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Current therapeutic strategy in Alzheimer's disease

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Abstract. – Alzheimer's disease (AD) is a chronic, progressive, neurodegenerative disorder that places a substantial burden on patients, their families, and society. Alzheimer's disease (AD) is the sixth leading cause of all deaths in the United States, and the fifth leading cause of death in Americans aged 65 and older. During the past years, several agents have been approved that enhance cognition and global function of AD patients, and recent advances in understanding AD pathogenesis has led to the development of numerous compounds that might modify the disease process. A wide array of antiamyloid and neuroprotective therapeutic approaches are under investigation on the basis of the hypothesis that amyloid beta (Aβ) protein plays a pivotal role in disease onset and progression and that secondary consequences of Aβ generation and deposition, including tau hyperphosphorylation and neurofibrillary tangle formation, oxidation, inflammation, and excitotoxicity, contribute to the disease process. Interventions in these processes with agents that reduce amyloid production, limit aggregation, or increase removal or vaccination and immunization might block the cascade of events comprising AD pathogenesis. Reducing tau hyperphosphorylation, limiting oxidation and excitotoxicity, and controlling inflammation might be beneficial disease-modifying strategies. Potentially neuroprotective and restorative treatments such as neurotrophins, neurotrophic factor enhancers, and stem cell-related approaches are also under investigation.

Key Words:
Alzheimer's disease, Inflammation, Hyperphosphorylation, Neuroprotection.

Introduction

Nearly a century has passed since Alois Alzheimer provided his meticulous description of the impaired cognitive performance and neuropathological analysis of his patient "Auguste". His observations still guide expanding efforts in both clinical medicine and basic research to uncover the pathogenesis of the brain degeneration and, ultimately, develop therapeutic interventions that prevent or slow progression of Alzheimer's disease. Alzheimer's disease (AD) is a challenging neurodegenerative disorder in elderly causing dementia characterized clinically by progressive memory loss and other cognitive impairments. The research in AD is expanding exponentially and currently aiming at clinical, cellular molecular, genetics and other therapeutic research approaches[1]. AD is not simply short memory loss but also results in other cognitive symptoms such as memory loss, disorientation, confusion, problems with reasoning and thinking and behavioral symptoms such as agitation, anxiety, delusions, depression, hallucinations, insomnia and wandering. Neuropathologically, presence of extra neuronal plaques and intraneuronal neurofibrillary tangles two characteristic lesions in post-mortem brain, where as clinically generalized progressive dementia are the major hallmarks of AD[2]. Increased neuronal iron in an active redox state, increased nitric oxide (NO) synthesis in microglia and abnormalities in mitochondrial genome are assumed as additional contributory sources. Also lipid peroxidation (LPO) a hallmark of oxidative tissue injury has been found to be elevated in the AD brain[3]. An understanding of these underlying mechanisms will certainly form the basis for devising better strategies for diagnosis, prevention and treatment. During the last couple of years, much has been learned about factors that may contribute to the onset of AD[3].

Antiamyloid Approaches

Proteolytic processing enzymatically of transmembrane amyloid precursor protein (APP)

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forms Aβ peptides. According to amyloid hypothesis these Aβ peptides initiate the process leading to neuronal dysfunction and death in patient suffering from AD. No anti-amyloid treatment options are currently available, but several are under active investigation.

Vaccination and Immunization Therapies

Studies so far have shown that passive transfer of Aβ monoclonal antibodies from vaccinated mice to AD model mice reduced cerebral amyloidosis[4,5]. Such effects are suggested to be mediated at least partially by reactive microglia that became activated to engulf antibody decorated Aβ via Fc receptor mediated phagocytosis[4]. Passive transfer of Aβ-antibodies discouraged the active immunization which is potentially unsafe and damages autoaggresive CD4+T cells response to show aseptic meningoencephalitis in a small percentage of patients. So, passive immunization has emerged as alternative to active immunization[4]. The first efficacy analysis conducted in a small subset of AN1792-treated patients showed antibodies generated against Aβ and significantly slower rates of decline in cognitive function and activities of daily living[6]. Recent studies of using combinations in 3X TG AD mouse model reported amelioration of behavioral deficits clearance of cerebral amyloidosis and reduction of soluble hyperphosphorylated tau proteins[7,8]. AN 1792 may have provoked brain inflammation in a small subset of treated individuals owing to the use of pro-inflammatory Th-1 adjuvant (QS-21)[9].

However, Aβ vaccine with a full length 1-40 administered by a different route (intranasal route) produced significant Aβ antibodies tities and have effectively reduced cerebral Aβ /plaque levels in the PDAAP mouse model[10,11]. The antibodies, thus, produced were largely of IgG1 and IgG2b isotypes widely recognized as the B- cells epitopes. Studies in which transcutaneous Aβ vaccination with a full length of Aβ along with cholera-toxin to PSAPP mouse model where brain to blood efflux was noted and reduction of cerebral Aβ levels by 50% have been reported. Furthermore, no induction of micro hemorrhage and nil aseptic inflammation which were earlier reported after passive Aβ immunization in AD mice were observed[12-14]. Several trials with passive immunization or vaccination with selective Aβ monoclonal antibodies are underway[15]. Immunoglobulin G (IgG) contains anti-Aβ antibodies, and passive immunization of AD patients with IgG has been tested in a preliminary clinical trial[16]. This novel approach in current theme of research and further detailed studies of this approach are planned.

β-Secretase Inhibitors

β-Secretase, a member of the pepsin family, is a membrane-anchored aspartyl protease[17]. Aβ is generated from APP by β and γ-secretase-mediated cleavage. The therapeutic potential of β-secretase inhibition, with limited mechanism-based toxicity, has been suggested by studies conducted in β-site APP cleaving enzyme 1 (BACE-1) knockout mice, which were shown to produce much less Aβ from APP[18,19]. Injection of the β-secretase inhibitor KMI-429 into the hippocampus of APP transgenic mice significantly reduced Aβ production *in vivo*[20]. Development of β-secretase inhibitors is challenging because of constraints of the active site; however, several small molecule agents are under active investigation[21].

γ-Secretase Inhibitors

Reductions in Aβ levels in the brain, cerebrospinal fluid (CSF), and plasma have been reported in rodents treated with the γ-secretase inhibitors DAPT[22-24]. Acute treatment with DAPT at a dose that reduced Aβ concentrations in the brain attenuated cognitive impairment in a transgenic mouse model of AD, with no effect on performance in controls[25]. This study suggested that cognitive impairments in AD might be associated with Aβ, potentially in advance of plaque formation, and might be reversible with acute pharmacologic treatment. In a randomized, controlled clinical trial conducted in 70 patients with mild to moderate AD, plasma Aβ 1-40 decreased by 38% with administration of LY450139 di-hydrate for 6 weeks, whereas CSF Aβ1-40 levels showed no significant change[26]. Treatment with the γ-secretase inhibitor was well-tolerated. Further investigation is needed to determine whether higher doses will yield more beneficial changes in Aβ concentrations without an increase in toxicity. Safety in γ-secretase inhibitor trials is closely scrutinized because agents with limited selectivity might affect proteins beyond Aβ-related γ-secretase, such as Notch, and might have deleterious effects on the gastrointestinal tract, thymus, and spleen[27].

γ-Secretase Modulators

Chronic CHF5074 treatment reduced brain b-amyloid burden, associated microglia inflammation and attenuated spatial memory deficit in

hAPP mice. This novel γ-secretase modulator is a promising therapeutic agent for Alzheimer's disease. In epidemiologic investigations, traditional nonsteroidal anti-inflammatory drugs (NSAIDs) have been associated with a significantly reduced risk of AD[28]. Negative findings from recent controlled clinical trials of individual NSAIDs suggest that protection against AD is not a benefit provided by the entire class[29]. Tarenflurbil (MPC-7869) modulates γ-secretase to produce less of the toxic form of Aβ (Aβ42) and more of the nontoxic shorter length peptide. Tarenflurbil reduces Aβ production by human cells and reduces plaque burdens in transgenic mouse modules of AD[30].

A phase II trial of tarenflurbil conducted in 207 patients with mild or moderate AD showed no overall benefit after 12 months of treatment[31]. In subgroup analyses, patients with mild AD who received the highest dose and had the highest blood levels of the drug exhibited a significant benefit in activities of daily living and global function. Furthermore, an analysis of adverse events showed a delay of almost a year in the onset of behavioral symptoms in the patients receiving tarenflurbil when compared with the placebo-treated group[32]. An ongoing phase III study is further testing the potential utility of this agent.

Antifibrillization Agents

Strategies targeting the fibrillary aggregates of Aβ protein are being explored. In neuronal cell cultures, the sulfated glycosaminoglycan mimetic tramiprosate (NC-531) was found to maintain Aβ in a non-fibrillar form and reduce Aβ42-induced cell death[33]. In transgenic mice, tramiprosate treatment significantly reduced amyloid plaque load and soluble and insoluble Aβ40 and Aβ42 levels in brain. In a 3-month, double-blind stage of a phase II trial conducted in 58 patients with mild to moderate AD, tramiprosate-treated patients exhibited dose-dependent reductions in CSF Aβ42 levels [34-35]. Treatment appeared to be well-tolerated, with no reports of serious adverse events. Nausea and vomiting were the most common side effects and occurred in 10% to 12% of patients. Results from the open-label extension of this trial suggested slowing of cognitive decline in patients with mild disease who were treated for up to 36 months. Two randomized, double-blind, placebo-controlled, 18-month, phase III clinical trials are underway in North America and Europe to further evaluate the safety and efficacy of this treatment in mild to moderate AD. Trials of other antifibrillization agents are planned. Other small molecule inhibitors of Aβ aggregation (scylloinositol; AZD-103) have been identified[36]. These stabilize Aβ in non-toxic non-fibrillar complexes. They rescue long-term potentiation in transgenic mice and reverse the memory deficit in rats observed after intra ventricular Aβ infusion. This method has promise for acute improvement in AD as well as reduction of the chronic effects of Aβ. Apolipoprotein E4 is an established risk factor for AD and might exert its effect through enhancing Aβ aggregation. Small molecules that alter the conformational structure of E4 to mimic E3 might ameliorate the E4 effect and are being assessed for their therapeutic potential[37].

Statins (Inhibitors of Cholesterol Biosynthesis)

A gene that is associated with AD is apolipoprotein E (APOE), which encodes for the lipid-binding protein APOE. This gene has three allelic variants: APOE2, APOE3 and APOE4. Individuals who have one or two copies of APOE4 are at higher risk for AD, whereas carriers of the APOE2 allele have a lower risk compared with the general population. Epidemiological studies, which indicate that there might be a connection between high cholesterol levels during mid-life and an increased risk of AD later in life. Moreover, people taking statins drugs that inhibit cholesterol biosynthesis have a remarkably reduced risk of developing AD[38,39]. These drugs are widely used and are safe for long-term treatment, so the possibility that they might prevent or delay the onset of AD has generated considerable excitement. Adding a further connection to the amyloid hypothesis, rabbits that were fed cholesterol had elevated brain Aβ levels and developed amyloid plaques. Furthermore, mammalian cells that were treated with statins produced much less Aβ 65,66 with similar results being observed in the plasma and cerebrospinal fluid of guinea pigs given high doses of statins[40]. Although the molecular mechanism of the effect of the statins on Aβ production is unclear, they might alter the ability of all three major secretases to cleave APP. Processing by α-secretase is apparently increased, whereas depletion of cholesterol inhibits γ-secretase activity in buoyant membrane micro domains.

It is possible that modulation of cholesterol levels might change membrane fluidity to affect these membrane-associated proteases and their APP substrates, and/or that changes in choles-

terol might affect Aβ deposition or clearance. Interestingly, by blocking the conversion of free cholesterol to cholesterol esters results in similar effects on Aβ production in cell culture[41,42]. This process is carried out by acyl-coenzyme A: cholesterol acyltransferase (ACAT) as part of the regulation of cholesterol homeostasis. Pharmacological inhibition or genetic mutation of ACAT results in a reduction of total Aβ as well as Aβ42, indicating that the effects of cholesterol on APP processing might be mediated through cholesterol esters, although a role for a more direct effect of free cholesterol cannot be ruled out.

A substantially reduced risk of AD has been reported in some (but not all) observational studies of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) used for the treatment of dyslipidemia[43,44] . The effect of statins on AD might not be mediated by their cholesterol-lowering properties. Rather, they might decrease Aβ by increasing processing of APP through the γ-secretase pathway[45,46]. In a recent randomized, double-blind, placebo-controlled pilot study in 63 individuals with normal cholesterol levels and mild to moderate AD, atorvastatin provided some clinical benefit after treatment periods of 6 months and 1 year[47]. However, several trials found no association with statin use and subsequent AD onset or cognitive decline[48,49]. Underscoring the need for further research with careful attention to study design and methodology. The U.S. National Institute on Aging (NIA) is sponsoring the Cholesterol Lowering Agent to Slow Progression (CLASP) of AD study to investigate the safety and effectiveness of simvastatin in slowing progression in patients with mild to moderate AD. Industry-sponsored, large phase III studies of atorvastatin in patients with mild to moderate AD are ongoing as well.

Peroxisome Proliferator-Activated Receptor Agonists

Insulin abnormalities and insulin resistance might contribute to the neuropathology and clinical symptoms of AD[50]. Transgenic mice fated to develop AD-type pathology are more likely to develop insulin resistance and have impaired regulation of insulin and glucose levels with age than wild-type mice[51]. The thiazolidinedione rosiglitazone increases peripheral insulin sensitivity through its effects on peroxisome proliferator-activated receptor-gamma (PPAR). In transgenic mouse brain, rosiglitazone has been shown to attenuate reductions in insulin-degrading enzyme (IDE) mRNA and decrease Aβ42 levels[52]. IDE's dual role in insulin degradation and Aβ metabolism might help explain the benefit of PPAR-γ agonists; reductions in insulin levels might liberate IDE to metabolize Aβ. Rosiglitazone-treated animals exhibited improved spatial learning and memory abilities when compared with controls. In a preliminary randomized, double-blind, placebo-controlled study conducted in 30 individuals with mild AD or amnestic mild cognitive impairment (MCI), patients receiving rosiglitazone exhibited better cognitive performance on selected instruments after 4 and 6 months than patients receiving placebo[53]. AD subjects without an APOE e4 allele showed significant improvement with rosiglitazone compared with those with E4. No change from baseline plasma Aβ levels was observed with rosiglitazone treatment at 6 months, but decreases were seen with placebo, suggesting a beneficial impact on disease progression. A second study found a similar response profile limited to those without the E4 genotype, Pioglitazone, another PPAR-γ-agonist, is also in clinical trials for mild-moderate AD[54,55]. Insulin itself might improve memory in AD. Craft[56] demonstrated that intranasal insulin resulted in improvement in delayed recall in AD and MCI subjects.

Metal-Protein Attenuating Compounds

Aβ and APP interact with the biometals zinc, copper, and iron, indicating these metals play a role in Aβ aggregation and cytotoxicity[57]. Metal-protein attenuating agents such as clioquinol were tested to determine their effects on Aβ activity in animal and human studies. A study of APP transgenic mice treated with clioquinol showed a 49% decrease in Aβ brain deposition after 9 weeks, without systemic toxicity[58]. Clioquinol was associated with subacute myelo-opticoneuropathy when administered as a systemic antibiotic in Japan in the 1950s to 1970s; studies of this drug were closely monitored for potential toxic effects. A pilot phase II clinical trial in 36 patients with moderately severe AD suggested that clioquinol might inhibit Aβ aggregation and reduce Aβ-related oxidative injury[59]. However, the statistically significant effects in this trial were observed only in the more severely affected subgroup of patients and were not maintained by the 36 week end point. Other metal chelators are being designed and tested in preclinical studies and clinical trials. A lipophilic metal chelator, molecule-XH1, with amyloid-binding and metalchelating moieties, reduced APP protein expression in human cells and attenuated brain Aβ amyloid pathology in APP transgenic mice[60]. The lipophilic metal chelator

DP-109 markedly reduced amyloid plaque burden in brains in APP transgenic mice[61] and epigallocatechin-3-gallate, the main polyphenol constituent of green tea, which has metal-chelating and radical-scavenging properties, produced significant reductions in iron-regulated APP and Aβ peptide in cell cultures[62].

M1 Muscarinic Agonists

The M1 subtype of muscarinic acetylcholine receptors potentially plays a role in AD via several mechanisms, including effects on Aβ peptide, tau hyperphosphorylation, and cholinergic function[63]. The M1 muscarinic agonist AF267B increased non-amyloidogenic APP processing *in vitro* and decreased brain Aβ levels *in vitro* and *in vivo*[64]. In a recently developed Alzheimer's mouse model characterized by both plaques and tangles, AF267B administration attenuated Aβ and tau pathologies in the hippocampus and cortex and improved some cognitive deficits[65]. Significant reductions in CSF Aβ were reported with the selective M1 agonist talsaclidine in a randomized, double-blind, placebo controlled study in 40 patients with AD[66]. Past studies with the M1 agonist xanomeline demonstrated limited cognitive and behavioral effects in AD patients[67].

Receptor for Advanced Glycation end Products-Related Mechanisms

Several molecules have been identified that affect brain Aβ through mechanisms not included in the previously described classes. The receptor for advanced glycation end products (RAGE) resides in cells of the blood vessel walls and transports Aβ across the blood-brain barrier. Inhibition of the RAGE-ligand interaction reduces Aβ accumulation in the brains of transgenic mice[68]. RAGE is a target for drug development.

Peripheral Sink Approaches

Gelsolin (GMI) has high affinity for peripheral Aβ, and administration of this agent results in binding of serum Aβ with creation of a "sink" pulling Aβ from brain to blood[69]. This approach might lead to reduced brain amyloidosis in humans. Some vaccination strategies are also achieved through "sink" mechanisms. The Nogo-66 receptor (NgR) is a receptor for myelin inhibitor proteins and participates in limiting brain injury related axonal growth. There is an inverse correlation between Aβ levels and NgR levels within the brain[70]. Administration of peripheral NgR reduces Aβ in the transgenic mouse brain, increases serum Aβ, and improves spatial memory[71]. Such approaches might be of value in humans with AD, and drug development targeting this mechanism is underway.

Neuroprotective approaches

Aβ appears to exert some of its neurotoxic effects through numerous secondary pathways, including tau hyperphosphorylation and neurofibrillary tangle formation, oxidation, inflammation, demyelination, and excitotoxicity. These processes are potential targets for neuroprotective therapies.

Table I. Drugs under clinical trial for the treatment of Alzheimer's disease.

Bapineuzumab-AAB001	Phase III	Binds and removes Aβ-peptide
CAD 106	II/IIa/IIb	Immunotherapy
AC1204	Phase II/III	Targets glucose hypo metabolism by providing ketone bodies as alternative source
Acc-001	II/IIa/IIb	Antibody vaccine
Affitope AD02/Mimotope Aβ (16)	II/IIa/IIb	Aβ (1-6) immunotherapy
α-Tochopherol	phase III	Destroys toxic free radicals
AZD 1446/TC-6683	II/IIa/IIb	Nicotinic (nAChR) receptor activator
BMS-708163	II/IIa/IIb	γ-secretases inhibitor
CERE-110/Nerve Growth Factor Gene Therapy	II/IIa/IIb	May reduce cholinergic cell loss in AD
Dimebon	Phase III	NMDA Inhibitor
DHA/Omega 3 fatty acid	Phase III	Modulation of presenilin
ELND005	Phase II/III	Prevent Aβ-oligomeric formation
I.V. Immunoglobulin/IvIg	Phase III	Reduces Aβ and improves cognition
MABT5102A	Phase I	Binds and remove Aβ that accumulates in brain
Nicotinamide	II/IIa/IIb	Inhibitor of sirtuins
PBT2	II/IIa/IIb	Inhibits oligomeric formation disaggregates plaques and neutralizes Aβ-toxicity
PF-04494700/TTP488	II/IIa/IIb	RAGE-inhibitor (receptor for advanced glycation end products)

Antioxidants

Oxidative injury is a common cause of cellular injury or death. Studies in a transgenic model of AD suggested that oxidative stress might be an important early event in the pathogenesis of disease[72]. Conflicting results have been reported in longitudinal studies of the putative free-radical scavenger α-tocopherol (vitamin E) in dementia-free elderly populations[73]. In a cross-sectional, prospective study of dementia in elderly individuals in Cache County, Utah, use of vitamin E and vitamin C supplements in combination was associated with reduced prevalence and incidence of AD[74]. In research conducted by the Alzheimer Disease Cooperative Study, the effects of vitamin E, selegiline (a monoamine oxidase B [MAO-B] inhibitor), the two agents in combination and placebo were compared on time to death, time to nursing home placement, progression to severe dementia, or a defined severity of impairment of activities of daily living in patients with moderate AD[75]. After adjusting for the severity of baseline cognitive impairment, there were significant delays in the onset of at least one of these four outcomes with vitamin E, selegiline, or combination therapy versus placebo after 2 years. In a recent double-blind study in patients with MCI, there were no significant differences between groups receiving vitamin E or placebo in the probability of progression to AD[76]. Several other antioxidants, including curcumin, are under investigation[77-79]. Concerns about cardiovascular risk of vitamin E have emerged, and the risk/benefit ratio of high dose vitamin E is being reexamined.

Astrocyte-Modulating Agents

The observation that Aβ plaques are surrounded by activated astrocytes that produce reactive oxygen and nitrogen species suggests that astrocyte activation might have a role in the pathogenesis of AD. In a rodent ischemia model, arundic acid (ONO-2506) decreased infarct size and improved neurologic outcome[80]. The astrocyte-modulating agent protected dopaminergic neurons against neurotoxicity in a mouse model of Parkinson's disease and prevented motor abnormalities by modulating astrocytic activation[81]. The astrocyte-modulating compound ONO-2506 is undergoing assessment in a phase II clinical trial of patients with mild to moderate AD.

Homocysteine-Lowering Strategies

Concentrations of the sulfur-containing amino acid homocysteine, previously associated with cardiovascular risk are reported to be higher in individuals with AD than in age-matched controls[82,83]. Among dementia-free elderly individuals participating in the Framingham study, development of AD was greater in participants with elevated homocysteine concentrations than those without elevations[84]. Evidence from APP transgenic mice suggested that increased homocysteine might. hinder DNA repair in neurons, rendering them vulnerable to Aβ-induced damage and a recent study in mice found that increased homocysteine levels might exert toxic effects on brain micro vessels and disrupt the blood-brain barrier[85,86]. A federally sponsored clinical trial of homocysteine lowering vitamin combinations is now in progress.

Anti-Inflammatory Agents

Certain Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have been shown to modulate γ-secretase activity in mammalian cells and in mice[87]. NSAIDs alter the specificity of γ-secretase, decreasing the production of Aβ42 and increasing the production of a shorter form of Aβ that terminates at residue 38. So, although the mechanism by which these compounds affect γ-secretase is unclear – for example, direct versus indirect effects their ability to reduce Aβ42 selectively and the fact that they are already known to be safe for human use has spurred plans for their use in clinical trials for the prevention and/or treatment of AD. Processing of APP by α-secretase does not lead to Aβ production. However, stimulation of α-secretase mediated processing of APP reduces Aβ formation, presumably by shunting more APP down this alternative pathway. Such stimulation can be accomplished by the activation of protein kinase C (PKC); for example, by treatment with phorbol esters. More practically, PKC can be activated by muscarinic M1- and M3-receptor agonists and such agents are considered reasonable candidates for AD therapy. Potential problems with this strategy might be undesirable side effects as a consequence of the chronic activation of muscarinic receptors[88-92]. The first demonstration that muscarinic agents can modulate APP processing[93,94]. Although microscopic evidence of inflammation has been observed in the brains of patients with AD[95], a series of controlled clinical trials demonstrated no significant benefit with prednisone[96], diclofenac[97] rofecoxib[98], nimesulide[99], or naproxen in slowing the rate of decline in AD. In a recent double-blind study of patients with MCI, rofecoxib did not significantly delay the diagnosis

of AD or improve cognitive or global function[100]. A disease-modifying effect of anti-inflammatory agents is plausible but not supported by currently available clinical trial data. Potential benefits of these agents must be weighed against risk of side effects including gastrointestinal bleeding.

NMDA-Receptor Antagonists

Cognitive decline in patients with AD has been linked with neuronal damage from excite toxicity caused by persistent over activation of NMDA receptors by glutamate. Both Aβ and over expression of tau proteins, appear to be triggers for the excessive activation of NMDA (N-methyl-D-aspartate) receptors and the resulting excite toxic pathway that leads to cell death[101,102]. The NMDA receptor antagonist memantine, approved by the Food and Drug Administration (FDA) for the treatment of moderate to severe AD, might reduce glutaminergic excitotoxicity and provide symptomatic improvement by affecting neuronal function in the hippocampus[103]. A randomized, double-blind, placebo controlled phase III study conducted in the U.S. in patients with moderate to severe AD showed significant improvement with memantine (20 mg/day) versus placebo in activities of daily living and neuropsychological outcomes[104]. No clinically relevant differences were observed in the safety profiles of memantine and placebo. In a second U.S. controlled clinical trial, memantine administered in combination with the cholinesterase inhibitor donepezil in patients with moderate to severe AD was also associated with significantly increased cognitive function, decreased decline in activities of daily living, and decreased incidence of new behavioral symptoms when compared with placebo[105]. Other NMDA receptors, including neramexane (MRZ 2/579), have demonstrated neuroprotective potential in preclinical investigations and are proceeding to clinical development[106] . In a double-blind, randomized, placebo-controlled phase II clinical trial conducted in 198 patients with moderate to severe AD, patients receiving neramexane therapy for 24 weeks had significantly greater improvement in activities of daily living compared with patients receiving placebo. However, no significant difference was demonstrated in measures of cognitive function.

AMPA-Receptor Modulators

Glutamate activation of alpha-amino-3-hydroxy-5-methyl- 4-isoxazole-propionic acid (AMPA) receptors is believed to mediate most fast synaptic neurotransmission in the brain. An investigation in mice demonstrated glutamate AMPA receptor involvement in the regulation of sensorimotor, affective, and cognitive functions[107]. Positive AMPA receptor modulators might have utility in a broad spectrum of neurologic pathologies, with tests in preclinical models suggesting potential neuroprotective effects and enhanced cognitive performance[108-112]. In a double-blind, placebo-controlled, phase II trial, the efficacy and safety of the AMPA receptor modulator CX516 were evaluated in 175 patients with MCI[113]. The CX516 treatment group did not demonstrate significant improvement in delayed recall of a 15-item list compared with the placebo group after 4 weeks of treatment. A subset analysis showed that the patients with the worst baseline memory impairment exhibited improvement in the delayed recall test with CX516 versus placebo. A significant difference in patient withdrawals was observed between the CX516 and placebo groups, primarily related to gastrointestinal side effects, but no treatment-related serious adverse events were reported in either group. Further development of this class of compounds is warranted.

Tau-related Therapies

Phosphorylation of tau proteins, critical for the production of intracellular neurofibrillary tangles, is dependent on Intracellular kinases such as glycogen synthase kinase 3 (GSK3) Lithium has been shown to reduce hyperphosphorylation of tau proteins by inhibiting GSK3 in cell culture and in transgenic mice[114-116]. Through its inhibitory actions on GSK3, lithium also blocks the accumulation of Aβ peptides in the brains of mice that overproduce APP[117].

This agent could prove beneficial by reducing the formation of both neurofibrillary tangles and amyloid plaques, but its toxicity in older adults might limit its use. A second mood stabilizer, valproic acid, has been reported to inhibit GSK3, and there is an ongoing NIA sponsored trial of valproic acid in mild to moderate AD. Tau hyper phosphorylation and formation of intracellular neurofibrillary tangles might be the principal cell death pathway in AD. Inhibitors of this process might be necessary to complement effects of anti-amyloid therapies. For example, a recent report demonstrated that transgenic mice with cerebral amyloidosis demonstrate a reduction in behavioral deficits when tau levels are lowered, without a concomitant lowering of the cerebral amyloid bur-

den. Drug discovery efforts are underway to identify viable therapeutic tau-modulating candidates.

Caspase Inhibitors

Caspase enzymes might represent an important link between amyloid plaques and neurofibrillary tangles in AD as well as being critical to cell death pathways. Neurons treated with Aβ peptide activate caspases, which trigger cleavage of tau and produce truncated forms of the proteins that rapidly and extensively assemble into abnormal filaments characteristic of the tangles found in AD[118]. Caspase activation is also required for apoptosis in forebrain neurons[119] and is increased in the brains of patients with AD[120,121]. Exposure of cortical cell cultures to a caspase-3 inhibitor blocked caspase-induced cleavage of tau[122]. In addition, caspase inhibitors have prevented neuronal damage or loss in animal models of head injury and stroke suggesting this approach might have utility in the treatment of AD[123,124].

Nicotine Acetylcholine Receptor Agonists

There are significant losses of some nicotine acetylcholine receptor (nAChR) subtypes on neurons in the hippocampus and temporal cortex of patients with AD, concurrent with significant increases in the number of astrocytes and astrocytes expressing the 7 nAChR subtype. The increased expression of 7 nAChRs on astrocytes is positively correlated with the number of neuritic plaques, suggesting a potentially important role for this receptor subtype in disease pathogenesis. Nicotine treatment of transgenic mice that over express Aβ with nicotine results in a reduction in cortical Aβ levels with short-term administration and a reduction in amyloid plaque formation and 7 nAChR expression with long-term administration[125] In animal models, nicotine produces enhanced performance on working memory tasks[126]. Galantamine, a cholinesterase inhibitor with nicotinic modulating properties, reduces APP metabolism in an animal model of AD[127]. The 4-2 nAChR partial agonist ispronicline (TC-1734) has demonstrated memory-enhancing properties in rat and mouse models, neuroprotective effects in studies in cultures and hippocampal slices, and a positive safety/tolerability profile in phase I clinical studies[128]. Phase II studies showed some improvement in cognitive function in ispronicline-treated patients with age-associated memory impairment and MCI; additional phase II studies in AD patients are underway.

Cholinesterase Inhibitors

Cholinesterase inhibitors augment cholinergic function in AD at the postsynaptic cholinergic neuron. This pharmaceutical class reduces acetylcholinesterase-induced destruction of acetylcholine in the synaptic cleft, increases the intrasynaptic residence time of acetylcholine, and facilitates interaction between acetylcholine and the postsynaptic cholinergic receptor. Cholinesterase inhibitors are used primarily as long-term symptomatic treatment for AD. Evidence derived from clinical trials[129], imaging[130] and basic science studies suggest that cholinesterase inhibitors might reduce APP processing and provide some degree of neuroprotection[131-135].

Most long-term clinical observations indicate the principal effect of cholinesterase inhibitors is symptomatic treatment with limited disease modifying activity[136].

Neuroprotective and Neurorestorative Approaches

Nerve growth factor (NGF) is a member of the neurotrophin family of polypeptides. Other members of this protein family exhibit similarities in structure and function and include neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and brain-derived neurotrophic factor (BDNF). Each of these growth factors plays an important role in normal neural development and maintenance of the mature central and peripheral nervous systems, including mediation of neuronal proliferation, differentiation, and neuronal survival[137] NGF, like other neurotrophins, promotes cell survival by signaling through specific tyrosine kinase receptors, thereby engaging internal cellular machinery to effectively block apoptosis from occurring in either a developing or damaged neurons. Given their survival-promoting properties, neurotrophins are considered potential therapeutic agents for neurodegenerative disease[138]. Specifically for AD, evidence from studies in mice suggests that NGF might play a significant role in maintaining neuronal integrity, as well as survival in response to injury of the basal cholinergic forebrain neurons A lack of endogenous NGF can lead to memory deficits, whereas NGF administration rescues neurons from injury-induced cell damage and leads to associated memory improvements[139,140]. Hence, NGF and NGF-related agents might have neurorestorative as well as neuroprotective properties. The impermeability

of the blood-brain barrier to exogenous NGF and other neurotrophins presents a significant challenge for evaluation of potential therapeutic benefits in AD. Strategies to circumvent this transport challenge are the surgical implantation of NGF-expressing cells (eg, gene therapy)[141] or administration of agents that or potentiate the endogenous production of NGF and other neurotrophins[142,143]. The nonpeptidic agent xaliproden (SR-57746), a neurotrophic factor enhancer (NTFE), demonstrates neurotrophic effects in several preclinical neurodegenerative *in vivo* and *in vitro* models[144]. Xaliproden activates endogenous neurotrophin synthesis, including NGF and BDNF, in rat cortical astrocytes[145]. In a rat model of the forebrain cholinergic neuron and memory deficits of AD, xaliproden reversed hippocampal choline acetyltransferase reduction (a measure of cellular activity and viability) and decreased behavioral disturbances[146]. Magnetic resonance imaging demonstrated the neuroprotective effects of xaliproden in this model Ongoing randomized, controlled, phase III trials are currently assessing effects of this agent on cognitive and global functions in patients with mild to moderate AD Cerebrolysin (FPF 1070), a peptide mixture with neurotrophic activity, enhances synaptic regeneration, reduces Aβ deposition, and ameliorates performance deficits in APP transgenic mice[147]. Randomized, double-blind, placebo controlled studies show that cerebrolysin infusions significantly improve activities of daily living and cognitive function[148,149]. Reported rates of adverse events are similar across treatment and placebo groups. Targeted delivery of human NGF by gene transfer prevents injury-induced degeneration of cholinergic neurons in adult monkeys[150,151]. In a recent phase 1 study, genetically modified, autologous fibroblasts producing human NGF were implanted into the forebrains of six patients with mild AD. After an average follow-up period of 22 months, no long-term postsurgical adverse events occurred, and the rate of cognitive decline appeared to be ameliorated. Clinical investigation of this approach is expected to continue.

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