Improved Treatment for Alzheimer’s by Enhancing Tyrosine Phosphorylation of the DAB1 Protein through Lauric Acid
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Improved Treatment for Alzheimer's by Enhancing Tyrosine Phosphorylation of the DAB1 Protein through Lauric Acid

Introduction

An Overview on Alzheimer’s

Just 10 years ago, the National Institute of Health stated that Alzheimer’s was the sixth leading cause of death (Liu, 2013). The NIH has just recently revised this fact to say that Alzheimer’s is the third leading cause of death, ranked just behind malignant neoplasms and myocardial infarctions. Furthermore, Alzheimer’s is the most common form of dementia that happens in the elderly. Dementia is linked with the loss of thinking, remembering, and reasoning as it disrupts a person’s daily life. In 1906, Dr. Alois Alzheimer noticed the brain tissue of a woman who had these symptoms (Domalaon et al., 2018). After she died, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called neurofibrillary, or tau, tangles).

These plaques and tangles in the brain are still considered some of the main features of Alzheimer’s disease. Another feature is the loss of connections between nerve cells (neurons) in the brain (Liu, 2013). Scientists have concluded that damage to the brain starts a decade or more before memory and other cognitive problems appear. During this preclinical stage of Alzheimer’s disease, people seem to be symptom-free, but toxic changes are taking place in the brain. Abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain, and once-healthy neurons stop functioning, lose connections with other neurons, and die. The damage initially appears to take place in the hippocampus, the part of the brain essential in forming memories (Mayo Clinic, 2018). As more neurons die, additional parts of the brain are affected, and they begin to shrink (see figure 1). By the final stage of Alzheimer’s, damage is widespread, and brain tissues have shrunk significantly (Mayo Clinic, 2018).
Alzheimer’s disease has three major stages that have symptoms relating to the loss of memory (Howell et al., 2000). Mild Alzheimer’s disease is the first stage. Problems often include wandering and getting lost, trouble handling money and paying bills, repeating questions, taking longer to complete normal daily tasks, and personality and behavior changes. People are often diagnosed in this stage. In moderate Alzheimer’s, damage occurs in areas of the brain that control language, reasoning, sensory processing, and conscious thought (Franco et al., 2011). As this progresses, memory loss and confusion grow worse and people begin to have problems recognizing family and friends. They may be unable to learn new things, carry out multistep tasks such as getting dressed, or cope with new situations. In addition, people at this stage may have hallucinations, delusions, and paranoia. People with severe Alzheimer’s cannot communicate and are completely dependent on others for their care (Dong et al., 2011). Near the end, the person may be in bed most or all of the time as the body shuts down (see figure 2).

**Causes of Alzheimer’s Disease**

Even though Alzheimer’s is such a life threatening disease, the definite cause is still not found. The disease is divided into 2 subtypes based on the age of onset: early-onset AD (EOAD) and late-onset AD (LOAD) (Niu et al., 2004). A combination of genetics, lifestyle, and environmental factors have shown to cause the early/late onset of Alzheimer’s. Most people with Alzheimer’s have the late-onset form of the disease, in which symptoms become apparent with an age later than 60 or 65 years (Dibattista et al., 2015). The apolipoprotein E (APOE) gene is involved in late-onset Alzheimer’s. This gene has several forms. One of them, APOE ε4, increases a person’s risk of developing the disease and is also associated with an earlier age of disease onset (Domalaon et al., 2018). Early-onset AD accounts for approximately 1% to 6% of all cases and ranges roughly from 30 years to 60 or 65 years.

APOE has major functions including the mediation of lipid transport from one tissue to the other, regulates lipid homeostasis, and modulates Aβ metabolism, aggregation, and deposition (Franco et al., 2011). APOE4 is linked to many different diseases including...
atherosclerosis but it’s major link is to Alzheimer’s Disease (AD). APOE is produced by the liver and macrophages which mediates cholesterol metabolism as well (Mayo Clinic, 2018). In the liver, specific astrocytes transport cholesterol to neurons by APOE receptors who are member of the LDLR family. From studies, APOE seems to regulate Aβ aggregation and deposition. Studies show that APOE4 causes the knockdown of the APOE gene (Niu, 2004). This causes Aβ aggregation and plaques to be formed. Not only this, but relationships of the DAB1 protein and APOE gene account for most case of Alzheimer’s along with the APOE4 allele (Mayo Clinic, 2018).

APOE is composed of 299 amino acids and has a molecular mass of ~34 kDa. Differences between the three APOE isoforms are limited to amino acids 112 and 158, where either cysteine or arginine is present: APOE2 (Cys112, Cys158), APOE3 (Cys112, Arg158), and APOE4 (Arg112, Arg158) (NeuroImage, 2015). The single amino acid differences at these two positions affect the structure of APOE isoforms and influence their ability to bind lipids, receptors and Aβ. Human and animal studies clearly indicate that APOE isoforms and DAB1 downregulation differentially affect Aβ aggregation and clearance (Howell et al., 2000). Several Aβ-independent functions are also associated with APOE isoforms. APOE4 and DAB1 downregulation on the APOE gene is what causes differences in interaction and causes and increase in aggregation (Franco et al., 2011). Genome-wide association studies have confirmed that the downregulation of APOE is the strongest genetic risk factor for AD. The presence of this is associated with increased risk for both early-onset AD and LOAD (Domalaon et al., 2018).

AD is associated with both functional abnormalities of the hippocampus and cortical atrophy in the memory network (see figure 3). Patients with AD who have a downregulation of APOE exhibit greater medial temporal lobe atrophy, particularly in the hippocampal area. Structural MRI studies found that, compared with normal gene function, those that have a downregulation of the APOE gene have accelerated age-related loss in cortical thickness and hippocampal volume that are tightly coupled to decline in cognitive performance (Franco et al., 2011). Functional MRI (fMRI) studies reported that APOE downregulation disrupts resting state fMRI connectivity and the balance between brain networks, in the absence of amyloid pathology. Such changes have been hypothesized to represent that downregulation of APOE is a great risk factor for AD (Domalaon et al., 2018).
The Role of VLDLR Gene

Studies suggest that VLDLR and APOER2 (LRP8) genes further lead to the downregulation of the APOE gene (Dibattista et al., 2015). These genes are downregulated because of messages from DAB1 protein (Dong et al., 2011). The DAB1 protein interacts with cadherin proteins on the receptor for VLDLR and APOER2, which causes tyrosine phosphorylation. When tyrosine phosphorylation occurs, more signals are being sent to maintain neuron signals and stability, preventing amyloid plaques to be formed in Alzheimer’s.

Connection between VLDLR, APOER2 (LRP8), and DAB1

Bioinformatics was used to examine if there is a connection between the VLDLR, DAB1, and LRP8 (APOER2). NCBI GEO2R BLAST, a bio-informatics tool, was used to find a dataset of VLDLR and its effect to Alzheimer’s. GSE8425 was found and had samples of VLDLR deficiency in the hippocampus. It was suggested that the samples lead to Alzheimer in patients. Six samples were given which were examined. Three of the samples were of a regular hippocampus and the other three were samples from VLDLR knockout. The three samples of the regular hippocampus were named control and the others were called VLDLR (NCBI Bank). Lower p values link to the increase of significance (Geo Accession Viewer). Further, VLDLR gene is highly downregulated in the VLDLR downregulated group rather than the control group (see figure 4).
Further, string-db, a bio-informatics tool was used to examine a connection between the three different genes - VLDLR, LRP8, and DAB1. The result suggests that DAB1 binds to the LRP8 and VLDLR protein on their cadherin tails and issues tyrosine phosphorylation of the DAB1 protein. This then sends signals to maintain neuron function in human body (see figure 5).

Research Question and Hypothesis

The purpose of this project is to examine if Lauric Acid could be proven to be a novel way of minimizing effects for the downregulation of a string with genes DAB1, VLDLR, and APOE; a gene which is the main link to Alzheimer’s. Unsaturated fatty acids and Lauric Acid exhibit similar patterns in structure. The research also suggests that the DAB1 protein bound to cadherin proteins on VLDLR which issued tyrosine phosphorylation of the DAB1 protein. This process later sent signals to the APOE protein in order to maintain neuron function. Thus based on the extant literature, the following research question and hypothesis is proposed:
**Research Question:** Does Lauric Acid when added to DAB1 protein lead to increase in tyrosine phosphorylation thus reducing the risk of Alzheimer?

**Hypothesis:** Adding Lauric Acid to the DAB1 protein will increase the intensity of tyrosine phosphorylation of cadherin proteins and DAB1 protein thus decrease the risk of Alzheimer’s because of the structural changes in the DAB1 protein.

**Materials Required**

**Step 1: Schrodinger** - List of 100 different fatty acids, Schrodinger biologics suite

**Step 2: Culture NIH-3T3 Cells** - Modified eagle’s medium, bovine calf serum, 0.25% (w/v) Trypsin, 0.53 mM EDTA solution, micropipettes, incubator, 3T3 cell line

**Step 3: Preparation of Plasmid** - 3T3 cells, buffer P1 (50mM Tris-Cl, pH 8.0, 10mM EDTA, 100μg/mL RNase A), buffer P2 (200mM NaOH, 1% SDS), buffer N3 - neutralization buffer for spin columns (4.2 M Gu-HCl, 0.9 M potassium acetate, pH = 4.8), centrifuge, QIA prep, buffer PB - binding buffer (5 M Gu-HCl, 30% isopropanol), buffer PE – wash buffer (10 mM Tris-HCl pH = 7.5, 80% ethanol), buffer EB - elution buffer (10mM Tris-Cl, pH = 8.5)

**Step 4: Transfection using PEI** - 3T3 cells, DMEM/10% FBS medium, plasmid, PEI

**Step 5: Crosslinking of Beads** - Elution buffer (0.1 M Glycine-HCL, pH = 2.5), binding buffer (0.1 M Sodium Phosphate buffer, pH = 8.0), cross linking buffer (0.2 M Triethanolamine, pH = 8.2), cross linker - Dimethyl Pimelimidate Dihydrochloride, blocking buffer (0.1 M Ethanolamine, pH = 8.2), immunoglobulin (in binding buffer), storage buffer (Phosphate-Buffered Saline), Amicon ultra .5 centrifugal filter units for volumes up to 500 microliters

**Step 6: Immunoprecipitation using Protein A Beads** - Immunoprecipitation buffer (150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA (pH 8.0), 0.2 mM sodium orthovanadate, 0.2 mM PMSF, 1% Triton X-100, 0.5% NP-40) , 3T3 cells, centrifuge, protein A beads, vortex, magnet

**Step 7: Protein Quantification** - 20μL of Copper Reagent,1000μL of BCA Reagent, 8 tubes, 40μL of BSA Standard, 500μL of immunoprecipitation buffer, pipette, DAB1 protein, ToxCast Assay Network (TCAN)

**Step 8: Combining Fatty Acid and DAB1 Protein by BSA** - DAB1 protein extracted 3T3 cell line, BSA, Lauric Acid, Sodium Palmitate

**Step 9: Combine DAB1 protein/ fatty acid mixture and Cadherin Protein and FRET** - DAB1 protein/ fatty acid mixture, Cadherin Protein, Nikon TE300 Eclipse Wide-Field microscope with computer, saline solution (pH = 7.4), FRET microscope

**Methods**
Step 1: Computational

Using Schrodinger’s construction, 100 fatty acid structures were made and the DAB1 protein and cadherin proteins were inputted. Using Schrodinger options like MM-GBSA and Protein Preparation, the ligand (fatty acid) and DAB1 protein were bound together. Further, this complex was bound to the cadherin proteins. Results were analyzed with the below equation and can be shown in Graph 1 in the “Results” section:

$$
\Delta G = \Delta H - T\Delta S
$$

- \(\Delta G\) = Gibbs free energy of each fatty acid
- \(\Delta H\) = Enthalpy (Total Heat)
- \(\Delta S\) = Entropy (Randomness)
- \(T\) = Temperature

Figure 6: Lauric Acid and DAB1 complex bound to the cadherin proteins displays the most spontaneity as it has the least Gibbs free energy in comparison to the other fatty acid complexes.

Step 2: Culture NIH-3T3 Cells

Frozen 3T3 cells were thawed in a 37°C water bath. 70% ethanol was used to decontaminate the cells. They were later transferred to a centrifuge tube containing 9.0 mL complete growth medium, and were spun down using a centrifuge at 125 rpm for 5-7 minutes. The cell pellet was resuspended and dispensed into a 75 cm² culture flask. The culture was incubated at 37°C with 5% CO₂ in atmosphere. Cells were later sub-cultured, trypsinized with 0.25% Trypsin and 0.53 mM EDTA, and re-suspended in fresh media in new culture flasks. The culture incubated at 37°C and the sub-culture was repeated every two days.

Step 3: Preparation of Plasmid

Plasmid was inoculated by adding it into 2-5 mL of LB medium. The mixture was incubated for 24 hours at 37°C, 300 rpm. The plasmid was harvested by centrifuging at 6000 rpm for 15 mins at 4°C. The pellet was resuspended in 10 ml of resuspension buffer (50 mM Tris-Cl, pH 8.0; 10 mM EDTA; 100 μg/ml RNase A), 10 ml of lysis buffer (200 mM NaOH, 1% SDS (w/v)) and neutralization buffer (3.0 M potassium acetate, 15–25°C; pH 5.5), inverted 4-6
times, and incubated on ice for 20 mins. The plasmid was centrifuged at 20,000 rpm for 30 min at 4°C. The supernatant was removed and kept in a tube. Micro-centrifuge tubes were equilibrated by applying 10 mL of equilibration buffer (750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v); 0.15% Triton X-100 (v/v)). The supernatant was added and washed two times with 30 mL wash buffer (1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)). 15 mL of elution buffer (1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol (v/v)) was added along with 10.5 mL of isopropanol and centrifuged for 15,000 rpm at 4°C for 20 mins. The supernatant was discarded and the above step was repeated with ethanol. DNA was later re-dissolved in 20 mL of elution buffer.

Step 4: Transfection using PEI

Further, cells were split in 5 ml DMEM/10% FBS medium (24 hrs before transfection). 3 ug total plasmid DNA was diluted in 200 µL of serum-free DMEM w/o phenol red. PEI (1 ug/µL) was added and mixed by vortexing.. The serum was incubated for 15 mins and 9ul DNA/PEI mix was added. The transfected cells were harvested at 48 hrs post-transfection.

Step 5: Crosslinking of Beads

Step 5a: Buffer Exchange for Antibody into Binding Buffer. An antibody (0.2 mg/mL concentration) was thawed under warm water. Contents of one vile were centrifuged for 15 mins at 14,000 rpm. 480 µL of Sodium Phosphate Buffer (0.1 M Sodium Phosphate buffer, pH = 8.0) was added to the resulting 20 µL and centrifuged. The concentrate was disposed into a new centrifuge tube and was centrifuged at 1000g for 2 mins.

Step 5b: IgG Binding to Protein A Magnetic Beads. 500 µL of 0.1 M Sodium Phosphate Buffer (binding buffer) was added to beads and were vortexed to re-suspend. The magnet was applied for 30 secs on the side and aspirated the supernatant. The beads were washed by adding 80 µL of Sodium Phosphate Buffer and 20 µg of DAB1 antibody to beads. The beads were mixed and incubated at 4°C with agitation for 30 mins. The magnet was applied and supernatant aspirated. The Sodium Phosphate Buffer step was repeated three times.

Step 5c: IgG Cross-Linking. 1 mL of cross linking buffer (0.2 M Triethanolamine, pH 8.2) was added to magnetic bead and antibody and vortexed to re-suspend. The magnet was applied and aspirated. The beads were washed and re-suspended by adding 1 mL of cross linking buffer with cross linker (25 mM DMP). The beads were mixed, incubated, applied to magnet, and aspirated. 1 mL blocking buffer (0.1 M Ethanolamine, pH 8.2) was added, vortexed, applied to magnet, and aspirated. The above step was repeated and incubated at room temp. for 1 hr. Further, it was washed with 1 ml of phosphate-buffered saline (PBS), vortexed, applied to magnet, and aspirated. The beads were washed and 1 mL of elution buffer (0.1 M Glycine-HCL, pH 2.5) was added, vortexed, applied to magnet, and supernatant was removed. Beads were stored in 100 µL of PBS storage buffer.

Step 6: Immunoprecipitation using Protein A Beads
**Step 6a: Cell lysis.** PBS were rinsed with a dish of confluent cells. The cells were lysed with 0.5 ml of immunoprecipitation buffer (150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA (pH 8.0), 0.2 mM sodium orthovanadate, 0.2 mM PMSF, 1% Triton X-100, 0.5% NP-40) and maintained at constant agitation for 30 mins at 4°C. The cells were scraped, sonicated on ice for 5 mins, and was repeated 4 times. The cells were centrifuged for 5 mins at 4°C to get crude cell lysate.

**Step 6b: Immunoprecipitation.** 25 μL of Protein A/G Magnetic Beads was added to 200μL of crude cell lysate. This was gently vortexed and incubated at 4°C for 2 hrs. A magnetic field was applied for 30 secs to pull the beads to the side of the tube. The protein was gently removed from beads.

**Step 7: Protein Quantification**
Working Solution was prepared by adding 20μL of Copper Reagent to 1000μL of BCA Reagent. Eight tubes were labeled 1-8. Stock Solution was created by adding 40μL of BSA Standard and 360μL of immunoprecipitation buffer. 256μL of Stock Solution was added to 144μL of immunoprecipitation buffer. From here, a 1:2 dilution was performed. 50μL from tube 8 was pipetted into a well of a 96 well plate. The same was done with the other tubes and protein. 100 μL of Working Solution was added into each well containing a sample. This was incubated for an hour at 37°C. OD was measured at 562 nm using a ToxCast Assay Network (TCAN), as shown in Graph 2 in results.

**Step 8: Combining Fatty Acid and DAB1 Protein by BSA**
18.4% fatty acid-free BSA was dissolved in lauric acid (room temp, 3 hrs). 9mM sodium palmitate was added and agitated (37°C), 6 hrs or overnight. DAB1 protein was treated with FFA/BSA mix (up to 48 hrs).

**Step 9: Combine DAB1 protein/ fatty acid mixture and Cadherin Protein and FRET**
From here, 1 mL of DAB1 protein was added to 0.5 mL of cadherin proteins. A Varian Cary Eclipse was later used to carry out FRET. The above sample was prepared three times to carry out three different samples. A control sample of 1.5 mL of DAB1 protein and was also prepared three times to measure three different samples. Results from the FRET Machine are shown in Graph 3.

**Results**
Graph 1: One can see the difference of Gibbs free energy with respect to various acids. The lower the Gibbs free energy, the more spontaneous the reaction is. However in the diagram displayed values are negative change of Gibbs free energy. Therefore, Lauric Acid displays the most spontaneous reaction. Made by Siya Goel using Excel.

Graph 2: A series of absorbance values were given based on the protein concentration in the assay from Step 7. A line of best fit was discovered and DAB1 protein concentration was 0.16 mg/ml based on absorbance. Made by Siya Goel using Excel.
Graph 3: Through results given by the FRET microscope, the average intensity of fluorescence was based on the concentration of protein. The intensity of DAB1 and Lauric Acid is higher than the intensity of just the DAB1 protein. The results shown are also statistically different due to the fact that the SEM error bars do not overlap. Made by Siya Goel using Excel.

**Equation 1**

Equation 1 shows the relationship between entropy change and intensity of fluorescence.

\[
\Delta S = -k \log \left( 1 + \frac{8 \pi n^2}{\lambda^2 B} \right)
\]

- \( \Delta S \) = Change of Entropy
- \( n \) = Index of refraction
- \( \lambda \) = Wavelength
- \( B \) = Intensity
- \( k \) = Boltzmann constant

Using Taylor’s series approximation and ignoring higher order terms:

\[
\log \left( 1 + \frac{8 \pi n^2}{\lambda^2 B} \right) \approx \frac{8 \pi n^2}{\lambda^2 B}
\]

\[
\frac{\Delta S_2}{\Delta S_1} = B_2 / B_1
\]

**Equation credited to Yahtovitch, 1980**

Equation 1 addresses entropy. Entropy is a commonly used metric for the measurement of brain complexity and addresses randomness and predictability. Greater entropy is associated with more complexity, more randomness, and less system order. The randomness and complexity has been suggested to decrease the risk of Alzheimer’s. Higher entropy also leads to a lower Gibbs free energy, thus leading to more spontaneity and decreased risk of Alzheimer’s. Made by Siya Goel using Excel.
Graph 4 shows the ratio of change of entropy with and without Lauric Acid with respect to concentration of the DAB1 protein. Due to Equation 1, the ratio should be less than 1 as $B_2 > B_1$. Therefore, the negative change in entropy with Lauric Acid is more than negative change without. Made by Siya Goel using Excel.

**Discussion and Conclusion**

The purpose of this research was to examine if adding Lauric Acid to DAB1 protein increases tyrosine phosphorylation thus reducing the risk of Alzheimer's. Both computational and experimental studies were conducted to test the hypothesis. The computational study suggested the most spontaneity between Lauric Acid and DAB1 complex when bounded to the cadherin proteins (see figure 6). During experimental study, when Lauric Acid was combined with DAB1 protein, the results suggested that increasing the concentration of Lauric Acid to DAB1 protein increases the intensity of fluorescence. Previous research has shown that an increase in fluorescence shows an increase in phosphorylation (Wang et al., 2011). One can also conclude that this intensity of fluorescence is measuring tyrosine phosphorylation because tyrosine fluoresces around the wavelengths of 290-320 nm (Guzow et al., 2004). The peak of fluorescence measured here has a wavelength of 306 nm. This further shows that tyrosine phosphorylation is increased when Lauric Acid is added to DAB1 protein, thus supporting the first part of hypothesis.

Entropy is a common used metric for the measurement of brain complexity and addresses randomness and predictability. Greater entropy is associated with more complexity, more randomness, and less system order. The randomness and complexity is associated with one’s ability to adapt to a changing environment. Loss of randomness and complexity has been suggested as the signal of aging and increase in risk of Alzheimer’s (Li et al., 2018). Higher entropy also leads to a lower Gibbs free energy, thus leading to more spontaneity and decreased risk of Alzheimer’s.

The second part of the hypothesis was also supported. Based on intensity of fluorescence, change in entropy was calculated. The results suggest the negative change in entropy using
DAB1 and Lauric Acid (ΔS₂) was more when compared with negative change in entropy using DAB1 (ΔS₁), thus suggesting more randomness and reduced risk of Alzheimer’s.

**Errors, Future Experiments, and Implication**

Some errors were observed throughout this research. The first was not conducting enough trials in the Carry Eclipse due to the lack of protein which may have skewed the results. Secondly, the only form of protein purification used was from the beads and an SDS-PAGE was not carried out. This further leads to the possibility that the protein was not fully purified. In the future, this research could be conducted again with more trials and an SDS-PAGE for more precise results. The experiment could also be tested using circular dichroism to show the precise structural changes, strengthening the current conclusion. In addition, experimenting on a cellular level using CRISPR to see if the conclusion still holds, would be relevant. If it does, then Lauric Acid can be tested on mice and later patients with Alzheimer’s, thus leading to an improved treatment of this deadly disease. Furthermore, Lauric Acid is found in coconuts. Food experiments could potentially be carried out to examine if coconut consumption decrease the symptoms of Alzheimer’s.

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**References**

Alzheimer's disease. Mayo Clinic. Retrieved December 8, 2018
https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447

Biolabs. Phage Display: Solution-phase Panning with Affinity Bead Capture. Retrieved November 11, 2018
https://www.neb.com/protocols/1/01/01/phage-display-solution-phase-panning-with-affinity-bead-capture


Chemical Thermodynamics. Retrieved November 13, 2018
http://chemed.chem.purdue.edu/genchem/topicreview/bp/ch21/gibbs.php


