Disease modifying strategies for the treatment of Alzheimer’s disease targeted at modulating levels of the β-amyloid peptide

M.N. Pangalos¹, S.J. Jacobsen and P.H. Reinhart
Wyeth Research, Neuroscience Discovery Research, Princeton, NJ 08543, U.S.A.

Abstract
AD (Alzheimer’s disease) is characterized neuropathologically by the presence of amyloid plaques, neurofibrillary tangles and profound grey matter loss. The ‘amyloid’ hypothesis postulates that the toxic Aβ (amyloid β) peptide, enzymatically derived from the proteolytic processing of a larger protein called APP (amyloid precursor protein), is one of the principal causative factors of neuronal cell death in the brains of AD patients. As such, methods for lowering Aβ levels in the brain are of significant interest with regard to identifying novel disease modifying therapies for the treatment of AD. In this review, we will review a variety of approaches and mechanisms capable of modulating levels of Aβ.

Introduction
AD (Alzheimer’s disease) is a progressive, degenerative brain disorder resulting in cognitive and behavioural decline and is the leading cause of dementia in the Western world. Much effort is currently directed at developing disease-modifying therapies that slow or halt the progression of the disease. A number of such therapies entering clinical trials are based on the ‘amyloid’ hypothesis, derived from one of the key pathological features of AD, the amyloid plaque. This hypothesis postulates that Aβ (amyloid β) peptides, enzymatically derived from the proteolytic processing of the transmembrane APP (amyloid precursor protein) (see [1,2]), are the principal cause of neuronal dysfunction and death in the brains of AD patients (Figure 1). Hence, agents that lower Aβ levels in the brain are of significant interest as potential disease modifying therapies for the treatment of AD. Several lines of evidence suggest that this small peptide is central to the progression of the disease process leading to neuronal loss and subsequent cognitive dysfunction. First, genetic studies in cases of familial AD have demonstrated that over 150 mutations in three autosomal dominant genes, APP, PS-1 (presenilin-1) and PS-2 are associated with increased production of Aβ (amyloid β) peptides by altering the proteolytic processing of APP [3]. Secondly, Down’s syndrome (trisomy 21) patients with three copies of APP, deposit increased levels of Aβ peptide resulting in amyloid plaque deposition and dementia (see [4]). Thirdly, ApoE4 (apoliprotein E4) genotype is one of the largest risk factors for developing late-onset AD, and has been shown to modulate both levels of Aβ peptide and the aggregation state and clearance of the peptide [5]. Finally, multiple reports have demonstrated the neurotoxic effects of fibrillar and soluble oligomeric species of Aβ peptides both in vitro and in vivo [6]. Irrespective of the cause (or toxic Aβ species), it is likely that during the disease process the balance between Aβ production and Aβ catabolism is lost, resulting in the slow accumulation of toxic Aβ species, amyloid deposition in the brain, and ultimately neuronal dysfunction and degeneration. We will review a range of current approaches and mechanisms capable of modulating the levels of Aβ to slow neuronal dysfunction and cognitive decline.

Modulating the proteolytic processing of APP and the generation of Aβ

α-Secretase
The predominant pathway by which APP is processed does not give rise to Aβ fragments, and hence, is referred to as the non-amyloidogenic pathway. The initial APP processing involves the cleavage of APP by α-secretase. Identification of proteins with α-secretase activity is ongoing, and currently includes a constitutive activity (ADAM (a disintegrin and metalloproteinase)10; [7]) as well as a PKC (protein kinase C)-regulated activity (ADAM17; [8]). Because the α-secretase cleavage site is within the Aβ-sequence of APP, enhanced cleavage at this site may represent a disease modifying strategy for AD as first postulated by Nitsch et al. [8]. A related strategy to increase the fraction of APP cleaved by α-secretase is to modulate the trafficking of APP to increase the likelihood of α-secretase cleavage. There is preliminary data that members of the SNX (sortin nexin family of proteins) can reduce the rate of APP endocytosis and increase sAPPα (soluble APP) production, possibly by exposing the APP substrate to ADAM10 for an extended period of time. Similarly,
strategies that increase the production of sADAM10 by inhibiting protease inhibitors such as TIMP1 and TIMP3, may represent therapeutically tractable approaches to shift further the bias of APP processing from the amyloidogenic to the non-amyloidogenic pathway. A related mechanism was identified by Li et al. [9], who screened 100,000 sequences from a human brain-derived cDNA library to identify cDNA sequences that can decrease β-cleavage and elevate α-cleavage. This group found that SUMO-2 (small ubiquitin-related modifier-2) significantly modulates APP processing to decrease τ-secretion from cells by 80%. Hence, the activation of SUMO-2 is a potential therapeutic target for a disease-modifying strategy in AD [11].

**β-Secretase**

A second pathway for the proteolysis of APP, referred to as the amyloidogenic pathway, involves the sequential action of β-secretase followed by γ-secretase (see Figure 1). In humans, two β-secretase genes have been identified, referred to as BACE-1 and BACE-2; however, only BACE-1 is significantly expressed in the brain. The therapeutic potential of BACE-1 inhibition was demonstrated by the findings that BACE-1 knockout mice develop normally, and appear to have completely abolished production of Aβ, suggesting that BACE-1 is the principal β-secretase in neurons [12–14]. Developing specific BACE inhibitors has been difficult, in part, because there appears to be a non-linear relationship between decreased BACE activity in vivo, and a reduction of Aβ in brain. Studies using heterozygous BACE-1 knockout animals have shown that a 50% decrease in BACE activity leads to a much smaller decrease (~15%) of brain Aβ levels. A further difficulty is the low brain-penetration of most inhibitors, probably due to the fact that many are substrates for P-glycoprotein, plasma membrane proteins that actively extrude a wide range of amphiphilic and hydrophobic drugs from cells, and important in preventing the accumulation of several drugs in the brain. Finally, crystallography of BACE-1 has revealed a large catalytic domain making it more difficult to identify small molecule transition-state analogues. This problem is further exacerbated if active BACE is a dimer, with a potentially even larger substrate-binding pocket [15]. Nevertheless, a number of small molecule inhibitors are close to entering clinical trials and should provide much needed efficacy and reliability information (Table 1).

Other than small molecule inhibitors, a novel approach for regulating production of Aβ is based on the intracellular expression of single chain antibodies (intrabodies) raised to an epitope adjacent to the β-secretase cleavage site of human APP [16]. Such intrabodies may be of potential therapeutic significance if appropriate delivery mechanisms, such as intranasal administration of phage expressing anti-β-site-directed antibodies, are shown to be safe in humans.

**γ-Secretase**

The product of β-secretase cleavage of APP becomes a substrate for the site-specific proteolysis by γ-secretase [2], generating two predominant Aβ peptides either 40 or 42 amino acids in length, and a short intracellular fragment AICD (APP intracellular domain) that may function as a transcriptional activator [17]. Because this processing step is proximal to the generation of Aβ peptides, the identification of specific γ-secretase inhibitors must be considered as one of the most promising strategies for a disease modifying treatment of AD. One potential problem with this target, however,
is that a variety of other proteins are also substrates of this enzyme complex, in particular processing of the Notch receptor may be inhibited by γ-secretase blockers [18]. Hence, γ-secretase inhibitors will probably need to be selective against Notch and other γ-secretase substrates. γ-Secretase is a hetero-oligomer containing at least four protein components, presenilin (PS-1/PS-2), nicastrin (NCT), APH-1 and PEN-2, in a high molecular mass complex of unknown stoichiometry [19]. The contributions made by each of the subunits are only just being unravelled; however, it is likely that each of the subunits may be an independent target for therapeutic intervention. A number of strategies for decreasing β-amyloid peptide by interference at the level of γ-secretase present themselves. The most direct pathway is the inhibition of the holoenzyme complex by brain-penetrant small molecule inhibitors. The potential usefulness of such inhibition has been demonstrated in a number of animal models, and also in early-stage clinical trials (Table 1).

A second strategy for lowering Aβ peptides is to modulate γ-secretase to shift bias away from the generation of the species of Aβ believed to be most toxic Aβ42. A number of modulators [including NSAID (non-steroidal anti-inflammatory drug)-like molecules] have the ability to increase the production of shorter Aβ species such as Aβ38, and decrease the production of Aβ42. For example, a subset of NSAIDs reduce secretion of the highly amyloidogenic Aβ42.

In a recent study, a correlation was found between Rho, Rho-associated kinase and the amount of Aβ42 produced in vitro [16]. Only those NSAIDs effective as Rho inhibitors lowered Aβ42. Thus, the Rho-Rock pathway may regulate APP processing, and a subset of NSAIDs can reduce Aβ42 through inhibition of Rho activity [16]. If such specific modulators can be identified, then they present an intriguing drug class that reduces the amyloid burden by altering the specificity of γ-secretase. Further points of intervention include altering the maturation of the γ-secretase complex, either by interfering with the protein trafficking or assembly of the four γ-secretase components.

### Active and passive immunization to lower Aβ levels in the brain

Perhaps, one of the most intriguing discoveries with regard to therapeutic approaches aimed at slowing or halting Aβ-mediated pathology, was by Schenk et al. in 1999 [20]. In this seminal study, preplaque PDAPP transgenic mice were immunized with aggregated Aβ42 for 11 months, to stimulate a polyclonal antibody response directed towards Aβ. At the end of the study, animals had significantly reduced amyloid deposits and neurtic pathology. Furthermore, Aβ immunization of older PDAPP animals, with pre-existing plaque pathology, also significantly reduced plaque load, suggesting that this treatment approach not only slowed the progression of amyloid deposits but perhaps even reversed it [20]. This work was extended by Bard et al. [21] who showed that chronic passive-immunization of PDAPP mice with antibodies directed against the Aβ peptide were effective in clearing amyloid plaques. Importantly, only antibodies binding aggregated Aβ in vitro reduced amyloid pathology, in contrast to antibodies recognizing only soluble forms of Aβ [22].

Several non-exclusive hypotheses have been proposed to account for the observed efficacy. The first is that anti-Aβ antibodies enter the brain, bind to amyloid plaques and recruit microglia via their cell surface expressed Fc-receptors [21]. The second proposed mechanism centres on the ability of anti-Aβ antibodies to inhibit Aβ fibrillogenesis or dissolve pre-existing amyloid fibrils in vitro [23,24]. Follow-up studies identified N-terminal amino acids 3–6 (EFRH) of the Aβ peptide as the minimally effective epitope for mediating these effects [25]. The third proposed mechanism is based on a ‘peripheral amyloid sink’ hypothesis [26], in which antibody-mediated sequestration of peripheral plasma Aβ shifts the equilibrium between CNS (central nervous system) and plasma Aβ, resulting in a net efflux of Aβ from the CNS into the peripheral circulation, where Aβ is degraded [27]. In support of this, Seubert et al. [28] made use of a monoclonal antibody, m266, that recognizes epitopes within the central domain of Aβ and binds to soluble Aβ but not amyloid plaques or fibrils [27,28]. Chronic administration of m266 resulted in a rapid increase in plasma Aβ levels with a concomitant reduction in brain Aβ levels. Furthermore, Dodart et al. [29] have reported that acute administration of m266 rapidly improved cognitive behaviour in APP transgenic mice, probably by altering brain levels of synaptoxic soluble or oligomeric species of Aβ. Overall, the preclinical data described above suggest that immunization approaches utilizing antibodies capable of binding either soluble Aβ, fibrillar Aβ or both soluble and fibrillar Aβ may be useful as treatment strategies for AD.
Despite the extensive preclinical data generated, the first human active-immunization trial, using a synthetic form of Aβ42, was halted due to a 6% incidence of meningoencephalitis [30]. Evidence to date suggests no correlation between the severity of encephalitis and the level of antibody titre produced by patients. In three post mortem cases examined thus far, patients had brain regions, particularly in the neocortex, almost completely devoid of amyloid plaques and with a concomitant presence of Aβ-phagocytosing microglia [31–33]. No effects were observed on either vascular amyloid deposits or neurofibrillary tangles, despite preclinical evidence, suggesting that passive immunization can attenuate early pathology in transgenic animals [34]. In addition, Hock et al. [35] published data from a small subset of AN1792-treated patients demonstrating a slowing in cognitive decline, as measured by ADAS-COG and MMSE scores, in those patients generating the highest antibody response. More recently, a complete analysis of all patients included in the AN1792 Phase 2 trial was presented, and, although AN1792 treatment had no effect on either ADAS-COG or MMSE scores, significant effects were reported in several cognitive tests associated with the NTB (neuropsychological test battery) and suggestive of a dose response when correlated with antibody titre [36]. Further examination of the two encephalitis cases post mortem revealed a marked CD4+ve T-cell infiltration suggestive of a T-cell response to Aβ [31–33]. Because T-cell epitopes have been mapped to the C-terminus of Aβ [37] and efficacy in preclinical studies appears to be correlated with N-terminal epitopes to Aβ, it may be possible to renew active clinical immunotherapy studies by targeting the N-terminal domain of Aβ, thereby circumventing potentially harmful T-cell responses in patients. At least one such improved ‘active’ immunization approach is in late stage preclinical development for the treatment of AD by Wyeth and Elan.

Although active immunization is likely to be less invasive and easier to administer to patients, passive immunotherapy using humanized monoclonal anti-Aβ antibodies does eliminate the risk of potential T-cell mediated responses to Aβ, and also allows dosing to be stopped quickly, should adverse events be observed. This is more difficult to do with an ‘active’ immunization approach where individuals may continue to respond to the immunogen, months after the last dose. Passive immunization clinical trials with humanized monoclonal antibodies are currently being pursued at Wyeth and Elan, and by Lilly with a humanized version of m266 (Table 1). The continued focus on active and passive immunization strategies raises expectations for the availability of a disease-modifying therapy for patients afflicted with this devastating neurodegenerative disorder.

**Modulating the catabolism of Aβ peptides**

Insufficient capacity for adequate clearance of brain Aβ had been proposed to account for a potential imbalance between normal Aβ formation and clearance resulting in the accumulation of pathogenic amyloid deposits in sporadic AD [38]. Several proteases involved in Aβ degradation have been identified that may potentially contribute to the normal physiological clearance of Aβ (for recent reviews, see [39]).

**NEP (neprilysin)**

NEP is a plasma membrane-bound, extracellular, metalloendopeptidase expressed in brain and demonstrated to hydrolyse Aβ42 in vivo [40]. Chronic overexpression experiments in transgenic mice [41], and data from knockout animals [42] confirm that NEP is a physiologically relevant protease degrading brain Aβ. The recent observation demonstrating a 50% reduction of cortical amyloid deposits in transgenic APP mice, following an intracerebral injection of a viral construct expressing NEP, provides further compelling evidence for a potential Aβ-clearance mechanism in vivo [43]. In addition, compelling data comes from a recent study by Sisodia and co-workers [44], who show that exposure of transgenic mice to an ‘enriched environment’ in combination with exercise, results in an elevation of brain NEP activity, and that this is correlated with a pronounced reduction in cerebral Aβ levels and amyloid deposits.

**Plasmin**

There is good evidence that the plasmin cascade can also modulate the clearance of Aβ [45]. Kinetic studies measuring the turnover rates of soluble and aggregated Aβ, evaluation of Aβ fibrils by electron microscopy and Aβ neuroprotection assays in rat cortical cultures, indicate that Aβ is a plasmin substrate [46]. As well as being a substrate, aggregated Aβ also up-regulates the expression of tPA (tissue plasminogen activator) in plaque-bearing transgenic APP mice and can activate the generation of plasmin by cleavage of plasminogen [47]. Plasminogen is expressed in brain [48], although, its activity appears reduced in AD patients [49]. Such decreased plasmin activity may explain reduced Aβ degradation and accumulation of amyloid pathology in AD [50], and strategies to elevate plasmin activity may be of therapeutic relevance.

**IDE (insulin degrading enzyme)**

IDE is a cytosolic metalloendopeptidase that hydrolyses numerous peptides with poor substrate selectivity and specificity, and was the first protease to be implicated in the proteolytic degradation of Aβ [51]. IDE isolated from human brain extracts, was demonstrated to cleave Aβ40 and Aβ42, preventing Aβ neurotoxicity and aggregation of Aβ in vitro [52]. In contrast to the reduction of soluble and insoluble Aβ levels, the reduction of amyloid burden and the improved survival rates of transgenic mice overexpressing IDE [41], IDE knockout mice demonstrate a clear elevation of brain Aβ levels [53]. Genetic association with late-onset AD supports the involvement of IDE in Aβ degradation [54].

Other Aβ42-cleaving peptidases including ECE (endothelin converting enzyme-1), A2M (α2-macroglobulin),
MMP9 (matrix metalloproteinase-9) and ACE (angiotensin-converting enzyme), have all been implicated in \( \alpha \beta \) degradation \textit{in vitro}, although, \textit{in vivo} evidence thus far is less compelling [55]. It is probable that several peptidases contribute to the degradation of \( \alpha \beta \) \textit{in vivo} and may participate in regulating both normal steady-state brain \( \alpha \beta \) levels with an appropriate balance of \( \alpha \beta \) formation and catabolism, and pathology with the accumulation of amyloid plaques in AD. Further understanding of \( \alpha \beta \) catabolism may lead to the discovery of novel strategies involving the therapeutically regulated \( \alpha \beta \) degradation.

**Conclusions**

There is a wealth of data supporting the ‘amyloid’ cascade hypothesis, making strategies that modulate \( \alpha \beta \) biosynthesis, steady-state levels or \( \alpha \beta \) catabolism attractive as therapeutic interventions. Many of these approaches have been shown to have profound effects on lowering amyloid plaque burden and improving cognitive deficits in animal models of AD. Nevertheless, validation of these approaches, and indeed the ‘amyloid’ hypothesis as a whole, will only be achieved following a clear demonstration of efficacy in the clinic. With the ever-increasing number of anti-amyloid therapies now entering clinical development (Table 1), we will soon have a better understanding of the role this pathway plays following a clear demonstration of efficacy in the clinic. With the ever-increasing number of anti-amyloid therapies now entering clinical development (Table 1), we will soon have a better understanding of the role this pathway plays following a clear demonstration of efficacy in the clinic.


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