LEADING ARTICLE

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Disease-Modifying Therapies in Alzheimer's Disease

How Far Have We Come?

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Abstract

Currently, there are no disease-modifying therapies available for Alzheimer's disease (AD). Acetylcholinesterase inhibitors and memantine are licensed for AD and have moderate symptomatic benefits. Epidemiological studies have suggested that NSAIDs, estrogen, HMG-CoA reductase inhibitors (statins) or tocopherol (vitamin E) can prevent AD. However, prospective, randomised studies have not convincingly been able to demonstrate clinical efficacy. Major progress in molecular medicine suggests further drug targets.

The metabolism of the amyloid-precursor protein and the aggregation of its Aβ fragment are the focus of current studies. Aβ peptides are produced by the enzymes β- and γ-secretase. Inhibition of γ-secretase has been shown to reduce Aβ production. However, γ-secretase activity is also involved in other vital physiological pathways. Involvement of γ-secretase in cell differentiation may preclude complete blockade of γ-secretase for prolonged times *in vivo*. Inhibition of β-secretase seems to be devoid of serious adverse effects according to studies with knockout animals. However, targeting β-secretase is hampered by the lack of suitable inhibitors to date. Other approaches focus on enzymes that cut inside the Aβ sequence such as α-secretase and neprilysin. Stimulation of the expression or activity of α-secretase or neprilysin has been shown to enhance Aβ degradation. Furthermore, inhibitors of Aβ aggregation have been described and clinical trials have been initiated. Peroxisome proliferator activated receptor-γ agonists and selected NSAIDs may be suitable to modulate both Aβ production and inflammatory activation. On the basis of autopsy reports, active immunisation against Aβ in humans seems to have proven its ability to clear amyloid deposits from the brain. However, a first clinical trial with active vaccination against the full length Aβ peptide has been halted because of adverse effects. Further trials with vaccination or passive transfer of antibodies are planned.

Dementia is among the leading causes of disability in developed societies affecting 8–10 million patients in the seven most industrialised countries.[1] While developed countries will see the prevalence of dementia double during the next 40 years, the prevalence of dementia will multiply three to four times in developing countries. Worldwide there are >24 million patients with dementia today, a figure

that will increase to >80 million patients in 2040.[2] Alzheimer's disease (AD) is the leading cause of dementia. AD severely interferes with participation in social activities and the risk of developing dementia is among the most eminent fears of elderly people.[3] Besides personal suffering, AD causes substantial financial burden on society. Estimations of the total cost of AD (direct and indirect cost) are as high as $US100 billion in 1997 in the US.[4] A postponing of the onset or the progression of the disease from a mild to a moderate clinical state would substantially reduce the number of AD patients in full-time nursing settings. Therefore, major efforts have been taken to investigate the neurobiology of AD, to delineate drug targets and to develop the methodology to perform clinical trials.[1,5]

1. Disease Modification and Clinical Trial Design

Disease-modifying therapies should interact with the disease process and lead to an arrest or slowing of neuronal loss and functional decline. Disease-modifying therapies should be introduced early in the course of a disease to achieve maximum benefit for patients. Improvement of sensitivity and specificity of early diagnosis of AD would facilitate these studies. Specifically, biomarkers from cerebrospinal fluid (CSF) or imaging studies have evolved during recent years and improved diagnosis.[6-8] In clinical trials, disease-modifying drugs should show increasing benefits throughout the duration of the trial. Therefore, differences in functional abilities may be small during the first month of treatment. However, the difference compared with placebo-treated patients should grow with time as a result of the slowing of disease progression. After withdrawal of a disease-modifying drug, functional abilities should be maintained as a result of preserved healthy tissue. In trials with a staggered start design, early onset of treatment should result in a better outcome compared with late onset of treatment.[9] Additionally, longitudinal measurements of biomarkers such as volumetric or metabolic brain imaging should suggest slowing of neuronal loss. An arrest of brain atrophy or a maintenance of glucose metabolism both possess some face value as surrogate markers for disease progression;[10] however, these surrogate markers remain secondary to the clinical outcome. Drug-induced changes in diagnostic biomarkers, for example, changes in CSF Aβ-peptides, are not validated as surrogate markers of disease progression and interpretation of findings may be difficult.

There are two major obstacles to proof of a disease-modifying effect in clinical trials: inadequate short periods of randomised, controlled treatment and substantial differences in withdrawal rates between treatment groups.

2. Current Drugs for Alzheimer's Disease (AD)

Available treatments for AD target the cholinergic system with acetylcholinesterase inhibitors (AChEIs) or the glutamatergic system with competitive blockade of the NMDA receptor with memantine.[11-14] An effect of AChEIs and memantine on the functional level (i. e. memory function, ability to perform activities of daily living) has been shown; however, the effect size has been low to moderate.[15] In the UK, a fierce debate concerning the recommendation to use or not to use these drugs accompanies the development of guidelines by the National Institute of Health and Clinical Excellence.[16] These guidelines will affect reimbursement of AChEIs and memantine by the National Health System. Because of the financial restrictions of the health systems, estimations of (cost-) utility will play an increasing role in the evaluation of drugs in most countries.

Despite many theoretical considerations suggesting that AChEIs or memantine may have a disease-modifying effect, only symptomatic effects of these compounds have been proven. Individual AChEIs have additional pharmacological effects besides the inhibition of acetylcholinesterase.[17,18] However, a clinical benefit of these additional effects has not been convincingly shown.[19] Crossover studies between two AChEIs are difficult to interpret because of 'regression to the mean' effects and a lack of appropriate control groups.[20] Head-to-

head studies of different AChEIs do not suggest major differences.[21] For AChEIs or memantine, an increase in differences compared with placebo groups for treatment periods >6 months has not been proven. Publications that present data for longer periods of treatment (>1 year) are usually uncontrolled, open-label follow-up studies. Comparison groups are often not parallel, randomised patients but rather historical controls or mathematically calculated progression curves (open-label studies). For example, data from 3 years of treatment with an AChEI have been presented in contrast to calculated progression curves for nontreated patients, whereas only data from 12 months' treatment are available from patients randomised to placebo control.[22] These data do not substantially support a disease-modifying effect.

Observations in placebo washout phases in some trials with AChEIs did not suggest a lasting treatment effect after drug withdrawal.[23] In some studies, patients from the placebo group switched at the end of the double-blind treatment phase to open-label AChEI treatment. These data are potentially helpful and represent a kind of staggered-start design. However, data from open-label, crossover studies are substantially biased by withdrawal rates and selectivity of patients who entered the open-label phase. A retrieved withdrawal design would greatly improve the ability of studies to demonstrate a disease-modifying effect and must aim to assess data from all randomised patients at the end of the study. The AD2000 study succeeded with a vigorous retrieval of data from withdrawal patients.[24] Although the study has several limitations, such as containing a crossover population, it showed a consistent positive effect of an AChEI (donepezil) on cognition and activities of daily living. In this study, the effect did not increase with time.

Currently, there are no clinical data to support preclinical speculation of a disease-modifying effect of AChEIs or memantine. Given the modest effects of these drugs, a call for a mandatory add-on design with AChEIs as a basal treatment in controlled clinical trials in AD is not supported by evidence that an early start of treatment with AChEIs is advantageous.

3. The Search for Disease Modifiers in AD

For this review, MEDLINE (2000–July 2006) has been searched with the terms 'Alzheimer*' and 'randomised clinical trial' or 'clinical trial phase I'. Additionally, a clinical trial register[25] has been searched for entries concerning 'Alzheimer*'. However, not all clinical trials with a potentially disease-modifying substance are listed in these databases. Abstracts of the International Conference on Alzheimer's Disease (ICAD, July 2006) have been searched for up-to-date information. Articles on test substances, which are thought to act mainly symptomatically (neurotransmitter substitution/modulation) or have the character of a nutritional supplement (fish oil, soya products, curcumin, dietary supplements with multiple components), or publications on substances with only minimal information on their putative mode of action have been excluded.

Suggestions for drug targets for disease-modifying agents may come from the following: (i) epidemiological observations; or (ii) new insights in neuropathology and pathophysiology. Drug targets may be embedded in the general amelioration of metabolic deficits, the modulation of neuroinflammation, the amyloid cascade or the *tau* pathology. Developments from epidemiological observations or preclinical research closely linked to the amyloid hypothesis have been included; however, this area is difficult to delineate. Therefore, some approaches for disease modification, which have not resulted in a registered clinical trial or seem not to be developed far enough to enter clinical research soon, may have been missed.

4. Pharmacoepidemiology

Signals from pharmacoepidemiological studies are powerful for detecting new beneficial effects of available drugs and essential for tracking rare adverse effects in a larger population than the restricted group of patients in phase III clinical trials.

Although phase IV studies with licensed drugs are recommended to follow up these effects in patients with new prescription drugs, adequate studies are rarely performed. Epidemiological studies mainly with cross-sectional data have suggested that NSAIDs, estrogens, HMG-CoA reductase inhibitors (statins) and tocopherol (vitamin E) may be beneficial to reduce the incidence of AD. However, bias of case selection and several other sources of error are inherent in epidemiological studies and subsequent clinical trials have often been disappointing.

4.1 NSAIDs and Neuroinflammation

More than 20 epidemiological studies, some with a follow-up design and a good estimation of NSAID use via prescription data from pharmacies have suggested that the prolonged intake of NSAIDs may be associated with a reduced incidence of AD.[26] The idea to test components of the inflammatory system was further supported by the detection of a whole orchestra of elements of inflammation in AD autopsies such as activated microglia, cytokines, complement protein and acute phase reactants.[27,28]

NSAIDs are a very heterogeneous group of substances which usually exert several modes of action (inhibition of cyclo-oxygenase [COX]-1, COX-2, activation of peroxisome proliferator-activated receptor [PPAR]-γ, modulation of Aβ production). Clinical trials with potent NSAIDs (indometacin) led to high withdrawal rates as a result of gastrointestinal toxicity.[29] Recent developments have tried limiting peripheral adverse effects by increasing brain/plasma ratios and the brain half-life time of NSAIDs.[30] In rodents, a derivative of indometacin has been shown to accumulate in the brain in higher doses while showing less peripheral toxicity. Further trials with COX-2 selective (celecoxib and rofecoxib) or unselective (naproxen) NSAIDs or other anti-inflammatory drugs such as dapsone, hydroxychloroquine and prednisone have not shown a beneficial effect.[31,32] A recent clinical trial with ibuprofen 800 mg/day (in combination with esomeprazole 20mg for stomach protection) did not show a significant difference to placebo after 12 months.[33] The positive effects of some NSAIDs in animal models of AD have to be understood and scrutinised more before further clinical trials are warranted.[34] One explanation of the data from epidemiological studies with NSAIDs is that some NSAIDs may interfere with the metabolism of the amyloid-precursor protein (APP) and the production of its 4 kDa fragment Aβ (see section 6). Selected NSAIDs lower the production of $A\beta_{1-42}$[35] eventually by shifting the cleavage site to $A\beta_{1-38}$ independent of COX-inhibition.[36] One suggested mode of action of NSAIDs on Aβ-cleavage involves the inhibition of the small guanosine triphosphatease Rho.[37] However, the way in which NSAIDs interfere with Aβ processing and whether different pathways are involved with different NSAIDs is unclear. If interfering with Aβ production is the important pathway for the beneficial effects of NSAIDs in epidemiological studies, selection of the right NSAID is crucial for clinical trials. Other recent suggestions on the involvement of NSAIDs in AD pathophysiology focus on differential effects of prostaglandin E receptors (EP1–EP4) on brain cells. EP2 stimulation in microglia may inhibit Aβ phagocytosis, while the role of EP3 in microglial and astroglial cells has not been established yet.[38-40] In neurons, EP1 stimulation may enhance neurotoxicity, whereas stimulation of other EP receptors may have neuroprotective effects.[41] Therefore, further research to delineate the role of individual EP receptor subtypes in defined cell populations may stimulate approaches to modulate microglial activity or neuronal cell death by selective EP receptor antagonists in contrast to global COX-inhibition by NSAIDs.

Flurbiprofen is a NSAID with multiple additional functions besides COX-inhibition. Flurbiprofen and its nitric oxide releasing derivatives have been tested in several conditions concerning the inhibition or modulation of microglial activity.[42] Both the R- and the S-enantiomer of flurbiprofen modulate γ-secretase (see section 6.2); however, the R-enantiomer has lost some potency concerning COX-inhibition.[43]

A further clinical study by Gordon et al.[44] focussed on neuroinflammation by using cyclophos-

phamide, a potent antimitotic drug and modulator of immune function. A randomised pilot trial suggested that intravenous pulse therapy with cyclophosphamide may be feasible in AD. Concentrations of 0.4 and 0.75 g/m^2 once a month were used in this trial.

4.2 Estrogens and Neurosteroids

In contrast to cross sectional epidemiological data,[45] there is no evidence from clinical trials that estrogens reduce the incidence or modify the course of AD.[46,47] A recently completed trial with low-dose estrogen transdermal applications showed no benefit in AD.[48] Another trial involving transdermal 17-β-estradiol and oral progesterone will end this year (2006).[49] The Women's Health Initiative Memory Study[50] even suggested an elevated risk for all causes of dementia in women on hormonal replacement therapy (HRT) after 65 years of age; however, the onset of HRT and the use of estrogen-progesterone combinations may have influenced the outcome. On the basis of preclinical data, early monotherapy with estrogen has been suggested to be beneficial in contrast to late initiation of HRT.[51] Estrogens are also part of the repertoire of endogenous antioxidants in the brain and treatment with homologous synthetic antioxidants, which lack binding to estrogen receptors, has been suggested.[52]

Neurosteroids such as pregnenolone or dehydroepiandrosterone have been found to be reduced in the brain in patients with AD.[53] Randomised clinical trials are needed to evaluate the potential of these substances in the treatment of AD. A phase II study with HF0220, an epiandrosterone preparation, commenced in 2006.[54]

4.3 Releasing Hormones for Gonadotropins and Growth Hormone

An imbalance of sexual hormones including a malignant influence of luteinising hormone has been suggested as a cause of AD.[55] The continuous application of the gonadotropin-releasing hormone leuprorelin (leuprolide) leads to a downregulation of luteinising hormone secretion. Preliminary results from a phase II study of 50 women with VP4896 (a novel formulation of leuprorelin acetate) for 48 weeks suggested some benefit with good tolerability.[56] A phase III study with an expected enrolment of 555 patients started in 2005.[57]

The age-associated decline of growth hormone has been suggested as a cause for a variety of age-associated diseases. The National Institute on Aging has sponsored a trial with tesamorelin (TH9507; human growth hormone releasing hormone) in healthy elderly and elderly individuals with mild cognitive impairment for 20 weeks.[58] Another growth hormone secretagogue, ibutamoren (MK-0677), was not superior to placebo in a 12-month clinical trial of 563 AD patients.[59]

4.4 HMG-CoA Reductase Inhibitors (Statins)

Statins are widely used to lower cholesterol levels and it has been suggested that they may modify the risk of developing AD. A recent study[60] on the incidence of AD in users of statins questioned older cross-sectional observations and suggested that statins had no particular benefits. Published smaller treatment studies concerning the use of statins in AD did not show a clear positive effect.[61,62] Other larger and longer-term studies[63,64] with patients receiving simvastatin and atorvastatin have been completed recently, but the results are not yet published. Several mechanisms of how statins may interfere with AD pathology have been discussed, including modulation of Aβ production, neuroinflammation or neuronal cell death.[65] Most probably, statins influence quite basic mechanisms of neuronal damage and a beneficial effect of statins has also been shown in animal models for stroke.[66] A recent randomised trial[67] with patients receiving atorvastatin 80 mg/day for 12 months suggested a benefit for patients with higher baseline cholesterol levels or an apolipoprotein-E4 genotype.

4.5 Tocopherol and Antioxidants

For a long time, oxidative stress has been suggested to be a major culprit of age-related disease.[68] Observational epidemiological studies suggest intake of antioxidant vitamins is beneficial. However,

these findings may be biased by other related differences in health behaviour. Well matched epidemiological studies were not able to confirm this benefit.[69-71] Despite the wide use of tocopherol, there are only two trials adequately addressing the effect of tocopherol in AD. Tocopherol was not effective in a prevention trial in mild cognitive impairment to reduce progression to AD nor clearly effective in patients with AD.[72-74] Since 2002 the PREADVISE (Prevention of AD by Vitamin E and Selenium) trial has recruited patients with an expected final enrolment of 10 400 people.[75] However, recent studies suggest a harmful effect of tocopherol in high doses for a variety of conditions including cancer.[76] In addition to tocopherol, a plethora of antioxidants have been suggested for the prevention and treatment of AD.[52] The National Institute on Aging in the US is sponsoring an ongoing phase I clinical trial to measure the effects of tocopherol, ascorbic acid (vitamin C), thioctic acid (alfa-lipoic acid) and coenzyme Q on markers of oxidative stress in AD.[77]

5. Neuronal Protection and Trophic Factors

5.1 Monoaminoxidase Inhibitors

It has been suggested that monoaminoxidase inhibitors (MAOIs) exert neuroprotective effects. MAOIs reduce the formation of toxic metabolites or oxygen radicals by blocking the enzymatic activity of MAO-A or MAO-B. Selegiline has been shown to exert some symptomatic benefits in clinical trials with AD patients; however, trials that demonstrate disease-modifying effects have not been performed.[78] MAOIs may have several functions besides enzyme inhibition. MAOIs of the propargylamine type such as rasagiline also influence amyloid metabolism and induces antiapoptotic genes such as *Bcl2* and *Bclxl*.[79,80] Interestingly, MAOIs have recently been shown to interact with histone-modifying enzymes in the cell nucleus, which promotes gene expression.[81] However, studies examining a disease-modifying effect of MAOIs in another neurodegenerative disorder, Parkinson's disease, have been difficult to interpret.[82] In these studies, a disease-modifying effect could not be separated from a symptomatic effect because of the prolonged inhibition of MAO after cessation of MAOI intake. A phase II trial in AD patients with rasagiline was commenced in 2004.[83] However, the short double-blind treatment phase of 1 year may limit the ability to detect a disease-modifying effect in this cohort of 345 patients.

5.2 Excitotoxicity and Calcium Channel Antagonists

Excitotoxicity describes the general phenomenon of neuronal cell death due to NMDA-receptor-mediated calcium influx. Excitotoxicity is not restricted to AD and may be even more relevant for stroke and traumatic brain injury.[84] While high-affinity blockade of NMDA receptors is strongly psychotomimetic, low-affinity modulation by memantine has been shown to be clinically suitable.[85] Symptomatic effects of memantine in moderate and severe dementia have been demonstrated.[86] However, a disease-modifying effect by NMDA modulators has still to be proven in clinical trials. The NMDA modulator *D*-cycloserine did not prove to be effective.[87]

Calcium influx via calcium channels is also part of the common final pathway of cell death in a variety of conditions. MEM1003 (an L-type neuronal calcium channel antagonist) has entered phase II of clinical testing in AD.[88] Results with the calcium channel antagonist nimodipine are restricted to short-term studies and are not completely published.[89]

5.3 Valproate and Related Compounds

Observations from clinical studies with valproate initiated for the treatment of behavioural symptoms in AD suggest some protective effects.[90] A related compound, arundic acid (ONO-2506PO), is being studied in approximately 600 patients.[91]

5.4 Trophic Factors

Neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor or small molecules that stimulate NGF release, have been

discussed as potential treatments for several brain diseases including AD.[92-95] Leteprinim potassium (AIT-082) increases the availability of NGF and has been tested in a phase I study sponsored by the National Institute on Aging, but larger studies have not been reported.[96] Xaliproden (SR57746A, phase III) and the related drug paliroden (SR57667B, phase II) are in clinical development.[97,98] Pilot studies with NGF gene therapy have been performed in a few patients and phase II studies are planned.[99] However, the inability of cholinergic neurons to utilise NGF in the face of an undisturbed endogenous NGF supply may limit the success of these strategies.[100]

6. The Amyloid Cascade

Amyloid metabolism starts with the synthesis of the APP, a transmembrane protein, which is cleaved by β- and γ-secretase leading to an amyloid-β peptide (Aβ) of 40–42 amino acids (see figure 1).[101] Extracellular, and perhaps also intracellular, Aβ exert neurotoxic effects.[102] Extracellular Aβ peptides cluster in a β-sheet structure to form amyloid plaques. Evidence for a leading role for the amyloid cascade theory comes from studies in human genetics. In different pedigrees with early-onset AD, mutations in APP, presenilin-1 (PS-1) or presenilin-2 (PS-2) alter Aβ production and lead to an autosomal dominant form of AD.[103] Transgenic animals harbouring these mutations have been shown to develop amyloid plaques and cognitive decline.

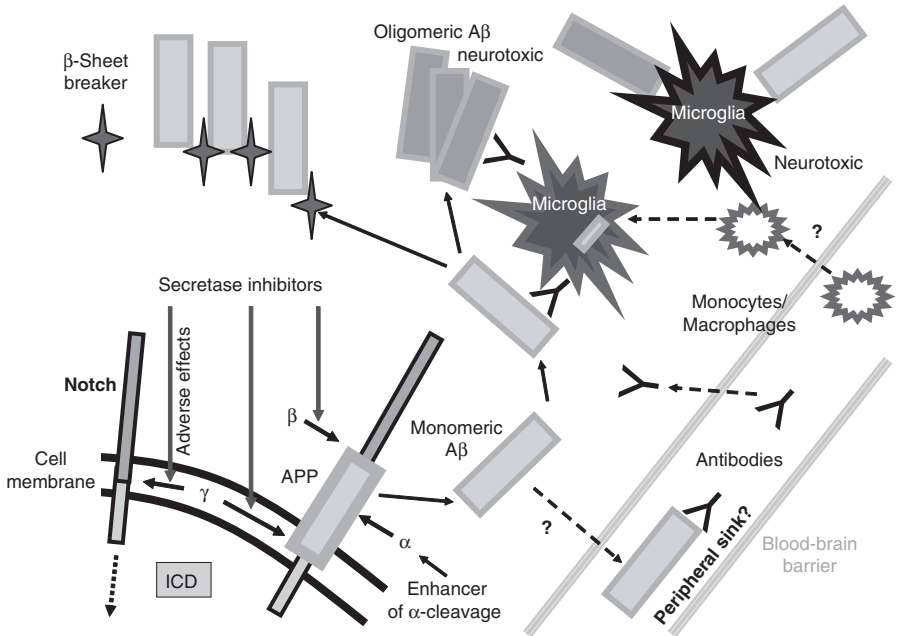

Fig. 1. Drug targets along the amyloid cascade. Three enzymes, α-, β- and γ-secretase cut the type-1 transmembrane protein amyloid-precursor protein (APP). The Aβ fragment of APP is formed by the activity of β- and γ-secretase. The activity of γ-secretase cleaves APP and other proteins such as notch inside the cell membrane and releases an intracellular domain (ICD). The ICD of notch has an essential physiological role and inhibition of notch cleavage causes severe adverse effects. A physiological role of the ICD of APP has not been established yet. The activity of α-secretase cuts inside the Aβ sequence. Inhibition of β- or γ-secretase or enhancement of α-secretase activity may be of therapeutic benefit. Intracerebral Aβ concentration may be connected to serum Aβ concentrations, although the process of translocation through the blood-brain barrier is unclear. Antibodies to Aβ may bind Aβ in the blood compartment and enhance clearance of Aβ from the brain (peripheral sink hypothesis). Antibodies from the serum seem to be able to cross the blood-brain barrier and stimulate Aβ degradation by microglial cells. After vaccination, blood-borne monocytotic cells may become activated and phagocyte Aβ after crossing the blood-brain barrier. In the absence of antibodies, microglia are unable to clear Aβ and this may enhance Aβ neurotoxicity. Oligomerisation of Aβ may be prevented with β-sheet breaker. Further details are given in section 6.

However, one should keep in mind that all of these models rely on APP overexpression. Mutant APP transgenic animals have been reported without neurodegeneration in the absence of APP overexpression.[104] Neuropathological investigation has suggested a modest correlation between the amount of amyloid plaque load and functional decline in AD.[105] However, Aβ-induced neurotoxicity may not depend on plaque formation and, therefore, tissue loss and amyloid plaques may not need to overlap anatomically.

6.1 β-Secretase Inhibitors

In 1999, β-amyloid cleaving enzyme-1 (BACE-1) was identified as a protease with β-secretase activity.[106] BACE-1 is an aspartyl protease and a type-1 integral membrane protein. In knockout animals, lack of BACE-1 abolishes Aβ generation in the absence of further abnormalities suggesting that blockade of BACE-1 may reduce progression of amyloid pathology without major adverse effects.[107] BACE-1 inhibitors are not easy to find because BACE-1 has a large catalytic side that may not avidly bind small molecules.[108] However, first reports show that naphthyl and coumarinyl biarylpiperazine derivatives may act as potent inhibitors of human BACE-1.[109]

6.2 γ-Secretase Inhibitors

The enzymatic activity of γ-secretase depends on a complex of at least four different proteins: PS-1 and PS-2, anterior pharynx defective-1, nicastrin and presenilin enhancer-2. The stoichiometric ratios of this heteromeric complex are still under debate.[110] The core protease function seems to be rooted in two presenilin molecules which are endoproteolytically cleaved in two fragments. The inhibition of intramembrane enzymatic cleavage of APP seems to be a straightforward target in the amyloid cascade. Although the γ-secretase generated intracellular domain (ICD) of APP might be involved in gene regulation, a firm physiological role for γ-cleavage of APP has not been established.[111,112] However, γ-secretase has a wide variety of substrates besides APP and cleaves several other type-1 transmembrane proteins inside their transmembrane sequence. Most notably, γ-secretase cleaves notch, which is crucially involved in the Wnt-pathway and is necessary for normal development. Cleavage of notch by γ-secretase is followed by translocation of the ICD in the nucleus. In the nucleus, the ICD of notch coactivates transcription of several target genes.[113] After embryogenesis, persistent activity of the notch pathway seems to be essential for the differentiation of mitotic cells, especially in the bone marrow.[114] While the notch pathway is well understood, it is unclear whether further γ-secretase substrates and their ICDs also have signalling character (see table I for further substrates). Inhibition of the catalytic centre of γ-secretase resulted in inhibition of APP processing and notch signalling, which has unwanted effects.[115] Although γ-secretase-inhibitors have been

Table I. Substrates of γ-secretase and their putative function[a]

Transmembrane type-1 protein	Function	Reference
APP	Cell adhesion (?)	111
Notch	Ontogenesis, proliferation	119
CD44	Hyaluron receptor	120
Nectin 1a	Cell adhesion	121
N- and E-cadherin	Cell adhesion mediated signalling	122
LRP	Endocytosis	123
ApoER2	Endocytosis	124
Megalin	Endocytosis	125
DCC	Chemoattractant	126

a Note that this list is steadily increasing and that some data from *in vitro* studies may not be relevant *in vivo*.

APP = amyloid-precursor protein; **ApoER2** = apolipoprotein E receptor 2; **DCC** = deleted in colorectal carcinoma; **LRP** = low-density lipoprotein receptor-related protein; **?** indicates that putative function is still under discussion.

reported to reduce Aβ levels in the brain of transgenic animals,[116,117] full reports on data on cognitive changes in animals have yet not been presented. Inhibition of the notch pathway and unwanted effects seem to reduce the feasibility of this approach.[118]

Double-knockout PS-1 and PS-2 mice are embryonically lethal, whereas conditional knockout leads to fast, progressive neurodegeneration in adulthood.[127] While it is unclear whether this neurodegeneration is a result of the loss of secretase activity, a massive disruption of PS-1 and PS-2 functions may result in severe adverse effects.[128]

Abstracts reporting results of a clinical phase 1 study of the γ-secretase inhibitor MK-0752 reported no serious adverse effects in humans given a single dose. This study also showed a reduction of Aβ levels in the CSF, which may be interpreted as a proof of principle.[129] Another γ-secretase inhibitor (LY450139) has been given to AD patients for 5 weeks and produced only a small, nonsignificant decrease in CSF Aβ levels.[130] LY450139 is now being investigated in a longer-term phase II study.[131]

A 12-month clinical trial with R-flurbiprofen (MPC7869), an NSAID-derived γ-secretase modulator,[43] showed some benefits in activities of daily living and psychiatric events in patients with mild AD (Mini-Mental State Examination score >19) taking 1600 mg/day.[132,133] A further phase III trial involving 800 patients has recently been started.[134]

6.3 Proteolysis of Aβ

6.3.1 α-Secretase Activator

α-Secretase cleaves APP within the Aβ sequence and thereby precludes the formation of Aβ. α-Secretase activity seems to be mediated by ADAM-10 (a disintegrin and metalloprotease-10) and tumour necrosis factor (TNF)-α converting enzyme.[135] Recently, activation of α-secretase has regained interest as a therapeutic drug target in AD.[136] Overexpression of ADAM-10 in transgenic animals led to a decrease of amyloid pathology, while the transgenic expression of a catalytic inactive form of the enzyme resulted in an increase of amyloid pathology.[137]

6.3.2 Metallo-Endopeptidases

The metallo-endopeptidase and type-2 membrane associated protein neprilysin (EC 3.4.24.11, also termed enkephalinase or CD10) seems to be a potent enzyme to degrade Aβ. Neprilysin knockout animals show an elevation of Aβ confirming a function of neprilysin *in vivo*. It has been suggested that a reduction of neprilysin activity by 50% is as effective as a PS-1 mutation in increasing Aβ load.[138] Neprilysin expression declines with ageing and is reduced in AD.[139] Neuropeptides are able to induce neprilysin and drugs acting on neuropeptid receptors may have an effect on amyloid catabolism.[140,141] Viral expression of neprilysin in transgenic animals resulted in a reduction of amyloid pathology.[142] Furthermore, metallo-endopeptidases such as insulin-like degrading enzyme (EC 3.4.24.56) or endothelin converting enzyme (EC 3.4.24.71) may also be able to degrade Aβ.[143,144]

While neprilysin, insulin-like degrading enzyme and endothelin all act on soluble Aβ, matrix metalloproteinase-9 is capable of degrading tightly bound fibril Aβ, which might be used for the development of further treatment strategies.[145]

6.4 Inhibition of Aβ-Aggregation

Aβ is primarily a soluble peptide that can be found in serum and cerebrospinal fluid.[146,147] Under certain conditions (concentration of Aβ, pH, ionic strength, oxygen radicals, presence of other proteins), Aβ is a β-sheet rich peptide prone to form oligomeres, especially under hydrophobic conditions.[148] Following the hypothesis that oligomeric Aβ is especially toxic to the brain, several approaches have been investigated to block this pathway. Congo red is well known from histological stainings and interacts with amyloid plaques. Furthermore, compounds such as anthracycline and melatonin can prevent Aβ aggregation *in vitro*.[149-151] Different peptides containing parts of the sequence of the amino acids 16–22 of Aβ have been tested to see if they inhibit oligomeric aggregation and further developments in this approach are expected.[152] A

modified $A\beta_{12–28}$ peptide that interfered with binding of Aβ to apolipoprotein E has been shown to reduce Aβ pathology in transgenic mice.[153] Because of the short half-life of peptides, non-peptidergic molecules have been designed that interact with the hydrophobic core sequence of Aβ.[154] Several molecules may act as a chaperone for Aβ. Underlying a similar approach, a synthetic glycosaminoglycan, 3-amino-1-propaneosulfonic acid (3APS, tramiprosate), has been developed and clinical trials are in progress.[155] More comprehensive phase III trials are currently recruiting.[156] In Europe, 930 AD patients will be treated for 18 months.[157]

6.5 Metal Chelators and Copper

Metal chelators have been suggested for the sequestration of zinc, iron or copper, which may stimulate both APP processing on the messenger RNA (mRNA) level and fibril formation on the protein level.[158,159] Clioquinol is an antimicrobial drug that inhibits Aβ fibrillation by interaction with copper ions.[160] Studies lasting for several weeks have been performed using clioquinol in AD patients; however, long-term use of clioquinol is known to cause severe CNS adverse effects.[161,162] Additional agents possessing a similar effect on brain copper concentrations as clioquinol have been developed.[163] However, the complex interaction of APP with copper has also led to the opposite suggestion, that is, to supplement oral copper for the treatment of AD.[164,165]

7. Peroxisome Proliferator-Activated Receptor-γ Agonists

Ibuprofen, indometacin and naproxen are among the five most prescribed NSAIDs, which potentially decrease the risk for AD.[166] Interestingly, they all activate the PPAR-γ,[167] a member of the nuclear hormone receptor family. PPAR-γ represents a ligand-activated transcription factor that forms heterodimers with the retinoid X receptor.[168,169] PPAR-γ either acts via binding to sequence-specific PPAR response elements (PPRE) in promoter regions of target genes or by interfering with other transcription factors critical for gene induction or upregulation.[170] In the brain, several anti-inflammatory effects of NSAIDs may be mediated through activation of PPAR-γ,[171] since PPAR-γ agonists protect neurons from cytokine- and nitric oxide-mediated cell death *in vitro*[172,173] and *in vivo*.[174] In addition, a direct effect of ibuprofen and synthetic PPAR-γ agonists on APP processing has been reported, demonstrating that Aβ-levels are downregulated by PPAR-γ-dependent mechanisms in cell culture.[175] More recently, it has been found that PPAR-γ regulates BACE1 mRNA, protein and activity levels via a PPRE.[176] In line with this finding, oral treatment of APPV717I transgenic mice with the PPAR-γ agonist pioglitazone or the NSAID ibuprofen reduced micro- and astroglial activation and decreased Aβ levels.[177] Preliminary data on glitazones in pilot clinical trials are available in the form of abstracts. Pioglitazone has been shown to be well tolerated but did induce oedema during an 18-month trial in AD.[178] In a 6-month trial, rosiglitazone showed positive trends in a *post hoc* analysis of choice reaction time,[179] but failed to show superiority in primary endpoints.[180]

8. Immunotherapy

8.1 Active Immunisation

Active vaccination against Aβ in transgenic animals that develop amyloid pathology resulted in a decreased accumulation of amyloid plaques.[181] Additional studies showed that passive immunisation by peripheral transfer of antibodies against Aβ led to binding of these antibodies to amyloid plaques in the brain. Bound Aβ-antibodies stimulated microglial phagocytosis of amyloid. However, whether this phagocytotic activity depends on the interaction of antibodies with the microglial Fc receptor or whether additional clearance mechanism exist is still unclear.[182-184] Antibodies against the middle portion of Aβ (amino acids 13–28, $A\beta_{13–28}$) also affected amyloid pathology without being able to bind to aggregated Aβ in amyloid plaques.[185] This result suggests that there might be an equilibrium between plaque-bound Aβ and soluble Aβ. In parallel to the

reduction of amyloid pathology, some studies found a reduction in the progression of cognitive dysfunction in vaccinated animals.[186,187]

Animal studies with an immunisation protocol combining $A\beta_{1-42}$ with the saponin QS-21 (AN1792; Alzheimer's disease vaccine) suggested that this approach may be suitable in humans. A phase II study with 300 vaccinated patients was halted in early 2002 because of symptoms of meningoencephalitis in 18 patients. While data analysis from patients who developed an antibody titre from one centre suggested positive effects for some clinical measurements, the interrupted trial as a whole did not establish the efficacy of this intervention.[188,189] Neuropathological findings in participants of this study point to a possible proof of principle in humans. Participants with and without encephalitis[190-192] exhibited much lower plaque pathology than expected. Only patients with encephalitis showed an infiltration of T cells. Activation of T cells by vaccination may be crucial for the development of meningoencephalitis. Microglial cells were activated in the vicinity of smaller plaques suggesting microglial involvement in plaque removal. Specially tailored immunisation procedures that avoid T-cell activation may be a feasible way forward. A change from the saponin QS-21, which triggers a prominent T helper cell (T_h)-1 response to aluminium salts, which then favours T_h2/B-cell responses, might already influence the risk of encephalitis.[193] Special immunoconjugates of aminoterminal Aβ fragments to carrier molecules have been tested successfully in animals.[194,195] Modified homologous Aβ peptides with disrupted or removed T-cell epitopes have been shown to induce a predominant synthesis of IgM accompanied by some behavioural improvement in transgenic mice.[196] Some observations in this context suggest that binding of antibodies or other molecules to Aβ in the bloodstream may drain Aβ from the brain, suggesting a peripheral sink hypothesis (see figure 1).[197] This peripheral sink function may depend on transport through the blood-brain barrier by mechanisms such as p-glycoprotein, which may in itself be a limiting factor.[198] Another modification of vaccination in AD may involve mucosal application of the antigen, which stimulates predominantly IgA production and less cellular immunity. Positive results have been shown in transgenic animals with intranasal application of Aβ.[199] Other approaches may use gene vaccination to bypass T_h1 activation.[200-202] Vaccination of middle-aged rhesus monkeys with Aβ in the presence of Freund's adjuvant, a strong promoter of T_h1 reactions, did not result in an autoimmune reaction.[203]

These findings suggest that active immunisation as a primary prevention or early secondary disease-modifying therapy may be achievable. The role of microglia/monocytes in vaccination trials deserves further attention. Although it is controversial whether resident microglia have the ability to phagocyte Aβ,[204,205] microglia seem to be crucial in the phagocytosis of Aβ after vaccination.[206] Recently, in transgenic animals with amyloid pathology, it has been shown that newly recruited bone marrow-derived monocytes/microglia enter the brain and phagocytose Aβ.[207] Invading macrophages and T cells have also been detected in some early forms of amyloid plaque pathology, which may open new avenues for the modulation of Aβ degradation.[208]

8.2 Passive Immunisation

Polyclonal antibodies against Aβ-peptides naturally occur in preparations of human immunoglobulins.[209] In a group of five patients, monthly infusion of intravenous human immunoglobulins (IVIgs) suggested that Aβ concentrations decreased in the CSF and rose in serum.[210] In a group of eight patients, IVIgs have been repeatedly administrated for up to 18 months. Results of this phase Ia/Ib study[211] suggest an arrest of cognitive decline in some patients. Despite the absence of a randomised control group these results point to a potential disease-modifying effect of IVIgs. Serum Aβ concentrations decreased after IVIg infusion, suggesting that naturally occurring anti-Aβ-antibodies in these preparations may bind Aβ and lead to clearance.[212] However, the prognostic value of changes in CSF or serum Aβ-concentrations is unclear. Moreover, the timepoint and methods of measuring Aβ concentra-

tions in the blood after infusion of IVIgs differed between studies and variation in Aβ measurement according to sample preparation are known.[213,214] A small phase II study[215] investigating the use of IVIgs in 24 patients for 1 year has been initiated.

Another method of passive immunisation may be the application of cell-culture generated monoclonal antibodies. In April 2005, a phase IIa trial with bapineuzumab (AAB-001; a humanised antibody against Aβ) has started and is expected to be completed in 2008. Repeated antibody administration is planed for 240 AD patients for approximately 2 years.[216]

8.3 Interferon-α

A dysregulation of the immune system has been suggested as one of the major factors in age-related vascular pathology and AD.[217] Oral ingestion of interferon-α results in fewer adverse effects than parenteral administration, but the same action on T cells. A clinical trial with oral interferon-α in AD patients for 1 year has been initiated.[218]

8.4 Inhibition of Tumour Necrosis Factor-α

TNFα has been shown *in vitro* to modulate Aβ-peptide toxicity in a concentration-dependent manner.[219] In mixed cultures of neurons and glial cells, microglial deficiency of TNFα secretion markedly attenuated Aβ-induced neurotoxicity.[220] Etanercept is a fusion protein that binds and inhibits TNFα. A phase I study[221] investigating etanercept by perispinal injection has been started in AD patients.

9. Peptide Mixtures

FPF-1070 is a peptide mixture that eventually reduces amyloid deposition by influencing APP phosphorylation and intracellular transport.[222,223] Clinical studies with FPF-1070 showed some positive effects after intravenous administration; however, higher doses may show fewer effects than lower doses.[224-226]

Colostrinin, a polypeptide mixture from ovine colostrum, is currently being investigated in phase II studies with oral administration.[227] Whether antibodies or trophic factors are present in these preparations and are able to enter the circulation, bind Aβ or cross the blood-brain barrier remains unclear.

10. *tau* Pathology

The formation of neurofibrillary tangles in AD involves hyperphosphorylation of *tau* proteins which build up to form paired helical filaments. Phosphorylation of *tau* is controlled by different kinases and phosphatases. The activity of protein phosphatase (PP)-2A may increase dephosphorylation of *tau*. PP-2A also inhibits kinases such as mitogen-activated protein kinases, which phosphorylate *tau*.[228] Cyclin-dependent kinase-5 (cdk5) is another kinase suggested to phosphorylate *tau* in AD. The cdk5 activator p25 is increased in the brains of AD patients where p25 and cdk5 are colocalised with neurofibrillary tangles. Therefore, inhibition of cdk5 may suppress *tau* phosphorylation and prevent tangle formation.[229]

Glycogen synthase kinase (GSK)-3β has also been suggested as a drug target to inhibit tangle formation.[230] This kinase is blocked by lithium, which has a long record as a mood stabiliser or for augmenting antidepressive therapy. Epidemiological data do not suggest a protective effect for lithium; however, lithium concentrations in long-term treatment of depression may be too low to inhibit GSK3β.[231]

Additional steps between hyperphosphorylation of *tau* and the formation of paired helical filaments may be suitable drug targets. Inhibitors of *tau* aggregation independent of phosphorylation have been found and tested in cell cultures.[232] However, animal studies to address *tau* phosphorylation and aggregation are hampered by a lack of adequate models.[233]

11. Conclusions

AD occurs mainly in patients >70 years of age and life expectancy is shortened by AD by up to 4–6 years. Although autopsy may underscore the results of proof of principle studies, reliable biological markers of disease progression would help to evaluate disease-modifying drugs during the treatment

phase.[234] In particular, interventions acting in the amyloid cascade may benefit from approaches to quantify amyloid in the living human brain with positron emission tomography or magnetic resonance imaging.[6] Activation of α-secretase or inhibition of β- or γ-secretase has been achieved in preclinical studies and clinical trials will follow. Active and passive immunisation has been shown to stop progression of neuropathology in several transgenic models of AD by different research groups in academia and industry. Unfortunately, meningoencephalitis was a serious adverse event in a first active vaccination trial,[189] and future vaccination trials will have to account for this risk. However, neuropathological data from this trial suggest that vaccination reduces amyloid pathology in humans in the absence of encephalitis.[192] While there is no disease-modifying therapy available so far, further placebo-controlled studies are warranted.

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