ABSTRACT

Accumulation of β-amyloid protein (Aβ) in the brain is one of the key neuropathologic features of Alzheimer’s disease. While current understanding of how Aβ is generated has grown there is still no approved treatment available that either prevents its formation or removes it from the brain. However, numerous treatment strategies are being tested that are aimed at each step of the amyloid pathway—from its initial liberation, to its early aggregation, and finally to the eventual formation of insoluble β-pleated sheets. Today, Aβ is the most accessible therapeutic target available for Alzheimer’s disease. In the process of developing treatments based on Aβ modification, there is a great deal to learn not only about Alzheimer’s disease but also about the normal function of the brain. This article discusses the current status of various strategies aimed at reducing amyloid burden in the brain.

INTRODUCTION

Alzheimer’s disease is the leading cause of dementia in the elderly. Prevalence estimates of Alzheimer’s disease in the United States range from 5.7% to 10% among people 65–85 years of age and 25% to 45% in those ≥85 years of age.1 There are 5.2 million Americans with Alzheimer’s disease in the US. While the clinical diagnosis of Alzheimer’s disease depends on recognition of typical cognitive deficits and behavioral disturbances, the neuropathologic hallmarks of the disease depend on detection of intra-cellular neurofibrillary tangles and extra-cellular amyloid plaques in the brain.2 The “amyloid hypothesis” is the most widely embraced theory for the causation of Alzheimer’s disease and posits that the deposition of amyloid is an early crucial event that in turn leads to a cascade of other neuropathologic changes that culminate in the final full-blown disease. An implication of this theory is that if amyloid could be somehow kept from forming or removed once in place, it might offer a specific treatment for the disease.

One of the keys to understanding the mystery of Alzheimer’s disease is elucidating the role of β-amyloid protein (Aβ). In the last 25 years research have moved from the first identification of Aβ as a significant component of the senile plaque of patients with the disease to an advanced understanding of many of the basic metabolic pathways that control the production of this protein. This article briefly reviews the current status of Aβ as a diagnostic marker,
discusses the current understanding of how Aβ is processed in the brain, and reviews the many potential therapeutic strategies focused on reducing or preventing the accumulation of Aβ in the brain.

THE PLACE OF AMYLOID IN ALZHEIMER DISEASE

The ultimate diagnosis of Alzheimer’s disease is based on neuropathologic criteria, with the accumulation of excessive Aβ being one of the key features. These criteria depend in part on such factors as the age-adjusted density of certain types of amyloid-containing plaques (neuritic vs. diffuse) in specific sampled brain areas.\(^3\)\(^5\) However, even though the quantification of Aβ plays a crucial role in diagnosis, Aβ accumulation in general is not specific since plaques are also seen in apparently normal aging. In fact, there is only a modest correlation between the amount of accumulated Aβ and the degree of cognitive impairment present in the individual case.\(^6\)\(^7\) Aβ also accumulates in the walls of arterioles and arterioles in the leptomeninges and cerebral cortex as cerebral amyloid angiopathy (CAA) in both Alzheimer’s disease and with aging.\(^8\)

The strongest evidence that Aβ plays a key pathologic role in the disease process is based on the rare familial cases of Alzheimer’s disease.\(^9\)-\(^13\) The three identified genes that produce early-onset, autosomal dominant Alzheimer’s disease each result in a significant increase in Aβ production. In addition, patients with Down syndrome have an extra copy of the amyloid precursor protein (APP) gene on chromosome 21 and very commonly have Alzheimer’s disease-like changes in the brain with advancing age. In these cases, overproduction of amyloid or abnormalities in its processing seem sufficient to cause the dementia syndrome. However, the role that amyloid plays in typical late-onset Alzheimer’s disease is much less clear.

The uncertain role of amyloid in common, late-onset Alzheimer’s disease is highlighted by reports of the presence of amyloid in the brains of cognitively intact individuals. It has been appreciated for some time that there exist a group of individuals who are cognitively intact at the time of autopsy, despite high levels of Aβ in their brains.\(^14\) Similarly, the emergence of compounds such as Pittsburgh Compound-B (PIB), which binds to fibrillar Aβ deposits in plaques and to amyloid angiopathy,\(^15\)-\(^17\) have allowed the detection of amyloid \textit{in vivo}. Studies have shown the presence of Aβ not only in the brains of those with a clinical diagnosis of Alzheimer’s disease but also in normal controls.\(^15,18\)-\(^20\)

The presence of amyloid in the brains of these patients without dementia implies either that amyloid can be present in normal patients without apparent cognitive impact or that it is present in people who have pre-clinical Alzheimer’s disease. Long-term follow up of these cognitively normal, PIB+ people will be necessary to resolve this dilemma.\(^20\) While the interpretation of these findings is currently being debated, it is clear that the presence of Aβ, even if necessary, does not appear sufficient to have a simple, immediate causative role.

On balance, however, Aβ remains the most accessible therapeutic target currently available. Given the enormous attention that Aβ has been given, it is not surprising that there are numerous approaches to disease management underway. This article discusses a number of these approaches and how they purport to address the amyloid hypothesis (Table).

FORMATION OF Aβ

Aβ is the end product of two sequential enzymatic cleavages of the integral membrane protein, APP (Figure).\(^21\)\(^22\) The generation of Aβ from APP occurs through the action of the enzymes β-secretase and γ-secretase. APP must first be processed by the β-secretase BACE (or BACE1; β site APP-cleaving enzyme) located on the extracellular side of APP, 28 residues from the membrane spanning region of APP. The next step in liberating Aβ is the action of the γ-secretase enzyme complex which contains four integral membrane proteins (one of which is presenilin). The known causes of familial Alzheimer’s disease are the result of mutation in APP or one of two presenilin genes.\(^13\) The end product of all known mutations leading to familial Alzheimer’s disease is an increase in the production of Aβ\(_1\). Altering the activity of β- and γ-secretase with the goal of reducing the formation of Aβ is therefore an attractive target for drug development.

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IVIg=intravenous immunoglobulin.

THERAPEUTIC STRATEGIES AIMED AT REDUCING OR ELIMINATING Aβ

Immunotherapy

One the most fascinating approaches to eliminating excess Aβ in the brain of those with Alzheimer’s disease is the attempt to do so by use of vaccines directed against Aβ or its components. This work began with the demonstration that human aggregated Aβ42 (AN1792) delivered to transgenic mice was effective in reducing cerebral Aβ plaque burden. This success led to the first human trial of AN1792 (with adjuvant) in 80 patients in the United Kingdom who had mild to moderate Alzheimer’s disease. Fifty-nine percent of the immunized patients developed antibodies against Aβ and adverse events were felt to be acceptable, though one patient developed meningoencephalitis that was diagnosed after death and 219 days after discontinuing from the study. A second trial was suspended early in its course when approximately 6% of participants developed meningoencephalitis. Given the early interruption of this trial, only limited follow-up of this cohort has been possible; however, after 1 year 19.7% of the subjects had developed the predetermined antibody response. These individuals did not differ from the non-responders on several standard measures of cognition, function, or global change but did perform better on a composite neuropsychological test battery. In addition, responders unexpectedly had greater brain volume loss on magnetic resonance imaging than non-responders. A variety of possible reasons for this volume loss have been proposed, including volume reduction due to removal of amyloid plaques, fluid shifts of cerebrospinal fluid associated with amyloid mobilization, or even loss of neurons. While any of these mechanisms could in part explain the volume loss, it is clear that substantial removal of amyloid did occur in some of these patients at long-term follow-up.

A 6-year follow-up of eight participants from this trial who were immunized and who were examined neuropathologically revealed that cortical Aβ loads were lower than in an unimmunized control group. The degree of plaque removal varied significantly with the mean antibody response attained during the treatment study period. Seven of the eight subjects had severe end-stage dementia at the time of death, including those with virtually complete plaque removal. Overall, there was no evidence of improved survival or time to severe dementia in the immunized group. While these results come from only a very small sample they demonstrate that immunization can lead to amyloid clearance but do not suggest that such clearance is clinically beneficial.

A recent analysis of the cerebral vasculature of these same patients has revealed a striking increase in the number of blood vessels containing Aβ42 in the cerebral cortex and leptomeninges (14- and 7-fold increase, respectively) compared to an unimmunized control group of patients with Alzheimer’s disease. Furthermore, in the patients who lived longest after immunization, there was a complete lack of CAA, mirroring the absence of plaques noted above. The authors suggest these findings support the hypothesis that Aβ immunization cause plaque Aβ42 to become soluble and then transit, at least in part, through the vessels of the central nervous system, resulting in a transient increase in CAA. These changes were associated with cortical microhemorrhages and microvascular lesion but not with CAA-related intracerebral hemorrhages.

One issue raised by these results is the timing and sequencing of the many neuropathologic processes that occur during the course of Alzheimer’s disease. It has been proposed that the toxic effects of Aβ are an early event in the pathophysiology of Alzheimer’s disease and once other neurodegenerative processes are initiated they may proceed even if Aβ is removed at a later point in time. This result makes the outcomes of ongoing immunotherapy critically important in order to determine if the overall approach will lead to meaningful response.

Given that the amyloid clearance induced by the AN1792 vaccine was felt to be due to antibody-based immunity while the cases of meningoencephalitis were thought to be due to cell-mediated immunity, another approach that is under active investigation is the use of monoclonal antibodies directed against Aβ (“passive immunization”). Passive immunization has several advantages compared to active immunization, including that monoclonal antibodies can be engineered to bind to Aβ at specific sites to limit activation of cell-mediated immunity, that the quantity of antibodies delivered to patients can be standardized and regulated, and that the infusion of antibodies can be stopped if side effects arise.

**FIGURE**

SECRETASE ACTIVITY ON THE AMYLOID PRECURSOR PROTEIN

APP = amyloid precursor protein; Aβ = β-amyloid protein.

Bapineuzumab is a humanized monoclonal anti-\(A\beta\) antibody currently in phase 3 trials. Results of a 234 patient phase II trial\(^{36}\) presented at the International Conference on Alzheimer’s Disease in 2008 were negative on pre-specified endpoints. However, in post-hoc analyses there was evidence of a statistically significant benefit in those patients who completed the trial and received all six planned injections as well as in the 33% of patients who lacked the ApoE4 allele (“noncarriers”). Twelve patients developed vasogenic edema, all in the treatment group and all of which resolved (one patient received steroid treatment). Ten of 12 patients with vasogenic edema were ApoE4 carriers and eight of the 12 were at the highest dose of the monoclonal antibody.\(^{37}\) Vasogenic edema can be detected on MRI as high-signal white matter intensities on fluid-attenuated inversion recovery sequences. The mechanism by which vasogenic edema occurred in these cases is not yet fully understood though changes in vascular permeability (ie, increased brain capillary permeability) associated with antibody-antigen interactions seem plausible. Vasogenic edema is often asymmetric or can be associated with transient symptoms such as increased confusion, lethargy, headache, and dizziness. The MRI changes of vasogenic edema have universally resolved over time with serial imaging. Phase III trials are being conducted in ApoE4 subgroups separately, using the lower doses tested in the Phase II trials, especially in the ApoE4 carriers, to limit the risk of the development of vasogenic edema.

Another variation of immunotherapy that has demonstrated some modest initial success is the use of intravenous immunoglobulin (IVIg), a pooled human IgG antibody preparation that contains 90% of all IgGs and IgG subclasses.\(^{38}\) Among these antibodies are those that target A\(\beta\). A small phase II trial\(^{39}\) with 24 patients with mild to moderate Alzheimer’s disease has reported positive results at 9 months on the primary outcome measures and phase III trials are underway.

### Preventing Formation of Fibrils

Considerable work has shown that not all forms of amyloid are equally toxic. As A\(\beta\) is generated from the processing of APP, it first exists in a soluble form that appears relatively non-toxic. It is when A\(\beta\) comes together to form oligomers (soluble aggregates of a few amyloid molecules joined together) on its way to fibrils that its toxicity first becomes manifest. Thus, methods to prevent the fibrillization of A\(\beta\) into insoluble \(\beta\)-pleated sheets have also been examined. One compound that is purported to be an inhibitor of fibrillogenesis, tramiprosate (a modification of the amino acid taurine),\(^{40}\) failed to demonstrate any clinical benefit in phase III trials and is no longer under development as a drug, though the manufacturer has introduced homotaurine as a nutraceutical.\(^{35}\)

### Secretase Inhibition

Inhibition of BACE is an appealing strategy since APP appears to be its single substrate. Knockout mice lacking BACE do not have detectable levels of A\(\beta\) and yet do not appear adversely affected. These two facts taken together suggest that compounds targeted at BACE that lower A\(\beta\) might be tolerated over a clinical time frame.\(^{36}\) However, clinical trials have lagged because of problems with blood-brain barrier penetration and bioavailability of compounds targeted at BACE.

Targeting \(\gamma\)-secretase as an alternative is challenging for a variety of reasons, including the facts that the enzyme structure is far more complex than BACE and is also responsible for processing other important transmembrane proteins—including Notch, which plays a crucial role in cell differentiation in many organ systems.\(^{37}\) While numerous potent \(\gamma\)-secretase inhibitors have been identified, their specificity for APP cleavage has been variable and therefore affects these other substrates. Therefore, any inhibition targeted at this enzyme must either be highly specific for APP or less specific but sufficiently transient in duration of effect to reduce levels of A\(\beta\) while simultaneously allowing some residual physiologic Notch functioning. \(\gamma\)-secretase inhibitors have reached clinical trials and at least one agent has demonstrated reduced plasma levels of A\(\beta\) with seemingly acceptable tolerability.\(^{38}\) LY450139, a \(\gamma\)-secretase inhibitor now in phase III trials, reduced plasma A\(\beta\)\(_{40}\) and A\(\beta\)\(_{42}\) concentrations after 20 weeks in a phase II trial.\(^{38}\)

Manipulation of the APP secretases is a rapidly evolving arena for research and it seems likely that numerous agents will be tested. Not only do such agents hold promise as possible treatments for Alzheimer’s disease, but they will also provide additional clues as to the true value of A\(\beta\) as a target for therapeutics.

### \(\gamma\)-Secretase Modulation

Given the potential problems involved with inhibiting \(\gamma\)-secretase, an alternative approach proposes to target the substrate, in this case the site on APP where \(\gamma\)-secretase acts, rather than directly targeting the enzyme.\(^{39}\) The intent is to administer a compound which blocks the cleavage site so that it is not available to \(\gamma\)-secretase, thereby preventing the formation of A\(\beta\). Such compounds have been described as “molecular cloaking devices” and reflect a new approach to therapeutics.\(^{40}\) This mechanism was proposed as the mechanism of action of tarenflurbil, which was being explored as a selective amyloid-lowering agent for Alzheimer’s disease.\(^{41}\) Modest benefits in a Phase II trial\(^{42}\) of tarenflurbil were not seen in an 18-month Phase III trial that failed to show efficacy on either primary endpoint.\(^{43}\) The compound is no longer in development though many other potential modulators may yet be tested.

### Zinc- and Copper-based Therapies

The recognition that both copper and zinc play a role in the process by which soluble forms of A\(\beta\) form oligomers has led to trials of compounds that can inhibit this process. The toxic oligomers of A\(\beta\) are dimers and trimers formed from A\(\beta\) monomers. This process is mediated in part by zinc and copper, which have been reported to be in high concentrations around excitatory N-
methyl-d-aspartate synapses.44-46 Clioquinol, and more recently PBT2, have been proposed as potential therapies based on their ability to complex with low affinity to metals without perturbing metal homeostasis.44 As such, these compounds attenuate metal-protein interactions without being metal "chelators." PBT2 was recently tested in a 78-patient safety trial.44 Patients treated with 250 mg of PBT2 had a dose-dependent and significant reduction in CSF Aβ42 concentration compared to placebo. Subjects taking 250 mg of PBT2 also showed significant improvements in category fluency and trail making part B compared to placebo. No serious adverse events were reported in subjects taking PBT2. Larger trials will be conducted.

CONCLUSION

Aβ is the target of multiple strategies designed to treat patients with Alzheimer’s disease. Although the trials of active immunization were terminated early due to safety issues, follow-up studies show benefit in clearing amyloid plaques. The promise of immunotherapy is being evaluated by clinical trials using passive immunization and IV Ig. Secretase inhibition is an attractive strategy which is being aggressively pursued, while γ-secretase modulators and metal protein attenuating compounds are also being explored for their ability to reduce amyloid burden. A key issue that remains to be solved is the appropriate timing of these potential interventions. It may be possible that removing amyloid at the point where dementia can be diagnosed clinically may be too late. Interventions targeted at the pre-clinical state may be required and will await better diagnostics. In the meantime, amyloid remains an attractive target for therapeutic intervention. PP

REFERENCES