Perspectives of Pharmacotherapy in Alzheimer’s Disease

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ABSTRACT—Alzheimer’s disease (AD) is the most common cause of progressive decline of cognitive function in aged humans, and it is characterized by the presence of numerous senile plaques and neurofibrillary tangles accompanied by neuronal loss. The senile plaques are composed of amyloid β-peptides (Aβ), 40-42 amino acid peptide fragments of the β-amyloid precursor protein. Genetic, molecular biological and neuropharmacological evidence support the ‘amyloid cascade hypothesis’ for the pathogenesis of the disease. We review the in vivo effects of various compounds on behavioral and neuropathological changes in the non-transgenic animal models of AD produced by continuous i.c.v. infusion of Aβ. These results support therapeutic strategies such as cholinergic therapy, anti-inflammatory agents, antioxidants and estrogen replacement therapy, as well as other cognition enhancers for the treatment of AD. In addition, the amyloid cascade hypothesis offers a number of potential targets for novel therapeutic strategies in AD. We believe that our non-transgenic animal model, as well as transgenic animal models, are useful for developing novel pharmacotherapeutics in AD.

Keywords: Alzheimer’s disease, β-Amyloid, Animal model, Learning and memory

Alzheimer’s disease (AD) is the most common cause of progressive decline of cognitive function in aged humans, and it is characterized by the presence of numerous senile plaques and neurofibrillary tangles accompanied by neuronal loss. The extracellular senile plaques are composed of amyloid β-peptides (Aβ), 40-42 amino acid peptide fragments of the β-amyloid precursor protein (APP). There are several APP isoforms, resulting from alternative splicing, ranging from 563 to 770 amino acid residues. The most abundant isoform, APP695, is predominantly expressed in neurons and lacks a Kunitz-protease inhibitor domain present in the APP751 and APP770 isoforms. Two major species of Aβ defined by their carboxy-terminus lengths are the Aβ1-40 ending at Val40 and Aβ1-42 ending at Ala42. The intracellular neurofibrillary tangles are composed of highly phosphorylated tau proteins (1, 2).

The amyloid cascade hypothesis of AD proposes that Aβ, especially Aβ1-42, triggers a neurotoxic cascade, thereby causing neurodegeneration and AD (Fig. 1) (1, 2). It was demonstrated that Aβ1-42, not Aβ1-40, is the initially deposited species. Diffuse plaques, the earliest forms of senile plaques, consist exclusively of Aβ1-42 (3). Aβ is known to possess neurotoxicity in vitro, and fibrillar but not soluble or amorphous-aggregated Aβ induces hyperphosphorylation of tau proteins and loss of microtubule binding in cultured neurons. The results suggest that amyloid fibril formation is a cause of abnormal tau phosphorylation in AD (4).

Gene mutations in AD

There are three distinct genes that cause AD: the APP gene on chromosome 21, presenilin 1 (PSI) gene on chromosome 14 and presenilin 2 (PS2) gene on chromosome 1. A small percentage of early-onset familial AD cases are due to mutations in the APP gene. A total of five mutations have been described in the APP gene that lead to AD. The effects of the APP mutations on APP processing have been studied extensively, and the results demonstrate that the mutations of APP lead to an increase in the levels of Aβ1-42 or the total Aβ levels. The majority of early-onset familial AD, however, appears to be caused by the PS mutations. More than 40 mutations in the PS genes have been reported. Molecular biological studies

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Fig. 1. The amyloid cascade hypothesis of Alzheimer’s disease and its pharmacological modulation. \(\text{A}\beta\) is derived through the processing of the precursor protein APP. PS1 is involved in \(\gamma\)-secretase-mediated proteolytic cleavage of the C-terminal transmembrane fragments of APP after their generation by \(\alpha\)- and \(\beta\)-secretases. \(\text{A}\beta_{1-42}\), not \(\text{A}\beta_{1-40}\), is the initially deposited species. \(\text{A}\beta\) possesses neurotoxicity in vitro, and fibrillary but not soluble \(\text{A}\beta\) induces hyperphosphorylation of tau proteins and loss of microtubule binding in cultured neurons. Oxidative stress is involved in its neurotoxicity. \(\text{A}\beta\) at very low concentrations (picomolar to nanomolar) directly inhibits various cholinergic neuronal functions (acetylcholine release and synthesis and choline uptake) independently of apparent neurotoxicity. The \(\text{A}\beta\) cascade could be modulated by various factors, including APOE, estrogens and neurotrophic factors.
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demonstrate that PS mutations alter APP processing, resulting in an increase of Aβ1–42 (1, 2). These findings support the amyloid cascade hypothesis of AD (1, 2).

There is an association between AD and apolipoprotein E genes (APOE) on chromosome 19. There are three major alleles of APOE: APOE2, APOE3 and APOE4. APOE4 is a susceptible gene of AD or risk factor in both sporadic and familial late-onset of AD. APOE binds to soluble Aβ in vitro and promotes amyloid fibril formation in an isoform-specific manner, with APOE4 promoting Aβ fibrillogenesis more potently than APOE3 (1).

Transgenic animal models of AD

The transgenic animal model of AD that shows extensive AD-like neuropathology was first generated using a platelet-derived growth factor (PDGF)-β promoter driving a human APP minigene encoding APP (Val17→Phe) mutation (5) associated with familial AD. The transgenic mice show numerous extracellular thioflavin S-positive Aβ deposits, neuritic plaques, synaptic loss, astrocytosis and microgliosis. Aβ deposition in these mice is associated with neuropil changes, but not with overt neuronal loss (6).

Transgenic mice overexpressing human APP751, which develop early AD-like histopathology with diffuse deposits of Aβ and aberrant tau protein immunoreactivity in some cases (7), exhibit age-dependent deficits in spatial learning in a water maze task and in spontaneous alternation behavior in a Y-maze (8). The transgenic mice expressing human APP695 containing the double mutation Lys670→Asn, Met671→Leu, which was found in a large Swedish family with early-onset familial AD, also show age-dependent deficits in spatial learning in a water maze task and in spontaneous alternation behavior in a Y-maze, Aβ elevation and amyloid plaques (9). In transgenic mice with the Swedish mutation of APP, the protein levels of both CuZn superoxide dismutase (CuZnSOD) and hemoxygenase-1 (HO-1), two markers of oxidative stress, are increased in the aged animals, suggesting an association of in vivo neurotoxicity of Aβ and oxidative stress (10). The “APP 23” transgenic mice with the Swedish mutation (11) develop almost exclusively congophilic plaques accompanied by neuritic changes and dystrophic cholinergic fibers, suggesting a causal link between Aβ deposition and cholinergic degeneration. Most importantly, plaques are immunoreactive with the Alz50 antibody for hyperphosphorylated tau proteins, reminiscent of early tau pathology in AD (11).

Aβ-induced learning and memory deficits

Several studies have demonstrated that intracerebral injection of Aβ causes brain dysfunctions as evidenced by neurodegeneration and an impairment of learning and memory (12–16), although neurotoxic effects of Aβ in vivo have been somewhat controversial. The in vivo neurotoxicity of Aβ is potentiated by co-injection with ibotenic acid (17) as previously described in vitro (1).

To mimic the slow evolution of AD, we have used the technique of a continuous i.c.v. infusion of Aβ with a mini-osmotic pump. In the initial study, Nitta et al. (12) demonstrated that a continuous i.c.v. infusion of Aβ1–40 at a dose of 300 pmol/day caused a significant impairment of spatial reference memory formation in a water maze task and a deficit of passive avoidance performance that was accompanied by a slight but significant reduction of choline acetyltransferase (ChAT) activity in the hippocampus. Accumulation of Aβ1–40 in the hippocampus and cerebral cortex was evident immunohistochemically following a 14-day period of infusion. Impairments of performance in the Aβ1–40-infused rats in both water maze and passive avoidance tasks were recovered 2 weeks after the cessation of the infusion although the reduction of ChAT activity and an increase in glial fibrillary acidic protein (GFAP) immunoreactivity were present 2 weeks after the cessation of infusion (13).

We carefully examined the effect of continuous i.c.v. infusion of Aβ1–42 on spatial reference and working memory in the water maze task (18, 19). The standard water maze task, in which a rat is required to locate a submerged platform, measures predominantly spatial reference memory. The performance of the Aβ1–42-treated rats in the standard water maze task was impaired significantly compared with that of Aβ40–1–infused rats, but the deficits were relatively small. The probe test, in which the platform is removed from the pool and performance is largely independent of swimming ability and speed, requires memory for the precise location of the platform, so it more reliably assesses the accuracy of spatial reference memory than the standard training. In the probe test, we observed a marked deficit in the Aβ1–42-treated rats compared with Aβ40–1–treated animals, providing additional evidence that continuous i.c.v. infusion of Aβ1–42 results in a significant impairment of spatial reference memory.

Working memory refers to memory based on new information gained within a testing session. By the repeated acquisition test of the water maze task, we demonstrated that accumulation of neurotoxic Aβ1–42, but not Aβ40–1, in the brain caused an impairment of spatial working memory, as evidenced by an increase in the escape latency in the 2nd trial without affecting the performance in the sample trial (1st trial).

Neurochemical and neurophysiological effects of Aβ in vivo

One of the fundamental features of AD is the widespread degeneration and dysfunction of the basal-
forebrain cholinergic system. Recent studies demonstrated that Aβ at very low concentrations (picomolar to nanomolar) directly inhibits various cholinergic neuronal functions (acetylcholine release and synthesis and choline uptake) independently of apparent neurotoxicity, suggesting a possible link between the Aβ burden and cholinergic dysfunction in AD (20). We showed, by using an in vivo brain dialysis technique, that KCl- and nicotine-induced increases in ACh and dopamine releases in the hippocampus/cerebral cortex and the striatum, respectively, are markedly impaired by the continuous infusion of Aβ1–40, although the basal levels of these neurotransmitters in the Aβ1–40-infused rats did not differ from those in vehicle-infused control animals (21). Harkany et al. (15) showed that bilateral injection of β-amyloid (Phe(SO3H)25–35 peptide, a metabolically stable analogue of Aβ25–35, into the rat nucleus basalis magnocellularis caused a reduction of cortical acetylcholinesterase (AChE)-positive projections. An in vitro experiment showed that Aβ25–35 activates tau protein kinase I / glycogen synthase kinase β, leading to an inactivation of pyruvate dehydrogenase, and causes an impairment of ACh synthesis without affecting ChAT activity (22). In light of these previous findings, it is suggested that dysfunctions of cholinergic and dopaminergic neuronal systems may be responsible, at least in part, for the Aβ-induced learning and memory deficits.

Long-term potentiation (LTP) in the hippocampus following a brief high-frequency stimulation is considered to be a synaptic correlate of memory. We prepared brain slices 10 or 11 days after the start of Aβ1–40 infusion, and a tetanic stimulation was applied to induce LTP. The enhanced response after the tetanic stimulation was maintained for more than 45 min in the control slices, whereas it declined rapidly to nearly baseline levels in the Aβ1–40-treated group (23). It is also reported that i.c.v. injection of Aβ1–40, Aβ1–42 or C-terminal fragment of APP greatly shortens the hippocampal LTP in vivo (24), although acute short-term treatment of brain slices with Aβ1–40 increased LTP in the hippocampus in vitro (25).

Pharmacotherapy of Aβ-induced learning and memory deficits

Although the exact pathogenesis of AD remains to be fully defined, several pharmacological strategies for the treatment of AD are under active investigation. These include the cholinergic therapy that is designed to increase cholinergic functions, anti-inflammatory agents, antioxidants and estrogen replacement therapy. The cholinergic therapy involves precursors of ACh, AChE inhibitors and muscarinic and nicotinic receptor agonists, etc.

Cholinergic therapy: Tacrine is the first AChE inhibitor that has been approved for treatment of AD. At present, three AChE inhibitors (tacrine, donepezil and rivastigmine) have been approved for the treatment of AD. It is reported that tacrine as well as (-)-nicotine ameliorate the Aβ25–35-induced impairment of performance of mice in the Y-maze, passive avoidance and water maze tests (14). Activation of sigma receptors, which results in an increase of ACh release in the cerebral cortex and hippocampus, also causes an improvement of performance in the Aβ25–35-treated mice (26). These results support the cholinergic therapy for AD.

Anti-inflammatory agents: Inflammation appears to contribute to the pathogenesis of AD since cytokines and other proteins associated with inflammation are found in the brains of AD patients but not in the brains of age-matched controls. Furthermore, epidemiological studies demonstrate that previous use of corticosteroid or non-steroidal anti-inflammatory drugs (NSAID) reduces the risk of AD (27). Netland et al. (16) recently demonstrated that continuous i.c.v. infusion of Aβ causes microglial activation and that indomethacin significantly attenuates the microglial response to Aβ, supporting the potential benefits of NSAID against AD. Recent evidence that a mitogen-inducible cyclooxygenase-2 is upregulated in AD brains represents a possible therapeutic target of NSAID. On the other hand, we found that the immunosuppressant FK-506 shows protective effects against Aβ1–40-induced impairment of performance in habituation and water maze tasks (unpublished observation).

Antioxidants: Several lines of evidence suggest that oxidative stress is important in the pathogenesis of AD and that antioxidants protect neurons from Aβ-induced toxicity (1). However, the bulk of the evidence for a neurotoxic role of Aβ comes from in vitro data, and it is not clear whether such toxicity exists in vivo.

We have recently provided the first in vivo evidence that oxidative stress is involved in Aβ-induced learning and memory impairments (18). We demonstrated that the repeated administration of antioxidants, idebenone and α-tocopherol, prevents learning and memory deficits in rats continuously infused with Aβ1–42 into the cerebral ventricle. We consider that clinical trials of antioxidants such as idebenone for the treatment of AD are warranted.

Estrogen replacement therapy: Epidemiological studies have indicated that the prevalence of AD after age 65 is two to three times higher in women than men, suggesting gender difference as a risk of AD. Studies also indicated that replacement therapy with estrogens in postmenopausal women delays the onset and decreases the risk of AD. Although the mechanisms by which estrogens affect the pathogenic processes in AD are still unknown, estrogens were demonstrated to modulate cholinergic neuronal activity, monoamine metabolism and the expression of brain-derived neurotrophic factor (BDNF)
mRNA in the brain. Estrogens have also been shown to attenuate excitotoxicity, oxidative injury and Aβ toxicity, to regulate APP metabolism, and to reduce the neuronal generation of Aβ (28).

We recently observed that the Aβ1-42-induced working memory deficits in a water maze task were significantly potentiated in ovariec tomized rats compared with sham-operated rats when mnemonic ability was examined 3 months after ovariectomy. Replacement therapy with 17-β-estradiol partially prevented some aspects of the Aβ1-42-induced impairment of performance in ovariec tomized rats (unpublished observation). These results indicate a potential role for estrogens in the development of Aβ-induced spatial working memory impairment in rats. Our findings provide evidence that the loss of estrogens at menopause may increase the risk of AD.

Other strategies: We have reported that repeated daily administrations of different types of cognition enhancers improve Aβ-induced learning and memory impairments. These include propentofylline (29), which stimulates the synthesis/secretion of nerve growth factor (NGF) in mouse astrocytes and inhibits gliosis induced by Aβ, an active fragment analog of arginine vasopressin NC-1900 (30) and neferacetam (19). It is noteworthy that repeated oral administration of neferacetam was started 7 days after the start of Aβ infusion, whereas the administration of propentofylline and NC-1900 was started 3 days before Aβ infusion. Although the mechanism of action of neferacetam is not completely understood, we speculate that it ameliorates the Aβ-induced learning and memory deficits at least in part by activating voltage-sensitive calcium channels and thereby improving dysfunction of cholinergic and dopaminergic neurons in this animal model of AD (19). In addition, modulation of GABAergic and nicotinic ACh receptor currents and stimulation of ChAT and glutamate decarboxylase activities and muscarinic ACh receptor binding may be involved in the effect of neferacetam. We consider that these three compounds may be useful for the treatment of patients with AD and that their clinical trials for the treatment of AD are warranted.

The anti-Aβ strategies in AD, which refers to intervention in the amyloid cascade of the disease, are the most attractive approach for the treatment of AD. The amyloid cascade hypothesis offers a number of potential targets for novel therapeutic strategies in AD. For instance, compounds that diminish or block the Aβ production and its aggregation/deposition are plausible as novel pharmacotherapeutics. Activation of the degradation/clearance of Aβ may provide some beneficial effects. Alternatively, inhibition of phosphorylation of tau proteins could be another strategy.

In conclusion, experimental evidence obtained with the non-transgenic animal models of AD produced by continuous i.c.v. infusion of Aβ supports the use of therapeutic strategies such as cholinergic therapy, anti-inflammatory agents, antioxidants and estrogen replacement therapy, as well as other cognition enhancers for the treatment of AD. In addition, the amyloid cascade hypothesis offers a number of potential targets for novel therapeutic strategies in AD. We believe that our non-transgenic animal model, as well as transgenic animal models, are useful for developing novel pharmacotherapeutics in AD.

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