Neuroprotective Strategies in Alzheimer's Disease

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Summary: In addition to strategies designed to decrease amyloid beta (Aβ) levels, it is likely that successful Alzheimer's disease (AD) therapeutic regimens will require the concomitant application of neuroprotective agents. Elucidation of pathophysiological processes occurring in AD and identification of the molecular targets mediating these processes point to potential high-yield neuroprotective strategies. Candidate neuroprotective agents include those that interact specifically with neuronal targets to inhibit deleterious intraneuronal mechanisms triggered by Aβ and other toxic stimuli. Strategies include creating small molecules that block Aβ interactions with cell surface and intracellular targets, down-regulate stress kinase signaling cascades, block activation of caspases and expression of pro-apoptotic proteins, and inhibit enzymes mediating excessive tau protein phosphorylation. Additional potential neuroprotective compounds include those that counteract loss of cholinergic function, promote the trophic state and plasticity of neurons, inhibit accumulation of reactive oxygen species, and block excitotoxicity. Certain categories of compounds, such as neurotrophins or neurotrophin small molecule mimetics, have the potential to alter neuronal signaling patterns such that several of these target actions might be achieved by a single agent.
Key Words: Alzheimer, neuroprotection, amyloid, stress kinase, neurotrophin.

Potential impacts of neuroprotection on AD

This review will focus on strategies targeted to neurons and designed to decrease their vulnerability to neurodegenerative mechanisms occurring in Alzheimer's disease (AD). Potential therapies intended to decrease amyloid burden[1,2] or inflammatory processes[3,4] have been covered in recent reviews. Implicit in neuroprotection is the concept of delaying onset or slowing progression of AD. The late onset of symptomatic impairment in the majority of AD cases creates a particularly high-impact opportunity for neuroprotective strategies that achieve even modest delays in disease onset. Delaying disease onset by only 2 years would have a marked impact on reducing prevalence and a 5-year delay would reduce AD prevalence by half.[5,6] Delaying onset by 10 years would, for the majority of individuals, eliminate symptomatic AD as a significant factor in advanced age. Once AD is present, delaying losses of independent activities of daily living or nursing home placement would markedly decrease costs associated with caregiver stress and nursing home care.

Will neuroprotection play a critical role in AD therapeutics?

Given the substantial body of evidence suggesting that the accumulation of amyloid beta (Aβ) is a major and early causative process in AD[7] it can be argued that treatments decreasing levels or availability of toxic forms of Aβ will constitute high-priority, first-tier treatment strategies while neuroprotective strategies focused on non-Aβ targets might play a supportive, less critical role. Promising approaches for decreasing Aβ levels include inhibition of Aβ generation, reduction of soluble Aβ levels and enhancement of Aβ clearance from the CNS.[1,8] While development of Aβ-based treatments follows logically from known Aβ mechanisms, a number of factors might limit the effectiveness of such treatments if applied in isolation. First, the degree to which Aβ levels need to be reduced to delay onset or slow progression of AD is unknown. If Aβ levels are several-fold above those capable of causing maximum rates of neural degeneration, a large proportionate reduction in levels by a "successful" drug candidate might be insufficient to slow degeneration. Second, the normal physiological func-

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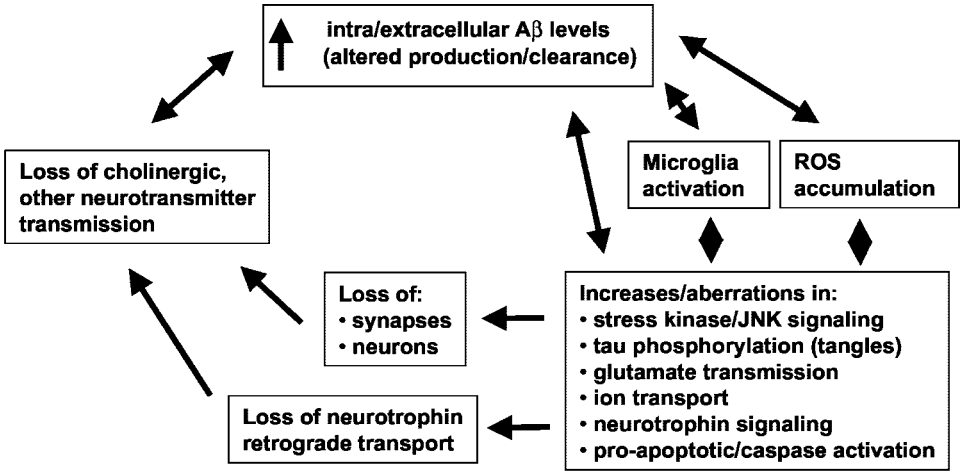

FIG. 1. Overview of pathophysiological processes occurring in AD. A perspective emphasizing the many mutually reinforcing pathological processes in AD suggests that neuroprotective strategies, inhibiting as many of these process as possible, will likely be required for successful therapy in AD in parallel to therapies reducing Aβ accumulation.

tions of Aβ, including its possible role as an antioxidant[9] remain unknown; disruption of critical functions might prove toxic. For example, *in vitro* studies suggest that excessive depletion of endogenously produced Aβ from culture medium leads to neuronal death.[10] Third, the ideal scenario would include the application of Aβ-based drugs in early stages of Aβ accumulation, i.e., years before onset of symptoms. This approach would require drugs of exceptionally low toxicity administered with difficult-to-achieve high compliance rates years before clinical manifestations begin. Fourth, Aβ-based therapies alone are unlikely to improve function or plasticity of damaged but still surviving neurons. Finally, although the bulk of current evidence points to Aβ accumulation as a critical primary causative factor in sporadic AD, a number of other potential mechanisms might constitute

TABLE 1. *Candidate Neurodegenerative Mechanisms in AD and Corresponding Therapeutic Neuroprotective Approaches*

Target Mechanism	Examples of Corresponding Therapeutic or Potential Therapeutic Development
Aβ interaction with binding targets	Neurotrophin small molecule mimetics binding to $p75^{NTR}$ might block Aβ-$p75^{NTR}$ mediated toxicity
Activation of stress kinase/JNK signaling	CEP-1347 inhibitor of stress kinase activation in clinical trials for Parkinson's disease Neurotrophin signaling blocks stress kinase signaling
Excessive tau phosphorylation and microtubule instability	GSK-3 inhibitors under development Valproate in AD trial underway Microtubule stabilizing drugs under development
Caspase activation	Minocycline caspase inhibitor in trials for ALS
Loss of synapses, neuronal death	Trial underway in which NGF-secreting fibroblasts are grafted to basal forebrain Neurotrophin mimetics under development
Loss of cholinergic function	ACIs in clinical use M1 agonists such as talsaclidine in clinical trials Neurotrophin mimetics under development
Generation of ROS	Vitamin E in trials for MCI Various antioxidants in MCI/AD trials Clioquinol metal chelator completed phase II trial
Glutamate excitotoxicity	Memantine NMDA uncompetitive antagonist in use in Europe for AD with FDA approval in US pending Other NMDA modulators under development

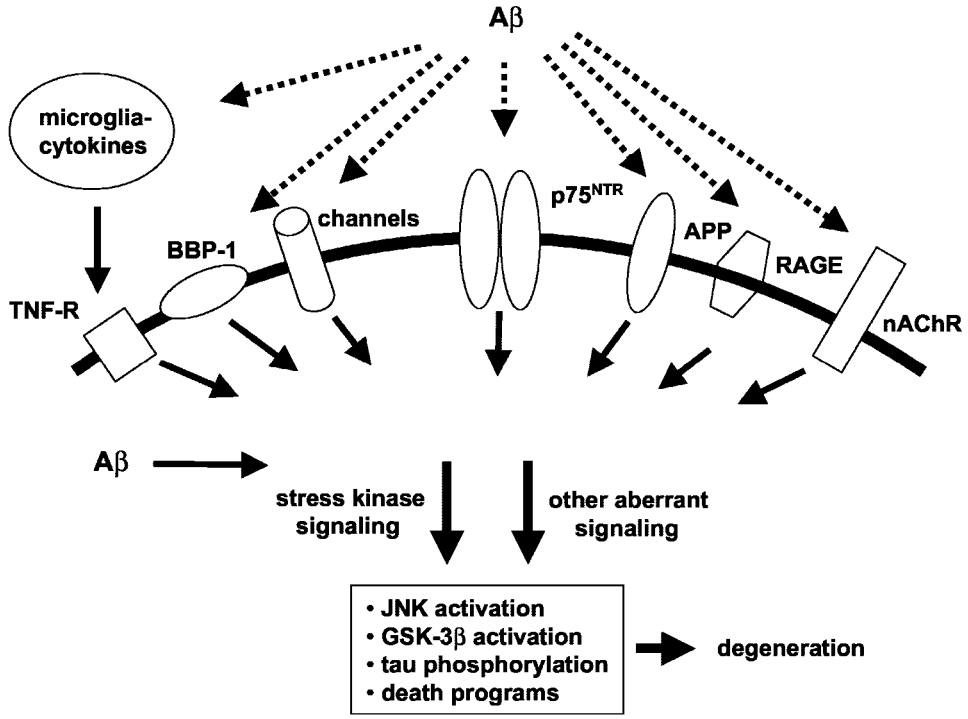

FIG. 2. Aβ binding targets and candidate associated neurodegenerative mechanisms. Extracellular Aβ interacts with a number of neuronal and glial cell surface receptors. Evidence suggests that many of these interactions promote stress kinase and other signaling triggering neurodegenerative processes. Intracellular Aβ is also likely to bind to one or more targets to contribute to neurodegenerative signaling.

important causative factors.[11] Such non-Aβ mechanisms might play even larger roles, or perhaps synergistic roles, as the disease progresses. Thus, it is likely that parallel application of neuroprotective strategies will play a vital role in delaying AD onset and slowing AD progression.

Neurodegenerative mechanisms point to potential neuroprotective strategies

Neurodegenerative mechanisms likely involved in AD are outlined in FIG. 1. While AD mechanisms are often outlined in linear terms of one pathophysiological process leading to the next, a more biological perspective might include multiple cycles and subcycles of self-amplifying neurodegenerative steps. Moreover, the pattern of relative contributions of different pathological cycles is likely to change as the disease progresses. This perspective encourages the view that one or more neuroprotective strategies, applied in parallel, will be required to successfully slow AD progression. Neuronal targets can be viewed from the perspective of those known to directly interact with Aβ, or alternatively, those found to be affected in AD and not necessarily interacting directly with Aβ. Many of these targets offer potential sites for therapeutic small molecules (Table 1).

Neuronal targets of Aβ

Evidence that either extracellular[12] or intracellular[13,14] accumulation of Aβ results in neuronal degeneration has encouraged identification of direct neuronal targets of Aβ that serve as candidates for mediating its toxicity (FIG. 2). Aβ has been reported to bind with relatively high affinity to a number of neuronal targets,[15] including the α7 nicotinic acetylcholine receptor (α7nAChR), the neurotrophin p75 ($p75^{NTR}$) receptor, cell surface amyloid precursor protein (APP), the receptor for advanced glycation end products (RAGE), and BBP-1, a G protein-coupled receptor. Except for α7nAChR, Aβ binding to these receptors leads to neuronal death. Intracellular binding targets of Aβ identified thus far include the endoplasmic reticulum Aβ-binding dehydrogenase (ERAB). A non-receptor based mechanism by which Aβ might affect neurons is suggested by its ability to form Ca^{2+}-permeable channels or to modulate ion-conducting channels, especially K^+ channels.[16] Application of compounds that block Aβ binding or that inhibit at proximal steps deleterious Aβ-induced signaling are potential neuroprotective approaches. Limitations of these approaches include the possibility that Aβ toxicity is mediated via multiple targets or that critical physiological functions of the target receptors or other proteins might be impaired. The ability of a given small molecule to confer neuronal protection by blocking such interactions will depend on the extent to which Aβ interaction with the intended target contributes to the degenerative process. A critical current challenge is to determine whether the predominant neurotoxic effects of Aβ can be narrowed to one or two pharmacologically accessible Aβ binding targets. Of

the above candidate targets, Kawasumi et al.[15] have argued that interaction with $p75^{NTR}$ is likely to play the predominant role in Aβ toxicity.

Neuroprotection via modulation of stress-activated protein kinase signaling

An alternative approach to neuroprotection that would not rely on identification and modulation of individual Aβ-target interactions is the identification and modulation of primary signaling pathways mediating Aβ toxic effects. Interestingly, studies of human AD brain, AD mouse model brain, and *in vitro* models of AD all point to the stimulation of stress-activated protein kinases, particularly c-Jun N-terminal kinase (JNK), as a critical early event in AD-associated neuronal degeneration.[17,18] Application of Aβ to cultured cortical neurons is associated with JNK activation and with subsequent downstream activation of caspases and expression of proapoptotic genes such as *bax*.[19,20] Immunostaining reveals JNK activation in degenerating neurons in AD brain,[21,22] including in association with intraneuronal Aβ accumulation and in association with tangle-like inclusions in entorhinal cortex before Aβ deposition.[23,24] Interestingly, evidence suggests that JNK activation also contributes to tau phosphorylation (discussed below). These findings, along with evidence *in vitro* that inhibition of JNK activation inhibits Aβ toxicity[20,25–28] and blocks caspase activation,[17,20] point to small molecule targets modulating stress kinase signaling and JNK activation as a high priority area for AD therapeutics. CEP-1347 is an example of a compound that inhibits stress kinase signaling and partially blocks Aβ induced neuronal degeneration.[17,29] CEP-1347 is currently undergoing clinical trials in Parkinson's disease and is a candidate agent for AD trials (Cephalon Inc., West Chester, PA; http://www.cephalon.com/research).

Neuroprotection via inhibition of aberrant tau phosphorylation

Excessive phosphorylation of the tau microtubule-associated protein in AD is thought to cause formation of insoluble tau filaments with resulting neurofibrillary tangles, disruption of microtubules, and subsequent neuronal dysfunction.[30] Although the mechanisms responsible for aberrant tau phosphorylation remain to be fully established, their elucidation has begun to point to novel protective strategies. Evidence that glycogen synthase kinase-3β (GSK-3β) phosphorylates tau has engendered considerable interest in GSK-3β inhibitors as neuroprotective agents.[31,32] The finding that valproate, a well-established epilepsy and mood-stabilizing medication, inhibits GSK-3β[33] has led to proposal that this drug might improve symptoms of or slow progression of AD.[34] Interestingly, lithium, another well-established mood stabilizer, has also been shown to inhibit GSK-3β.[31] Evidence that JNK contributes to tau phosphorylation suggests that inhibition of JNK activation might promote the parallel beneficial effects of inhibiting tau phosphorylation along with cell death signaling.[23,35] The large number of kinases found to be capable of phosphorylating tau, including extracellular signal-regulated kinase (ERK) and cyclin-dependent kinase 5 (Cdk5), among others,[32] introduce a number of potential small molecule targets. The presence of multiple candidate targets raises the important questions of which kinases play a critical pathophysiological role in AD, and if more than one is involved, how many would have to be targeted to prevent pathological tau phosphorylation. Another recent neuroprotective approach emerging from microtubule studies is the development of small molecules that stabilize microtubules and prevent Aβ-induced cytoskeletal disruption and toxicity.[36]

Neuroprotection via inhibition of caspase activation

A number of observations have raised the possibility that caspases contribute to neuronal degeneration in AD, although the actual extent to which caspase-mediated cell degeneration or death occurs in AD remains to be established.[37] In AD brain, activated caspases are found in association with neurofibrillary tangles.[38] As described above, activation of JNK promotes caspase activation.[20,29,39] *In vitro* studies demonstrate that application of Aβ induces caspase activation and that caspase inhibitors can block Aβ-induced cell death.[40,41] Consistent with a role for caspases in relatively distal steps of cell death pathways, caspase inhibitor-protected cells have been found to survive in an atrophic and metabolically compromised state in which there is decreased protein synthesis, glucose uptake, and mitochondrial activity.[29,42] An analysis of neuronal death induced by nerve growth factor (NGF) withdrawal from sympathetic neurons demonstrated that caspase inhibition resulted in neurons that were more atrophic and had decreased metabolic function compared with those rescued via blocking of stress kinase activation using CEP-1347.[29] These authors concluded that caspase inhibitors are unlikely to constitute an effective therapy in chronic neurodegenerative settings since caspase inhibitor rescue of neurons might result in neurons surviving but in a dysfunctional state. In contrast to this view, the finding of caspase activation in early stages of AD and in association with neurofibrillary tangles suggests that caspases might serve as a link between senile plaques and tangles and contribute to early as well as terminal steps of cell death.[38] Minocycline, a tetracycline-type antibiotic known to inhibit caspases, is currently under trials for ALS (NINDS) and might be considered for trials in AD.

Ligand-receptor mechanisms promoting neuroprotective signaling

A number of growth factors and other ligands acting via known receptors, as well as peptides acting via un-

known targets, have been found to protect neurons from Aβ toxicity and other deleterious mechanisms relevant to AD. These factors include neurotrophins (discussed below), insulin-like growth factor-1,[26,43] basic fibroblast growth factor,[44] estrogen,[45] activity-dependent neurotrophic factor,[46] and Humanin.[47] Given the long-standing, large body of work assessing neurotrophins in the context of AD, the recent entry of neurotrophins into clinical trials, and the space limitations of the present review, we will focus primarily on neurotrophins.

Neuroprotection via neurotrophins

The extensive overlap in signaling pathways regulated by neurotrophins and those likely to be involved in AD degeneration along with the expression of neurotrophin receptors by neurons undergoing degeneration point to neuroprotective applications for neurotrophins. The major role of neurotrophins in synapse stabilization and function[48] along with emerging evidence that synaptic failure is a critical early process in AD[49] further adds to interest in neurotrophins as candidate therapeutic agents. In the context of AD, NGF and brain-derived neurotrophic factor (BDNF) have been of particular interest. Neurotrophins each bind to a dual receptor system, consisting of $p75^{NTR}$ along with one of the Trk tyrosine kinase receptors. NGF binds to TrkA and BDNF to TrkB.[50,51] Neurotrophin receptors are expressed by neuronal populations particularly vulnerable in early stages of AD. $p75^{NTR}$, TrkA, and TrkB are each expressed by basal forebrain cholinergic neurons, hippocampal pyramidal neurons and layer V cortical neurons.[50,52–56]

Neurotrophin signaling is directly relevant to the aberrations in core signaling mechanisms, likely contributing to neuronal dysfunction and degeneration in AD. Neurotrophin binding to Trk receptors activates at least three fundamental pathways, including phosphatidylinositol-3-kinase (PI3K)/Akt kinase, mitogen-activated protein (MAP) kinase, and phospholipase Cγ (PLCγ) signaling.[50,51] Activation of the PI3K/Akt pathway inactivates SEK1 and ASK1, two key activators of JNK and other stress kinases, suppresses JNK activation, inhibits pro-apoptotic members of the Bcl-2 family, activates CREB and IκB kinase/NF-κB signaling to promote survival and protects cultured neurons from Aβ toxicity.[26,29,39,57] NGF-induced MAP kinase signaling blocks formation of reactive oxygen species (ROS) (see discussion below).[58] $p75^{NTR}$ acts in coordination with, and independently of, Trk receptors to modulate neurite integrity, neuronal size, and neuronal survival. In the context of different ligands or different cell types, $p75^{NTR}$ signaling promotes either neuronal survival or death.[59,60] $p75^{NTR}$-ligand interactions provide a good example of the principle that different ligands acting at a given receptor can differentially modulate its function; for example, binding of different neurotrophins to $p75^{NTR}$ can elicit different patterns of signaling.[50,61] Recent observations that levels of "pro-NGF," a precursor form of NGF, are doubled in parietal cortex harvested from AD patients,[62] along with the finding that pro-NGF might bind with greater affinity to $p75^{NTR}$ compared to NGF and promote death via binding to $p75^{NTR}$,[63] add additional complexity to potential mechanisms by which $p75^{NTR}$ signaling regulates trophic status.

In paradigms in which ligand-induced activation of $p75^{NTR}$ prevents neuronal death, evidence suggests that $p75^{NTR}$ signaling prevents death via either the PI3K/Akt pathway and/or NF-kB activation.[59,60] In view of these signaling studies and evidence that different ligands can differentially affect $p75^{NTR}$ signaling, it is of interest that synthetic mimetics of the loop 1 region of the NGF protein were found to prevent death of dorsal root ganglia sensory neurons.[64] Preliminary data in our laboratory suggest that application of NGF loop 1 mimetics to cultured hippocampal neurons results in activation of PI3K/Akt signaling and prevents cell death. In earlier studies, addition of NGF to cultured E18 rat hippocampal neurons was found to up-regulate $p75^{NTR}$ expression and potentiate Aβ neurotoxicity.[12] Current studies will determine if NGF loop 1 mimetics prevent rather than promote Aβ toxicity.

Within the neurotrophin protein family, NGF has been the most extensively studied with respect to its ability to confer neuroprotection in *in vivo* models of AD. NGF administration to multiple models of basal forebrain cholinergic neuron atrophy, including post-cholinergic neuron axotomy, trisomy 16 mice, aged rodents, and aged primates demonstrates a potent effect in reversing atrophy, up-regulating cholinergic function, reversing age-related cognitive impairment and increasing density of cortical cholinergic innervation.[50,65] Interestingly, *in vitro* studies point to the possibility that NGF might promote non-amyloidogenic secretory processing of APP.[66] In AD brain, NGF levels in the basal forebrain are reduced, while levels in the hippocampus target region have been reported as either unchanged or increased.[67] This shift in NGF distribution, along with direct evidence of impaired retrograde transport of NGF in a mouse model of AD,[68] suggests an impairment of retrograde transport of NGF. These findings raise the possibility of a degenerative cycle in which degeneration leads to a critical lack of NGF reaching the neuronal soma with deficiency of somal NGF leading to further degeneration.[69] Interestingly, a transgenic mouse model in which chronic NGF deficiency is created via the expression of NGF antibodies demonstrates degeneration of basal forebrain cholinergic neurons, tau hyperphosphorylation associated with neurofibrillary pathology, and accumulation of Aβ plaques.[70] This model points further to a potential degenerative cycle incorporating accumulation of Aβ, neuronal degeneration and loss of

neurotrophin function, and further Aβ accumulation. Augmentation of neurotrophin function might serve to both decrease Aβ accumulation and render neurons less vulnerable to Aβ toxicity.

Application of NGF protein as a therapeutic agent for AD faces a number of critical limitations typical of protein ligands, including limited blood brain barrier and intraparenchymal tissue penetration and a short half-life. Moreover, NGF interaction with its dual $p75^{NTR}$/TrkA receptor system elicits a wide range of biological actions beyond those preventing neural degeneration including sprouting of sympathetic fibers and up-regulation of pain transmission.[71] In the limited clinical experience assessing NGF actions in AD patients, NGF was administered to three patients via intraventricular infusion over a period of up to three months.[72] No significant improvement in cognition was detected and patients experienced back pain and weight loss. In an ongoing phase I trial, NGF is being delivered to the basal forebrain via intraparenchymal grafting of autologous fibroblasts engineered to secrete NGF.[65] Another neurotrophin-based therapeutic approach is the development of NGF small molecule mimetics.[73–75] Such mimetics acting selectively at $p75^{NTR64}$ or TrkA[76] receptors have been shown to prevent neuronal degeneration *in vitro* and activate partially distinct patterns of intracellular signaling cascades, compared with those activated by NGF. These findings point to the possibility of creating compounds with preferred pharmacological properties and bioavailability that are capable of preventing neuronal degeneration without stimulation of the entire range of NGF effects.

Cholinergic strategies for neuroprotection

An early pathological process in AD consists of degeneration of basal forebrain cholinergic neurons along with their projections to hippocampal, cortical, and limbic targets.[77] Losses in certain other neurotransmitter systems might occur as a secondary result of reduced cholinergic innervation and function. Strategies involving application of acetylcholinesterase inhibitors (ACIs) and cholinergic agonists have largely been focused on improving cognitive and neurobehavioral symptoms in AD rather than slowing underlying neuronal degeneration. It is of interest to note, however, that recent clinical observations, along with emerging insight into reciprocal interactions between Aβ production and cholinergic function, suggest that cholinergic-based therapies might in fact have neuroprotective effects.

Results of ACI trials have raised the question of whether ACIs might slow disease progression.[78–80] Patients initially placed in placebo groups appeared to have lost cognitive function that was not restored after starting ACIs in open-label extension phases. The open-label design of the extension phase of these trials, however, would preclude any formal conclusion regarding slowing of progression. In a study of cognitive function of patients who had dropped out of an ACI trial, individuals who had initiated and then discontinued ACI treatment demonstrated less subsequent cognitive loss compared with placebo-treated patients.[81] These findings again raise the interesting scenario of slowed disease progression; however, a critical caveat is that patients discontinuing the medication cannot be assumed to represent a random sample from the total study population. Detection of slowed disease progression requires study designs that incorporate strategies such as delayed treatment with subsequent blinded follow-up or random discontinuation of drug with adequate washout periods. In the case of ACIs, their recognition as a standard of care in AD limits such trial designs. Current trials of ACIs applied to mild cognitive impairment (MCI) will determine whether ACIs can delay progression to AD, although distinguishing between symptomatic *versus* disease-slowing effects will remain a challenge.

There are several potential mechanisms by which ACIs or cholinergic agonists might slow underlying disease progression including decreased Aβ production and/or reduction of neuronal vulnerability to Aβ toxicity.[66,82] M1 muscarinic acetylcholine receptors (mAChR) are primarily localized postsynaptically to cortical cholinergic nerve terminals. M1 mAChR signaling activates protein kinase C (PKC), increases secretory processing of APP and down-regulates production of Aβ.[83–86] Cholinergic stimulation has also been found to: reduce tau phosphorylation;[87] protect neurons from Aβ[88] and promote neurotrophin release.[89,90] Chronic M1 agonist treatment in clinical trials has been shown to reduce Aβ levels in cerebrospinal fluid of AD patients.[91,92] Current goals in the development of cholinergic-based strategies in neuroprotection include the development of M1 agonists with adequate bioavailability, potency, and receptor selectivity.[82]

The known inhibitory effects of Aβ on the synthesis and release of Ach and on cholinergic signaling point to the possibility of a degenerative feedback loop in which Aβ impairs Ach release, which leads to altered APP processing, increased Aβ levels and disrupted neurotrophin regulation.[16,66,77] These processes in turn lead to further increases in Aβ production, further loss of neuronal function and further decline in Ach release. Given a potential neurotrophic role of cholinergic neurons on target hippocampal and cortical neurons and the effect of cholinergic input on APP regulation, it has been proposed that cholinergic degeneration might lead to secondary degeneration in a wide range of non-cholinergic target systems.[90] From the perspective of these mechanisms, it is plausible that drug strategies designed to up-regulate cholinergic function might slow degeneration.

Oxidative stress and antioxidant neuroprotective strategies

The role of oxidative stress, an imbalance between the production and detoxification of oxidative reaction products, continues to be the subject of extensive research in AD.[93,94] While oxidation products accumulate to some degree in normal brain, their levels increase with age and are substantially greater in AD, including in its early stages.[95] Excessive levels of hydrogen peroxide and ROS such as hydroxyl free radical and superoxide lead to formation of oxidization products including oxidized proteins, lipid peroxides, advanced glycosylation end products (AGEs), and DNA adducts. Protein and lipid oxidation leads to loss of critical enzyme functions, including those regulating glutamate transport, which results in excitotoxicity due to excessive extracellular glutamate, and to the loss of ion-transporting ATPases, causing a disruption of calcium ion homeostasis and impaired mitochondrial function.[96] Oxidative stress triggers degenerative signaling, including activation of stress kinases (including JNK) and caspases.[25,28] Sources of oxidative stress in AD include impaired mitochondrial metabolism and Aβ-associated sources.[97,98] Addition of vitamin E to neuronal cultures inhibits Aβ-induced toxicity, protein oxidation, and JNK and $p38^{MAPK}$ activation.[28,93] Studies in AD transgenic mice (Tg2576) revealed elevated peroxidation occurring several months before detectable Aβ accumulation and amyloid plaque formation.[99] Further supporting a causal role for oxidative stress in amyloid-induced pathology, administration of the antioxidant curcumin to these mice led to reduced oxidative stress and amyloid pathology.[100] Aβ itself, in particular when binding Cu^{2+} or Fe^{3+} and forming certain types of aggregates, may be a primary source of ROS.[98] Cu^{2+} and Zn^{2+} promote aggregation of human Aβ and chelation of these metals renders the structure of Aβ aggregates less compact and less resistant to turnover. Clioquinol (CQ), a retired hydrophobic antibiotic with brain-penetrating and Cu/Zn-chelating properties, is a potential agent for inhibiting Aβ accumulation and decreasing ROS production.[98,101] In the Tg2576 transgenic mouse model, CQ led to reduced amyloid accumulation and improved behavioral scores.[102] Clinical phase II efficacy testing of this agent is currently completed, though the results are not yet published.[101]

In human antioxidant studies, vitamin E is one of the most extensively studied antioxidant agents. Data from cross-sectional and longitudinal studies assessing the relationship between vitamin E consumption and AD risk have led to conflicting results. Two prospective epidemiological cohort studies of AD found that diets containing higher levels of vitamin E were associated with lower odds of developing AD.[103,104] A surprising finding was that neither study was able to identify an association between AD incidence and use of vitamin E supplements. In commentary on these studies, Foley and White[105] pointed to the limitations of observational studies and raised the possibilities that clinical status before the onset of dementia might have influenced diet, recollection of diet, and/or supplement use. In addition, it is possible that predicted vitamin E content of food might have served as a marker for the presence of other compounds that actually conferred the neuroprotective effects. In a prospective cohort study, Luchsinger et al.[106] were unable to detect an association between antioxidant vitamin use and AD risk. Finally, a pioneering trial with vitamin E supplementation of 2000 IU per day for moderate-stage AD patients led to a small but significant delay in reaching the endpoints of institutionalization, loss of major activities of daily living, or death, but did not delay loss of cognitive performance.[107] These findings have encouraged current trials in which vitamin E is being given to individuals with MCI with the hope that antioxidant therapy administered at earlier stages of disease might have a greater impact on outcomes.[108] Other antioxidant compounds have been studied in terms of delaying AD onset, slowing progression, or improving cognitive function.[99] European trials in small numbers of AD patients with idebenone, a centrally active antioxidant and analog of coenzyme Q, suggested improved cognitive scores, with efficacy similar to ACIs.[109] To date, there is no clear body of definitive data, derived from adequately controlled prospective trials of sufficient size and duration, that suggests a given antioxidant compound delays the onset of cognitive loss or slows its progression in AD.

Modulation of NMDA receptor function

Oxidative stress, accumulation of Aβ and other mechanisms lead to neuronal energy deficits in AD which in turn can result in excessive neuronal depolarization with a subsequent excess of extracellular glutamate, evoking further depolarization. Persistent depolarization leads to activation of NMDA receptors and deleterious increases in intracellular Ca^{2+}.[110,111] Olney et al.[112] proposed that an early event in AD pathophysiology consists of increased sensitivity to glutamate-induced excitotoxicity secondary to effects of Aβ accumulation, oxidative stress, and/or energy metabolic dysfunction. Aβ has been shown to inhibit glutamate uptake by synaptosomes and glia.[16] The observation that free-radical scavengers block these effects is consistent with a model in which Aβ inhibits glutamate uptake via oxidative damage.[113] The potentially synergistic multiple effects of Aβ on glutamate function, including enhancing its release, preventing its uptake, and increasing neuronal vulnerability, along with the degenerative feedback cycle of excess glutamate, excess depolarization and intracellular Ca^{2+}, with subsequent further glutamate release and loss of

multiple neuronal functions, points to a prominent role for excitotoxicity in AD.

These findings suggest that modulation of glutamate receptors might serve as a neuroprotective strategy in AD. Memantine is an NMDA channel uncompetitive antagonist that preferentially blocks channel opening and neuronal death due to excessive exposure to glutamate while allowing physiological activation required for long-term potentiation (LTP).[114,115] Administration of memantine to rats has been found to block neuronal degeneration caused by injection of $A\beta_{1-40}$ raising the possibility that memantine can protect against Aβ-induced degeneration.[116] In a recent clinical trial, a comparison of AD patients treated with memantine *versus* placebo showed that after 26 weeks, patients treated with memantine demonstrated a significantly reduced decline in scores measuring overall clinical impression of change and activities of daily living.[117] Determining whether these effects were due to an actual slowing of underlying disease progression or were merely the result of symptomatically improved function will require further clinical and animal studies.

Summary

Given the many potential limitations of isolated Aβ-based therapies, it is likely that effective AD therapeutics will include parallel strategies that confer neuroprotection against deleterious forms of Aβ and other agents and processes causing neuronal dysfunction and degeneration. Mechanisms underlying the onset and progression of AD are likely to consist of a number of interacting events including the following: excessive accumulation of Aβ, oxidative stress, deleterious stress kinase/JNK signaling, aberrant tau phosphorylation, excitotoxicity, disruption of neurotrophin signaling, loss of synapses, neurites and neurons, and loss of cholinergic and other neurotransmitter function. Given the many layers of potential integration and the mutually reinforcing nature of these processes, it seems unlikely that clinically achievable modulation of a single process will prevent onset or significantly slow AD.

These candidate underlying mechanisms of neuronal degeneration point to a number of therapeutic strategies currently at various stages of development. A number of agents with the potential to provide neuroprotective effects (including ACIs, memantine, and antioxidants) are already clinically available; however, results of additional clinical testing will be required to determine if any of these are capable of delaying onset or slowing underlying disease progression. Other compounds not in widespread clinical use but undergoing clinical trials in AD and/or other neurodegenerative disorders include nerve growth factor, valproate and other GSK inhibitors, various nicotinic agonists, the CEP-1347 stress kinase inhibitor, minocycline as a caspase inhibitor, and metal chelators. Some compounds, such as neurotrophin small molecule mimetics, might prove successful in addressing multiple underlying disease mechanisms in parallel. Taken together, the work reviewed here points to a promising emerging picture of successful, mechanism-based, neuroprotective strategies for AD.

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