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CURRENT AND EXPERIMENTAL THERAPEUTICS OF ALZHEIMER DISEASE

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An enhanced understanding of the neurobiology and neurochemistry of Alzheimer disease, combined with provocative epidemiologic studies, has led to a plethora of new approaches of treatment. It has been estimated that between 50 and 60 drugs are in or entering clinical trials in Alzheimer disease (1). These approaches range from symptomatic and palliative, to preventive and disease altering. Although no magic bullets have been unveiled, it is clear that since the early 1990s, the therapeutics of Alzheimer disease have inexorably proceeded along a rational course, generating an increasing number of compounds that have entered the marketplace and have shown benefit for patients and caregivers.

ACETYLCHOLINESTERASE INHIBITORS

The first drug approved in the United States and Europe with an indication to diminish the intensity of the core symptoms of Alzheimer disease, namely, problems in memory, praxis, and language, was tacrine (Cognex). A few years thereafter, approval through most of Europe and the United States was granted to donepezil (Aricept). In 1999, rivastigmine (Exelon) received approval in Europe and was approved in the United States in 2000. Most recently, galantamine (Reminyl) was approved in Sweden for the treatment of Alzheimer disease. This drug, previously available in Austria under the trade name Nivalin for a host of other indications, is now awaiting approval throughout the rest of Europe and the United States.

Although all these drugs are cholinesterase inhibitors, the mechanism of cholinesterase inhibition and other properties of the compounds make them far less than equivalent. Cholinesterase inhibition can be mediated through numerous different mechanisms, characterized as reversible, irreversible, or pseudoirreversible. Additionally, the relationship among cholinesterase, acetylcholine, and the cholinesterase inhibitor could be either competitive or noncompetitive. The specificity of cholinesterase inhibitors can also vary, with differing affinity for butyrylcholinesterase. Finally, these drugs can also differ in the degree to which they modulate the sensitivity of nicotinic receptors.

The group of cholinesterase inhibitors also differs among classical pharmacokinetic and pharmacodynamic parameters. Degree of protein binding, duration of action, and drug interactions discriminate among the drugs in this class. All these specific differences are delineated in this chapter.

Tacrine

Tacrine (Cognex) is a noncompetitive reversible inhibitor of both butyrylcholinesterase and acetylcholinesterase. In fact, its specificity for butyrylcholinesterase is greater than for acetylcholinesterase. It is of the acridine class. The bioavailability of the drug is variously estimated between 17% and 33%, its peak plasma level occurs relatively rapidly within 1 to 2 hours, and it has a serum half-life of $1\frac{1}{2}$ to 2 hours. The drug has a protein binding of about 75%, and it is metabolized by numerous cytochrome pathways including 1A2 and 2D6. Four times a day dosing is required. The efficacy of tacrine was established in a series of placebo-controlled, double-blind studies (2–4). These studies ultimately led to United States Food and Drug Administration (FDA) approval based on tacrine's ability to improve the core symptoms of Alzheimer disease as reflected in the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) (5) and in the assessment of global change by the clinician (6–9).

Tacrine has been associated with a high frequency of elevated liver transaminases. At some time in their course of drug administration, approximately 50% of patients receiving tacrine will develop elevation in liver transaminases (10). However, with discontinuation of the drug or even lowering of the dose, most such elevations return to baseline.

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Indeed, it is even possible to readminister tacrine to many patients who previously had "transaminitis," with a subsequent benign course. Given these data, it is not surprising that fatal hepatotoxicity has been extremely rare (11). Extensive experience with tacrine has led to the conclusion that rechallenge with tacrine is possible, unless patients have had jaundice, eosinophilia, or other signs of a hypersensitivity reaction. Nonetheless, frequent monitoring of liver enzymes is a necessary concomitant of administration of tacrine, as is relatively slow drug titration. The most efficacious doses of tacrine are between 120 and 160 mg per day. Patients do not often achieve this dose, and even in those patients in whom a dose of 120 mg is obtained, the minimal time to reach that level is 12 weeks. For all these reasons, tacrine is no longer actively promoted, and it is rarely used.

Donepezil

Donepezil (Aricept) is a piperidine cholinesterase inhibitor that is reversible and has both competitive and noncompetitive features. It is 100% bioavailable, and it reaches peak plasma levels between 3 and 5 hours after administration. The drug has not been shown to have any interaction with food. It is highly protein bound. The drug is metabolized by the cytochrome system, specifically 2D6 and 3A4. Donepezil has a long serum half-life, estimated to be between 70 and 80 hours. The consequence of these characteristics is that donepezil requires only a once-daily dose (12).

Large placebo-controlled, multicenter, randomized trials have established the statistically significant effects of donepezil on ADAS-cog and clinical global measures (13–17). The effective doses are 5 and 10 mg administered once per day. Some trials do not show a superiority of 5 mg over 10 mg, although other data would suggest greater superiority for 10 mg (17).

The major adverse events that are associated with donepezil administration are those that can be anticipated from drugs that increase cholinergic activity. These include nausea, vomiting, and diarrhea. To minimize these effects at the higher, more effective, dose of 10 mg, a titration schedule in which patients remain at the 5-mg dose for 6 weeks, before being raised to the 10-mg dose, is recommended.

Other adverse events are less common, but they are also explicable by the cholinomimetic properties of donepezil. The drug has been associated with bradycardia and syncope as well as some sleep disturbance. Increased cholinergic activity is well known to produce bradycardia, and the cholinergic system can have profound effects on sleep architecture including increasing arousal (18–22).

Rivastigmine

Rivastigmine (Exelon) is a carbamate that inhibits both acetylcholinesterase and butyrylcholinesterase. Its mechanism of action at cholinesterase is termed pseudoirreversible,

meaning that although it binds to the cholinesterase-like irreversible inhibitors do, it is metabolized by cholinesterase, the enzyme it is inhibiting. This circumstance produces a truly "pseudoirreversible" state and accounts for rivastigmine's half-life of 10 hours, far shorter than would be expected from an irreversible cholinesterase inhibitor. Irreversible inhibitors are active for as long as the time necessary to regenerate cholinesterase, between 2 and 4 weeks. Rivastigmine's bioavailability is approximately 40%, and its time to peak concentration can be as rapid as half an hour or as long as 2 hours. There is some interaction with food in its absorption. Its binding to plasma proteins is approximately 40%. Its metabolism is totally nonhepatic, and it can be presumed to have minimal drug interactions. Based on its pharmacokinetic and pharmacodynamic characteristics, the drug is given twice a day, with total doses ranging from 6 to 12 mg.

Efficacy of the agent has been established (23). However, only the higher doses of rivastigmine (doses higher than 6 mg) were shown to be efficacious in two pivotal studies, in both ADAS-cog and global measures. High doses also demonstrate efficacy compared with placebo in activities of daily living, as reflected on the progressive deterioration scale. However, for some patients, it is difficult to achieve these high doses. Despite slow dose titration that took up to 12 weeks, approximately 25% of patients receiving more than 6 mg per day of rivastigmine withdrew from the study, and substantially more had some gastrointestinal complaints. The side effects predominantly occurred during dose escalation.

Galantamine

Galantamine is an alkaloid-derived, reversible, competitive acetylcholinesterase inhibitor. It occurs naturally in certain plants. It is relatively selective for acetylcholinesterase, with far less activity at butyrylcholinesterase. The drug is also an agonist at allosteric nicotinic sites, a mechanism of action that it has in common with benzodiazepines that have a similar mechanism of action at the γ-aminobutyric acid$_A$ receptor. Activity at this site facilitates release of acetylcholine (24). The drug is less than 10% protein bound, it has a very high bioavailability, and it interacts with food such as to decrease its maximum concentrations (25). Twice-daily dosing is supported by approximately a 9-hour half-life (26). The drug is metabolized in the liver by 2D6 and 3A4 (27).

A series of placebo-controlled, randomized, double-blind studies established the efficacy of galantamine. A dose of 24 mg per day is recommended, although both 16 and 32 mg have been shown to also be efficacious (28–30). A significant difference between drug and placebo has been found on the traditional psychometric and global measures, as well as measures of activities of daily living, ADAS-ADL scale, and behavior, Neuropsychiatric Inventory (NPI).

As with other cholinomimetics, the most common ad-

verse events are gastrointestinal. These effects are dose related and occur predominantly when the drug's dose is increased. Gastrointestinal side effects can be minimized by dose titration of 8 mg every 4 weeks up to the 16- to 24-mg dose.

Other Cholinesterase Inhibitors

Both metrifonate and extended-release physostigmine have been studied in patients with Alzheimer disease. Indeed, attempts have been made to register these drugs for approval in the United States market, without success. Both drugs have been associated with some degree of efficacy (31–34). Metrifonate is an organophosphate that is a prodrug for its major metabolite, dichlorvose, which binds irreversibly to acetylcholinesterase. Physostigmine is a reversible inhibitor of both acetylcholinesterase and butyrylcholinesterase.

Development of both these drugs has been stopped, but for different reasons. Metrifonate has been associated with muscle weakness and a possible risk of respiratory muscle dysfunction leading to death. Organophosphate-related delayed neurotoxicity has been well described and has been linked to the binding of a phosphorylated metabolite of organophosphates to neurotoxic esterase (35). A likely related symptom, characterized as a myasthenic-like problem, has also been well described (35), and it seems similar to the problems that had led to the failure of metrifonate to reach the marketplace. Extended-release physostigmine has had a substantial association with nausea and vomiting, with 47% of patients reporting these symptoms during a 12-week trial (31). Unless additional work is done with these compounds to modify this adverse event profile, it is unlikely that either of these drugs will be available for routine use in the clinic.

Course-Altering Properties of Cholinesterase Inhibitors?

That cholinesterase inhibitors are efficacious in the palliative treatment of Alzheimer disease is now beyond question. A far more intriguing issue is whether these drugs alter the course of the disease. Here the data are far more tentative.

The effect of cholinomimetic activity on the processing of amyloid precursor proteins (APP) in various cell culture lines has been studied (36,37). Cholinergic stimulation apparently increases the production of nonamyloidogenic APP fragments. In all animals in which potentially amyloidogenic fragments of APP are increased as a consequence of lesioning in various neuronal populations, some, but not all, cholinomimetics normalize that process and diminish the production of amyloidogenic fragments (38). It has also been suggested that the toxicity of beta-amyloid peptide (Aβ) itself on neurons is diminished by some cholinesterase inhibitors (39,40).

Nicotinic stimulation may be particularly relevant in al-

tering the processes of neurodegeneration. Many epidemiologic studies have demonstrated that the relative risk of Parkinson disease is diminished among smokers compared with nonsmokers (41). However, prevalence studies, not incidence studies, among smokers versus nonsmokers suggest a neuroprotective effect in Alzheimer disease (42,43). The absence of incidence data is problematic for the imputation of any epidemiologic data to support the notion that smokers are less likely to be affected by Alzheimer disease than nonsmokers. Still, nicotinic stimulation has been found to protect neurons from β-amyloid induced neurotoxicity (44, 45), as well as to enhance the secretion of nonamyloidogenic forms of APP (46).

Ultimately, the question whether cholinomimetic activity, through nicotinic, muscarinic, or other unknown mechanisms, may alter the course of Alzheimer disease rests on clinical data. Such data are scanty and largely indirect. *Post hoc* analyses of patients who participated in the pivotal tacrine studies indicated that patients able to tolerate more than 80 mg per day of the drug had a substantial delay in placement in nursing homes, of the magnitude of approximately 450 days (47). Clearly, there are multiple interpretations of this observed phenomena that need not invoke the effect of tacrine on the progression of Alzheimer disease.

Acetylcholinesterase itself is present in plaques. This enzyme has been shown to enhance the aggregation of β-amyloid into the more fibrillar form that is deposited in plaques (48–51). Antibodies to cholinesterase blocks Aβ aggregation *in vitro* (52). Whether such effects on aggregation are produced by cholinesterase inhibitors, as occurs with antibodies directed at the cholinesterase molecule *in vitro,* has not been shown. It is possible that the aggregating effects of cholinesterase are facilitated by sites in the enzyme that are totally unaffected by cholinesterase inhibition. Alternatively, cholinesterase inhibition could alter cholinesterase in such a way as to diminish aggregating properties.

Two paradigms that could offer some insight into course-altering properties of cholinesterase inhibitors have been termed *delayed start* and *drug withdrawal.* In the delayed start paradigm, an agent that would alter the course of Alzheimer disease would be expected to have a greater effect in patients who have been started on the drug at time zero than a matched control group started 6 months later. In every study with cholinesterase inhibitors reported to date using a related but flawed delayed start procedure, placebo-treated patients who were given cholinesterase inhibitors 6 months after the group of patients on the drug did not catch up on cognitive measures to the patients who were treated with the drug from the start of the study. However, the interpretability of these data is limited because at the time of switchover from placebo to drug, the studies were no longer blinded. Furthermore, self-selection for switchover, or retention on drug, could occur and further confound these data. In the withdrawal paradigm, patients receiving the drug are randomly discontinued from the drug

on the completion of the trial and compared with the placebo group. Occasionally, this paradigm has demonstrated continued efficacy for some, but not all, cholinesterase inhibitors (52–54). Here too, methodologic problems limit an unequivocal interpretation of these data.

Taken together, no carefully conducted, adequately powered studies address the question of whether cholinesterase inhibitors, at any course in the illness, delay progression. Until such studies are carried out, only tantalizing pieces of the puzzle are open to interpretation. However, as interesting as this question is to clinical neuroscientists, it may be relatively moot to caregivers who struggle with patients with Alzheimer disease. To such people, the nuances of whether plaque and tangle formation may be slowed, and neurons kept alive, are less relevant than the question whether time to a particular milestone of the disease can be delayed by cholinesterase treatment. In fact, such an outcome can occur even if this class of compounds has a solely palliative effect. It can be argued that simply improving the patients' cognitive capacity increases the likelihood that a patient will be maintained at home. Additionally, the effects of some of these compounds, if not all, on such problematic noncognitive behaviors (55) can also lead to a better outcome. This seems increasingly likely given that the cholinesterase inhibitors appear to have their most robust effect in middle-stage disease, or perhaps even later (56–58). This result is completely compatible with postmortem findings of cholinergic parameters that find the cholinergic deficit to be most apparent in middle- or later-stage disease and to be not present in the earliest stages of illness (59).

VITAMIN E AND ANTIOXIDANTS

The production of free radical species has been considered a mediating event for many forms of neuronal death or damage. Initiating events as diverse as glutamate-induced neurotoxicity, ischemia, apoptosis, and Aβ neurotoxicity all can produce oxidative stress with free radical production (60). Thus, the use of antioxidants and free radical scavengers in the prevention, or delay in the progression, of Alzheimer disease is not without a reasonable rationale. Vitamin E, in part because of its accessibility, has received greatest attention among compounds in this class. Furthermore, *in vitro* cell studies in various cell culture preparations indicate that vitamin E can have a protective effect on β-amyloid–induced neurotoxicity (61).

A carefully conducted double-blind, placebo-controlled, multicenter investigation of the effect of vitamin E and selegiline provided some support for the efficacy of both these agents in altering the progression of Alzheimer disease (62). In this trial, patients with moderate to severe Alzheimer disease received either 2,000 IU per day of vitamin E, 10 mg per day of selegiline, or the combination of vitamin E and selegiline. An additional treatment arm exposed patients only to placebo. This was a 2-year trial in which the primary outcome measures were nursing home placements, death, or the loss of a well-defined activity of daily living. Cognitive change was also evaluated. All three antioxidant groups showed a statistically significant beneficial effect on all outcome measures except cognition. Surprisingly, a favorable effect on cognition was not found for any agent. Unfortunately, despite randomization, subjects in the treatment arms significantly differed in baseline Mini-Mental State Examination scores. Consequently, the significant results were only obtained when a covariant technique was used to adjust for the difference in baseline cognition across the treatment arms. This circumstance, combined with the negative effect on cognition, raises questions regarding the robustness of these antioxidant treatments. Nonetheless, the inclusion of 2,000 IU per day of vitamin E in the treatment regimen of patients with Alzheimer disease has become relatively commonplace.

Vitamin E ingestion is not without potential toxicity. Thrombophlebitis has been reported in adults in doses far less than the 2,000 IU recommended for patients with Alzheimer disease (63). Coagulopathy can be another vitamin E–associated adverse event (64–67). Interactions between vitamin E and oral anticoagulants are a real possibility and emphasize the need for monitoring prothrombin times in patients who receive this combination. In contrast to the widespread use of vitamin E, selegiline has not become a routine part of Alzheimer disease therapy because selegiline was not found to be superior to vitamin E, nor was there any benefit of combining vitamin E and selegiline to either drug alone. The adverse event profile for selegiline is far more extensive than vitamin E, and it includes hypotension with subsequent falls, as well as sleep disturbance, psychosis, agitation, and confusion. The potential for a serious interaction between selegiline and antidepressants commonly used to treat comorbid depression in patients with Alzheimer disease further limits the potential utility of selegiline.

ANTIINFLAMMATORY AGENTS

Inflammatory processes have well been characterized in the Alzheimer brain. Elevations in cytokine, acute-phase proteins, complement, and activated microglia are all present in Alzheimer disease brain (38,68–70). Of potential significance is that the complement cascade can be activated by Aβ, ultimately leading to the induction of the membrane attack complex, which can be neurotoxic (71–73). These postmortem findings are given increased meaning by epidemiologic studies that also impute a role for inflammatory mechanisms in Alzheimer disease. The use of nonsteroidal antiinflammatory drugs (NSAIDs) well before the onset of Alzheimer disease has been associated with a decreased incidence of Alzheimer disease in late life. Studies of siblings with differential exposure to NSAIDs reveal a profound delay in the onset of Alzheimer disease in the sibling with exposure to these agents (74–77).

Particular interest has centered on the inhibition of cyclooxygenase in Alzheimer disease. Although the inflammatory reaction in the Alzheimer brain appears quite broad, a rationale nonetheless exists for inhibition of cyclooxygenase, especially cyclooxygenase 2 (Cox-2). Cox-2 levels are elevated in hippocampal neurons from postmortem examination of patients with Alzheimer disease (78). Additionally, Cox-2 expression is up-regulated in the frontal cortex of the patient with Alzheimer disease. The severity of Alzheimer disease neuropathology correlates with Cox-2 levels (78) and β-amyloid increase expression of Cox-2 in neuroblastoma lines.

Given these data, it is not surprising that numerous antiinflammatory agents are being, or have been, tested in patients with Alzheimer disease. With the extensiveness of the inflammatory response in the Alzheimer disease brain, a relatively nonspecific antiinflammatory drug such as prednisone seemed a rational approach to treatment. A large, multicenter, double-blind study in which an initial dose of up to 20 mg of prednisone, followed by a maintenance dose of 10 mg for 1 year, was conducted. No evidence of efficacy in delaying the progression of Alzheimer disease was found. Indeed, patients receiving prednisone were more likely to develop behavioral worsening as well as glucocorticoid-related medical adverse events. Although it is conceivable that a higher dose of prednisone was necessary, the administration of such a dose would seem impossible, based on the medical problems encountered with relatively modest doses of prednisone (79).

Diclofenac, another antiinflammatory agent, was investigated in a 25-week randomized, double-blind, placebo-controlled trial in patients with mild to moderate Alzheimer disease. The patient withdrawal rate from the study was exceedingly high, and it limited the interpretability of the results. Nevertheless, efficacy of this agent was not found. Conversely, indomethacin administered in a 6-month trial was reported to be efficacious, but here, too, the dropout rate was excessive, compromising both the interpretability of the results as well as the ultimate utility of this drug (80).

The most positive results obtained to date from large-scale studies derive from the clinical trials with propentofylline. This drug is an inhibitor of microglia activation. A series of studies demonstrated improvement in global functioning, cognitive measures, and activities of daily living compared with placebo (81–83). However, the effects were exceedingly modest, and attempts to obtain approval for an Alzheimer disease–related indication in the European community have so far been unsuccessful, because the extent of drug effect has not been deemed to be sufficient to warrant approval.

Numerous trials with selective Cox-2 inhibitors are currently ongoing. These results are eagerly awaited. However, to date, despite the relatively compelling rationale for testing antiinflammatory agents in Alzheimer disease, results have not been encouraging. The apparent contradiction between epidemiologic studies showing benefit from prior exposure to NSAIDs and treatment studies with NSAIDs could reflect the period in which NSAIDs were administrated. Conceivably, such drugs will have no effect, or even an adverse effect, once Alzheimer disease has developed, but they may still be effective in delaying onset by drug administration before patients are symptomatic. Hence, a full test of the antiinflammatory approach in Alzheimer disease will require additional studies.

ESTROGEN

As with antiinflammatory agents, the basis for estrogen therapy in Alzheimer disease, in part, derives from epidemiologic studies. One such study, the Baltimore longitudinal study of aging, followed 500 women, of whom half were estrogen users, for approximately 16 years. The relative risk of developing Alzheimer disease in the women who were taking estrogen was approximately halved (84). A similar result was obtained in an Italian longitudinal aging study (85). Other epidemiologic surveys have reached similar conclusions (86). The plausibility of these results are enhanced by the finding that estrogen replacement therapy was associated with higher cognitive test scores in healthy elderly women over the age of 65 years, compared with a cohort not receiving such treatment (87,88). There is, however, one large 15-year follow-up study of approximately 800 elderly women in which no relationship between estrogen replacement therapy and a host of neuropsychological test scores was found (89).

That estrogen replacement therapy may have a positive effect on the development of Alzheimer disease, or cognition in general, is supported by a series of studies investigating the actions of estrogen on neuronal tissue. For example, ovariectomized rats treated with estrogen show preservation of the integrity of hippocampal neurons and their dendritic arborization (90). Furthermore, activity of choline acetyltransferase is augmented by estrogen treatment (91,92). Estrogen may also have antioxidant activity, may facilitate processing of APP toward a nonamyloidogenic pathway, and may promote cell survival (92,93). Hence, some role for estrogen in the therapeutics of Alzheimer disease is a reasonable proposition.

Two studies examined the effect of estrogen on both the course and symptoms of Alzheimer disease. Estrogen replacement therapy for 1 year did not slow disease progression among women with mild to moderate Alzheimer disease who had previously undergone a hysterectomy (94). In another randomized, double-blind, placebo-controlled parallel group study, no effects of estrogen on cognitive symptoms was noted (95). Conversely, some benefit of a transdermal estrogen preparation was noted in an 8-week treatment trial in a very small group of women. Furthermore, positive results were found in a few, but not all,

neuropsychological tests (96). Given the effect of estrogen on cholinergic parameters, of note is a retrospective analysis of patients previously exposed to tacrine in the pivotal trials leading to the approval of that drug. Women taking estrogen replacement therapy had a significantly greater response on all outcome measures than those female patients receiving tacrine who were not receiving estrogen replacement therapy. These data raise the possibility that estrogen replacement therapy may augment the cognitive effects of cholinesterase inhibitors (97).

Selective estrogen-receptor modulators (SERMs) have been designed to have agonistic effects on some organ systems and antagonistic effects on others. Should estrogen replacement therapy have beneficial effects in preventing Alzheimer disease, delaying its progress, treating its symptoms, or augmenting other therapies, a SERM with agonist activity in the brain, but without effect on reproductive organs, would have obvious therapeutic potential, including administration to male patients. Many SERMs are currently being tested in numerous conditions. However, as yet no reports of studies on the role of these agents in any aspects of Alzheimer treatment have been published.

GINKGO BILOBA

The broad use of vitamin and herbal preparations, facilitated by their general availability without prescription, encouraged a placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia (98). This extract, termed *Egb761,* was tested in a 52-week study of mild to severely demented outpatients with various forms of dementia including Alzheimer disease and multiinfarct dementia. One-third of all patients entered into the study did not provide 52-week endpoint data. A small and statistically significant effect was found on the ADAS-cog, but no effect was found on the Clinical Global Index (CGI). Thus, by a prior standard set by the FDA to establish efficacy of an agent in Alzheimer disease (statistically significant drug effect on both a psychometric and a global measure), Egb761 would not have met this standard for receiving an indication for the treatment of Alzheimer disease. Nonetheless, this compound continues to be widely used, even though it has been reported to cause spontaneous bleeding and it may interact with anticoagulant and antiplatelet aggregating agents (99).

APPROACH TO ALTERING AMYLOID DEPOSITION

Increasingly, amyloid deposition is seen as one of the earliest components, if not the earliest, of the pathologic process in Alzheimer disease, as well as an initiating event in neuronal death (100,101). Furthermore, the elucidation of the cellular consequences of the various mutations associated with Alzheimer disease supports the notion of the centrality of amyloid production in the pathophysiology of Alzheimer disease. Specifically, regardless of whether a mutation occurs in the amyloid precursor protein gene, presenilin 1 or presenilin 2, all mutations increase the concentrations of $A\beta1$-42 in brain, plasma, or cell culture media. A similar outcome is associated with the apolipoprotein E-4 allele compared to E-2 or E-3 (101–103). The well-documented toxicity of $A\beta$, particularly in the aggregated form, adds to the growing consensus that altering $A\beta$ production or deposition is a viable approach to the therapeutics of Alzheimer disease.

There are numerous theoretic approaches to altering the $A\beta$ concentrations in the brain of patients with Alzheimer disease. The activities of both β- and γ-secretase are necessary to cleave APP into the $A\beta$ fragments that constitute amyloid plaques. Conceivably, inhibiting either γ- or β-secretase could alter the production of $A\beta$. Alternatively, enhancing the activity of α-secretase could result in the preferential cleavage of APP to nonamyloidogenic forms. Yet another approach focuses on enhancing the breakdown or clearance of $A\beta$ in the brain. This approach adopts the view that inflammatory mechanisms in the Alzheimer brain are potentially beneficial and facilitate the removal of $A\beta$ from the brain. Finally, the enhanced toxicity of aggregated $A\beta$ encourages therapeutics designed to block the aggregation of $A\beta$. All these approaches are in various stages of clinical development.

Numerous groups have cloned and characterized β-secretase, also termed β-amyloid cleavage enzymes (BACE) (104–109). The success of this effort encourages combinatorial chemistry and screening efforts designed to identify small lipophilic compounds that could inhibit BACE activity and thereby limit $A\beta$ production. The logic of this approach is unquestioned, but the presence of relatively high levels of BACE in the pancreas leads to the question of the role that BACE may play in biological functions whose activity, if inhibited, could cause significant adverse events. Conversely, to produce meaningful changes in the course of Alzheimer disease, or simply to delay the disease onset, safe levels of brain BACE inhibition may readily exist.

Although γ-secretase has not yet been cloned, a γ-secretase inhibitor is currently in clinical trial (104). However, the intimate relationship between presenilins and γ-secretase could have implications for the ultimate safety of this approach. If, in fact, presenilins influence the critically involved Notch pathway (37), a host of potential adverse effects could arise from inhibiting the activity of presenilins. Still, elucidation of the clinical effects of the γ-secretase inhibitors will be eagerly awaited.

Transgenic mice overexpressing $A\beta$ have been used as a vehicle to determine whether inoculation with the $A\beta$ peptide could produce an immune response that would alter $A\beta$ concentrations in a mouse brain (110). Animals inoculated before the deposition of substantial amyloid deposits in the

brain subsequently displayed little amyloid deposition. Even more remarkably, animals in which amyloid deposition had already begun demonstrated an apparent diminution in amyloid plaque load following inoculation. Behavioral data now confirm that Aβ peptide immunization reduces cognitive impairment and plaques in animal models of Alzheimer disease (111–113). Vaccination with Aβ protects transgenic mice from the learning and age-related memory deficits that normally occur in the mouse model of Alzheimer disease. During testing for potential deleterious effects of the vaccine, all mice performed superbly on the radial-arm water maze test of working memory. Later, at an age when untreated transgenic mice show memory deficits, the Aβ-vaccinated transgenic mice showed cognitive performance superior to that of the control transgenic mice and, ultimately, performed as well as nontransgenic mice (111).

Based on these exciting results, Aβ inoculations are beginning in humans. A major question that these trials will eventually answer is whether elderly persons can generate an adequately robust immune response to Aβ inoculation that will extend to the brain. Additionally, the concern that the adjunctive procedures necessary to generate an immune response to a peptide that already exists in healthy humans would also produce an autoimmune response must be considered. Still, results obtained in transgenic mice are so dramatic that it is essential that Aβ inoculations proceed at least preliminarily in humans.

There are numerous theoretic possibilities to altering the aggregation of Aβ fibrils into their more toxic aggregated form. Congo red, a dye that readily binds Aβ, has been used as a prototypical molecule for the development of analogues that would enter the brain, bind to Aβ, and inhibit aggregation (102,103). Other approaches have included the development of antibodies directed specifically at Aβ or small molecule ligands (114) that also can block aggregation (115,116). Acetylcholinesterase has been found to augment Aβ aggregation, and antibodies to acetylcholinesterase can, *in vitro,* decrease aggregation (50). Indeed the Alzheimer plaque contains numerous proteins, many of which may facilitate aggregation of Aβ. Developing compounds that preferentially bind these plaque-containing molecules could decrease Aβ aggregation.

SUMMARY

Since the early 1990s, remarkable progress has been made in the current and experimental therapeutics of Alzheimer disease. An area that was recently characterized by therapeutic nihilism can now be regarded with real optimism. It would seem highly likely that the next decade of progress should show the development of compounds that move beyond palliation and could actually either delay onset or substantially alter the course of the illness in such a manner as to bring new hope to the patient with Alzheimer disease.

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