Do amyloid-lowering strategies work clinically?

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Nowadays, the amyloid hypothesis, introduced more than 20 years ago, is the most widely accepted hypothesis explaining the development of Alzheimer's disease (AD) [Hardy *et al.* 1992]. According to this hypothesis, that has undergone some alterations during the last two decades, the increased production of the amyloid- β peptide ($A\beta$) from the amyloid precursor protein (APP) and the accumulation and deposition of this $A\beta$ in the brain will lead to oxidative stress, neuronal destruction and finally the clinical syndrome of AD [Hardy *et al.* 2002]. Together with the presence of intraneuronal tangles, consisting of phosphorylated tau-proteins, amyloid plaques form the hallmark of the pathological diagnosis AD. $A\beta$ consists of two major forms, $A\beta_{40}$ and $A\beta_{42}$, depending on whether the C-terminus of the protein ends at the 40th or the 42nd amino acid. $A\beta_{42}$ is the predominant form found in the brain parenchyma of AD patients. $A\beta_{40}$ is mostly found in the cerebral vasculature as part of 'cerebral amyloid angiopathy'. $A\beta_{42}$ has a tendency to cluster into oligomers. Oligomers can form $A\beta$ -fibrils that eventually will form deposits called amyloid plaques. Several lines of evidence have converged to demonstrate that soluble oligomers of $A\beta$, but not plaques, monomers or insoluble amyloid fibrils, may be responsible for synaptic dysfunction in the brains of AD patients [Selkoe, 2008]. In transgenic and other models of co-expressed $A\beta$ and tau, $A\beta$ oligomer formation precedes and accentuates tau-related pathology, which is consistent with the hypothesis that formation of neurofibrillary tangles is downstream of $A\beta$ aggregation. As a result of this process, tau-proteins will fold into intraneuronal tangles, which results in cell death [Rankin *et al.* 2008]. Progressive neuronal destruction will lead to

shortage in, and imbalance between, various neurotransmitters (e.g. acetylcholine, dopamine, serotonin) and to the cognitive deficiencies seen in AD.

Evidence for the association between altered amyloid production and the development of AD is found in the relationship between mutations in genes associated with APP processing and the risk of developing AD [Bertram *et al.* 2008]. Mutations in the APP-gene at chromosome 21 will lead to AD. Also mutations in the presenilin genes (PS-1 and PS-2) will lead to early AD, possibly by altering the function of gamma-secretase protease, one of the cutting enzymes of APP. Evidence linking $A\beta$ to sporadic AD is less extensive. It is remarkable that the number of amyloid deposits in the brain does not correlate well with the degree of cognitive impairment that the patient experienced in life. Given this weak correlation, some say that the presence of $A\beta$ is merely a marker for the presence of AD and not the driving force in pathophysiology.

There is no doubt that alterations in APP processing, leading to the production of excess $A\beta$, are associated with the development of AD. It is therefore not surprising that influencing the processes involved in amyloid formation has become the Holy Grail for the pharmaceutical industry. In the 1990s, the focus was mainly on the development and clinical evaluation of the cholinesterase inhibitors (ChEIs) - donepezil, rivastigmine and galantamine - drugs that restore neurotransmitter imbalances resulting from neuronal cell death. The focus in the last decade has been on developing drugs that

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decrease the production, or increase the clearance of, A β [van Marum, 2008]. Compared to the ChEIs, one might expect larger and longer-lasting effects on clinically relevant outcomes (e.g. cognition, global functioning and activities of daily living) from these disease modifying drugs.

One of the interventions considered most promising is immunological clearance of A β . Both active (monoclonal antibodies) and passive (vaccination) immunization therapies are being studied. One of the first active vaccination trials was initiated using human beta amyloid A β ₁₋₄₂ (AN-1792) in conjunction with a T-helper adjuvant (QS-21). Unfortunately, in 2002 the phase II trial with AN-1792 in mild-to-moderate AD patients was discontinued because of the occurrence of meningoencephalitis in 6% of the participants [Gilman *et al.* 2005]. In this study, 59 (19.7%) of the 300 AN-1792-treated patients developed the predetermined antibody response. On MRI, these antibody responders showed a greater brain volume decrease, greater ventricular enlargement and a nonsignificant greater hippocampal volume decrease than placebo patients, suggesting that the immunoresponse had resulted in clearance of amyloid plaques [Fox *et al.* 2005]. It was therefore very disappointing that for almost all predetermined cognitive and functional outcome measures, no statistically significant differences were found between responders and nonresponders. Recently, 6-year follow-up results of the population that started in 2000 in the phase I trial with AN-1792, including postmortem brain examinations, were published [Holmes *et al.* 2008]. Immunization seemed to initiate a long-term process, with postmortem evidence of plaque removal 5 years after the last injection. In the survivors, there was evidence of persistently raised serum antibodies to A β correlating with the initial mean antibody response. However, all but one of the individuals who died during the follow-up phase had clear end-stage dementia before death, including the two individuals with the highest mean antibodies to A β and almost complete elimination of plaques. Other features of the disease (e.g. the accumulation of tau) remained even in areas of amyloid clearance. This implies that progressive neurodegeneration can occur in AD despite removal of plaques.

The results from the phase II trial studying the effects of passive immunization with the monoclonal antibody bapineuzumab in mild-to-moderate AD were presented in 2008 at the ICAD in Chicago [<http://www.elan.com/News/Bapineuzumab.asp>]. Statistically significant effects of bapineuzumab on predefined clinical outcomes could only be found in the subgroup of patients negative for the APO-E4 gene. The magnitude of these effects, however, was not much larger than the effects found for ChEIs in AD. A large phase III study has been started this year.

Intervention in processes involved in aggregation of A β into oligomers or plaques has also yielded disappointing results so far. The drug glycosaminoglycan 3-amino-1-propanesulfonic acid (3APS, tramiprosate, Alzhemed[®]) was designed to interfere with the binding of glycosaminoglycans and A β therewith preventing conformational transitions that lead to the assembly of oligomers, protofibrils, and fibrils. Despite promising results in a phase II study, disappointing interim results from the phase III trial, in which no significant effects could be found on clinical endpoints led to early abortion of the study in 2007 [Aisen *et al.* 2006]. Other researchers have focused on the role of cofactors in the aggregation of A β . Results from a phase II study with PBT2, a metal-protein attenuating compound that affects Cu²⁺-mediated and Zn²⁺-mediated toxic oligomerisation, were recently published [Lannfelt *et al.* 2008]. PBT2-treated patients had a dose-dependent and significant reduction in CSF A β concentration compared with those treated with placebo. Of all cognition tests, only two executive function component tests of the NTB showed significant improvement over placebo in the PBT2 group.

Interventions in the production of A β from APP have also shown little clinical effects. The nonsteroidal anti-inflammatory drug (NSAID) tarenflurbil, a selective amyloid-lowering drug (SALA) has been studied in patients with mild-to-moderate AD. The purpose of SALAs is to shift the γ -secretase cutting point in order to produce shorter, nontoxic A β fragments. After positive results of tarenflurbil in a phase II trial in patients with mild-to-moderate AD, the phase III trial failed in finding positive effects and was stopped in 2008 [Wilcock *et al.* 2008].

Another protease involved in APP cleaving is β -secretase or BACE1. BACE1 activation leads to an increased production of A β . Pro-inflammatory mediators can increase BACE1 mRNA whilst, opposite to this, BACE1 can be down regulated by certain NSAIDs. The protective mechanism by which NSAIDs inhibit BACE1 involves activation of the peroxisome proliferated activated receptor- γ (PPAR- γ) [Heneka *et al.* 2007]. It is not surprising that this finding has led to studies in AD with PPAR- γ agonists currently used in diabetes mellitus. A small study in 2005 in 30 subjects with mild AD or amnesic mild cognitive impairment provided preliminary support that rosiglitazone might offer a novel strategy for the treatment of cognitive decline associated with AD [Kulstad *et al.* 2005]. This was followed by a larger trial in which 518 patients with mild-to-moderate AD were treated for 6 months with rosiglitazone [Watson *et al.* 2006]. Treatment was associated with a statistically significant improvement in cognition only in patients that did not possess an ApoE4 allele. Patients with ApoE4 did not experience any improvement in cognition or function.

In conclusion, drug trials that have investigated amyloid synthesis or deposition have not given us what was hoped for – results, at best, do not exceed the results from 6-month duration trials with the ChEIs. The problem so far is that, although current drugs clearly affect amyloid metabolism, these changes do not result in large clinically significant effects. The best efficacy data that have been published come from trials studying drugs that are not primarily associated with A β metabolism. The effects of dimebon on patients with mild-to-moderate AD were published this year and exceeded those from ChEI trials [Doody *et al.* 2008]. The mechanism of dimebon in AD has not been fully elucidated. The authors suggest that it exerts its effects at the level of the mitochondria, which could enhance neuronal function. Promising preliminary data were also presented at this year's ICAD conference for Rember (methylene blue), which is believed to disrupt aggregation of tau-proteins [Gura, 2008].

Although the amyloid hypothesis has not yet brought us a breakthrough in treatment of AD, it would be incorrect to reject it. At this moment, no alternative hypothesis is available that could replace it with the same body of evidence. Disappointing results from current trials do not

prove that the amyloid hypothesis is wrong. The precise pathophysiological events that cause AD are not yet known. The role of tau and its relationship with amyloid metabolism is not fully known. Also, the role of many other cofactors (e.g. cholesterol, metals) must be further elucidated. It can also be assumed that the presence of A β in itself is not a driving factor in AD pathophysiology, but merely a byproduct of a process that has been initiated earlier in the cascade and cannot be stopped by simply removing A β . Therefore, the focus should be more on intervening in APP processing, but even this may be too far downstream in the cascade. For therapeutic strategies to move more upstream in the cascade of AD pathophysiology or in the timeline of the disease, much more knowledge is needed. In most AD-trials, we see a broad range of response – some patients show no clinically relevant response at all while others show large responses. Identifying responder characteristics from these studies is important for further drug development. Until now, the role of pharmacogenetics is only briefly touched on. From several studies we have learned that APO-E genotype is partly responsible for the effects of amyloid-lowering drugs. In the future, genotyping patients may help in selecting which patient benefits most from a certain therapy. Current studies also suggest that starting a therapy once the diagnosis AD has been made is bound to fail since the process of neurodegeneration has started already. The finding of reliable biomarkers for AD that can help us diagnose the disease at an early stage is crucial for further drug development [Dubois *et al.* 2007]. If a therapy can be initiated before the clinical syndrome of dementia has presented, amyloid-lowering drugs may show more efficacy. The effects of drugs like dimebon should however keep us alert that, besides the pathway from APP to amyloid plaque, more routes leading to the final clinical syndrome of AD may exist.

Conflict of interest statement

None declared.

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