The Current Status of Drugs Related to Amyloid β-protein in Alzheimer's Disease

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Abstract. Alzheimer's disease(AD) is the main cause of dementia, and the main patient group is the elderly, patients' cognitive ability will even degrade to difficult to live independently. According to the WHO 2020 statistics on dementia, there were about 50 million patients in that year. With the progress of the global aging, the number of AD patients will increase rapidly, which is becoming a global public health problem.Currently, the main drugs for AD are cholinesterase inhibitor, N-methyl-D-aspartic acid receptor inhibitor, and aducanumab which is open to the public and enters the Phase IV test in 2021. However, the current understanding of the mechanism of AD is still insufficient. In this article, we will introduce one of the current hypotheses for the pathogenesis of AD, Amyloid β-protein (Aβ) and aggregations, and describe a potential Aβ drug, Fc fusion protein, so that to provide more theoretical basis for the diagnosis and treatment of AD.

Keywords: Alzheimer's disease, Amyloid β-protein, Fc fusion protein, Clinical Treatment.

1. Introduction

Dementia is a neurologic syndrome, the World Health Organization estimated that the dementia patients was almost 50 million in 2020 with a increasing trend to add 10 million patients in each year; and report showed that dementia is caused by several reasons but Alzheimer's disease (AD) is the main reason with 60%-70% dementia patients [1]. The AD mainly affect elderly individuals, because of the ageing of populations worldwide and the difference between biological definition and clinical criteria, the number of AD patients will increase faster than other dementia patients, which makes great burden to medical, psychological, social, economic, and other industries.

The AD was found by a German psychiatrist Alois Alzheimer in 1907, one of his psychopath had serious loss of cognitive functions and unpredictable behaviour. After examining this patient's brain, Alois Alzheimer found the amyloid plaques and loss of neurons in the brain. According to anatomy research, the AD patients' brain can be observed massive shrinkage of brain, especially in cerebral cortex and hippocampus which is link with comprehension and memory.

In clinical, dementia patients have obvious decline in cognitive functions which link to ability of individuals to live, including language, behavioral, cognitive and memory. In order to recognize AD from other dementia, the National Institute on Aging-Alzheimer's Association revised the newest clinical criteria in 2016 mentioned that probable AD patients need to make magnetic resonance imaging for atrophy measurement, measure the metabolic activity of neuronal injury, and measure amyloid level in brain by positron emission tomography and cerebrospinal fluid [2].

In Pathology, AD patients have two cardinal features: amyloid plaques and neurofibrillary tangles (NFTs) which are the mainly targets of current researching drugs. Meanwhile, there are many pathological neural phenomena, such as microglial activation and neuropil threads. The amyloid plaques are the accumulation of abnormally folded Amyloid β-protein (Aβ), which is a common misfolded protein. In AD brain, the amyloid plaques will not degenerate as normal, this accumulation will adhere to nerve cells to block neurotransmitter and even cause cell apoptosis. The NFTs is formed by hyperphosphorylated tar proteins which begin in entorhinal cortex and hippocampus of the medial temporal lobe in normal condition. When tau is hyperphosphorylated, it will be unable to bind microtubules and clumps together into helical filaments, paired helical filaments will compose NFTs which always parallel to neuronal and synapse loss.

This article will introduce the clinical treatment of the Alzheimer's disease, especially focusing on the Aβ Cascade hypothesis and biopharmaceutics which are still under research.

2. Alzheimer's disease and Amyloid β-protein (Aβ)

Aβ is derived from Amyloid Precursor Protein (APP) cleavage by β-secretases and γ-secretases. The Aβ can be generated and eliminated continuously in normal condition. But in AD brain, the ability of Aβ degradation has a decrease and break the balance of Aβ production and elimination, finally cause Aβ accumulation. Which is the amyloid plaque found in AD brains.

The accumulation of Amyloid peptide will block in synapsis cleft affect Ach binding to receptor to transmit, which is one of the speculates for why AD patients lack of cognitive ability to individually [3]. Dineley test the ability of Aβ bind with AchR by recording the result of two-electrode voltage clamp in Xenopus Oocytes, used nicotine (which is a receptor agonist at nicotinic acetylcholine receptors) as a control group [3]. The result is Aβ42 can competitively inhibit about 53.2% of the nicotine, which means Aβ42 will combine Ach receptors to cause dementia symptoms [3].

The Aβ also cause nerve cell ER stress and Unfolded Protein Pesponse (UPR) finally cause cell apoptosis which is the AD symptom, loss massive neurons. UPR is the mechanism for protecting the cell against the misfolded toxic aggregated proteins. When UPR is activated, it will increase transcription of the UPR responsive genes and decrease normal protein synthesis to reduce protein loads. However, if the amount of unfold proteins is out of control, UPR will lead pyroptosis which will cause sterile inflammation in brain and damage to other nerve cells. The Aβ will combine with Grp / Bip and dissociates from PERK and ATF-6, leading from PEPK to elF2a phosphorylation, leading the ATF-6 cleavage and activation in Golgi [4]. Then the p-elF2a will activate and increase the level of ER chaperones, like Grp78 / Bip which has the ability to increase the protein folding capacity [4]. In normal brains, this pathway of UPR can deal with the error cleavage of APP, but in AD, this ability is overload and finally cause cell death.

3. Clinical treatment and Aβ Cascade Hypothesis treatment

3.1. Clinical treatment

3.1.1 Ach and AchEi drugs

Ach is an organic chemical, secreted by cholinergic neurons and synthesized from choline and acetyl-coenzyme. In Central Nervous System (CNS), cholinergic neurons acts in memory, attention, sensory and many neural processes [5]. Under physiological conditions, Ach will be inactivated by Acetyl cholin Esterase (AchE), to keep Ach production and elimination in a dynamic equilibrium. In AD brain, the binding and production of Ach is blocked by Aβ peptide, while the AchE is still working, cause the level of Ach between nerve cells especially in CNS is much lower than normal, which affects AD patients cognitive functions [3]. And some researches also illustrate that cholinergic neurons in AD brain is dropping to 100 thousand from about 500 thousand in normal brains [5]. Thus, rising the level of Ach is the target of AD treatment.

The inhibitors of AchE (AchEi), such as donepezil, galantamine, rivastigmine, can increase the level of Ach in synapsis cleft. Using standard measurement in patients mental state and daily living activities to compare donepezil continued treatment with discontinued treatment for 52 weeks, continued donepezil treatment can significantly improve cognitive functions ($P<0.001$) [6]. The previous researches have proved AchEi drugs is beneficial [7]. Patients under rivastigmine treatment in 2 years can significantly enhance cognitive behaviors and reduce mortality [5, 7]. But considering to the selective and the ability of penetrating to the blood-brain barrier (BBB), when AchEi are used in CNS, it always cause side effect in peripheral nervous system. The side effects including, Hypertension, Atrioventricular block and other gastro-intestinal tract response.

3.1.2 NMDA drugs

The another clinical drug for AD is memantine, which is a kind of non-competitive inhibition of NMDA receptor. NMDA receptor, a sub-receptor of glutamic acid in the CNS, is located in the postsynaptic membrane of excitatory neurons. When the NMDA receptor is excited, its coupled ion channel will open, involving sodium, potassium, and calcium. Previous studies have suggested that the NMDA receptor is related to such behaviors as memory and learning. Memantine can bidirectionally adjust the content of Glu acid; when the excessive secretion of Glu acid leads to overexcitation of nerve cells and causes cell death, memantine can reduce the excitability of nerve cells; if the glutamic acid secretion is insufficient, it will cause the patient to concentrate, learning and memory difficulties, memantine can reduce the glutamic acid content required for exciting nerve cells. Studies have shown that memantine has a good cognitive improvement effect on severe Alzheimer's patients. In the experiment, the memantine group showed a more significant cognitive improvement effect than the placebo group ($P<0.001$) [7].

Both of the current clinical methods for AD can only alleviate symptom in the behavioral and psychological, but can not cure the original problems and help patients recovery.

3.2. Aβ Cascade Hypothesis and Aβ drugs

3.2.1 Aβ Cascade Hypothesis

Aβ is from mistakenly cut out of APP. APP is cut out to cell-membrane–bound fragment by β-site APP–cleaving enzyme 1 (BACE), and the final fragments to form Aβ is cut by γ-secretase. The mainly members of Aβ is Aβ1-40, the proportion accounts up to 90%. The neurotoxicity of Aβ1-42 may cause synaptic clefts block and nerve cell death, is considered to be one of the pathogenic causes of AD [3]. Preclinical studies have proved that Aβ is the main component of amyloid deposition. When Aβ accumulates it will form oligomers, protofibrils, fibrils at first, this accumulations will form senile plaques, which is one of the pathological signs of AD brain [8]. Studies have shown that monomeric Aβ has a low toxicity to nerve cells, but when they are accumulated it will harm to cells and lead to AD. Therefore, in order to eliminate protein deposition, the current drug studies targeting Aβ have mainly focused on three aspects: reducing the generation of Aβ, prevention Aβ aggregation, and promotion Aβ clearance.

3.2.2 Aβ drugs and targets

Based on the generation mechanism of Aβ, the currently studied drug targets mainly act on BACE1 to reduce the differentiation of APP into Aβ by inhibiting the target. BACE inhibitors such as verubecestat were designed to reduce the production of Aβ in the early AD phase [9], but the results in the Phase III study were unsatisfactory. In the Phase III study of verubecestat, the level of Aβ deposit in CSF of experimental group was significantly reduced, with the content reduced by about 94% when compared with that of placebo group [9]. However, the cognitive ability of patients was not improved, and even the condition deteriorated. Subsequent studies proposed new speculation that the reason for the experimental failure was that the dosing time needed to be advanced. Even in the preclinical stage, Aβ might have deposited or damaged the nervous system due to other pathogenic reasons. In this case, the therapeutic effect of interfering with Aβ synthesis was limited.

ELND005 (scyllo-inositol) is an amyloid anti-aggregation agent that binds to Aβ42 and inhibits aggregation to reduce the synthesis of Aβ fibrils in the AD brain [10]. Because of decrease in Aβ aggregation, the protein plate will also be reduced and alleviate ER stress of nerve cells, eventually protect nerve cells from apoptosis. Early experiments have proved that scyllo-inositol can significantly reduce the content of Aβ42 ($P<0.01$), but the cognitive improvement of patients was not significant [10].

Currently, there are two main methods for promotion Aβ clearance drugs, namely the addition of Amyloid-degrading enzyme (ADE) and Immunotherapy. ADE mainly includes neprilysin (NEP), endodermin converting enzymes, and inselysin [11]. Previous mouse experiments showed that if NEP-related gene was knocked out, the mice would show obvious amyloid plaques symptoms, and

also showed obvious learning and memory disorders in the Morris water maze test of behavioral experiment [11]. Currently, ADE is considered a new feasible route to reduce amyloid plaques and prevent Aβ accumulation.

Immunotherapy mainly includes two treatment methods of active immunity and passive immunity. The advantage of active immunity is that it can reduce the times of medicine taking, which is relatively affordable for chromc patients. Vaccine drugs can have long-term effects even after they are taken and produce the expression of corresponding antibodies. Although The First Human Active Vaccine IS AN 1792 was discontinued due to adverse reaction in 2002, 20% of the vaccinated patients developed Aβ antibody after 1–3 doses, and patients with antibody after autopsy showed a real decrease in amyloid plaques. Subsequent studies with CAD106, ACC-001, V-950, and several Aβ vaccines are still ongoing in Phase II and Phase III of the clinical program [10].

On the other hand, passive immunity refers to that immunologic proteins are artificially produced through monoclonal antibodies (mAβ) and the like and directly administered to exert immunologic effects. The advantage of this treatment is that it does not need to interfere with the immune cells to produce the therapeutic effect, and the immune damage caused by the large number of activated immune cells by vaccine drugs is avoided [10]. However, the downside is that artificial immune preparations are relatively expensive, and patients with AD need frequent dosing for a long time, which is an economic burden for patients.

3.2.3 Monoclonal antibody drugs

MAβ is an artificial synthetic protein with the same structure as the natural immunoglobulin (Ig). In general, mAβ consists of four light chains and two heavy chains, with the heavy chains binding to the light chains through disulfide bonds. The whole protein assumes the Y shape and has a molecular weight of 150kDa. The presence of the v part of the antibody y structure determines the complementarity-determining of the antibody specificity.

Region can be targeted by modifying this part of the heavy-chain part to obtain different affinities for different proteins, for example, Gantenerumab has higher affinity for Aβ oligomers (0.6 nM) and Fibrols (1.2 nM) but lower affinity for Aβ monomers (17nM) [8]. Therefore, it can be targeted to aggregated Aβ in the brain. The stem portion of mAβ, also known as FC region, binds to proteins on cell membranes, allowing antibodies to reach cells or cross barriers [12].

There are only two guesses as to why mAβ can clear Aβ, which is uncertain. The first idea is that Aβ binds to the amyloid plaques or Aβ oligomers antigen antibodies and induces microglia to phagocytize and degrade Aβ aggregates [9]. The second hypothesis is that the antibody reduces intra-neurological Aβ to achieve a reduction in Aβ aggregate content. Studies have demonstrated that antibodies can bind to Aβ domain of APP, resulting in Aβ not being isolated by the enzyme [9].

Because the IgG protein was the most abundant of the five antibodies, it was used as a mAβ sample. And mAβs currently work well and are being validated in Phase II and III clinical trials include Solanezumab, Gantenerumab, BAN 2401, and Aducanumab. However, it is regrettable that solanezumab and gantenerumab found in the Phase III experiment that the safety problems of long-term drug use failed to achieve the expected experimental results, and they stopped their research and development in 2016 and 2020 respectively. BAN2401 is still in Phase III trials and analysis is not expected until 2024. Fortunately, in the Phase III experiment, solanezumab delayed the development of dementia by about 34% in the Mini Mental State Examination (the test showed a decrease in scores), suggesting that the drugs acting on Aβ aggregation could alleviate the progression of AD [13]. Aducanumab has also successfully entered phase IV clinical trials. Mab obtained by reverse translational medicine from plasma extract of healthy elderly volunteers by aducanumab has achieved significant elimination of plaques in AD model mice [14]. In the Phase III clinical trials, aducanumab has been proved to reduce the degree of cognitive decline in patients, especially the high dose (10mg/kg) can significantly reduce protein pressure in the brain and improve the condition [14]. However, high dose aducanumab also brings new safety problems, such as ARIA, headache, and infection [14]. The specific treatment and adverse reactions require further observation in subsequent clinical trials.

3.2.4 Fc Fusion proteins drugs

The Fc fusion protein is composed of a new protein that modifies and connects Fc fragments on the basis of natural proteins. Because Fc fusion proteins usually have high affinity, the Fc fragment is related to the protein's transmembrane ability and can be targeted to specific tissues after special modification. Therefore, although there are few precious studies, it is an available research direction for CNS drugs such as AD.

The transferrin receptor (TfR) is the focus of research on BBB-related transmembrane transport because TfR is highly expressed on BBB and participates in ligand-independent endocytosis [15]. Therefore, designing the linkage of Fc fragment with macromolecular drugs using TfR as the target can help macromolecular drugs more easily enter brain lysate.

In the previous studies, a fusion protein targeting the inhibition of BACE1 was designed and tested, and the synthesized protein has a Fab moiety against BACE1 and an FC fragment that can bind to hTfR. The test results showed that the fusion protein had high affinity for hTfR and a certain selectivity (100nm to human and 2μm to cynomolgus). For the decomposition of Ab, ATV was demonstrated to be approximately 50% reduced in Amyloid plaques compared to controls treated with IgG only [15]. When Ab entered BBB, brain lysate was observed by Super-Resolution Confocal Microscopy (SRCM) with BACE1 antibody as the control group. After 24h culture, the signal of ATV group was slightly 30 times that of the control group. These tvs can be used as evidence of future binding native Ig [15]. Compared with traditional drugs and mAb, the TV-based Fc fusion protein has a smaller volume and higher ability to penetrate through BBB [15]. At the same time, because the ability of TV to penetrate through BBB is only through the modification of Fc fragments, the remaining two complete Fab fragments can respectively act on two different targets. Therefore, the fusion protein has better specificity, and it is even possible to prepare a BI-specific drug to provide the possibility for future multi-target therapy.

4. Conclusions

Alzheimer's disease is a disease that mainly occurs in the elderly population. The disease always manifests as dementia, whose patients' cognitive and learning and memory abilities are lost year by year.In the later stage, the patients can hardly live independently, which is a great burden for individuals and society. At present, the only clinically used drugs are AChE inhibitor and NMDAR inhibitor, both of which can only delay the symptoms of patients in a limited time, but cannot recover or stop the development of AD, while the research and development of new drugs are slow. At present, the main research directions of new drugs include secretion inhibitors based on Aβ, aggregation blockers, drugs that promote and induce degradation of amyloid plaques, and drugs that act on tau protein.

Only Aducanumab has entered the market for Aβ-targeted drugs and Phase IV clinical trials have been conducted. For the drugs stopped in the early stage of the study, through the analysis of experimental records, it is considered that the new Aβ needs to have a better ability to penetrate BBB, and increase the drug content in the brain. A higher dose of drug concentration at the target site may help to eliminate Aβ. Second, we need to have a better selection ability for Aβ because the cognition of its function is not yet complete, and some drugs may cause safety problems after long-term use. Analysis suggests that due to insufficient drug specificity, in addition to the pathogenic Aβ and aggregations, other protective Aβ are also removed, and adverse reactions include ARIA, headache, and infection. Based on the above problems, Fc fusion protein may provide a new direction for drug research because compared with monoclonal antibodies, Fc fusion protein has smaller size, higher membrane permeability, lower immunogenicity, and better targeting with complete CDR region.

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