Affibody-Mediated Reduction of Amyloid Burden on Aged Mouse Model of Alzheimer's Disease

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Introduction

Alzheimer's disease (AD), the world's leading cause of dementia, affects over 5.8 million Americans, and this number is projected to increase to 14 million Americans within 30 years. Unless an effective treatment is created, the cost of AD will dramatically increase to 1.1 trillion dollars by 2050 (Alzheimer's Association, 2019). AD is a neurodegenerative disease generally characterized by extracellular amyloidogenic plaques and intracellular neurofibrillary tangles (NFT). The amyloid β (Aβ) plaques are a result of improper cleavage of amyloid precursor protein (APP) by β- and γ-secretase, leaving a toxic form of Aβ (42 amino acids long) that has a higher propensity to aggregate. Normally Aβ is cleaned out from the brain by microglia, but these longer insoluble Aβ peptides instead are unable to be internalized by microglia and thus begin to aggregate into insoluble plaques (Minati, 2008). These plaques then accumulate within the synapses of neurons, interrupting cell communication. As the pathology progresses, the severity of cognitive impairment tends to escalate with patients being susceptible to falls, infections, mobility issues, and aspiration pneumonia.

There are few therapies in which significant improvements are seen, therefore an effective method of treating patients is essential. Current treatments, cholinesterase inhibitors and memantine, are only symptomatic. Many of these symptomatic treatments are only effective if taken early in the development of AD. However, detection of AD can be difficult in the early stages since the symptoms are not severe. Modern approaches are seen with active and passive immunization targeting the AD pathology by interfering with their formation (Citron, 2010; Karran et al., 2011). Passive immunization, unlike active immunization, involves the administration of pre-made antibodies into patients in order to stimulate the immune system to neutralize a targeted antigen. Several monoclonal anti-Aβ antibodies showed promising results of reducing Aβ burden and producing cognitive improvements within transgenic animal models (Wilcock et al., 2004). However, efficacy in the animal models did not transfer over to human clinical trials (Schilling et al., 2018, Boutajangout et al., 2019). Still the amyloid hypothesis is the prevailing theory, that the Aβ plaques are drivers of Alzheimer's disease progression. Yet, a more serious issue brain swelling (edema) arose in the clinical trials for a number of patients, especially those patients with a gene called APOE4, associated with accelerated cognitive

decline. It is currently believed to be a side effect of Aβ clearance, as even clinical trials that targeted different mechanisms all exhibited this side effect. As a result, newly engineered scaffold proteins called affibodies have been designed to lack immunoglobulin-related effector functions, hopefully leading to safer treatments (Vazquez-Lombardi et al., 2015).

Recent research has focused on using affibody scaffolds, which are smaller than antibodies, to stimulate an immune response against the Aβ plaques. The affibody molecule has a three helical scaffold domain, high solubility, and facilitates engineering of multimeric proteins that have bispecific properties, which can be valuable for therapeutic designs (Bass et al., 2017). Furthermore scaffold proteins can be genetically linked to an albumin binding domain (ABD) allowing for an increased in vivo circulation time which is crucial for therapeutic treatments (Boutajangout et al., 2019). A second generation Affibody molecule ABY-025 is currently being evaluated as an imaging agent targeting HER-2 expressing tumors. More recently, an affibody compound has been used to target the complement protein C5 to treat inflammatory conditions (Vazquez-Lombardi et al., 2015). Within a certain study, Z_{SYM73}-ABD represents a sequestering affibody molecule that has been able to prevent the development and progression of Aβ-related pathology (Boutajangout et al., 2019). Z_{SYM73}-ABD is able to produce a unique structure while binding to monomeric Aβ; a tunnel like cavity is created by two affibody units with identical disulfide links (Hoyer et al., 2008). While it is known that Z_{SYM73}-ABD can successfully lower amyloid burden, as well as not produce unwanted neuroinflammation or microhemorrhage in the brain when administered before pathology was observed in mice models (Boutajangout et al., 2019), there is little research that shows what impact affibody Z_{SYM73}-ABD once pathology is already developed within mouse models.

It is speculated that Z_{SYM73}-ABD may have various applications towards different proteins involved with neurodegeneration (Boutajangout et al., 2019). Inspired by these results and due to the fact that no effective treatment of AD exists, Z_{SYM73}-ABD is being studied to understand its role in aged transgenic mice, where AD pathology is vastly present, that have shown success in cognitive tests conducted outside of this report. Microgliosis and Astrogliosis was also examined in order to observe any side effects of over inflammation.

Materials and Methods

Histology

Sections previously mounted on slides from the brain of treated and control animals, which were stained with different antibodies: (i) mixture of anti-Aβ mAbs 6E10 (Biosource, Camarillo, CA, USA) and 4G8 (Biosource); (ii) polyclonal anti-glial fibrillary acidic protein (anti-GFAP) antibody; or (iii) anti-Iba-1 antibody. 6E10 and 4G8 are mAbs that recognize Aβ and stain both pre-amyloid and Aβ plaques. The staining was performed with a combination of the antibodies, as each labels a portion of amyloid plaques, while the combination labels all plaques. GFAP is a component of the glial intermediate filaments that form part of the cytoskeleton in astrocytes and is often employed as a marker of astrocyte activation. Iba-1 is a commonly used marker for microglial activation at both early and later stages of plaque development. All procedures were performed by an individual blinded to the experimental study as previously described (Boutajangout et al., 2009, 2017; Scholtzova et al., 2009, 2014, 2017; Liu et al., 2014). Briefly, slides were incubated with primary mouse monoclonal anti-Aβ antibodies 6E10 and 4G8 (Covance Research Products Inc., Denver, PA, USA) at 1:1,000 dilution for 3 h, secondary biotinylated mouse anti-mouse IgG antibody for 1 h at 1:2,000 dilution, and subsequent avidin-peroxidase complex for 30 min at the same dilution. The slides with sections were thereafter reacted in 3, 3-diaminobenzidine tetrahydrochloride with nickel ammonium sulfate (Mallinckrodt, Paris, KY, USA) color intensification solution. GFAP immunostaining was performed by incubation with primary polyclonal anti-GFAP (Dako Inc., Carpinteria, CA, USA) at a 1:1,000 diluent composed of 0.3% Triton X-100, 0.1% sodium azide, 0.01% bacitracin, 1% bovine serum albumin (BSA), and 10% normal goat serum in PBS for 3 h, followed by secondary biotinylated goat anti-rabbit antibody (Vector Laboratories Inc., Burlingame, CA, USA) for 1 h at 1:1,000 dilution. Iba-1 immunohistochemistry was performed similarly to that for GFAP staining with the exception that a secondary goat anti-rat antibody was used (1:1,000, Vector Laboratories Inc., Burlingame, CA, USA).Reactive astrocytosis was rated on a scale of 0–4. The rating was based on a semi-quantitative analysis of the extent of GFAP immunoreactivity (number of GFAP immunoreactive cells and complexity of astrocytic

branching), as previously published (Goñi et al., 2013). The assessment of the Iba-1 immunostained sections was based on a semiquantitative analysis of the extent of microgliosis (0, none; 1, a few resting microglia; 2, moderate number; 3, numerous ramified/phagocytic microglia; 4, high number of microglia) in increments of 0.5, as previously reported (Scholtzova et al., 2009; Liu et al., 2014). Sections were analyzed per animal by an investigator who was blinded to the treatment status of the mice.

Results

In order to assess the effects of Z_{SYM73}-ABD as a reversing protein of pathology, levels of AB burden within the hippocampus and cortex were examined in tissue samples from mice that were previously sacrificed for another project. Images of cortex and hippocampus areas show that lower levels of stained amyloid is seen (A-D). Staining showed a trend towards lower levels of AB burden in the cortex and a significant decrease in levels within the hippocampus for the Z_{SYM73}-ABD treated mice when compared to the control group (Figure 1E/F).

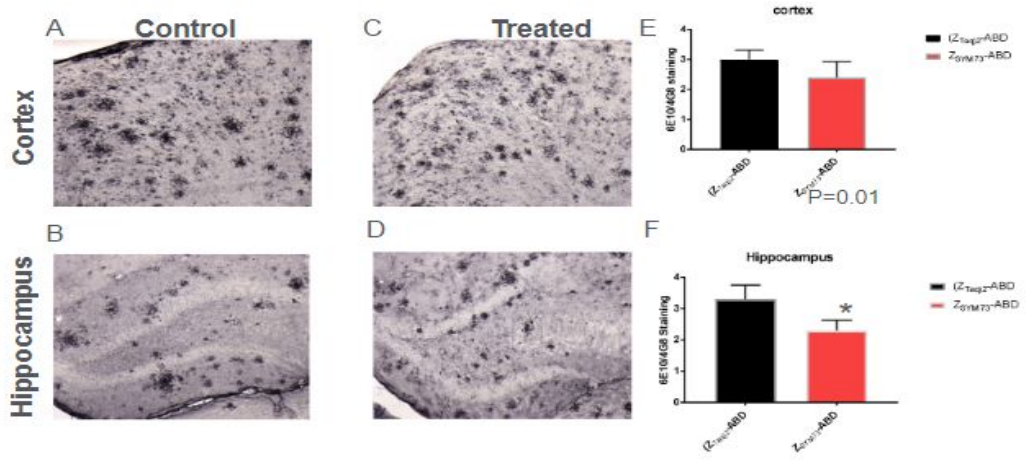

Figure1- Amyloid beta burden was examined with histology. Semi-quantitative analysis showed statistically significant reduction of the amyloid burden in the hippocampus (p=0.01), and trend towards reduction in amyloid burden in the cortex. Brain sections treated with ZSYM73-ABD (red) and Ztaq2-ABD (black) were stained with antibodies 6E10/4G8 as represented by the immunohistochemical images (A-D) and analysis (E and F). When sections were viewed under a microscope, treated mice were seen to have less stained amyloid beta (A-D).

Brain inflammation was also a necessary component to stain for in order to confirm that Z_{SYM73}-ABD does not stimulate excess inflammation. Therefore, serial sections were stained with Anti-GFAP or Anti-Iba1 antibodies which react to astrocytes and microglia, respectively. Reactive astrocytosis was rated on a scale of 0–4. The rating was based on a semi-quantitative analysis of the extent of GFAP immunoreactivity (number of GFAP immunoreactive cells and complexity of astrocytic branching). Microscopic images show that there was no significant increase in A significant decrease in GFAP staining was observed within the cortex and hippocampus within Z_{SYM73}-ABD treated mice (Figure 2 E/F). However, when the Z_{SYM73}-ABD group is compared to the control group, no significant difference in microgliosis is observable (Figure 3 E/F).

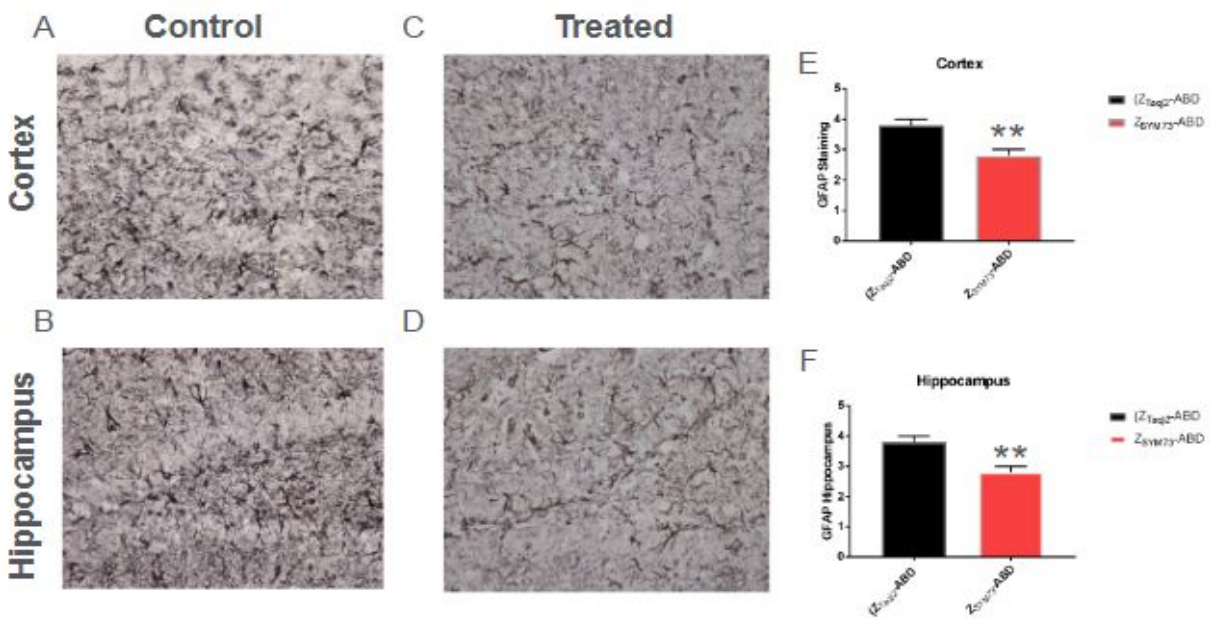

Figure 2- Treated mice sections showed lower visible astrogliosis (A-D). Semi-quantitative analysis showed statistically significant reduction of glial fibrillary acidic protein (GFAP) in the cortex (p=0.03) and in the hippocampus (p=0.0038) respectively (E and F).

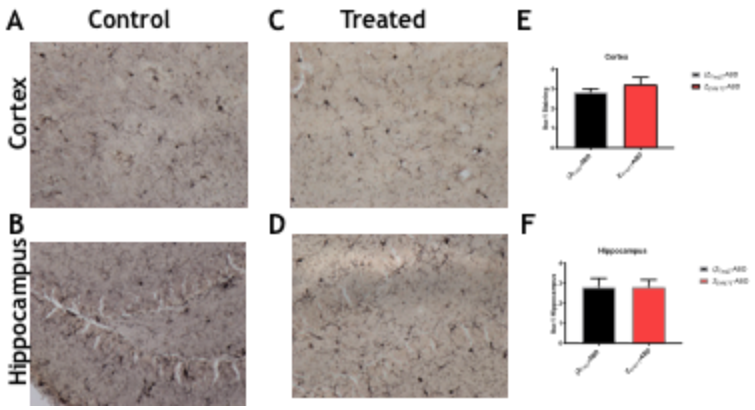

Figure 3- Treated mice sections showed visibly less microgliosis than control sections (A-D). Semi-quantification performed in the cortex and in the hippocampus shows a little reduction of microgliosis in ZSYM73-ABD affibody treated mice, but it was not statistically significant in either area (E and F).

Discussion

In this study we present a therapeutic approach towards reversing the pathology of AD using the Z_{SYM73}-ABD affibody molecule. Due to its capability of sequestering AB peptides, thus inhibiting the proteins ability to aggregate, we went forward in testing the molecules capability to reduce already existing amyloidogenic pathology. We found that within aged double transgenic APP/PS1 mouse models, being intraperitoneally injected twice a week, a significant decrease of AB burden was produced due to the Z_{SYM73}-ABD molecule. This suggests a direct correlation between the success in cognitive tests to the use the affibody molecule. Showing that passive immunization with affibody molecules may prove to be a successful treatment for AD.

It is important to note that no immune-related side effects were seen. Generally, antibodies would cause excess activation of immune responses. However, Z_{SYM73}-ABD lacks such binding domains that would in turn activate these responses, supported by the fact that GFAP and IBA1 staining levels had no increase in levels of astrogliosis or microgliosis. This being said, the Z_{SYM73}-ABD molecule shows that affibody based immunization is very promising as a treatment for AD. The small size of the affibody molecule (6 kDa) is beneficial as well since it can be administered in larger quantities, than antibodies, in the same volumes.

Conclusion

In this study we provide concrete evidence that the affibody molecule can replace monoclonal antibodies within passive immunization to treat Alzhiemer's disease. With the decrease of amyloid beta plaques, neurons in the brain can properly communicate with no interference; this reverses the cognitive decline that occurs in AD. This provides evidence that affibody Z_{SYM73}-ABD should be further studied to further understand its mechanism in targeting amyloid beta. We should also take this to clinical trials and begin conducting on actual humans. For future studies, levels of microhemmorrohaging should be examined in the cortex and hippocampus to examine these side effects. As the amount of people that are living with AD increases and the amount of money spent on AD patient care as well, it is crucial to create a vaccination or cure. Affibody ZSYM73-ABD has proven successful within transgenic mice, however, for future steps, the scaffold protein will be needed to be tested within clinical trials to make sure it is beneficial to humans with AD.

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