accumulation is the primary factor in AD, and the rest of the disease process results from an imbalance between Aβ production, accumulation and Aβ clearance.

Reduction of Aβ production

Aβ is generated from APP by two proteases, β-secretase and γ-secretase (Figs. 1, 2). A third protease, α-secretase, which competes with β-secretase for the APP substrate, interferes with the production of Aβ (Figs. 1, 2). Therefore, three strategies to reduce Aβ have been proposed: inhibition of β-secretase, inhibition of γ-secretase and stimulation of α-secretase.

β-Secretase inhibition

β-Secretase was identified as the transmembrane aspartic protease beta-site APP-cleaving enzyme 1 (BACE1) [36–39], which, together with its homologue BACE2 [40], forms a new branch of the pepsin family. Although both enzymes exhibit many of the characteristics expected for β-secretase, it has been quite convincingly demonstrated that BACE1 is in fact the major β-secretase responsible for Aβ generation in the brain [41–43]. In contrast to BACE1, BACE2 is more highly expressed in peripheral tissues, but also to some extent in the brain [39, 44, 45]. Interestingly, BACE1 immunoreactivity is increased around amyloid plaques in AD brains compared with nondemented controls; the level of BACE1 is elevated in AD brain homogenates [46–48], but it remains to be established whether this apparent BACE1 overexpression has an important role in the pathogenic cascade of AD.

From the therapeutic point of view, BACE1 appears to be a promising drug target, since genetic ablation of the BACE1 gene in mice does not seem to be associated with any gross abnormality [41–43]. Small interfering RNAs, which downregulate gene expression through enhancement of messenger RNA (mRNA) degradation at the post-transcriptional level, targeted to the BACE1 gene, reduced BACE1 expression and Aβ production in cultured cell studies [49, 50]. Moreover, BACE1 deficiency

could prevent the learning and memory impairments and cholinergic dysfunction observed in a transgenic mouse model for AD [51].

These results have encouraged the development of β-secretase inhibitors as drugs for AD therapy. However, it has been difficult to identify small molecules that act as β-secretase inhibitors and possess the desired pharmacokinetic properties for AD therapy. Recently, a smaller compound of effective β-secretase inhibitor, KMI-429, was synthesized [52]. KMI-429 reduced the level of soluble and insoluble Aβ40, and soluble and insoluble Aβ42, in both APP transgenic and wild-type mice [53]. KMI-429 may potentially permeate the blood-brain barrier, and may be an effective therapeutic reagent for AD [53].

γ-Secretase inhibition

γ-Secretase is an unusual transmembrane protease complex consisting of at least four proteins, presenilin, nicastrin, anterior pharynx (APH1), and presenilin enhancer 2 (PEN2) [54]. Overexpression of these four proteins is sufficient to produce observable γ-secretase activity in yeast [55], but reconstitution from purified components has not yet been accomplished, and the structure of the active site remains unknown. One of the fundamental concerns about therapeutic secretase inhibition is that secretases are likely to have other protein substrates, and inhibiting the cleavage of these substrates could have detrimental effects. The identification of the γ-secretase components and the generation of gene-targeted models led to the identification of several γ-secretase substrates other than APP, including notch receptor 1 (NOTCH1) [56], the Notch ligands delta-like protein 1 (DELTA1) and Jagged2 (JAG2), v-erb-a erythroblastic leukaemia viral oncogene homologue 4 (ERBB4) and others [54]. The knockout of the γ-secretase component PS1 showed a lethal phenotype similar to a *NOTCH1* knockout [56]. γ-Secretase inhibitors reduced thymocyte numbers and blocked thyomocyte differentiation in organ culture systems in a NOTCH1-dependent manner [57, 58]. Therefore, notch-related side effects of total γ-secretase inhibition interfere with generating therapeutically useful potent γ-secretase inhibitors. However, certain non-steroidal anti-inflammatory drugs (NSAIDs) can modulate γ-secretase cleavage without blocking Notch cleavage, and NSAIDs can reduce Aβ42 [59]. NSAIDs directly modulate γ-secretase or its substrate, because the effect is observed in cells that lack the NSAID targets cyclo-oxygenase I (COX1) and II (COX2), and the effect can be demonstrated in a cell-free assay [60]. Recently, a small G protein, Rac1, was demonstrated to stimulate γ-secretase-mediated APP processing, and a new small molecule, EHT 1864, which blocks the Rac1 signaling pathways, decreases Aβ production by inhibiting the γ-secretase dependent cleavage of APP, without affecting Notch cleavage, *in vitro and in vivo* [61]. In the future, γ-secretase might emerge as a viable drug target, if more potent compounds that target this Aβ42 modulator mechanism could be generated.

α-Secretase stimulation

α-Secretase stimulation by M1 muscarinic receptor agonists has been investigated [62]; and treatment of AD patients with M1 agonists has been reported to decrease cerebrospinal fluid levels of Aβ42 [63]. α-Secretase pathway stimulation leads to a reduction of APP formation through effects mediated by cell-surface receptors [64]. However, α-secretase pathway stimulation also changes the metabolism of various other membrane proteins, although the potential side effects of this approach are not known.

Promotion of Aβ degrading catabolic pathway

Promotion of the Aβ degrading catabolic pathway has become a major target for anti-amyloidogenic therapy. The activation of proteases involved in Aβ degradation could contribute to Aβ removal from the brain. Such proteases include insulin-degrading enzyme [65], endothelin-converting enzymes [66], plasmin [67, 68] and neprilysin (NEP) [3, 69]. Among these peptides, NEP plays the largest role in the degradation of Aβ [65, 66, 69]. NEP levels in regions vulnerable to senile plaque development, such as the hippocampus and midtemporal gyrus, are particularly low in sporadic AD patients, compared with those in age-matched controls [70, 71]. In normal aging mice, NEP levels were selectively decreased in regions highly vulnerable to AD pathology [72], and, in human, NEP decreases uniformly in AD and in normal aging [73]. In addition, cerebrospinal fluid (CSF) NEP activity is reduced in prodromal AD [74]. Thus, age-related decline of NEP activity in these specific regions is likely to promote the pathogenesis of sporadic AD. In transgenic mice studies, high NEP levels at an age before the appearance of amyloid plaque deposition led to the cleavage and removed of Aβ, and consequently to a delay of amyloid plaque deposition [75]; however, high NEP levels in aged transgenic mice that had already formed numerous amyloid plaques could not effectively reduce brain Aβ levels or remove the existing amyloid plaques from brains [76]. These findings indicate that NEP may prevent or delay the onset of AD, although it may be ineffective after onset. Recently, somatostatin has been reported to regulate the metabolism of brain Aβ42 through upregulation of neprilysin activity, suggesting that somatostatin receptors may be pharmacological targets for the prevention and treatment of AD [77].

Plasmin has also been reported to contribute Aβ degradation [67, 68]. Aβ can stimulate tissue plasminogen ac-

tivator (tPA), which activates plasminogen, resulting in plasmin generation and subsequent Aβ degradation *in vitro* and *in vivo* [67, 68]. However, tPA could also exert detrimental effects, such as Aβ neurotoxicity and tau phosphorylation [78].

Immunotherapy for Aβ

It was reported that selected monoclonal antibodies raised against Aβ inhibited the *in vitro* aggregation of Aβ and that the inhibitory effect appeared to be related to the localization of the antibody-binding sites and the nature of the aggregating agents [79]. Moreover, it was also shown that site-directed monoclonal antibodies against various Aβ fragments selectively disaggregated the fibrillar and amyloid-like assemblies, and reduced Aβ neurotoxicity [80]. Schenk et al. and others investigated the reduction in the deposition of Aβ in transgenic mice following immunization with pre-aggregated Aβ42 [81], and passive administration of antibodies raised against Aβ [82]. Both active immunization and passive administration of Aβ antibodies attenuated amyloid plaque deposition, neuritic dystrophy, astrogliosis and behaviour deficits in transgenic animals [81–87]. Although the mechanism of plaque clearance after active or passive immunization has not been established as yet, three different hypotheses have been tested: (i) small amounts of anti-amyloid antibodies reach amyloid deposits in the brain and trigger a phagocytic response by microglia [82], (ii) anti-amyloid antibodies act as a peripheral sink for soluble Aβ species, leading ultimately to the resolution of brain deposits depending on the dose of Aβ (peripheral sink) [83, 84], and (iii) anti-amyloid antibodies reach amyloid deposits in the brain and clear them directly through interaction of the antibody with the amyloid deposit [85].

On the basis of preclinical findings, a multicenter, randomized, double-blind, placebo-controlled trial of the vaccination with Aβ42 was performed; however, this trial was interrupted because of meningoencephalitis in 6% (18 of 300) of the immunized patients [88–90]. Some patients with meningoencephalitis underwent autopsy that showed infiltration of lymphocytes in the leptomeninges, in perivascular spaces and within the parenchyma [91, 92]. The brains from these patients contained only a few plaques, lacked plaque-associated dystrophic neurites and astrocyte clusters, and showed Aβ immunoreactivity associated with microglia in some regions that were devoid of plaques [91, 92]. These findings resemble the changes that were observed after Aβ immunotherapy in the transgenic mouse models. This implies that the immune response was generated against the peptide-elicited clearance of Aβ plaques in the AD patients. The investigations of clinical effects of vaccination with Aβ42 trial showed that cognitive performance was better and CSF tau levels were lower in antibody responders than placebo cases, suggesting a downstream neuropathologic benefit of targeting Aβ [89]. In the reports of brain magnetic resonance image from the same study, antibody responders had greater brain volume loss but better cognitive performance compared with placebo cases [90]. In this report, the volume loss in responders may be due in part to amyloid removal [90]. These findings indicate that Aβ immunotherapy may be useful in AD. Further trials with anti-Aβ antibodies and the development of more sophisticated Aβ immunotherapies without such side effects are ongoing [93].

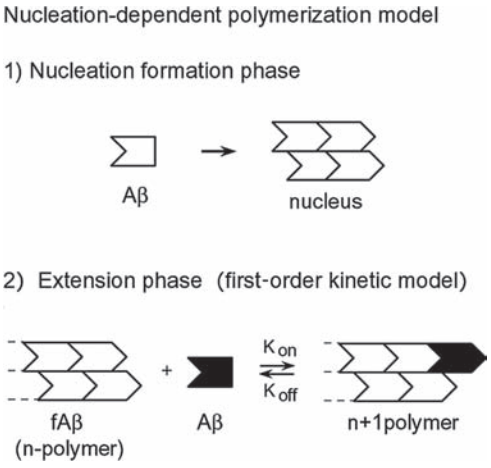

Figure 3. Nucleation-dependent polymerization model.

Inhibition of Aβ aggregation and destabilization of aggregated Aβ

Over the past decade, various compounds have been demonstrated to interfere with Aβ aggregation in several different assay formats; as an *in vitro* model, a nucleation-dependent polymerization model (Fig. 3) was frequently used since it is thought to model the mechanism of Aβ aggregation leading to the formation of Aβ fibrils (fAβ) [94]. In this section, we review recent developments re-

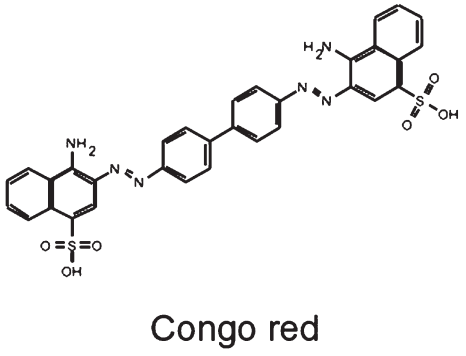

Figure 4. Structure of Congo red.

garding molecules that inhibit the formation and destabilization of fAβ.

Congo red

Congo Red (CR) (Fig. 4) has been reported to inhibit the toxicity of Aβ40 by blocking fibril formation and by binding to preformed fibrils and reducing their toxicity [95]. For Aβ42, it was shown that CR blocked the toxicity of fibrils but did not prevent their formation [95]. In another study, CR was reported to increase monomer/oligomer ratios in cell culture by stabilizing the Aβ monomer [96].

Cu/Zn chelators

Aβ possesses selective high- and low-affinity Cu^{2+} and Zn^{2+} binding sites [97, 98]. Several chelators were shown to inhibit and reverse Zn/Cu-induced aggregation of Aβ40 and Aβ42 *in vitro* [97–99]; one example is clioquinol (iodochlorhydroxyquin, 5-chloro-7-iodo-8-hydroxyquinone) (CQ), a quinone that selectively binds Zn^{2+} and Cu^{2+} with greater affinity than it binds Ca^{2+} and Mg^{2+} [99]. A 49% decrease in brain Aβ deposition was reported in a blinded study of APP2576 transgenic mice treated orally for 9 weeks with CQ [99]. This was accompanied by a modest increase in soluble Aβ (1.45% of total cerebral Aβ), whereas APP, synaptophysin and glial fibrillary acidic protein levels were unaffected [99]. Moreover, those authors tested CQ in a clinical intervention (a pilot phase 2), and found that the effect of treatment was significant in the more severely affected AD group [100].

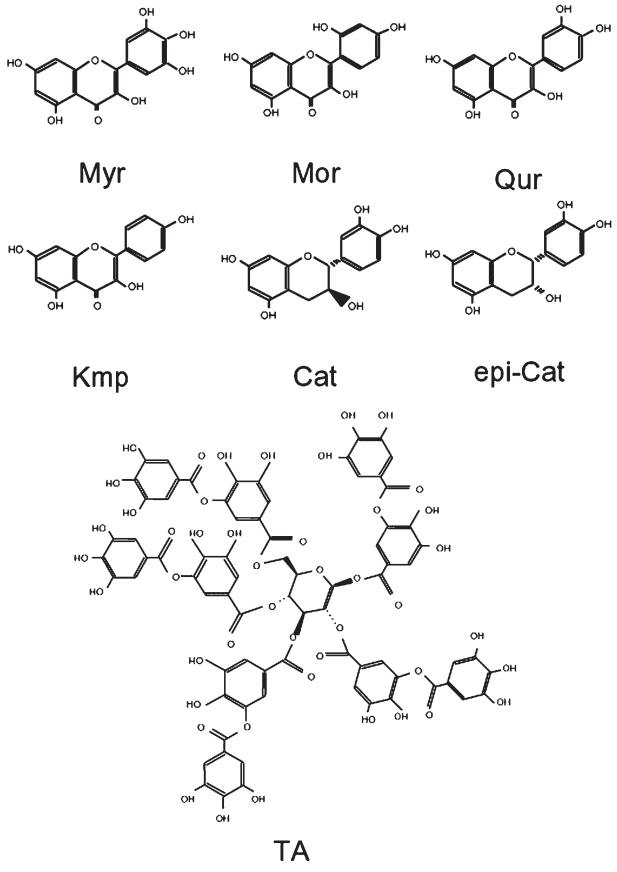

Figure 5. Structures of myricetin (Myr), morin (Mor), quercetin (Qur), kaempferol (Kmp), (+)-catechin (Cat), (–)-epicatechin (epi-Cat) and tannic acid (TA).

Polyphenols

Recently, several studies have suggested that many kinds of natural polyphenols may have anti-amyloidogenic effects. We examined the effects of wine-related polyphenols, myricetin (Myr), morin (Mor), quercetin (Qur), kaempferol (Kmp), (+)-catechin (Cat) and (–)-epicatechin (epi-Cat) (Fig. 5) on the formation, extension and

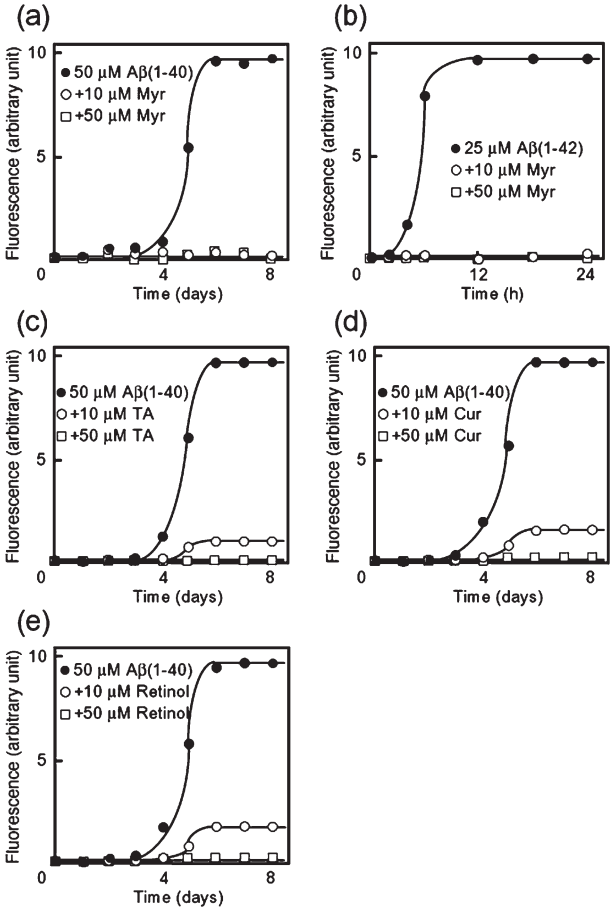

Figure 6. Effects of Myr (*a*, *b*), TA (*c*), Cur (*d*) and retinol (*e*) on the kinetics of fAβ(1-40) (*a*, *c–e*) and fAβ(1-42) (*b*) formation from fresh Aβ(1-40) and Aβ(1-42), respectively. Reaction mixtures containing 50 μM Aβ(1-40) (*a*, *c–e*) or 25 μM Aβ(1-42) (*b*), 50 μM phosphate buffer, pH 7.5, 100 μM NaCl, and 0 (λ), 10 (○) or 50 mM (□) of Myr (*a*, *b*), TA (*c*), Cur (*d*) or retinol (*e*), were incubated at 37 °C for the indicated times. Each figure is a representative curve from 1 of 3 independent experiments.

destabilization of fAβ *in vitro* [101]. All of the examined polyphenols dose-dependently inhibited the formation of fAβ from fresh Aβ40 and Aβ42, as well as their extension (Figs. 6a, b, 7a, b, 8, and data not shown) [101]. Moreover, these polyphenols dose-dependently destabilized preformed fAβs (Figs. 9a, b, 10, and data not shown) [101]. Cell culture experiments suggested that Myr-treated fAβ is less toxic than intact fAβ [101]. However, few reports on the effects of polyphenols on the fAβ burden *in vivo* are available at present.

Tannic acid (TA; Fig. 5) is a water-soluble polyphenol which differs from most other natural phenolic compounds in its ability to precipitate proteins such as gelatin from solution [102]. TA is commonly found in a large array of higher plant species of both herbaceous and woody types [103]. It can accumulate in large amounts (often more than 10% of dry weight) in particular organs or tissues that can include almost all plant parts: bark, wood, leaves, fruits and roots [103]. In our studies, TA

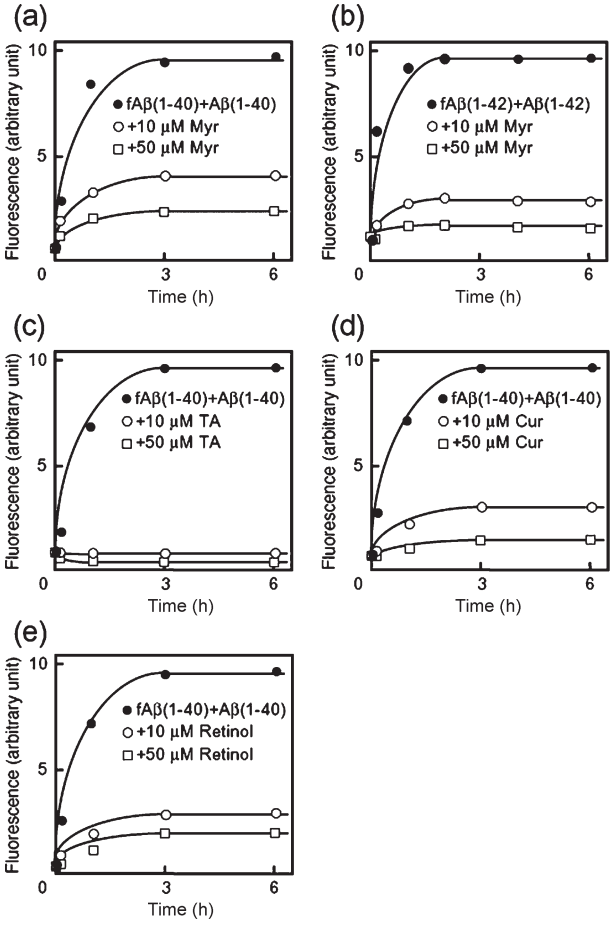

Figure 7. Effects of Myr (*a*, *b*), TA (*c*), Cur (*d*) and retinol (*e*) on the kinetics of fAβ(1-40) (*a*, *c–e*) and fAβ(1-42) (*b*) extension. Reaction mixtures containing 10 mg/ml (2.3 μM) sonicated fAβ(1-40) (*a*, *c–e*) or fAβ(1-42) (*b*), 50 μM Aβ(1-40) (*a*, *c–e*) or Aβ(1-42) (*b*), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (λ), 10 (○) or 50 μM (□) of Myr (*a*, *b*), TA (*c*), Cur (*d*) or retinol (*e*) were incubated at 37 °C for the indicated times. Each figure is a representative curve from 1 of 3 independent experiments.

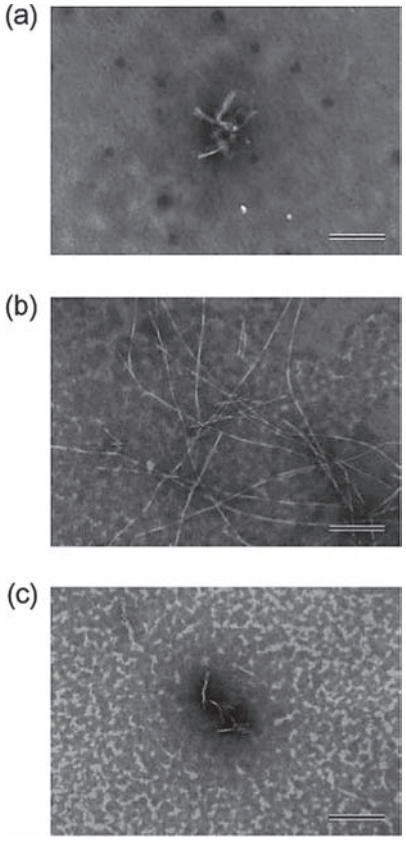

Figure 8. Electron micrographs of extended fAβ(1-40). Reaction mixtures containing 10 mg/ml (2.3 μM) fAβ(1-40), 50 μM Aβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (*b*) or 50 μM Myr (*a*, *c*), were incubated at 37 °C for 0 (*a*) or 6 h (*b*, *c*). Scale bars indicate 250 nm.

dose-dependently inhibited fAβ formation from fresh Aβ, in addition to destabilizing preformed fAβ *in vitro* (Figs. 6c, 7c, 9c, and data not shown) [104]. The potency of the anti-amyloidogenic and fibril-destabilizing effects of these polyphenols is in the order TA > Myr = Mor = Qur > Kmp > Cat = epi-Cat.

Furthermore, we examined the effects of nordihydroguaiaretic acid (NDGA), which is a pure compound isolated from the creosote bush, *Larrea tridentate*, curcumin (Cur), which derived from turmeric in yellow curry spice, and rosmarinic acid (RA), which is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae (Fig. 11). In our study, NDGA, Cur and RA dose-dependently inhibited the formation of fAβ from Aβ40 and Aβ42, and their extension, and destabilized preformed fAβs (Fig. 6d, 7d, 9d, and data not shown) [105, 106]. The potencies of the anti-amyloidogenic and fibril-destabilizing effects of these compounds are similar.

In addition to our studies, several other reports suggest that Cur, RA and NDGA could be key molecules for the development of therapeutics for AD. Cur protects PC12 and human umbilical vein endothelial cells from Aβ in-

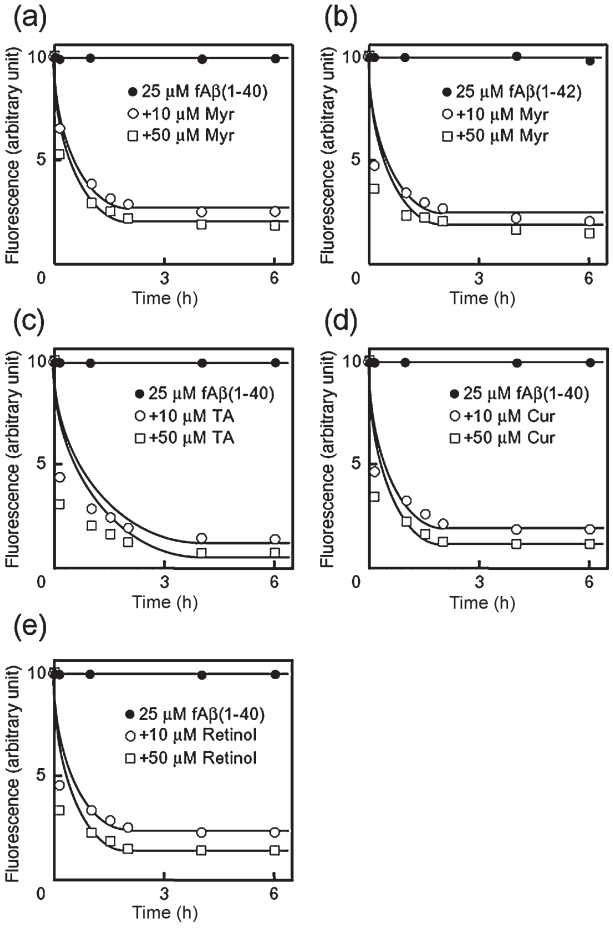

Figure 9. Effects of Myr (*a*, *b*), TA (*c*), Cur (*d*) and retinol (*e*) on the kinetics of fAβ(1-40) (*a*, *c–e*) and fAβ(1-42) (*b*) destabilization. Reaction mixtures containing 25 μM fAβ(1-40) (*a*, *c–e*) or fAβ(1-42) (*b*), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (λ), 10 (○) or 50 μM (□) Myr (*a*, *b*), TA (*c*), Cur (*d*) or retinol (*e*) were incubated at 37 °C for the indicated times. Each figure is a representative curve from 1 of 3 independent experiments.

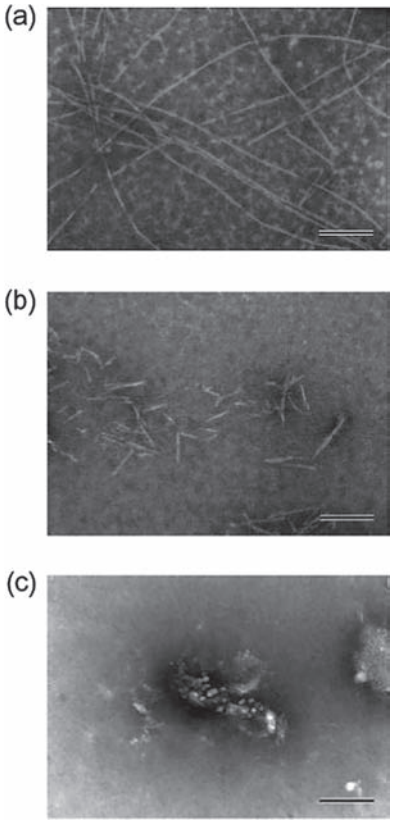

Figure 10. Electron micrographs of destabilized fAβ(1-40). Reaction mixtures containing 25 μM fAβ(1-40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl and 50 μM Myr were incubated at 37 °C for 0 (*a*), 1 (*b*) or 6 h (*c*). Scale bars indicate 250 nm.

sult as a result of its strong antioxidant properties [107]. NDGA prevents the cytotoxicity of Aβ to cultured rat hippocampal neurons by suppressing Aβ-induced accumulation of reactive oxygen species and intracellular free Ca^{2+} [108]. In an *in vivo* study with Tg2576 APPSw transgenic mouse, Cur suppressed indices of inflammation and oxidative damage in the brain, and low, nontoxic doses of Cur decreased the levels of insoluble and soluble Aβ and the plaque burden in many affected brain regions [109]. Moreover, Cur inhibits the formation of Aβ oligomers and fibrils, binds plaques and reduces plaque burden [110].

Nicotine

Previous reports suggested an inverse relationship between smoking and AD [111, 112]. This beneficial effect of smoking has been attributed to the protection of neurons by nicotine against Aβ toxicity [113, 114]. We and another group reported that nicotine inhibited fAβ40 and fAβ42 formation [115, 116], and destabilized preformed fAβ40 and fAβ42 [116]. It has also been reported that chronic nicotine treatment reduces Aβ deposits in the brain of Tg2576 mice [117]. In addition, there was a selective reduction in extractable Aβ in nicotine-treated mice; cortical insoluble Aβ40 and Aβ42 levels were lower, but there was no significant change in soluble Aβ40 or Aβ42 levels [117].

Rifampicin and tetracyclines

Rifampicin (RIF) and tetracyclines (TCs) are effective against the bacterium *Chlamydia pneumoniae*, which is recognized as a common cause of upper and lower respiratory infections (Fig. 12). RIF and TCs have also been shown to interfere with the accumulation of Aβ and the subsequent development of fAβ [105, 118–121], and to exert fibril-destabilizing effects *in vitro* [105, 120]. Similarly, it was shown that doxycycline not only inhibited

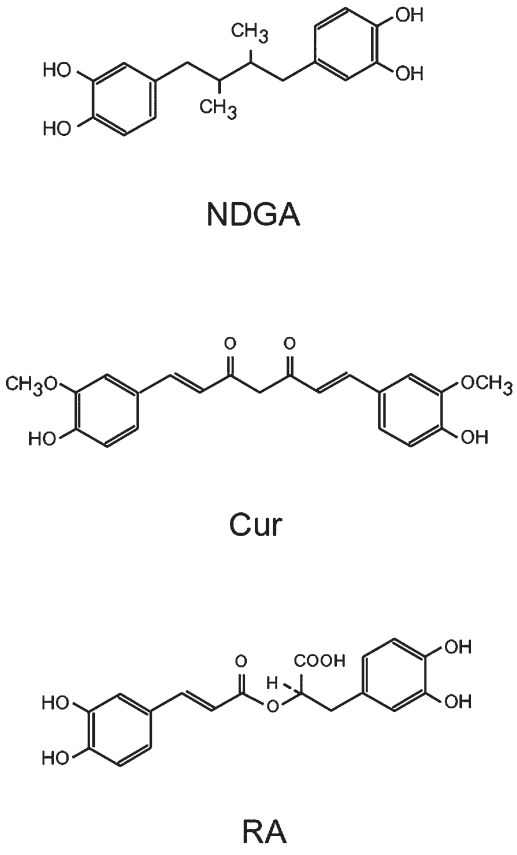

Figure 11. Structures of nordihydroguaiaretic acid (NDGA), curcumin (Cur) and rosmarinic acid (RA).

fAβ42 formation but also disassembled the preformed fibrils *in vitro* [121]. Clinically, a 3-month course therapy of RIF and doxycycline reduced cognitive worsening at 6 months of follow-up in patients with mild to moderate AD in a randomized, controlled trial [122].

Vitamins

A variety of antioxidant vitamins, such as vitamin E (DL-α-tocopherol) [123–127], vitamin C (ascorbic acid) [128, 129] and vitamin A [130, 131], have been suggested to reduce the oxidative stress associated with AD. Some groups reported that the concentrations of vitamins A, C, E and β-carotene in plasma, serum or cerebrospinal fluid are lower in AD patients than in controls, suggesting that these antioxidant vitamins and carotenoids may have been consumed as a result of excessive production of free radicals [132–135]. Clinically, treatment with these vitamins has been reported to delay the progression of dementia [130, 136–140].

Several mechanisms have been postulated to explain how these vitamins exert such effects, including the protection of neurons from Aβ-induced neurotoxicity [123–129]. Recently, we examined the effects of vitamin A (retinol, retinal and retinoic acid), β-carotene and vitamins B2, B6, C and E on the formation, extension and destabilization of fAβ *in vitro* [141]. Among them, vitamin A and β-carotene dose-dependently inhibited the formation of fAβ from fresh Aβ, as well as their extension (Figs. 6e, 7e and data not shown) [141]. Moreover, they dose-dependently destabilized preformed fAβs (Fig. 9e and data not shown) [141]. However, few reports describing the effects of vitamins on the fAβ burden *in vivo* are currently available.

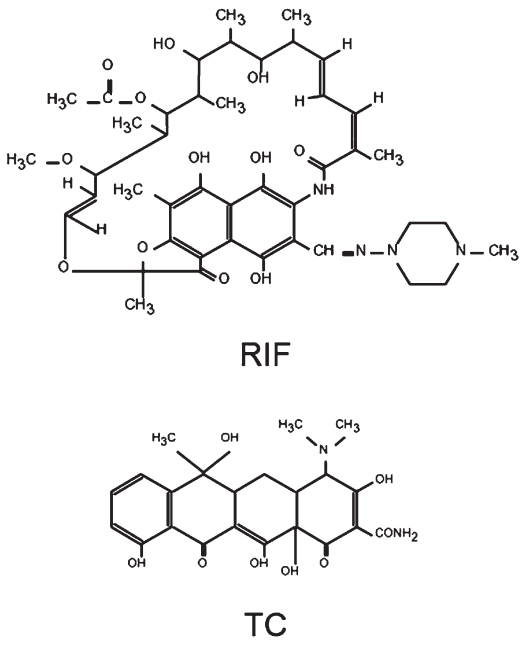

Figure 12. Structures of rifampicin and tetracyclines.

Melatonin

Melatonin is a pineal hormone secreted during the dark phase of the circadian cycle (Fig. 13) [142]. Various studies have shown that melatonin is decreased during the aging process [142] and that patients with AD have more profound reductions of this substance [143–145]. It has been shown that melatonin inhibits the formation of β-sheets and amyloid fibrils *in vitro* [146, 147]; however, in our *in vitro* study, melatonin exhibited no significant destabilizing activity toward preformed fAβ40 or fAβ42 [105]. Recently, it was reported that the administration of melatonin partially inhibited the expected time-dependent

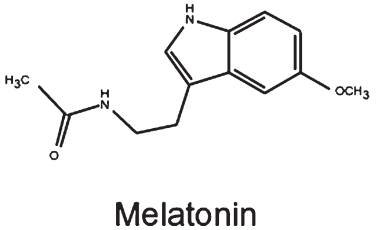

Figure 13. Structure of melatonin.

elevation of total brain Aβ levels, reduced the abnormal nitration of proteins, and increased survival in a transgenic mouse model of AD [148]. These results suggest that melatonin should be explored as a disease-modifying agent in AD. No controlled, double-blind studies are currently available concerning the preventive or therapeutic potential of melatonin in AD.

β-Sheet breaker peptide

Several *in vitro* studies have shown that β-pleated sheet Aβ fibrils are neurotoxic [95, 149, 150]. It has been postulated that fibrillogenesis can be inhibited by short peptides partially homologous to Aβ that contain residues acting as β-sheet breaker peptides and that an 11-residue β-sheet breaker peptide (iAβ11) binds to Aβ with high affinity and inhibits amyloid formation *in vitro* [151]. In addition, those authors demonstrated that a 5-residue β-sheet breaker peptide (iAβ5) inhibits Aβ fibrillogenesis, disassembles preformed fibrils *in vitro* and prevents neuronal death induced by fibrils in cell culture [152]. They described a modified iAβ5 with improved pharmacological properties, a high rate of penetration across the blood-brain barrier and the ability to induce a dramatic reduction in amyloid deposition in two different transgenic AD models [153]. Furthermore, they reported for the first time a significant increase in neuronal survival and a decrease in brain inflammation associated with the reduction of amyloid plaques [153]. These results suggest that iAβ might be candidates for AD therapy focused on reducing amyloid deposition. Recently, those authors assessed the neuroprotective effect of the chronic intraperitoneal administration of an iAβ5 on the rat behavioral deficit induced by intrahippocampal fAβ injection [154]. One month after the injection, animals showed a partial reduction of the amyloid deposits and a decreased astrocytic response around the injection site [154]. More important, the authors reported that following the iAβ5 treatment, hippocampal-dependent spatial learning paradigms, including the standard Morris water maze and a working memory analysis, showed a significant prevention of the impairments induced by Aβ deposits in the dorsal hippocampus [154]. Thus, it is possible that the administration of iAβ may be useful as a therapy for AD patients.

Heparan sulphate

Heparan sulphate proteoglycans (HSPGs) play an important role in amyloidogenesis through interactions with various proteins, and they are associated with the earliest stages of the formation of amyloid plaques [155]. It has been shown that heparan sulphate and sulphate ions accelerate fAβ formation *in vitro*, suggesting that small molecules which may interfere with heparan sulphate/amyloid interactions might prove useful as potential amyloid therapeutic agents [156]. It has been reported that small molecule anionic sulphonates or sulphates such as sodium 1,3-propanediol disulphates (1,3-PDS) inhibit the *in vitro* acceleration of fAβ formation by heparan sulphate, and lead to disassembly of preformed fAβ [157]. We also ascertained that 1,3-PDS and poly(vinylsulphonic acid, sodium salt) destabilize preformed fAβ at millimolar order *in vitro* [105]. It was speculated that small-molecule anionic sulphonates or sulphates may also be involved in binding the specific site of Aβ [157]. A low molecular weight heparin, which is a highly sulphated structural analogue of heparan sulphate, reduced Aβ plaque, cytotoxicity and proinflammatory activity in a transgenic mouse model of AD [158].

Conclusion

We review here various disease modification strategies for inhibiting the accumulation of Aβ in the brain. Some candidate therapies based on these strategies have been demonstrated to have anti-amyloidogenic effects *in vivo* as well as *in vitro*, and clinical trials are ongoing. Further understanding of the mechanisms underlying their anti-amyloidogenic effects will facilitate the development of more refined preventives and therapeutics for AD.

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