

Therapeutic Perspectives in Alzheimer's Disease

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Received: July 29, 2005; Accepted: August 31, 2005; Revised: September 05, 2005

Abstract: It is now almost a century ago that Alois Alzheimer first presented his results in public. Main characteristics of Alzheimer's disease (AD) are massive cerebral accumulation of amyloid, composed of fibrillary aggregates of the Amyloid beta peptide (A β) and intracellular accumulation of abnormally phosphorylated tau protein associated with widespread neurodegeneration. The clinical picture is characterized by progressive and irreversible dementia, which is eventually fatal. To date, there is no cure for this severe disease affecting more than of 30 million individuals worldwide. In the last decades, the treatment of Alzheimer patients was mainly focusing on symptomatic strategies. Based on the augmented knowledge about the mechanisms underlying the pathology of AD, particularly the molecular causes and consequences of AD, different therapeutic approaches arose and recently, treatment with Statins, NSAIDs and A β vaccines reached the level of clinical trials, showing some indication of efficacy already. According to actual evaluations, these approaches have realistic chances to become established as therapeutic routine in AD within the next 10 years. We will review here some of the most promising novel approaches to cure and prevent rather than to treat the symptoms of AD.

Keywords: Alzheimer's disease, amyloid A β , secretases, presenilin, lipids, cholesterol, therapy, NSAIDs.

FUNDAMENTALS OF THERAPY IN AD

Regarding the treatment of AD, there are two self-evident strategies to address the molecular processes leading to AD. One could either (1) try to prevent the mis-regulated molecular events and to alter consequences thereof or (2) to approach the already existing symptoms by mildening if not reverting them. We will here mainly focus on the first possibility.

The first strategy includes the non-negligible problem that a very early diagnosis is desirable. Such an early diagnosis cannot be provided by the commonly accepted psychiatric procedures, as those apply long after molecular 'symptoms' have emerged already. Similarly to how studies on familial AD (FAD) helped to reveal the general principles of AD pathogenesis, studying the clinical course in FAD patients will allow discovery of presymptomatic markers and further development for use in general clinical diagnostics. But, even the identification of hippocampal atrophy as one of the earliest abnormality that distinguishes AD patients 2-3 years ahead of the first clinical symptoms [1-3] would provide a diagnosis still based on events that are needed to be seen as consequences of the early molecular processes in AD pathogenesis. Thus, the development of diagnostic procedures which will assay the misled molecular processes underlying AD has an important potential in AD therapy, but will not be of subject in this article. As long as such an early diagnosis will not be available, the preventive approach has to be used to treat the general population or those perceived as patients with increased risk for AD. In other words, many, if not the majority of the patients exposed to preventive therapy will be treated for a disease they are not likely to develop at all. Current estimates predict that life-time risk is

some 30% [4-6], with definite increase of this value for the elderly population. This scenario sets specific demands for preventive therapies, the most critical and important one being that a potential therapy needs to be very safe. The difficulties this involves are exemplified by AD trials that had prematurely terminated due to safety concerns. However, prevention is a reasonable goal and is done with success e.g. in prevention of atherosclerosis. The high incidence of AD in the elderly population, the social and economic consequences for the affected individuals and societies suggest that prevention should be considered for AD as the ultimate goal.

The second approach self-evidently suggests to treat the neurological and psychiatric symptoms, i.e. with choline esterase inhibitors etc. [7]. This strictly symptomatic attempt has the clear disadvantage to counteract the very late symptoms of the dementia without affecting the actual causes of the disease or the disease progression at all. While this seems on first sight less favorable, it still represents the best established and available option and will continue to remain important either alone or in combination with preventive treatments.

In practical terms, the differences between prevention, curative treatment of clinical stage AD and symptomatic treatment are more ambiguous. The preventive treatments are expected to show a variable degree of effectiveness in clinical AD, at least during the early stage of the disease. On the other hand, some symptomatic treatments may turn out to have some benefits in disease progression, although evidence for this remains scarce [8].

Mechanistically, the earliest possible intervention is based on the augmenting evidence, that the over-production and accumulation of Amyloid beta 42 (A β 42) is one of the initial, if not *the* most important molecular step leading to AD. Studies on the infrequent familial forms of the disease (FAD), especially with presenilin mutations from FAD

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cases, indicate increased production of A₄₂ to be invariably associated with FAD and to cause AD [9-13]. All other molecular hallmarks of the disease are mainly supposed to occur in consequence of this event [14].

PREVENTIVE TREATMENT

Currently, the most promising therapeutic approaches are mainly characterized by their preventive potential. Thus, primarily patients in mild and pre-clinical stages of the disease would benefit from these therapies so far. As mentioned above, such preventive treatment requires an early diagnosis. A number of previous and recent clinical studies addressed the beneficial effect of certain medications on cognitive decline and mental status in AD patients. As such, studies are long-ranging an adequate accumulation of comprehensive data and results may still take some more years. Furthermore, there will be even more time needed until the *protective* potentials of such treatments can be estimated.

The starting point of preventive treatment needs to be set as early as possible. The last 10 years before the age of onset of clinical symptoms provide the best chance for a preventive therapy. Any reduction of Amyloid burden in general, and the prevention of A₄₂ generation in particular, are most effective in this time period. The progressive neurodegeneration is in an early stage and may be functionally reversible, at least up to a certain degree. Furthermore, the disruptions observed in biochemical (A₄₂, tau), pathological (decline of cerebral volume), and cognitive (MCI, depression) respect already point to AD at this early stage of the dementia.

ACHIEVABLE RESULTS

Regarding the current state of diagnosis and the perspectives of preventive treatment, it seems likely, that this kind of treatment may be applied to a population of patients that actually incorrectly diagnosed or that does not benefit from this treatment for different reasons. Thus, it is required that such therapies meet extreme high safety criteria. On the other hand, economic aspects may limit the availability of a broad treatment for entire risk groups in the population.

Recent research suggests that small changes in A₄₂ production may already postpone the onset of disease by years [15]. The mean age of disease onset is approx. 80 years [4]. If a preventive treatment could delay disease progression by 10 years, it would drastically reduce the number of less severe forms of AD and almost eliminate severe (later) stages of this disease. As a typical aging related disease, AD competes with other causes of death. Thus, the benefit from AD treatment is rather likely an increase in life quality than an increase in life expectancy.

THERAPEUTIC STRATEGIES

Despite the still ongoing discourse, A₄₂ load in the brain appears to be a crucial parameter in AD pathology and its reduction could mainly be achieved by: (1) decreasing A₄₂ production, (2) enhancing A₄₂ clearance, or (3) constricting A₄₂ aggregation and its precipitation into plaques or fibrils [16]. One factor of uncertainty here is that the large fibrillary A₄₂ depositions which form the amyloid plaque may not represent the toxic A₄₂ species, but rather small soluble A₄₂

oligomers. Dissolving plaques may transiently increase the concentration of the toxic A₄₂ form.

Secretase Inhibitors

A₄₂ is a proteolytic fragment of amyloid precursor protein (APP), a protein of unclear function. Two prominent proteins are directly involved in the generation of A₄₂ peptides: γ -secretase (BACE) and β -secretase (presenilin). The BACE I-catalyzed cleavage of APP initiates A₄₂ generation and the resulting C-terminal fragment C99 - but not full-length APP - is a substrate for β -secretase, a ubiquitous multimeric protease. The β -secretase cleavage results mainly in A₄₀ and A₄₂ peptides. The active center of β -secretase complex is formed by presenilin 1 or presenilin 2 (PS1 or PS2). The double knock-out of PS1 and PS2 results in complete loss of β -secretase activity and A₄₂ production [17].

The direct involvement of these two proteases in A₄₂ generation made them an important and prominent target for drug research [18].

Inhibitory drugs targeting BACE are under investigation. But, there are several aspects making BACE a difficult target. BACE is an aspartatic protease with broad distribution, but especially abundant in the brain. The discovery of the crystal structure of BACE with one of its inhibitors [19] may open new access to targeted drug design in this field, but shows that BACE has a very large binding pocket with several sub-sites, making it a difficult target for the generation of highly specific inhibitors. However, BACE remains a promising target for AD treatment. BACE knock-out mice show only minor abnormalities [20], which is a first indicator that BACE inhibition may have less undesirable consequences than inhibition of β -secretase. The search for BACE inhibitors will certainly benefit from the large amount of data available for other aspartatic proteases and from the list of inhibitors that have been developed against them, particularly the HIV1 protease [21]. The other protease generating A₄₂ seems to be as promising as BACE. The β -secretase cleavage determines the ratio in which A₄₀ and A₄₂ are produced. This comparable small decision features one of the most important parameters in AD - the amount of A₄₂ generated. Only this A₄₂ species is involved in AD pathology, and changes in A₄₂ production caused by presenilin mutations in FAD perfectly matches disease onset, which in very severe cases can occur even before the fourth decade [15]. The major complicacy in respect to therapeutic inhibition of β -secretase is its function in many other proteolytic events involved in important cellular processes, i.e. Notch signaling. Genetic and biochemical studies revealed that PS function interferes with a broad range of cellular processes [22]. Initial attempts by almost all major pharmaceutical drug companies to target β -secretase resulted in highly specific inhibitory molecules active in low nano- and some even at pico-molar concentration and high toxicity in animal studies. This was accompanied by a transient increase in A₄₂ production [23, 24]. More recent progress shows that at least some problems could be overcome. The Lilly compound LY 450139 was used in a 70 AD patient trial, which helped to define the safe dose, but the cerebrospinal fluid A₄₂ concentration could not be reduced as expected [25]. An interesting feature of LY 450139 and some other β -secretase inhibitors is that they target APP

cleavage more effectively than e.g. Notch 1 cleavage. There might be several aspects to this, but one of the most promising ones is that presenilin is only one of the four proteins present in the γ -secretase complex [26]. This might be used to design γ -secretase inhibitors which specifically inhibit APP cleavage, but have little or no effect on the cleavage of other γ -secretase substrates. While this may be the currently favored route, Merck Sharp & Dohme reported that even a single dose of their compound F reduced brain soluble A β by 90% and plaque load by 60% (reported 2005). These truly remarkable results were obtained with an inhibitor that shows no selectivity between Notch and APP cleavage inhibition.

Taken together, direct inhibition of the proteolytic activity of A β -generating proteases is the most consequential approach, but for a number of reasons, this path is spiked by as much trap holes as it is full of opportunities.

Lipids AND AD

Until recently, little indication existed suggesting a link between Alzheimer's disease, the proteases that process APP and homeostasis of cholesterol and other lipids. But, this picture changed dramatically in recent years [27-30]. APP processing and the proteases involved are sensitive to cholesterol and cholesterol trafficking as cellular and biochemical studies showed [31-39]. Studies *in vivo* revealed that cholesterol feeding increases whereas cholesterol lowering by medication decreases A β 42 production and amyloid burden [34, 40-42]. Likewise, the relevance of cholesterol homeostasis in Alzheimer's disease was further substantiated in epidemiological studies revealing that hypercholesterolemia and high blood pressure are risk factors in AD [43, 44]. This led to preliminary clinical trials with cholesterol lowering drugs [45-49].

The correlation between apolipoprotein E (ApoE) allele frequency and AD risk [50, 51], the early increase in brain cholesterol levels during AD progression [52], and studies on lipid composition in AD brains showing age-dependent changes in cholesterol levels [53] further support the importance of cholesterol and lipids in AD.

It is well documented now that cholesterol and ganglioside GM1 enhance γ -secretase mediated A β production *in vitro* [31, 33, 34, 38, 54], whereas inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase with statins reduces A β levels in cerebrospinal fluid and brain tissue [34]. Analogous results were found using the cholesterol synthesis inhibitor BM15.766. BM15.766 inhibits the HMG-CoA reductase, which catalyzes the final step of cholesterol biosynthesis. Treatment with this substance decreases amyloid load in transgenic mice [42]. In cell culture, depletion of cholesterol with cyclodextrin leads to a reduced level of A β generation [31]. It has been reported that cholesterol esters stimulate non-amyloidogenic APP degradation [55]. This degradation pathway is catalyzed by γ -secretase (ADAM10), which has been shown to be activated by low cholesterol levels [39]. This is of particular importance, because α - and γ -processing of APP exclude each other molecularly. In respect to therapy, this would mean that lowering cholesterol levels inhibits the amyloidogenic

pathway, whilst the non-amyloidogenic processing is activated the same time, revealing a double beneficial effect.

Furthermore, it has been recently shown that certain steps of APP processing are sensitive to alterations in sphingolipid levels. Inhibition of serine palmitoyltransferase (SPT) causes increased A β 42 secretion along with an increased release of other cleavage products [56].

Thus, the A β generating machinery is highly responsive to small alterations in lipid composition [31, 33, 34, 38], and even small changes that are achieved by altering the standard mouse diet to a cholesterol enriched diet cause increased cerebral amyloid load [40, 41] and increase accumulation of A β in animal brains [57]. Furthermore, research on other targets in lipid homeostasis led to patent on inhibitors of soluble phospholipase A (sPLA).

The molecular mechanisms underlying these observations are yet not completely understood. Cholesterol, sphingomyelin (SM) and gangliosides play an important part in the biology of rafts - lipid micro domains that introduce lateral heterogeneity into the membrane layer and accumulate specific proteins. Rafts are involved in modulating cellular function and cellular structures [58-61] including APP processing [54, 62-67]. BACE I and γ -secretase or PS respectively are present in rafts [62, 63, 65-68].

Taken this together, it may provide an explanation for the lipid sensitivity of the proteolytic cascade of APP processing. Although several aspects remain unclear, the accumulating evidence strongly points towards a link between lipids, membrane composition, APP processing secretases, and AD etiopathology that is substantial and comprehensive.

Statin Treatment

The most advanced approach to treat AD by addressing lipids is the application of statins, effective HMG-CoA reductase inhibitors. Cholesterol metabolism and its homeostasis are well-characterized processes in lipid metabolism. The links between cholesterol metabolism and AD have been already discussed. Briefly, hypercholesterolemia and the ApoE allele ϵ 4 as risk factors provide strong epidemiological correlation between cholesterol and AD, and numerous *in vitro* and *in vivo* studies have investigated the mechanisms behind the epidemiological observations (see above).

While the link between statins, cholesterol and A β has been established in animal models, additional factors may have to be considered for the human situation. Statins have anti-inflammatory potential [69] and since inflammation, vascular pathology, and other aspects contribute to AD, this may add beneficial side effects to the findings discussed below. Nevertheless, there are recent US patents on statins and their therapeutic potential in AD.

Based on mechanistic evidence [31, 34] a number of epidemiological studies were initiated addressing a possible risk modulating effect of statin use [45, 48, 49]. The outcome was spectacular. Statin use correlated with strongly reduced risk for AD. However, already the first study raised the point that patients may need to be treated for a significant time before relevant effects could be observed [49]. Later studies

gave contradictory results, but could not provide an answer to exposure time or drug dosage correlation to risk [70, 71].

Meanwhile, two prospective high dosage clinical AD trials, one dose response trial with healthy volunteers and several low dosage or short treatment trials have been published [46, 47, 72-75]. The dose response trial showed a dose dependent reduction in plasma A β levels, while cerebrospinal fluid levels were not investigated. An interesting aspect of this study is that controlled release lovastatin was used [46]. This is important, because in rodents, statin levels decline sharply following the initial dose and the brain half-life averages depending on hydrophilicity of the drug several minutes to few hours. The human situation remains currently unknown. Some of the low dosage or short duration trials reported altered APP metabolism but no further indications of a beneficial effect [75]. The high dosage simvastatin trial reported for AD patients in the initial stage of the disease a direct correlation between decreased brain cholesterol metabolism and decreased cerebrospinal fluid A β levels. This correlation was found for patients in the advanced stage of the disease. For mild AD patients, slightly increased cognitive performance was found and cognitive performance was stable for the entire treatment group, while performance decreased as expected in the placebo group [47]. Very recently, results from the high dosage atorvastatin trial were published [72]. Unlike in the other study, the focus was here on cognitive performance, but cerebral APP was not investigated. Similar to the first study, the treated patients performed better, than those in the placebo group. These strongest differences were found after six month of treatment and a tendency towards a decline was observed at the end point of 12 months [72].

All of these studies, each involving only small numbers of patients, were rightfully labeled by the authors as preliminary studies. Several large prospective clinical trials are currently on the way to address these points in more detail.

Non-Steroidal-Anti-Inflammatory Drugs - NSAIDs

Inflammatory events are assumed to contribute to the pathological cascade of AD. Activated microglia and reactive astrocytes are found in the proximity of amyloid plaques along with cytokines and complement components [76, 77]. Reduced incidence and slower progression of AD have been reported in epidemiological studies [78-80] and preliminary clinical trials [81]. In addition, a beneficial effect of chronic use of NSAIDs is provided by a recent prospective study, revealing a reduced risk for AD under permanent medication with NSAIDs [82]. In a mouse model, ibuprofen showed to reduce plaque pathology and amyloid deposition [83].

Even though it seems self-evident that the effective mechanism of NSAIDs is due to their anti-inflammatory properties, some confusing observations persist. Based on the observation that prednisone or hydroxychloroquinone do not display protective activity [84, 85], it appears likely, that not only inflammation is involved in this. This gets further substantiated by the fact that despite classical targets of NSAIDs are the cyclooxygenases (COX) 1 and 2, other inhibitors of COX 2 show only minor effects in pilot clinical

trials [86]. More recent *in vitro* studies revealed unexpected promising specificity of a subset of NSAIDs: ibuprofen, indomethacin and sulindac sulphide selectively inhibited the generation of A β 42 (but not A β 40) independently of their anti-inflammatory activity and without interfering with Notch cleavage [87, 88]. Thus, NSAIDs seem to act at the roots of AD pathology, which places NSAIDs in the "*ivy league of anti-amyloidogenic drugs*" [16, 89].

Mechanistically, NSAIDs work differently from all other A β targeting molecules. Instead of reducing β -secretase activity, they shift the balance between A β 40 and A β 42 towards the non-pathological A β 40. Because NSAIDs increase the amount of A β 38, it is assumed that the β -secretase site-specific cleavage is shifted by two amino acids [90]. In respect to therapy, it is important to note that some NSAIDs shift the ratio in the opposite direction, i.e. *towards* pathological A β 42. This may explain the controversial nature of the epidemiological data. For future therapy, even more interesting is the fact that the β -secretase modulating aspect of NSAIDs is apparently independent of COX 1 and COX 2 inhibition [87, 88, 91]. A future AD-NSAID may therefore be designed as a molecule that exhibits no COX-inhibition and reduces A β 42 levels in favor of smaller and presumably less pathogenic A β species. Little is known about the physiological consequences of shifting the A β 40/42 ratio, or the equivalent ratios of other β -secretase substrates. If this does not reveal undesired side-effects, novel NSAIDs may provide very interesting therapeutic opportunities.

Amyloid Clearance

Solubilization of A β

Once amyloid deposits are formed either due to excessive A β overproduction or other subsequent cellular events, removal of A β gains in importance. This task comprises the (re)solubilization of A β and its actual and final removal and/or degradation.

The factors and conditions contributing to the different stages of A β fibrilization are not entirely identified yet, but it seems obvious, that interfering with A β aggregation could be the first step of A β clearance. A β has been shown to bind to Cu and Zn, both of which reduce A β tendency to aggregate and reduce A β toxicity in certain assays [92, 93]. Cu or Zn chelators can solubilize A β in AD brain tissue, and in a mouse model, these substances have been evaluated to resolve amyloid plaques [94]. An approach treating animals with the Cu-chelating antibiotic Clioquinol led to substantial reduction of amyloid load and to general improvement in health. Therefore clinical trials for AD treatment with this combinative medication are currently attempted [95]. Recently, the phase II/III trial was halted due to impurities in the formulation. Now a modified compound (PBT-2) is in phase I trial. Copper seems to be an especially interesting target. Another approach pursued by other groups is to increase, rather than to reduce copper levels. What seems to be a contradiction may in fact be a related mechanism [96]. As for the clioquinol, a clinical trial is currently ongoing.

One of the long standing approaches that falls into this area is to prevent fibril formation, e.g. by molecules that break the ordered amyloid structure [97, 98]; recently, this

approach has been redesigned to take the toxic property of small A oligomers or ADDL (A -derived diffusible ligands) into account. Several other substances like anthracycline, anionic sulfonates, rifampicin or Congo Red have previously been shown to bind to A and to prevent its aggregation *in vitro* [99-103]. Beyond that, non-fibrillogenic A homologues can bind to it and break the formation of β -sheet structures [104, 105]; derivatives of those were designed to improve solubility, stability and ability to cross the blood brain barrier [106].

A degradation

There are numerous cellular processes that may be involved in A degradation, i.e. phagocytosis and intra- or extracellular proteolysis. A list of proteolytic enzymes have been recently identified to degrade A in different assays and *in vivo* models, giving insulin degrading enzyme (IDE) [107, 108], neprilysin [109, 110], plasmin [111-113], angiotensin-converting enzyme (ACE) [114], and endothelin-converting enzyme (ECE) [115] estimable functions in A catabolism. However, answers to numerous questions remain to estimate whether these proteases are possible targets for therapeutic approaches in AD.

A immunization

Another major treatment approach of major importance is A immunization. Using the immune machinery to eliminate already accumulated A monomers, oligomers or aggregates is as appealing as self-evident approach. Consequently, many studies focusing on the utilization of antibodies rose against A peptides. Primarily, immunization of mice with synthetic A 1-42 lead to an antibody response associated with a remarkable clearance of A deposits. Furthermore, such vaccination prevented mice from amyloidosis if applied very early in life [116] providing prophylactic potential. Subsequent research proved and expanded this approach revealing that A vaccination is able to reduce amyloid burden in brain and to improve cognitive deficits in rodents [117, 118]. Passive application of monoclonal anti-A antibodies revealed the same amyloid-clearing effect in mice [119]. However, the vaccination against A of patients with mild to moderate AD resulted in some 6% cases developing an inflammatory reaction in the central nervous system resembling a postvaccinal meningoencephalitis [14, 120]. Therefore, this Phase II clinical trial was suspended, but a closer analysis revealed that in the small subgroup analyzed first at least a subset of patients who had developed high titers of amyloid removing antibodies showed a decelerated cognitive and behavioral decline [121]. Later, the evaluation of the full patient sample revealed a correlation of increased amyloid removing capacity, reduced tau levels, and also a decreased brain volume [122]. A few individual brains could be analyzed so far and are in line with reduced amyloid pathology. Not as promising were the results from neuropsychological assays, but overall towards a positive trend [123].

Further research needs to be done on redesigning the vaccines to avoid toxicity and unspecific over-stimulation of the immune system. Unlike in the study above, the focus now is on specific A epitopes or the generation of a

conformation specific antibody response. Again A oligomers are one of the prime candidates here [124].

CURRENT AND FUTURE DEVELOPMENTS

Taken together it appears to be clear, that none of the aforementioned possible strategies *alone* will get into clinical routine for AD treatment in the next 10 years. Therefore, combinations of strategies will characterize the clinical treatment of AD in the oncoming years. Important to mention is that also symptomatic treatment would assuredly become combined with curative strategies medicating patients in later stages of the disease. In principle, most of the aforementioned approaches bear the possibility to become combined with other strategies, i.e. an A vaccination in later stages of the disease may be accompanied by statin medication to slow down *de novo* production of amyloid β - parallel to its removal; or a treatment with NSAIDs may be supported by particular dietary suggestions to early-stage AD patients in respect to certain lipids to further reduce the distress of new A synthesis.

Available for clinical use already now would be statins and NSAIDs. Whereas statins have recently been very successfully used in clinical trials thus providing promising prospect, the limited information about efficacy, dosing and safety concerns for NSAIDs however, preclude for now their large scale use.

For a successful treatment, a diagnosis as early as possible is essentially required. This is of particular importance for all preventive approaches, but curative strategies will certainly benefit from this, too. Consequently, future research should not only focus on the mechanisms that underlie AD pathology; rather, all biochemical and molecular pathways that will become linked to AD in future will need to be evaluated for their potential to provide early and easy accessible diagnostic markers for the disease.

Close to nothing is known about the physiological function of A. Knock-out mouse models of AD and animal studies with A targeting strategies show that lowering A has at least no major consequences. Since already moderate A lowering is expected to sufficiently reduce the risk for AD, or to significantly affect AD progression, the therapeutic window appears to be comfortably wide. Each of the therapeutic approaches brings additional potential problems. Targeting β -secretase activity or shifting β -secretase cleavage site specificity may run into problems due to the wide range of β -secretase substrates. For BACE, few substrates are yet identified, but similar obstacles may occur. Statins are well established and comparatively safe drugs, but strongly affect the patients' lipid homeostasis. In many cases, this may actually be desirable, but may exclude some patients from this kind of treatment. Dissolving amyloid plaques may face intermediate generation of high levels of toxic small A aggregates and the immunization trial highlighted problems for this approach.

There is currently no wonder drug available that promises to cure AD in a week. In fact, curing AD in later stages may never become possible due to the difficulties to revert to neurodegeneration, once it is established. The goal of AD research for the oncoming years will be, to develop

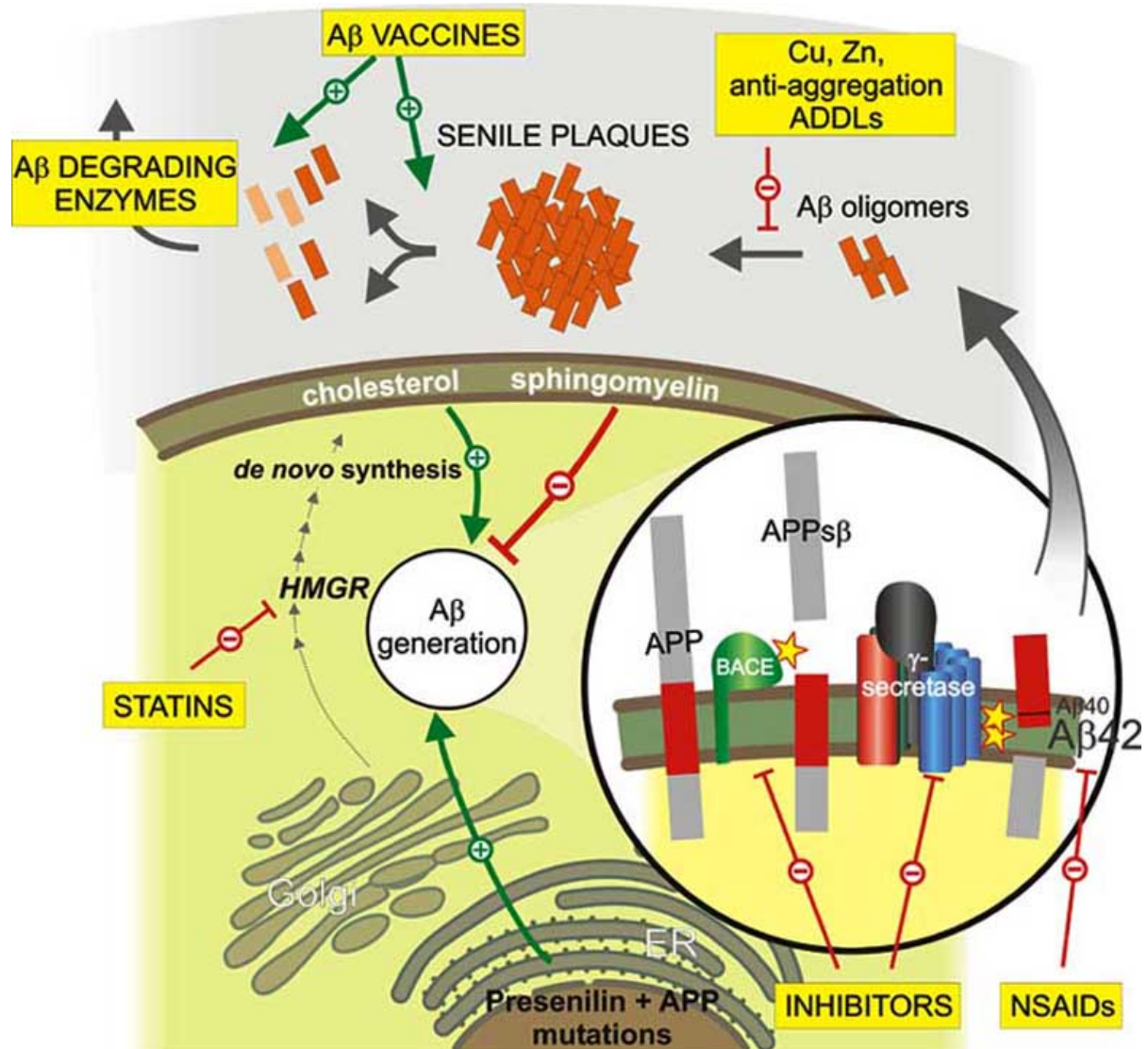


Fig. (1). Based on the augmented knowledge about the mechanisms underlying the pathology of AD, particularly the molecular causes and consequences of AD, different therapeutic approaches arose in recent time. The figure is depicting the main molecular players in early pathology of AD.

therapeutic strategies that treat the disease as early as possible. If drug safety permits, this should apply even before clinical symptoms become evident, providing the basis for these first generation drugs and pro-drugs that target the cause of the disease, and not the symptoms.

At the end of the day, selecting the best approaches will possibly go by the two criteria of safety and effectiveness, with the first one likely to be significantly more important than the second.

REFERENCES

- [1] Hardy J. Toward Alzheimer therapies based on genetic knowledge. *Annu Rev Med* 2004; 55: 15-25.
- [2] Barnes J, Scahill RI, Schott, JM, Frost C, *et al.* Does Alzheimer's disease affect hippocampal asymmetry? Evidence from a cross-sectional and longitudinal volumetric MRI study. *Dement Geriatr Cogn Disord* 2005; 19 (5-6): 338-44.
- [3] Schott JM, Fox NC, Frost C, Scahill RI, *et al.* Assessing the onset of structural change in familial Alzheimer's disease. *Ann Neurol* 2003; 53 (2): 181-8.
- [4] Helmer C, Joly P, Letenneur L, Commenge D, Dartigues JF. Mortality with dementia: results from a French prospective community-based cohort. *Am J Epidemiol* 2001; 154 (7): 642-8.
- [5] Jagger, C, Andersen K, Breteler MM, Copeland JR, *et al.* Prognosis with dementia in Europe: A collaborative study of population-based cohorts. *Neurologic Diseases in the Elderly Research Group. Neurology* 2000; 54 (11 Suppl 5): S16-20.
- [6] Ramarosan, H, Helmer C, Barberger-Gateau P, Letenneur L, Dartigues JF. [Prevalence of dementia and Alzheimer's disease among subjects aged 75 years or over: updated results of the PAQUID cohort]. *Rev Neurol (Paris)* 2003; 159 (4): 405-11.
- [7] Forstl H, Kurz A. Clinical features of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* 1999; 249 (6): 288-90.
- [8] Capsoni S, Giannotta S, Cattaneo A. Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. *Proc Natl Acad Sci USA* 2002; 99 (19): 12432-7.

- [9] De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. *J Cell Sci* 2000; 113 (Pt 11): 1857-70.
- [10] Tanzi R. A genetic dichotomy model for the inheritance of Alzheimer's disease and common age-related disorders. *J Clin Invest* 1999; 104 (9): 1175-9.
- [11] Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol* 2001; Rev 81 (2): 741-66.
- [12] Sisodia SS, St George-Hyslop PH. gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? *Nat Rev Neurosci* 2002; 3 (4): 281-90.
- [13] Younkin SG. Evidence that A beta 42 is the real culprit in Alzheimer's disease. *Ann Neurol* 1995; 37 (3): 287-8.
- [14] Selkoe DJ. Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann Intern Med* 2004; 140 (8): 627-38.
- [15] Duering M, Grimm MO, Grimm HS, Schroder J, Hartmann T. Mean age of onset in familial Alzheimer's disease is determined by amyloid beta 42. *Neurobiol Aging* 2005; 26 (6): 785-8.
- [16] Dominguez DI, De Strooper B. Novel therapeutic strategies provide the real test for the amyloid hypothesis of Alzheimer's disease. *Trends Pharmacol Sci* 2002; 23 (7): 324-30.
- [17] De Strooper, B. Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. *Neuron* 2003; 38 (1): 9-12.
- [18] Vassar R, Citron M. Abeta-generating enzymes: recent advances in beta- and gamma-secretase research. *Neuron* 2000; 27 (3): 419-22.
- [19] Hong L, Koelsch G, Lin X, Wu S, *et al.* Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. 2000; *Science* 290 (5489): 150-3.
- [20] Luo Y, Bolon B, Damore MA, Fitzpatrick D, *et al.* BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time. 2003; *Neurobiol Dis* 14 (1): 81-8.
- [21] Gruninger-Leitch F, Schlatter D, Kung E, Nelbock P, Dobeli H. Substrate and inhibitor profile of BACE (beta)-secretase and comparison with other mammalian aspartic proteases. *J Biol Chem* 2001; 7: 7.
- [22] Marjaux E, Hartmann D, De Strooper B. Presenilins in memory, Alzheimer's disease, and therapy. *Neuron* 2004; 42 (2): 189-92.
- [23] Behr D, Shearman MS. Gamma-secretase inhibition. *Biochem Soc Trans* 2002; 30 (4): 534-7.
- [24] Best JD, Jay MT, Otu, JF Ma, *et al.* Quantitative measurement of changes in amyloid-beta(40) in the rat brain and cerebrospinal fluid following treatment with the gamma-secretase inhibitor LY-411575 [N2- [(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1- [(7S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo [b,d]azepin-7-yl]-L-alaninamide]. *J Pharmacol Exp Ther* 2005; 313 (2): 902-8.
- [25] Siemers E, Skinner M, Dean RA, Gonzales C, *et al.* Safety, Tolerability, and Changes in Amyloid beta Concentrations After Administration of a gamma-Secretase Inhibitor in Volunteers. *Clin Neuropharmacol* 2005; 28 (3): 126-132.
- [26] Edbauer D, Winkler E, Regula JT, Pesold B, *et al.* Reconstitution of gamma-secretase activity. *Nat Cell Biol* 2003; 5 (5): 486-8.
- [27] Casserly I, Topol E. Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. *Lancet* 2004; 363 (9415): 1139-46.
- [28] Puglielli L, Tanzi RE, Kovacs DM. Alzheimer's disease: the cholesterol connection. 2003; *Nat Neurosci* 6 (4): 345-51.
- [29] Simons K, Ehehalt R. Cholesterol, lipid rafts, and disease. *J Clin Invest* 2002; 110 (5): 597-603.
- [30] Hartmann T., Cholesterol. Abeta and Alzheimer's disease. *TINS* 2001; 24 (11S): 45-48.
- [31] Simons M, Keller P, De Strooper B, Beyreuther K, *et al.* Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci USA* 1998; 95 (11): 6460-4.
- [32] Mizuno T, Nakata M, Naiki H, Michikawa M, *et al.* Cholesterol-dependent generation of a seeding amyloid beta-protein in cell culture. 1999; *J Biol Chem* 274 (21): 15110-4.
- [33] Wahrle S, Das P, Nyborg AC, McLendon C, *et al.* Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. *Neurobiol Dis* 2002; 9 (1): 11-23.
- [34] Fassbender K, Simons M, Bergmann C, Stroick M, *et al.* Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 2001; 98 (10): 5856-61.
- [35] Burns M, Gaynor K, Olm V, Mercken M, *et al.* Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production *in vivo*. *J Neurosci* 2003; 23 (13): 5645-9.
- [36] Runz H, Rietdorf J, Tomic I, de Bernard M, *et al.* Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci* 2002; 22 (5): 1679-89.
- [37] Tschape JA, Hammerschmid C, Muhlig-Versen M, Athenstaedt K, *et al.* The neurodegeneration mutant lochrig interferes with cholesterol homeostasis and Appl processing. *EMBO J* 2002; 21 (23): 6367-76.
- [38] Yamazaki T, Chang TY, Haass C, Ihara Y. Accumulation and aggregation of amyloid beta-protein in late endosomes of Niemann-pick type C cells. *J Biol Chem* 2001; 276 (6): 4454-60.
- [39] Kojro E, Gimpl S, Lammich S, Marz W, Fahrenholz F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10. *Proc Natl Acad Sci USA* 2001; 98 (10): 5815-20.
- [40] Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC. Diet-induced hypercholesterolemia enhances brain A beta accumulation in transgenic mice. *Neuroreport* 2002; 13 (4): 455-9.
- [41] Refolo LM, Pappolla MA, Malester B, LaFrancois J, *et al.* Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* 2000; 7 (4): 321-31.
- [42] Refolo LM, Pappolla MA, LaFrancois J, Malester B, *et al.* A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 2001; 8 (5): 890-9.
- [43] Kivipelto M, Helkala EL, Laakso MP, Hanninen T, *et al.* Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ* 2001; 322 (7300): 1447-51.
- [44] Pappolla MA, Bryant-Thomas TK, Herbert D, Pacheco J, *et al.* Mild hypercholesterolemia is an early risk factor for the development of Alzheimer amyloid pathology. *Neurology* 2003; 61 (2): 199-205.
- [45] Rockwood K, Kirkland S, Hogan DB, MacKnight C, *et al.* Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. *Arch Neurol* 2002; 59 (2): 223-7.
- [46] Buxbaum JD, Cullen EI, Friedhoff LT. Pharmacological concentrations of the HMG-CoA reductase inhibitor lovastatin decrease the formation of the Alzheimer beta-amyloid peptide *in vitro* and in patients. *Front Biosci* 2002; 7 a50-9.
- [47] Simons M, Schwarzler F, Lutjohann D, von Bergmann K, *et al.* Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: A 26-week randomized, placebo-controlled, double-blind trial. *Ann Neurol* 2002; 52 (3): 346-50.
- [48] Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. *Lancet* 2000; 356 (9242): 1627-31.
- [49] Wolozin B, Kellman W, Russeaux P, Celesia GG, Siegel G. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch Neurol* 2000; 57 (10): 1439-43.
- [50] Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer disease. *Proc Natl Acad Sci USA* 1995; 92 (11): 4725-7.
- [51] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; 261 (5123): 921-3.
- [52] Cutler RG, Kelly J, Storie K, Pedersen WA, *et al.* Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci USA* 2004; 101 (7): 2070-5.
- [53] Wood WG, Schroeder F, Igbavboa U, Avdulov NA, Chochina SV. Brain membrane cholesterol domains, aging and amyloid beta-peptides. *Neurobiol Aging* 2002; 23 (5): 685-94.
- [54] Zha Q, Ruan Y, Hartmann T, Beyreuther K, Zhang, D. GM1 ganglioside regulates the proteolysis of amyloid precursor protein. *Mol Psychiatry* 2004; 9 (10): 946-52.
- [55] Puglielli L, Konopka G, Pack-Chung E, Ingano LA, *et al.* Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. *Nat Cell Biol* 2001; 3 (10): 905-12.

- [56] Sawamura N, Ko M, Yu W, Zou K, *et al.* Modulation of amyloid precursor protein cleavage by cellular sphingolipids. *J Biol Chem* 2004; 279 (12): 11984-91.
- [57] Sparks DL, Scheff SW, Hunsaker JC 3rd, Liu H, *et al.* Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol* 1994; 126 (1): 88-94.
- [58] Simons K. and Vaz WL. Model systems, lipid rafts, and cell membranes. *Annu Rev Biophys Biomol Struct* 2004; 33: 269-95.
- [59] Simons K. and Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; 1 (1): 31-9.
- [60] Simons K. and Ikonen E. How cells handle cholesterol. *Science* 2000; 290 (5497): 1721-6.
- [61] Hakomori S. and Handa K. Glycosphingolipid-dependent cross-talk between glycosynapses interfacing tumor cells with their host cells: essential basis to define tumor malignancy. *FEBS Lett* 2002; 531 (1): 88-92.
- [62] Vetrivel KS, Cheng H, Lin W, Sakurai T, *et al.* Association of gamma-secretase with lipid rafts in post-golgi and endosome membranes. *J Biol Chem* 2004; 279(43): 44945-44954.
- [63] Cordy JM, Hussain I, Dingwall C, Hooper NM. and Turner AJ. Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. *Proc Natl Acad Sci USA* 2003; 100 (20): 11735-40.
- [64] Marlow L, Cain M, Pappolla MA. and Sambamurti K. Beta-secretase processing of the Alzheimer's amyloid protein precursor (APP). *J Mol Neurosci* 2003; 20 (3): 233-9.
- [65] Ehehalt R, Keller P, Haass C, Thiele C. and Simons K. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol* 2003; 160 (1): 113-23.
- [66] Riddell DR, Christie G, Hussain I. and Dingwall C. Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. *Curr Biol* 2001; 11 (16): 1288-93.
- [67] Parkin ET, Turner AJ. and Hooper NM. Amyloid precursor protein, although partially detergent-insoluble in mouse cerebral cortex, behaves as an atypical lipid raft protein. *Biochem J* 1999; 344: Pt 1 23-30.
- [68] Lee SJ, Liyanage U, Bickel PE, Xia W, *et al.* A detergent-insoluble membrane compartment contains A beta *in vivo*. *Nat-Med* 1998; 4 (6): 730-4.
- [69] Crisby M, Carlson LA. and Winblad B. Statins in the prevention and treatment of Alzheimer disease. *Alzheimer Dis Assoc Disord* 2002; 16 (3): 131-6.
- [70] Rea TD, Breitner JC, Psaty BM, Fitzpatrick AL, *et al.* Statin use and the risk of incident dementia: the Cardiovascular Health Study. *Arch Neurol* 2005; 62 (7): 1047-51.
- [71] Zandi PP, Sparks DL, Khachaturian AS, Tschanz J, *et al.* Do statins reduce risk of incident dementia and Alzheimer disease? The Cache County Study. *Arch Gen Psychiatry* 2005; 62 (2): 217-24.
- [72] Sparks DL, Sabbagh MN, Connor DJ, Lopez J, *et al.* Atorvastatin for the treatment of mild to moderate Alzheimer disease: preliminary results. *Arch Neurol* 2005; 62 (5): 753-7.
- [73] Ishii K, Tokuda T, Matsushima T, Miya F, *et al.* Pravastatin at 10 mg/day does not decrease plasma levels of either amyloid-beta (Abeta) 40 or Abeta 42 in humans. *Neurosci Lett* 2003; 350 (3): 161-4.
- [74] Hoglund K, Wiklund O, Vanderstichele H, Eikenberg O, *et al.* Plasma levels of beta-amyloid(1-40), beta-amyloid(1-42), and total beta-amyloid remain unaffected in adult patients with hypercholesterolemia after treatment with statins. *Arch Neurol* 2004; 61 (3): 333-7.
- [75] Sjogren M, Gustafsson K, Syversen S, Olsson A, *et al.* Treatment with simvastatin in patients with Alzheimer's disease lowers both alpha- and beta-cleaved amyloid precursor protein. *Dement Geriatr Cogn Disord* 2003; 16 (1): 25-30.
- [76] Akiyama H, Barger S, Barnum S, Bradt B, *et al.* Inflammation and Alzheimer's disease. *Neurobiol Aging* 2000; 21 (3): 383-421.
- [77] McGeer EG. and McGeer PL. Brain inflammation in Alzheimer disease and the therapeutic implications. *Curr Pharm Des* 1999; 5 (10): 821-36.
- [78] Rich JB, Rasmusson DX, Folstein MF, Carson KA, *et al.* Nonsteroidal anti-inflammatory drugs in Alzheimer's disease. *Neurology* 1995; 45 (1): 51-5.
- [79] Breitner JC, Gau BA, Welsh KA, Plassman BL, *et al.* Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study. *Neurology* 1994; 44 (2): 227-32.
- [80] McGeer PL, Schulzer M. and McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 1996; 47 (2): 425-32.
- [81] Rogers J, Kirby LC, Hempelman SR, Berry DL, *et al.* Clinical trial of indomethacin in Alzheimer's disease. *Neurology* 1993; 43 (8): 1609-11.
- [82] T Veld BA, Ruitenber A, Hofman A, Launer LJ, *et al.* Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N Engl J Med* 2001; 345 (21): 1515-21.
- [83] Lim GP, Yang F, Chu T, Gahtan E, *et al.* Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. *Neurobiol Aging* 2001; 22 (6): 983-91.
- [84] Van Gool WA, Weinstein HC, Scheltens and Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. *Lancet* 2001; 358 (9280): 455-60.
- [85] Aisen PS, Davis KL, Berg JD, Schafer K, *et al.* A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology* 2000; 54 (3): 588-93.
- [86] McGeer PL. Cyclo-oxygenase-2 inhibitors: rationale and therapeutic potential for Alzheimer's disease. *Drugs Aging* 2000; 17 (1): 1-11.
- [87] Weggen S, Eriksen JL, Sagi SA, Pietrzik CU, *et al.* Abeta42-lowering nonsteroidal anti-inflammatory drugs preserve intramembrane cleavage of the amyloid precursor protein (APP) and ErbB-4 receptor and signaling through the APP intracellular domain. *J Biol Chem* 2003; 278 (33): 30748-54.
- [88] Weggen S, Eriksen JL, Sagi SA, Pietrzik CU, *et al.* Evidence that nonsteroidal anti-inflammatory drugs decrease amyloid beta 42 production by direct modulation of gamma-secretase activity. *J Biol Chem* 2003; 278 (34): 31831-7.
- [89] De Strooper B, Konig G. An inflammatory drug prospect. *Nature* 2001; 414 (6860): 159-60.
- [90] Weggen S, Eriksen JL, Das P, Sagi SA, *et al.* A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 2001; 414 (6860): 212-6.
- [91] Combs CK, Johnson DE, Karlo JC, Cannady SB. and Landreth GE. Inflammatory mechanisms in Alzheimer's disease: Inhibition of -amyloid-stimulated proinflammatory responses and neurotoxicity by PPAR agonists. *J Neurosci* 2000; 20 (2): 558-67.
- [92] Bush AI, Pettingell WH, Multhaup G, d Paradis M, *et al.* Rapid induction of Alzheimer A beta amyloid formation by zinc. *Science* 1994; 265 (5177): 1464-7.
- [93] Huang X, Cuajungco MP, Atwood CS, Hartshorn MA, *et al.* Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. *J Biol Chem* 1999; 274 (52): 37111-6.
- [94] Cherny RA, Atwood CS, Xilinas ME, Gray DN, *et al.* Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 2001; 30 (3): 665-76.
- [95] Bush AI. The metallobiology of Alzheimer's disease. *Trends Neurosci* 2003; 26 (4): 207-14.
- [96] Treiber C, Simons A, Strauss M, Hafner M, *et al.* Clioquinol mediates copper uptake and counteracts copper efflux activities of the amyloid precursor protein of Alzheimer's disease. *J Biol Chem* 2004; 279 (50): 51958-64.
- [97] Hilbich C, Kisters-Woike B, Reed J, Masters CL. and Beyreuther K. Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease beta A4 peptides. *J Mol Biol* 1992; 228 (2): 460-73.
- [98] Hilbich C, Kisters-Woike B, Reed J, Masters CL. and Beyreuther K. Aggregation and secondary structure of synthetic amyloid beta A4 peptides of Alzheimer's disease. *J Mol Biol* 1991; 218 (1): 149-63.
- [99] Tomiyama T, Shoji A, Kataoka K, Suwa Y, *et al.* Inhibition of amyloid beta protein aggregation and neurotoxicity by rifampicin. Its possible function as a hydroxyl radical scavenger. *J Biol Chem* 1996; 271 (12): 6839-44.
- [100] Pappolla M, Bozner P, Soto C, Shao H, *et al.* Inhibition of Alzheimer beta-fibrillogenesis by melatonin. *J Biol Chem* 1998; 273 (13): 7185-8.
- [101] Merlini G, Ascari E, Amboldi N, Bellotti V, *et al.* Interaction of the anthracycline 4'-iodo-4'-deoxydoxorubicin with amyloid fibrils:

- inhibition of amyloidogenesis. *Proc Natl Acad Sci USA* 1995; 92 (7): 2959-63.
- [102] Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, *et al.* Arresting amyloidosis *in vivo* using small-molecule anionic sulphates or sulphates: implications for Alzheimer's disease. *Nat Med* 1995; 1 (2): 143-8.
- [103] Lorenzo A. and Yankner BA. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc Natl Acad Sci USA* 1994; 91 (25): 12243-7.
- [104] Soto C, Sigurdsson EM, Morelli L, Kumar RA, *et al.* Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nat Med* 1998; 4 (7): 822-6.
- [105] Soto C, Kindy MS, Baumann M. and Frangione B. Inhibition of Alzheimer's amyloidosis by peptides that prevent beta-sheet conformation. *Biochem Biophys Res Commun* 1996; 226 (3): 672-80.
- [106] Wisniewski T. and Frangione B. Immunological and anti-chaperone therapeutic approaches for Alzheimer disease. *Brain Pathol* 2005; 15 (1): 72-7.
- [107] Qiu WQ, Walsh DM, Ye Z, Vekrellis K, *et al.* Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998; 273 (49): 32730-8.
- [108] Vekrellis K, Ye Z, Qiu WQ, Walsh D, *et al.* Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J Neurosci* 2000; 20 (5): 1657-65.
- [109] Iwata N, Tsubuki S, Takaki Y, Watanabe K, *et al.* Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nat Med* 2000; 6 (2): 143-50.
- [110] Iwata N, Tsubuki S, Takaki Y, Shirotani K, *et al.* Metabolic regulation of brain Abeta by neprilysin. *Science* 2001; 292 (5521): 1550-2.
- [111] Tucker HM, Kihiko M, Caldwell JN, Wright S, *et al.* The plasmin system is induced by and degrades amyloid-beta aggregates. *J Neurosci* 2000; 20 (11): 3937-46.
- [112] Ledesma MD, Abad-Rodriguez J, Galvan C, Biondi E, *et al.* Raft disorganization leads to reduced plasmin activity in Alzheimer's disease brains. *EMBO Rep* 2003; 4 (12): 1190-6.
- [113] Ledesma MD, Da Silva JS, Crassaerts K, Delacourte A, *et al.* Brain plasmin enhances APP alpha-cleavage and Abeta degradation and is reduced in Alzheimer's disease brains. *EMBO Rep* 2000; 1 (6): 530-5.
- [114] Hu J, Igarashi A, Kamata M. and Nakagawa H. Angiotensin-converting enzyme degrades Alzheimer amyloid beta-peptide (A beta); retards A beta aggregation, deposition, fibril formation; and inhibits cytotoxicity. *J Biol Chem* 2001; 276 (51): 47863-8.
- [115] Eckman EA, Reed DK. and Eckman CB. Degradation of the Alzheimer's amyloid beta peptide by endothelin-converting enzyme. *J Biol Chem* 2001; 276 (27): 24540-8.
- [116] Schenk D, Barbour R, Dunn W, Gordon G, *et al.* Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999; 400 (6740): 173-7.
- [117] Morgan D, Diamond DM, Gottschall PE, Ugen KE, *et al.* A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000; 408 (6815): 982-5.
- [118] Janus C, Pearson J, McLaurin J, Mathews PM, *et al.* A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000; 408 (6815): 979-82.
- [119] Bard F, Cannon C, Barbour R, Burke RL, *et al.* Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 2000; 6 (8): 916-9.
- [120] Selkoe DJ. and Schenk D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* 2003; 43 545-84.
- [121] Hock C, Konietzko U, Streffer JR, Tracy J, *et al.* Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* 2003; 38 (4): 547-54.
- [122] Fox NC, Black RS, Gilman S, Rossor MN, *et al.* Effects of A{beta} immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. *Neurology* 2005; 64 (9): 1563-72.
- [123] Gilman S, Koller M, Black RS, Jenkins L, *et al.* Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* 2005; 64 (9): 1553-62.
- [124] Klyubin I, Walsh DM, Cullen CA, Lemere WK, *et al.* Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity *in vivo*. *Nat Med* 2005, 11 (5): 556-61.