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Using Antibodies to Target Specific Proteins in Vaccines for Modern Alzheimer's Treatments

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Abstract - Alzheimer's Disease (AD) has grown over the past two decades in an unprecedented fashion and continues to be the leading cause of dementia worldwide. Currently, the disease is perceived to be a result of an accumulation of the β-amyloid peptide (Aβ) in congruence with the tau protein. Genetic and biochemical research suggest that the creation of the Aβ peptide plays a pivotal role in the eventual development of AD. The difficulty with treating AD is that plaques are physiologically generated from the body itself and generally beneficial to the central nervous system (CNS) and in synaptic physiology. Thus, the body does not recognize the Aβ peptide as a threat, which leads to the possibility of AD. A majority of current treatments utilize medications to cope with the disease, but the use of vaccines is fairly rare within the industry. In this paper, a possible solution for the future of Alzheimer's treatment is proposed by promoting the use of antibody-based vaccines that target specific proteins and analyzing their effectiveness.

Index terms - Alzheimer's, Antibody, Beta-amyloid, Medication, Plaque, Tau protein, Vaccine

1 Introduction

Alzheimer's Disease, now the sixth leading cause of death in the United States, is projected to cost the nation $227 billion by 2050, and costs could rise to as high as $1.1 trillion [1]. The disease is most prevalent between the sixth and seventh decades of life, and is generally recognized by the existence of plaques in the brain of potential patients [2]. The biological structure of the amyloid precursor protein (APP) is similar to that of single-pass transmembrane proteins. Splicing the APP transcript generates 8 isoforms, 3 of which appear with great frequency: the 695 amino acid form, the 751 amino acid form, and the 770 amino acid form. The Aβ peptide is generated physiologically from sequential amyloid precursor protein (APP). The enigmatic nature of APP lies in its physiological function, as the protein possesses both positive and negative effects. Under healthy conditions, the Aβ and tau proteins actually demonstrate beneficial physiological impacts, improving cognitive function and synaptic density [3]. The proper Aβ function appears to have antimicrobial activity while the tau protein stabilizes microtubules and secures neuronal integrity and stimulus transfer. The neurological processes affected in AD are so damaging because of their necessity to the CNS. AD disrupts communication between neurons, resulting in loss of function and cell death. When a neuron receives a signal from other neurons, an electrical charge travels through the axon and releases neurotransmitter chemicals across the synapse [4]. During the primary stages of AD, these connections between neurons and the neurons themselves are destroyed, including the entorhinal cortex and hippocampus. As the disease progresses, other areas are affected in the cerebral cortex, including those responsible for language, reasoning, and social behavior. $A\beta_{42}$ and neurofibrillary tangles as a result of the tau protein are thought to be the main causes of the disease.

A variety of current methods exist to target different aspects of AD, the most common method being some form of medication. One negative effect AD has on the physiological functions of brain is displayed in disappearance of the neurotransmitter acetylcholine, which sends signals to other cells, aiding in memory, thought, and judgement. Cholinesterase inhibitors increase the amount of acetylcholine available in the brain, essentially restoring some neurological functions. However, this medication's effectiveness dwindles, as the amount of acetylcholine produced is not enough to counter the amount lost [5]. Generally, the medication begins at a low dosage and increases as time goes on to decrease side effects. Common examples of cholinesterase inhibitors are available depending on the severeness of the disease: Donepezil (otherwise known as Aricept, approved to treat all stages of AD), Galantamine (Razadyne, approved for mild to moderate AD), and Rivastigmine (Exelon, approved for mild to moderate AD). For later stages of AD, Memantine (Namenda), a neurotransmitter which regulates the activity of glutamate, is approved for use by the FDA. The chemical makeup of each medication is more clearly expressed in fig. 1.

Another treatment for AD on the market is the use of NMDA(N-methyl-D-aspartate) receptor antagonists, a class of drugs that, simply put, inhibits the ability of glutamate to connect to cells. Patients who have AD may experience a surplus of glutamate, causing nerve cells to be overloaded with calcium. As a result, these cells are damaged at a faster rate. In order to prevent this cell damage, NMDA receptor antagonists still allows crucial signals to pass through cells, but does not allow a surplus of glutamate to attach to nerve cells. The aforementioned medication, memantine (Namenda), is an example of an NMDA receptor antagonist used in congruence with cholinesterase inhibitors. Due to their neurological effectiveness, NMDA receptor antagonists are also being studied in other diseases such as Parkinson's and amyotrophic lateral sclerosis (ALS).

These AD medications are often administered to patients of higher ages, mainly due to a change in the ratio of A**β**42 in different parts of the CNS. During the early stages of AD, the concentration of A**β**42 in the cerebrospinal fluid (CSF) begins to diminish, while the concentration of A**β**42 in the brain increases, indicating a decrease in A**β** transport from the brain as time elapses [3]. Contrasting to the majority of current solutions, a more innovative solution to AD treatment and prevention lies in using vaccines to counter the disease; this approach targets the plaque directly, nullifying the need for the heavy medications currently on the market. A more intuitive approach to treatments for AD involve the use of immunotherapy; Use monoclonal and polyclonal antibodies to attenuate the growth of AD plaques in the brain.

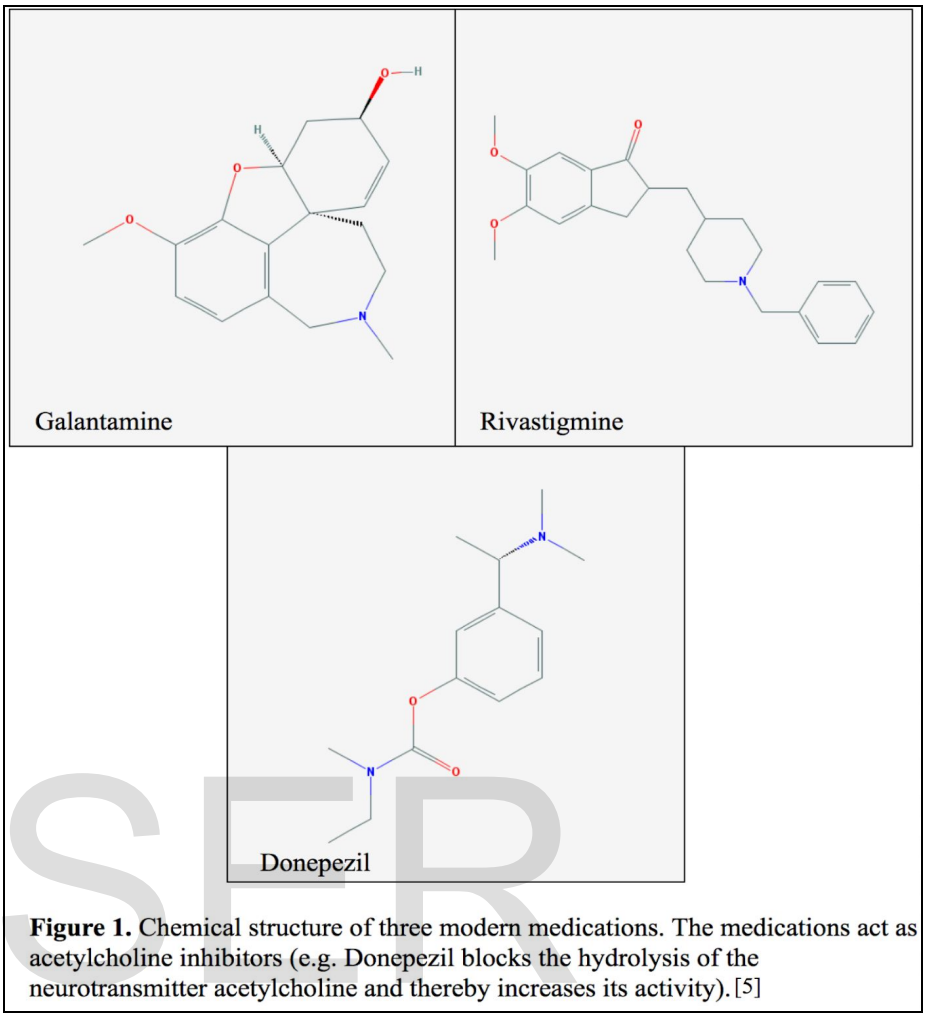


Figure 1. Chemical structure of three modern medications. The medications act as acetylcholine inhibitors (e.g. Donepezil blocks the hydrolysis of the neurotransmitter acetylcholine and thereby increases its activity). [5]

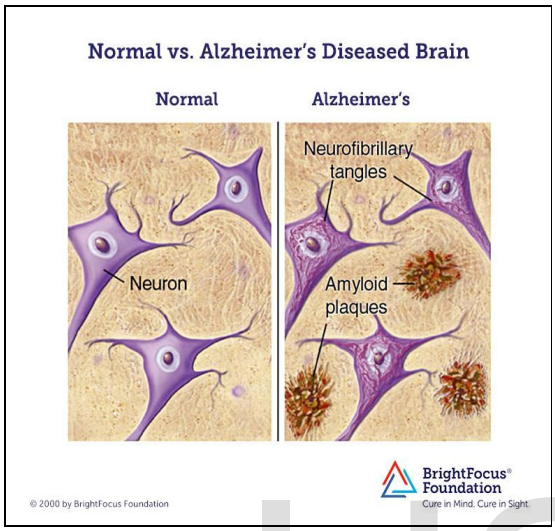
2 Methodology

Rather than creating a new medication to prevent or treat AD, a vaccine that focuses on using monoclonal and polyclonal antibodies can be tested for a possible solution. The two aggregates for AD, A**β** and the tau protein, each have made advances in vaccine possibilities. For instance, the pharmaceutical company Novartis has targeted amyloid **β** in a recent vaccine through the use of bacteriophage based VLP technology [2]. Taking the name of CAD106, the vaccine displays A$\beta_{1\text{-}6}$ on its surface through chemical coupling and was highly immunogenic for the induction of antibody in transgenic mice. However, this vaccine was notably less effective in reducing A**β** in older mice than in younger mice. Other companies, including Merck and Co. and United Biomedical have adopted a similar method with small variations in the carrier and/or peptide and adjuvant used for vaccine generation. The weakness with these vaccines is that there is no clinical data that supports the notion that a decrease in A**β** leads to a regression in AD. Additionally, as demonstrated by the tests with transgenic mice, the vaccine is not optimized for the elderly and indicates that AD must be detected at an earlier stage.

The aforementioned vaccine targets A**β**, but ignores the possibility that the tau protein plays an equal or greater role in the progression of AD. After being demonstrated in studies for immunization in mouse brains, A**β** vaccine clinical trials were held using Aβ 1–42 and QS-21 adjuvant that promotes cytotoxic T-cell response [6]. Autopsies from the trial demonstrated plaque clearance but the existence a majority of tau pathology continued. One of the cohorts demonstrated a positive relationship between the presence of antibodies that recognized A**β** in tissue selections and a less pronounced cognitive decline [7]. These specific A**β** trials are promising, but they have clearly expressed that singular focus on A**β** is not sufficient to treating AD.

Similarly, the development of tau therapeutics has had considerable success in showing cognitive improvement for AD patients. In a placebo-controlled, escalating-dose phase IIa clinical trial, thirty patients with mild to moderate AD were treated for 20 weeks with tideglusib (NCT00948259). These trials showed a positive trend on the MMSE and ADAS-Cog tests [8], and resultantly, a second, 6 month phase IIB trial with 308 patients with mild to moderate AD was created (NCT01350362). Tideglusib (NP031112, NP-12, a GSK-3**β** inhibitor)[9] demonstrated its safety in the trial but missed its primary endpoint and even some secondary endpoints, thus showing no clinical benefit [10]. It is worth noting however that the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) granted the compound orphan drug status and was also evaluated in a 1-year study in 146 patients with PSP (NCT01049399). As in the aforementioned A**β** trials, the tau clinical trials for AD treatment showed positive results, but still could not create a tangible correlation between treating AD patients and inhibiting the tau protein.

A more intuitive approach to creating Alzheimer's vaccines is to target both tau and A**β** simultaneously. Rather than using only tau or A**β** based antibodies, a combinatory vaccine can be utilized as a possible solution to finding AD treatments. Although scarce in number, modern AD treatments are adopting this concept. U.S. researchers say they have developed an "exceptional" universal vaccine platform, called MultiTEP, that inhibit the aberrant forms of A**β** and tau proteins [11]. This method immunizes patients with a single anti-A**β** vaccine, then an anti-tau vaccine if the disease progresses. In essence, the vaccine can treat patients at earlier stages of AD or even healthy people at the risk of AD. The process for using antibodies to target both tau and A**β** protein is fairly similar to any other antibody-based vaccine.



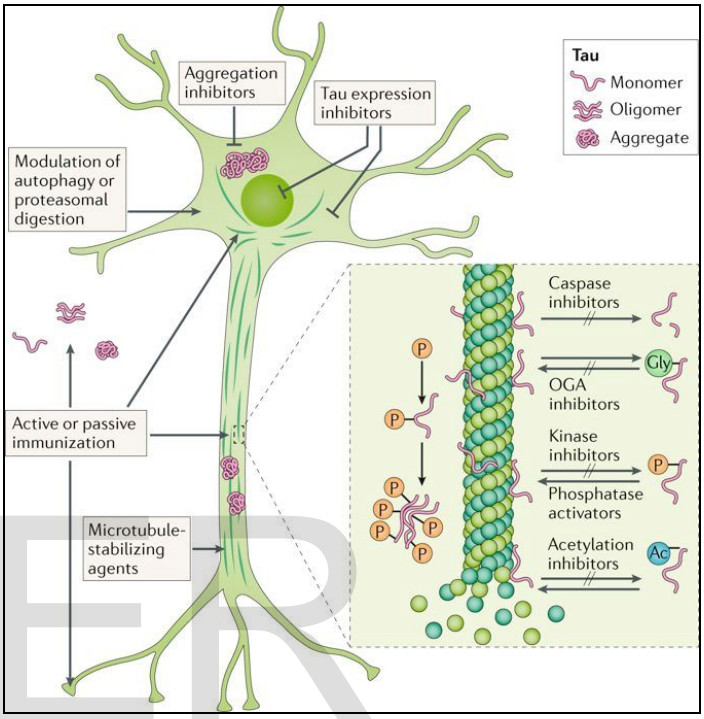


Figure 2 [12], [13]. Visual model of locations of neurofibrillary tangles and A β plaques. The model on the right describes a typical antibody process for the tau protein. This process is extremely similar to amyloid antibodies, the only main difference being the antibody used to target beta-amyloid.

As shown in fig. 2, the process of using antibodies can be applied to modern Alzheimer's vaccines. However, rather than using a singular antibody to isolate tau or A β , using a vaccine that contains inhibitory properties for both aggregates could potentially increase the effectiveness of AD vaccines. The process is fairly similar to other vaccines and quite straightforward: inject the vaccine, generating antibodies, which bind to the tau and A β proteins, inhibiting their function and essentially nullifying their negative effects [13]. The challenge lies in finding antibodies that will continue to generate after the vaccine injection. Although fairly scarce in its existence, research is being conducted in antibodies that exhibit such properties. For instance, in a recent report published in 2017, the effects of immunization with tau antibodies 43D against tau 6–18 and 77E9 against tau 184–195 on tau and amyloid-β (A β) pathologies and cognition in triple-transgenic (3×Tg)-AD mice at mild to moderate stages of the disease were observed [14]. The table below demonstrates with more specificity the antibodies used:

Antibody/Drug	Type	Specificity	Phosphorylation sites	Source/reference
43D[a]	Monoclonal	Tau		Covance (Princeton, NJ, USA)
77E9[a]	Monoclonal	Tau		Covance
R134d	Polyclonal	Tau		[15]

GAPDH	Polyclonal	GAPDH		Santa Cruz Biotechnology (Dallas, TX, USA)
pS199	Polyclonal	p-tau	Ser199	Invitrogen (Carlsbad, CA, USA)
pT205	Polyclonal	p-tau	Thr205	Invitrogen
12E8	Monoclonal	p-tau	Ser262/356	Dr. D. Schenk, Elan Pharmaceuticals (South San Francisco, CA, USA)
PHF1	Monoclonal	p-tau	Ser396/404	Dr. P. Davies, Albert Einstein College of Medicine (Bronx, NY, USA)
APP	Polyclonal	APP		New York State Institute for Basic Research in Developmental Disabilities
4G8	Monoclonal	APP/A β		BioLegend (San Diego, CA, USA)
Iba1	Polyclonal	Iba1		Abcam (Cambridge, MA, USA)
Iba1	Polyclonal	Iba1 for IHC		Wako Chemicals (Richmond, VA, USA)
Verubecestat		A β		Merck & Co.

Table 1 [14], [15], [16]*:* Various sites of tau and beta-amyloid vaccine trials. *Abbreviations: APP* Amyloid precursor protein, *A* β Amyloid-β , *GAPDH* Glyceraldehyde 3-phosphate dehydrogenase, *IHC* Immunohistochemistry, *PHF1* Paired helical filament 1, *pS199*Phospho-tau (Ser199), *pT205* Phospho-tau (Thr205), *p-tau* Phosphorylated tau

The results of this research demonstrate that both 43D and 77E9 antibodies rescued spatial memory and short-term memory impairments in 3×Tg-AD mice. This beneficial effect of 43D and 77E9 antibodies on cognitive performance was sustained for up to 3 months on the last dose. Furthermore, the data was collected from a variety of professional and educational sites, leading one to realize that the data collected was consistent and replicated reliably. Thus, modern research has demonstrated that using antibodies that target both tau and A**β** in vaccines has large potential both as a prevention method and as a treatment. Patients can be injected with either two vaccines simultaneously (one for tau and another for A**β**) or with a single vaccine that targets both tau and A**β**.

3 Conclusions and Future Applications

Finding a treatment for Alzheimer's disease is undeniably paramount in recent years with the rise of the disease in unprecedented rates. Although AD research continues to advance and prosper, using vaccines that target both tau and A β should

be considered in the forefront of possible AD treatments. This paper not only aims to demonstrate the effectiveness of such a vaccine, but also to recognize that such methods are not being regarded with the same importance as traditional treatments. With motivated researchers and advanced techniques, developing a vaccine for AD is closer than many realize. Developing a vaccine that utilizes antibodies that target both tau and Aβ is only a single step toward the creating a reliable treatment for AD. Furthermore, this research on using vaccines that target specific plaques in AD can be researched by using polyclonal antibodies that have the capability of regenerating without periodic injections. By continuing to find AD treatments through antibody vaccines, the search for a potential cure for AD can be much more straightforward.

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