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Inhibition of Amyloid-β Induced Signaling as an Approach to Prevent Neurofibrillary Tangle Formation and Cure Alzheimer's Disease

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Abstract

Alzheimer's Disease is a devastating neurodegenerative disease and is the most prevalent cause of age-related dementia worldwide. Numerous resources are invested in the development therapies based on the removal of amyloid-beta plaque; however, insignificant results are yielded in late-stage clinical trials. Here we propose a treatment for AD through the inhibition of amyloid-beta (Aβ) oligomers induced signaling pathway, which prevents the phosphorylation of tau protein and the formation of neurofibrillary tangle, a pathologic hallmark of AD. High-throughput screening and mouse trials are respectively designed for binding inhibitor discovery and behavioral testing. The results of the experiments will provide important data regarding the potential chemicals for the inhibition of amyloid-beta (Aβ) oligomer binding and their effectiveness in AD treatment.

Keywords

Alzheimer's Disease; Tau protein; Amyloid-beta oligomer; Neurofibrillary tangle.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that accounts for 60 to 70 percent of the 50 million dementia cases worldwide [1]. Researches suggest that amyloid-beta (Aβ) plaques and tau-positive neurofibrillary tangles are the two major histopathological hallmarks of AD [2] and represent the two most promising targets to treat the disease.

The leading hypothesis for AD, amyloid cascade hypothesis, posits causation by amyloid-β peptide deposition in the aging brain [3]. It has been the focus of research for academia and pharmaceuticals company in the past two decades and billions of dollars have been invested. However, the removal of Aβ plaques had yielded little or no cognitive improvements in clinical trials. This leads to a major shift in the field from focusing on insoluble amyloid-beta plaques to soluble amyloid-beta oligomers (AβO). The amyloid-beta oligomers (AβO) hypothesis proposed that ligand-like AβOs instigated the brain's physiological changes leading to AD [4]. This paper concerns with AβO signaling pathway, which is illustrated in Figure 1.

Hypothesis: Tau-positive neurofibrillary tangles in AD brain result from the aggregation of phosphorylated Tau protein. If the AβO signaling pathway is inhibited, kinase Fyn could not phosphorylate Tau and lead to Tau detachment; neurofibrillary tangles formation is prevented.

Multiple approaches can be used to inhibit signaling. For example, knocking out the gene that codes for any protein component of the pathway. However, the proteins may carry out other critical physiologic functions in the brain and complete protein inhibition would produce adverse side effects. Thus, inhibition of amyloid-β oligomer binding is considered to be an option with higher feasibility. This leads to the hypothesis: *Inhibition of Amyloid-β oligomer binding (in Aβ induced signaling*

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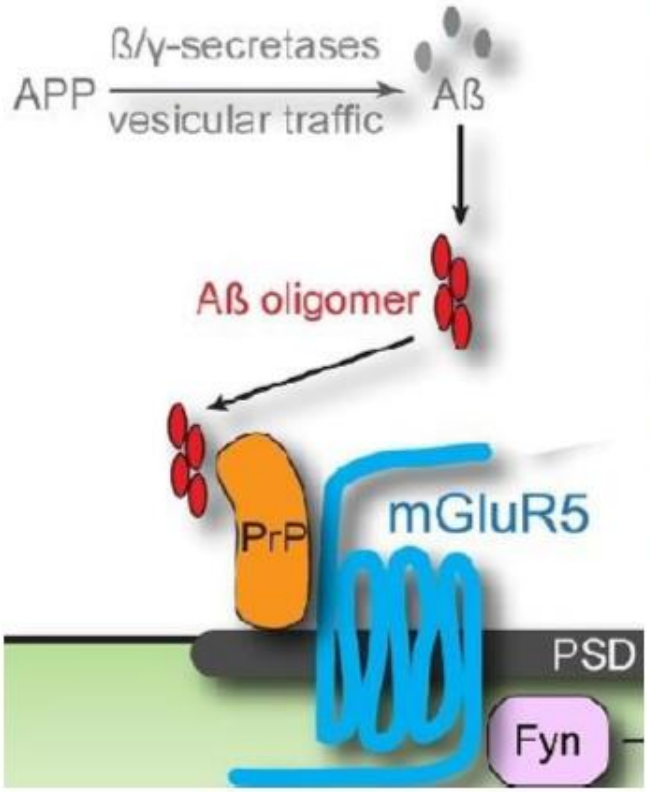
pathway) would prevent neurofibrillary tangle formation and cure Alzheimer's disease. Our proposed experiments aimed to prove that whether AβO binding inhibition is feasible; if so, can this inhibition 1) prevent neurofibrillary tangle formation, 2) provide cognitive improvement, and 3) potentially cure AD.



In AD, Amyloid Precursor Protein is cleaved by beta-secretase and gamma-secretase. Amyloid-beta monomers are produced and assemble into oligomers, which are soluble. Aβ oligomers binds to cellular prion protein, which triggers mGluR5-dependent signaling events [5]. Kinase Fyn is then activated. It physically associates with Tau (a protein maintains the stability of microtubules) and phosphorylate Tau at tyrosine near the N-terminus. resulting in Tau detachment from microtubules and neuronal dysfunction.

Figure 1 AβO signaling pathway[5]

2. Experiment Design

2.1 Experiment 1: Binding Inhibition

In order to test the hypothesis, high-throughput screening is conducted to discover the chemical that is able to inhibit the interaction between AβO and cellular prion protein. Different concentrations of the same type of chemical are applied to prevent excessive or insufficient drug effects.

Independent variable: type of chemical

Dependent variable: interaction between AβO and cellular prion protein

Control variables:

1) Region of hippocampus from the same type of mouse

2) Surface area of the section

3) Access to chemical (duration: 5 hours; volume: keep the solution 1 μm above the upper surface of the hippocampus section)

Procedure:

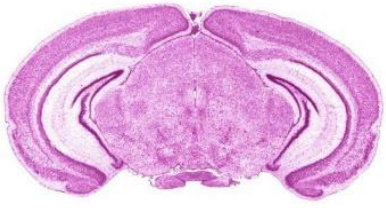


Figure 2 Coronal section of mouse hippocampus[6]

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1) In a 48-well dish, place a 13-μm thick coronal section of mouse hippocampus in the center of each well.

2) Dissect green fluorescent protein (GFP) into N-terminal GFP

3) fragment and C-terminal GFP fragment. Fuse the two fragments with AβO and cellular prion protein respectively.

4) Add each specific inhibitor selected from chemical library to two wells of the dish but with concentrations of 5 nanomolar and

5) 10 nano molars respectively. In one of the wells, add no chemical to serve as the control group.

6) Observe under a fluorescence microscope to determine whether the inhibition is effective.

a) Green fluorescence observed

This indicates that the N-terminal GFP fragment and C-terminal GFP fragment reconstitutes to form fully functional GFP that shows fluorescence. This means that AβO and cellular prion protein (which are fused with the two GFP fragments respectively) interacts, indicating the chemical's ineffective inhibition. Green fluorescence is expected to be observed in the control group and the groups with ineffective chemical.

b) Green fluorescence not observed

This indicates that the reconstitution of GFP fragments did not occur, meaning that the interaction between AβO and cellular prion protein is effectively inhibited by the drug. Green fluorescence is not expected to be observed in the group with effective chemical.

7) Repeat the steps above until effective inhibition is observed.

The discovery of inhibitor leads to the question of whether Tau protein phosphorylation is inhibited as well, which is the ultimate objective of intervening Aβ induced signaling pathway. The question can be answered by observing dendritic spines. A Golgi-Cox staining analysis revealed that, with increased levels of phosphorylated tau protein, mouse dendritic spines were significantly reduced [7]. Thus, if the dendritic spines remain intact, tau protein phosphorylation is also inhibited in the group with effective AβO binding inhibition.

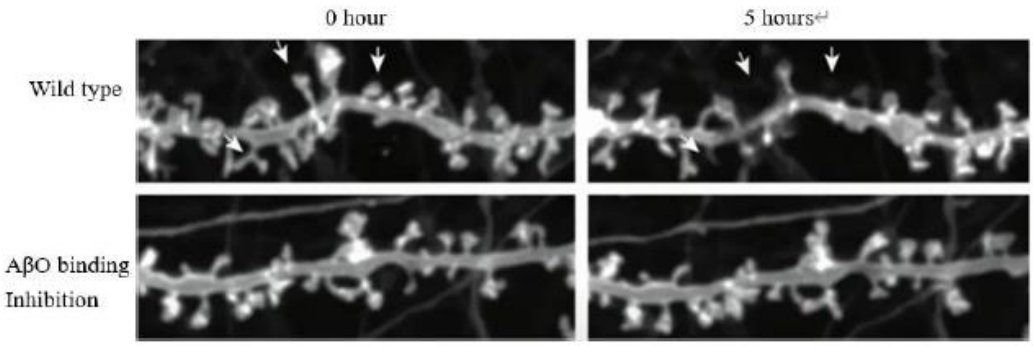

Figure 3 Neurotoxicity of phosphorylated tau protein [8]

In the wild type, 5 hours after AβO addition, loss of dendritic spines can be observed (see arrow pointing). By contrast, in the group with AβO binding inhibition, dendritic spines remain intact 5 hours after AβO addition.

If the chemical discovered in Experiment 1 can effectively inhibit AβO binding and prevent tau protein phosphorylation, further experiments should be conducted to test whether the chemical can alleviate symptoms of AD and provide cognitive improvement.

2.2 Experiment 2: Mouse Trial

This experiment aims to provide a behavioral perspective on the role of AβO-induced signaling pathway in AD.

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Independent variable: dosage of the chemical discovered

Dependent variable: learning and memory of 3xTg-AD mouse

Control variables:

1) Age and weight of the 3xTg-AD mouse

2) Gender ratio (1:1)

3) Access to chemical (one dose every 12 hours)

Procedure:

1) Place 15 male and 15 female 3xTg-AD mice in 30 individual cages. Divide the mice into 3 groups, with 5 male and 5 female mice each. (Mice would be ordered from The Jackson Laboratory https://www.jax.org/strain/004807)

2) Feed two groups of mice with the chemical discovered in Experiment 1, with the amount results in cerebrospinal fluid concentration of 5 and 10 nanomolar respectively. In the other group, feed no chemical to serve as the control group.

3) Let each of the 30 mice perform the Morris water maze (MWM) task.

4) Average the results of each group and plot graphs titled "Escape Latency (sec) versus Block of 5 Trials".

Possible experiment results:

Result A: The result of the mouse's MWM task over trials is the same as Figure 4A.

A better path selection with shorter lengths can be observed as the number of trials increases. This indicates that the subject is able to learn from past training and recall its memory in new trials. The data collected would produce the black line in Figure 4B, reflecting the adoption of a focal search strategy that significantly reduces escape latency. The data suggest that the discovered chemical is able to provide cognitive improvement and potentially cure AD; the hypothesis is proved.

Result B: The result of the mouse's MWM task over trials remains the same.

This is expected to be the result of the control group, as the subjects are AD mice. The length of the path selected by the mouse is the same or close to the one in Figure 4A TRIAL 1, as the number of trials increases. The data collected would produce the grey line in Figure 4B, with little or less significant decrease in escape latency over trials, compared with the black line. This indicates that little or no learning activity occurred; the discovered chemical fails to provide cognitive improvement and cannot cure AD; the hypothesis is disproved.

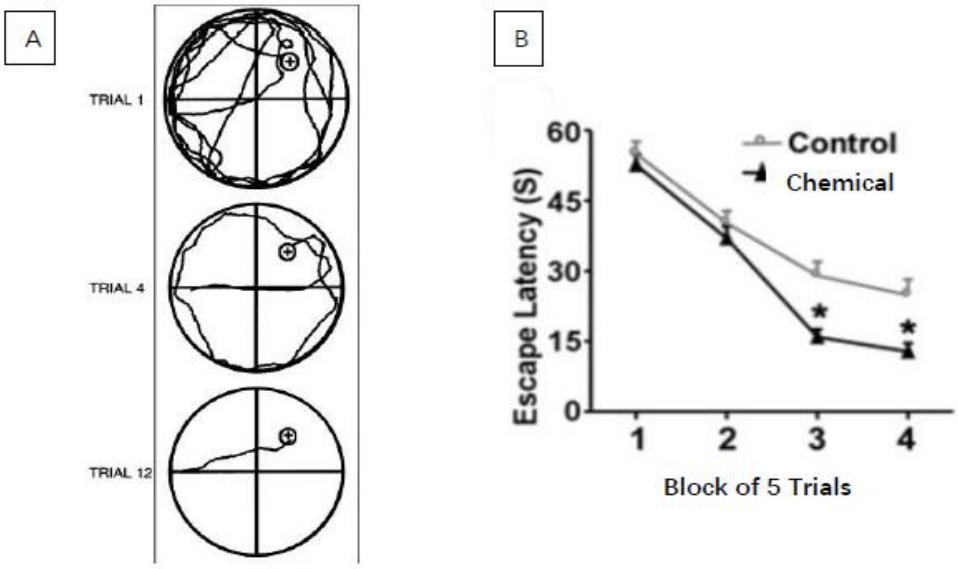

Figure 4 Result of Morris water maze tasks[9,10]

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3. Summary and Conclusions

The hypothesis of this paper is *Inhibition of Amyloid-β oligomer binding (in Aβ induced signaling pathway) would prevent neurofibrillary tangle formation and cure Alzheimer's disease.*

If the result is *Result A* for both of the experimental groups in *Experiment 2: Mouse Trial*, the hypothesis is **proved**. The chemical discovered in *Experiment 1: Binding Inhibition* is able to 1) inhibit AβO binding and prevent neurofibrillary tangle formation; 2) provide cognitive improvement and potentially cure AD. This also highlights tau protein's critical role in the pathogenesis of AD. As Amyloid-beta is not inhibited in the experiments, neurofibrillary tangle formation may be the only cause of AD. This provides an alternative approach for AD drug development; enormous resources have been spent in the pharmaceutical industry to treat human AD through Aβ removal but failed. Dog trials and clinical trials can be conducted in the future to further test the effectiveness and tolerability of the discovered chemical.

If the result is *Result A* for one of the experimental groups in *Experiment 2: Mouse Trial*, the hypothesis is **proved**. However, the dose level in one of the groups is not well tolerated in the mice. Further experiments need to be conducted to assess the tolerability and safety of taking the discovered chemical orally.

If the result is *Result B* for both of the experimental groups in *Experiment 2: Mouse Trial*, the hypothesis is **disproved**. The chemical discovered in *Experiment 1: Binding Inhibition* is 1) able to inhibit AβO binding and prevent neurofibrillary tangle formation but 2) unable to provide cognitive improvement or cure AD.

This indicates that neurofibrillary tangle formation may not be the (only) cause of AD; physiologic changes such as Aβ plaque formation may also impair cognitive functions. Also, the discovered chemical may not specifically target the interaction between AβO and cellular prion protein; it may have other adverse side effects that impair the learning or memory of the mice.

However, it is also possible that the dose levels in both of the groups are not well tolerated in the mice (or the chemical amount is excessive or insufficient) that produced no net effect. In this case, the hypothesis can be proved by dose level adjustment. It is also possible that the discovered chemical is only effective in certain stages of AD; future experiments can be conducted using mice with mild, moderate, and severe stage AD.

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