SPECIFIC AIMS

The blood-brain barrier (BBB) is a central nervous system line of defense against infections that may preside outside of the body. The BBB has a tough anatomical barrier that is constituted of "astrocytic feet around capillaries, thicker basement membrane, and tight junctions between endothelial cells" [2]. The highly selective permeability of the BBB only allows essential substances such as oxygen and hormones while other molecules such as nutrients and glucose can only cross with the help of transport proteins. Because the BBB disallows passage of antibiotics and bulky antibodies, the physiology of BBB is believed to be involved in many brain diseases such as multiple sclerosis, meningitis, Alzheimer’s, and epilepsy for it has prevented the treatment of such debilitating medical conditions.

More specifically, scientists believe that patients with Alzheimer's disease (AD) have an altered BBB. Studies suggest that a breakdown of the BBB membrane may cause AD by increasing the amounts of beta-amyloid and incidentally causing the vascular degeneration. This precedes a neuronal degeneration and dysfunction of the brain over the course of time [6]. Scientists at the University of Southern California and the director of the Center for Neurodegeneration and Regeneration discussed the role of BBB and neurodegeneration in AD. Important substances involved in this relationship include the receptor for advanced glycation end products (RAGE) and apolipoprotein E (APOE). RAGE is a primary transporter of beta-amyloid into the brain across the BBB. There are two predominant isoforms of amyloid β that are associated with the pathology of AD: Aβ-42 and Aβ-40 residue. The main residue found in the amyloid plaques of Alzheimer’s patients is Aβ-42 and is known to be the more amyloidogenic form of the peptide due to Aβ-42 hydrophobic property [3]. The presence of advanced glycation end (AGE) products that bind to the receptor- RAGE has been implicated in many diseases. This results in abnormal levels of inflammatory chemicals, oxidative stress markers, and endothelin-1, a vasoconstrictor [5]. The BBB has continued to be a challenge for scientist endeavoring to target immunotherapy to the cranial for AD patients. A study conducted by Donahue (et al) suggested that AD is associated with the relative distribution of LRP-1 because RAGE LRP-1 receptors were seen in high abundance in human hippocampus. A similar study by Sasaki (et. al) supported the hypothesis that amyloid β protein (Aβ) is taken up via RAGE and is degraded through the lysosomal pathway in astrocytes. The presence of advanced glycation end (AGE) products and the receptor-controlled interactions (RAGE) may contribute to neuronal dysfunction among AD patients.

To decrease the activity and possible progression of AD due to the presence of AGE distribution after the binding of Aβ to RAGE, we must specifically target the receptor ligand and the main amyloid beta formations that are present amongst all AD patients. We predict that a protein complex immunotherapy drug that targets both RAGE and AB-42 will be a more effective treatment for AD than vaccination for AB 1-42 alone. One such receptor blocker that has been identified to be of interest by Berislav Zlokovic, M.D., Ph.D., and his colleagues is the FPS-ZM1 inhibitor. It is an incredibly small molecule that is able to bypass the BBB and get into the brain to block RAGE from recognizing Aβ and further proliferating the distribution of it. Aβ-42 immunization resulted in the clearance of amyloid plaques in patients with Alzheimer’s disease but did not prevent progressive neurodegeneration [8]. Therefore, solely immunizing against Aβ-42 does not have high efficacy of decreasing Aβ-42. We believe that a combination of the inhibitor FPS-ZM1 and immunotherapy against Aβ-42 could potentially be effective in preventing the progressive nature of AD because the protein complex of RAGE and it’s binding antigens will be directly targeted in the form of an immunotherapeutic. In Aim 1, we propose to experiment with 60 amyloid precursor protein (APP) transgenic mice ranging at various ages below 3 years old and a control group of 60 mice. These transgenic mice will be specifically designed to accumulate amyloid beta – 42 rapidly in their brains. In Aim 2, we will then purify and isolate the antibodies that were produced in response to Aβ-42. In Aim 3, we will then deliver the FPS-ZM1 drug and Aβ -42 antibody to deliver as a passive immunotherapeutic in vivo to another set of transgenic mice that have Alzheimer’s disease.

The immunotherapeutic will be delivered intravenously, as this is the method we believe will have the highest likelihood of getting pass the BBB. Part 1 of the aqueous immunotherapeutic will consist of the inhibitor drug, which will theoretically block RAGE receptors from binding to amyloid beta.
proteins implicated in the generation and progression of AD. Part 2 will consist of the Aβ-42 antibodies. We believe that this approach will neutralize amyloid beta-42 specifically with the assistance of the Aβ-42 antibodies and the inhibitor will target the other forms of the amyloid beta before it can entirely the brain by binding to RAGE. This design is in the form of a combined preventative and passive therapeutic; it attempts to target the problem of amyloid aggregation both in the blood and brain. Thus, we will test for the presence of Aβ-42 in the AD mice both in their blood and brain. With this design, we can assess at what levels the therapeutic may decrease amyloid beta-42 and assess cognitive function and retention of memory. We will also conduct maze runs on the mice and observe their response to footshock to observe improvements in cognitive function in their behavior. Previous studies have shown that the RAGE/Aβ complex is immunogenic; therefore the addition of Aβ-42 specific antibodies could potentially decrease AGE distribution. In addition, the inhibitor will prevent Aβ binding to the receptor site of RAGE. We will extensively analyze the amyloid beta levels of the transgenic mice. The mice will be safely euthanized via strict protocols of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. **We predict that this immunotherapy will slow down AD neurodegeneration thereby improving cognitive function and memory.**

**Aim 1:** To manufacture and inject APP transgenic mouse models with Aβ-42 antigen

**Aim 2:** Will then purify to isolate antibodies produced against Aβ-42

**Aim 3:** Will then intravenously deliver Aβ-42 specific antibodies and linked drug immunotherapeutic to the AD mouse models (60 AD mice total). The team will then thoroughly study beta amyloid levels in mice brain and blood. Will then humanely euthanize mice at the end of the study.

**BACKGROUND AND SIGNIFICANCE**

This research is significant because we are specifically targeting a receptor called RAGE that shuffles beta amyloid into the brain, indicating that it can bypass the BBB without harming the barrier. Aβ in the brain degrades cognitive function in AD individuals. Target immunotherapy can potentially help thousands of Alzheimer’s patients stop the progression of neurodegeneration. Alzheimer’s disease (AD) is a debilitating disease that causes progressive loss of memory and important brain functions. As the disease progresses, problems with language, mood swings, irritability, and confusion develop. AD affects around 3 million people per year and accounts for 60 to 80 percent of dementia cases. With 5 million Americans aged 65 and older expected to have Alzheimer’s Disease, this is an expensive medical burden for the society and patient’s families. Current Alzheimer’s treatments can only temporarily slow the worsening progression of dementia symptoms and improve the quality of life for the patients and their caregivers. Today, there is a global need for ways to stop the disease, delay its onset, and prevent it from developing. Our treatment plan is a novel approach that aims to target the RAGE/Aβ complex with inhibitors that can block RAGE receptors located on neuronal membranes and amyloid plaques circulating in the blood. Dr. Berislav Zlokovic, a neuroscientist now at USC was one of the first scientists to identify RAGE’s involvement in Alzheimer’s disease nearly a decade ago. Zlokovic identified that RAGE is a shuttle that transports amyloid beta from the blood into the brain. Zlokovic and his colleagues studied thousands of compounds for anti-RAGE activity and specified three that appeared to be the most promising. Chemists then studied the molecular structure of the anti-RAGE molecule to recreate candidates that mimic the design. The candidate that appeared to be the most promising was FPS-ZM1; it appeared to be most effective at blocking the receptors from binding amyloid beta. It successfully reversed amyloid deposits, restored healthy blood flow into the brain, and reduced inflammation. However, using a sensitive ELISA assay, the scientists demonstrated that FPS-ZM1 does not inhibit Aβ-40 and Aβ-42 binding to another important Aβ receptor, the LDL receptor–related protein-1 (LRP), which is the major clearance receptor for Aβ at the BBB. FPS-ZM1 molecule is small enough to bypass the BBB and decrease AGE distribution as shown in clinical studies. Therefore, RAGE is a key therapeutic target that must be neutralized in order to decrease Aβ activity in the brain. A recent study has proven that the anti-RAGE inhibitor decreased the levels of

![Molecular structure of FPS-ZM1, a RAGE inhibitor](image-url)
amyloid beta in the brain because of reduced effect of RAGE. FPS- ZM1 successfully prevented the binding of AB. Figure 1 indicates the structure of the molecule. We speculate that using a combination of FPS-ZM1 molecules and immunotherapy to target the Aβ antigen, specifically amyloid beta-42 we can lower AGE activity in the brain. This study will be conducted on mice, however with high efficacy and safety assessment, we are eager to test on AD patients one day.

A study conducted by Webster (et. al) hypothesized that a protein complex immunotherapeutic that targets both RAGE and Aβ1-42 will be more effective than a vaccine for Aβ1-42 alone. Incidentally, Zlokovic and his colleagues have discovered a BBB permeable inhibitor of RAGE that will block the beta – amyloid receptors. Since amyloid beta-42 is one of the most common antigens we want to use this as our preliminary target. We understand that antibodies are excessively difficult to pass through the BBB; we intend to deliver our immunotherapeutic intravenously for the best probability to bypass the inflamed BBB using a prepared RAGE/Aβ complex. A combination of an inhibitor can prevent beta amyloid from even getting into the brain, which is essential because once it is in the brain build up can be debilitating. An immunotherapeutic delivered intravenously constituting of the drug linked with Aβ-42 antibodies may potentially be able to bypass the inflamed BBB while also infiltrating the blood of the mice with the Aβ-42 antibodies and neutralizing the plaques. This form of an immunotherapeutic could specifically target the predominant form of the peptide and can potentially reverse and prevent neurological degradation. The drug inhibitor will be able to inhibit the bypass of various beta amyloids. We intend to deliver an aqueous combinational immunotherapy for the RAGE/Aβ complex. Nonetheless, we hypothesize that in order to fully access and neutralize RAGE, a proposed target inhibitor and anti-Aβ-42 must be created to ensure the degradation of the plaque. When RAGE is blocked, other molecules that cause inflammation in the brain are also inhibited. Figure 2 displays this hypothesis in a microscopy analysis in which FPS-ZM1 inhibitor suggests that it can reduce cranial inflammation the most, which can reduce pathology in AD individuals and improve cognitive performance.

It is clear that something needs to be done about the degenerative disease. Viable treatment options are available to slow down the aggregation of AB deposits, however we believe that a more target based immunotherapy that targets the RAGE/Aβ complex specific antigens that are known to cause the most aggregation of Aβ42 deposits will be the most helpful. This novel immunotherapeutic aims to combine the most efficient RAGE inhibitor and Aβ-42 antibody.
AIM 1: We will generate Aβ-42 in 60 APP mouse models to produce Aβ-42 antibodies that will be utilized in the immunotherapeutic.

AIM 2: After we have identified the antibodies specific to the Aβ-42 we will move on to the manufacture phase. Pacific Immunology will produce the large-scale number of antibodies needed for the experiment. Furthermore, we will produce the anti-RAGE model- FPS-ZM1. This would require a full analysis of the compound and colleagues specializing in chemistry to recreate it in order to administer it to the 60 transgenic mice. FPS-ZM1 will bind to the V domain of RAGE and inhibit Aβ protein binding at specific receptor – RAGE. We understand that the polar chloride atom on the electron-poor aromatic group of FPS-ZM1 may enhance its binding to the receptor [1], contributing to its higher affinity as a Aβ/RAGE blocker in comparison to other inhibitor candidates such as FPS2.

AIM 3: Mouse models that have been designed to be AD positive will then be intravenously injected with the aqueous drug and antibody combination.

This combinational therapy will be administered as an aqueous immunotherapeutic to the AD mice with the goal of improving not only their cognitive impairment, but memory as well since this has remained an elusive goal that has not been met yet. The therapeutic will be delivered in vivo for six months on a sample size of 60 mice. After vaccinations are given daily, the mice will be monitored and will be tested for any potential memory improvement (retention) and the levels of amyloid-beta clearance upon the delivery of targeted FPS-ZM1 and Aβ-42 antibodies.

To be specific, retention amongst the mice will be tested using footshock method in a maze. Prior to the beginning of the trial in the maze the mice will not be allowed to explore it. There will be specific sections of the maze that will contain a slight shock once they cross it. We will test to see if over the course of the trial maze runs, the transgenic AD mice can retain the memory of the location of the footshock in order to avoid it in the maze while on the therapeutic treatment. Beta amyloid-42 levels will be measured in the mice brain after they are safety euthanized.

ANTICIPATED RESULTS AND FUTURE DIRECTIONS

We anticipate improved cognitive function and retention amongst the mice with the delivery of a protein immunotherapeutic that targets both the RAGE and beta amyloid antigen complex. The inhibitor drug, FPS-ZM1 will bind to RAGE, preventing it from transporting beta amyloid that is associated with neurodegeneration. Beta-amyloid 42 antigens will be targeted with target specific antibodies in the liquid immunotherapeutic.
We believe there will be decreased levels of beta-amyloid 42 in the brains of the 60 mice with delivery of designed protein vaccine and we will assess that by measuring Aβ-42 levels specifically. This will be tested over the course of 6 months on mice models via behavioral observation in a maze experiment. We will introduce mice to different mazes over the course of 6 months and introduce footshock to assess their memory retention of the location of the footshock and how well they can accomplish and figure out the course of a maze. We will be measuring variables such as time it took to accomplish the maze and number of times the AD mouse models stopped for a period of time.

We believe that the antibodies circulating in blood could attack the beta amyloid-42 before it ever reaches the BBB, which is crucial because the inhibitory drug cannot stop Aβ-42 binding to other receptors such as LRP. LRP receptors will uptake Aβ-42 and Aβ-40 that is why the goal of our immunotherapeutic is to stop the problem before it can reach the BBB. Meanwhile, FPS-ZM1 will be working at the BBB membrane inhibiting the passage of Aβ for those that are attempting to be shuttled inside the cranial cavity. We anticipate some antibodies to be diffused into the brain as well since the BBB will be characteristically inflamed due to the nature of the disease.

In the future, a continuation of this study will entail targeting Aβ-40 as well, since this is also another form of beta amyloid that can be present in the brains of AD individuals. In this future study, we can compare which antibody therapeutic decreased the presence of beta amyloid the most – either antibody Aβ-40 or Aβ-42 when delivered as a combination with Aβ/RAGE treatment.

TIMELINE

YEAR 1:

With the protein immunotherapy, we suspect successful inhibition of RAGE via FPS-ZM1 and decreased levels of Aβ-42 oligomers in the 60 tested mice.

One year for identifying and developing targeted proteins to Aβ-42. This one year will also include manufacturing of FPS-ZM1 molecules to be included in the immunotherapeutic.

Year 2: First 6 months - delivery of the immunotherapeutic and physical experimentation on the mice. At the end of month 6 will euthanize mice. Next 6 months - examination of mice’s brain to assess amyloid beta concentration

BUDGET

Anti-RAGE inhibitor manufacture...$3,000

Anti- Aβ-42 manufacture...$4,000

Injection tools...4,000

Mice...$1,100

Labor...$27,900

CITATION

1. Deane, Rashid, Itender Singh, Abhay P. Sagare, Robert D. Bell, Nathan T. Ross, Barbra Larue, Rachal Love, Sheldon Perry, Nicole Paquette, Richard J. Deane, Meenakshisundaram Thiyagarajan, Troy Zarcone, Gunter Fritz, Alan E. Friedman, Benjamin L. Miller, and Berislav V. Zlokovic. "A


