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The Role of Galanin, Conjugated Linoleic Acid, 12-O-Tetradecanoylphorbol-13-acetate and Molecular Docking of Treatments on Beta-Secretase 1 Enzyme: Effects of Inflammation on Alzheimer's Disease

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Abstract

Obese Alzheimer patients are at double the risk for memory loss compared to non-obese Alzheimer patients. Therefore a correlation between obesity and neuronal function as it correlates to memory loss pathology was investigated. This study also investigated the role of inflammation by exacerbating this pathology. MTT assay was used on 3T3 differentiated and undifferentiated cells treated with galanin, and its antagonist, M40, conjugated linoleic acid, and lipopolysaccharide to determine percent survival. LDH assay was used on 3T3 differentiated cells treated with Aβ40 and tetradecanoylphorbol acetate to determine cell cytotoxicity. Oil red staining of immune cells treated with galanin, M40, and conjugated linoleic acid were used to enhance cell imaging of lipid accumulation. 3T3 differentiated cells treated with conjugated linoleic acid in MTT assay had a 23.20% greater cell survival than 3T3 undifferentiated cells. In the LDH assay, there were 653.63 cells treated with Aβ40 and 652.63 cells treated with tetradecanoylphorbol acetate. Galanin treated cells in oil red staining had 6.18% fat which indicated cell accumulation and an inflammatory response of cells which is similar to the response in the brain. M40, toll-like receptor 4, and somatostatin were molecularly docked to the beta-secretase 1 enzyme and results indicated that somatostatin had the greatest ligand efficiency. Somatostatin had a ligand efficiency of -0.13 and a binding energy of -14.9986. Compared to the other treatments, the results suggested that somatostatin had the greatest binding effects and had the most significant role on the binding to the Alzheimer's disease enzyme. To further evaluate Alzheimer's disease and obesity, testing ginseng, berberine, and polychlorinated biphenyls on Aβ40 is pertinent. This will help elucidate how other dietary supplements and environmental contaminants that can affect the human body when ingested, influence the risk of Alzheimer's disease. This study provided a potential method in minimizing the risk of Alzheimer's disease in which future research would also be conducted.

Keywords: Obesity; Alzheimer; Inflammation

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Introduction

Alzheimer's disease and obesity are two of the most prevalent diseases in the United States. As of 2020, the neurodegenerative disease tops as the fifth leading cause of death in Americans [1], and more than 1.9 billion people worldwide are considered to be obese [2]. As both of these diseases are common to be present in individuals, there is a relationship between the two. Obesity is a risk factor for Alzheimer's disease, thus with a strong prevalence of obesity in billions of individuals globally, this puts countless individuals at risk for being diagnosed with the detrimental neurodegenerative disease in the future [3]. Galanin (GAL), a 29/30 amino acid long neuropeptide that is found in the hypothalamus and mediates several physiological processes such as food intake, is greatly involved with the risk of obesity. This neuropeptide is correlated with obesity as it is

in a positive feedback loop when presented with a high-fat diet. In this positive feedback loop, galanin is released when an individual ingests food containing high fat. After galanin is released, the neuropeptide enhances the individual's desire to consume additional foods with high fat, consequently leading to a positive feedback loop [4]. Obese individuals have higher levels of galanin present in their central nervous system compared to others who are not obese [5]. This is due to galanin reducing insulin resistance by increasing Glucose Transporter Type 4 (GLUT4) contents. Galanin promotes GLUT4 from intracellular membranes to plasma membranes in adipocytes [6]. M40 is the receptor-ligand antagonist for galanin [7] and when bound to the receptor, M40 penetrates the cell membrane and inhibits the complementary receptor from binding. This results in a downstream pathway and decreases fat accumulation production. Similarly, Conjugated Linoleic Acid (CLA) also decreases fat production as it is a common weight-loss dietary supplement that is sold at nutrition stores across the nation. CLA is composed of isomers of linoleic acid and a conjugated double bond [8]. CLA inhibits glucose from entering the adipocytes and increases lipolysis significantly in human adipocytes. The co-activator complex, PPAR, will bind to an additional ligand and activate the Peroxisome Proliferators (PPRE). PPRE activates the target gene in order to translate the gene to form β oxidation (the process to break down fatty acids). An increase in β oxidation will increase the fat metabolism rate and reduce adipocyte accumulation [9]. Treatments with opposing effects of M40 include somatostatin, Toll-Like Receptor 4 (TLR4) Lipopolysaccharide (LPS), and 12-O-Tetradecanoylphorbol-13-acetate (TPA). Somatostatin is a growth hormone inhibiting hormone that is located in various parts of the body, particularly the pancreas. In the pancreas, somatostatin is responsible for regulating the secretion of insulin and glucagon [10]. However, a recent study has indicated that when exposed to high levels of glucose, somatostatin inhibits the secretion of insulin and thus results in cell proliferation and fat accumulation [11]. TLR4 is a member of the toll-like receptor family and identifies bacterial lipopolysaccharides. After identifying the lipopolysaccharide, this receptor then activates a pro-inflammatory response [12]. LPS acts on Toll-Like Receptor 4 (TLR4) to activate the NF-κB pathway and increase cytokines such as interleukin-8 [13]. An increase in cytokines increases Aβ in the brain of Alzheimer's disease patients [14]. Comparably, TPA also induces inflammation as it is a phorbol ester that induces protein kinase C inflammatory response resulting in tau protein aggregation [15,16]. TPA stimulates increased amyloid precursor protein (APP) mRNA and APP transcription as it is involved in the proliferation and differentiation of cells [17]. Somatostatin, TLR4, LPS, and TPA represent the inflammation of fat cells present in obese individuals. M40, somatostatin, and TLR4 were the chosen ligands for the beta-secretase 1 enzyme protein. This enzyme is significant in Alzheimer's disease pathology as it is widely known for cleaving the amyloid precursor protein which then releases the amyloid-beta peptides to form into amyloid plaques. Within the amyloid plaques, neurofibrillary tangles begin to form, all of which are major biomarkers for Alzheimer's disease. Currently, there have been no successful cures discovered for Alzheimer's disease [18]. This highlights the importance of this study's purpose. First, to examine GAL and CLA's roles in adipocyte inflammation using 3T3-L1 and RAW 264.7 immune cells. Second, to determine the role of GAL production on fat inflammation and increased risk of Alzheimer's Disease. Finally, to identify the relationship of adipocyte proliferation or inflammation on amyloid plaque formation and TPA. Results would determine if the treatments can reduce Alzheimer's disease risk by reduction of Aβ production. Next in order to elucidate the biological interactions between certain ligands and proteins specific to obesity and Alzheimer's disease, molecular docking was performed to identify which ligand involved in the obese processess would have the greatest binding potential to the BACE1 enzyme protein involved in Alzheimer's disease processes. It is hypothesized that somatostatin will result in having the lowest binding energy and highest ligand efficiency to BACE1 because somatostatin directly influences fat inflammation in comparison to the indirect relationships of the other ligands. M40 is hypothesized to result in having the highest binding energy and lowest ligand efficiency due to its function as the galanin receptor antagonist and overall prevent fat inflammation.

Materials and Methodology

Identifying Effects of GAL, CLA, and TPA on Inflammation

Materials: The 3T3-L1 was obtained from ATCC CL-173, USA, and was grown in vitro in DMEM (Dulbecco's Modified Eagle Medium) cell culture media from Mediatech Inc. 96 and 6 well cell culture plates were purchased from Corning Inc. DMSO (dimethylsulfoxide) was purchased from Fisher Chemical. Formalin solution was purchased from Sigma-Aldrich. Isopropanol was purchased from Fisher Scientific. Oil Red O Staining Kit was purchased from Sigma Aldrich. MTT powder was purchased from Fisher Scientific. MTT and LDH assay kits were purchased from ThermoScientific. Galanin powder was purchased from Sigma-Aldrich. Aβ40 was purchased from Sigma-Aldrich. Hematoxylin 3 fixative was purchased from Sigma-Aldrich. TPA was purchased from Sigma-Aldrich. Trypsin was purchased from Fisher Scientific. LPS was purchased from Fisher Scientific. M40 was purchased from Fisher Scientific. CLA dietary supplement capsules were purchased from GNC. All cells were incubated at 37°C and 5% CO_2.

Cell Culture: Old media was poured out and 4 mL of trypsin was added to 3T3 cells in order to detach the cells from the flask. After waiting 4 minutes and 5 mL of fresh media was added, the solution was centrifuged at 3,400 rpm. Media was poured out and 10 mL of regular media was added to the untreated 3T3 cells and 10 mL of differentiated media to the differentiated 3T3 cells. The cells were homogenized in the solution and 100 µL was added to each well in the 96 well plate. The MEM media was changed once to twice a week. The cell cultures were placed in an incubator set to 37°C and 5% CO_2.

MTT Assay: MTT assay was used to test for cell metabolic activity

which would determine the number of viable cells present in the cell plates. The control had no treatment and experimental treatments were treated with the varying chemicals studied. Using a 96-well plate, 3T3 cells were removed from the cell culture flasks, suspended and homogenized in DMEM medium. 100 µL of the cell suspension solution (about 10,000 cells/well) was added to each well. After cell plates were incubated for two hours, the cells were treated with 1 µM and 10 µM of Aβ40, GAL, M40, CLA, and 10 µM of LPS. The cells were incubated for 24 or 48 hours. In preparation of the MTT stock solution, 10.0 mg of yellow MTT powder was added. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was dissolved with 2 mL of Phosphate Buffer Solution (PBS) and vortexed to allow complete solubilization. 10.0 µL of the MTT solution was dispensed into each well, and formazan crystals were allowed to form during incubation. Following an incubation period of two hours at 37°C and 5% CO_2, 30 µL of media was removed by pipetting and 70.0 µL of DMSO was added to break down cellular membranes and dissolve the formazan product. After adding DMSO, the plate was placed in the incubator for 10 minutes to aid in the solubilization process. The resulting solution for each well was then analyzed spectrophotometrically at a wavelength of 595.0 nm using the BioTek microplate reader.

LDH Assay: 96-well plates containing 3T3 cells were prepared and treated with their respective chemicals following the same protocol previously mentioned for the MTT assay preparations. 0.6 mL of LDH buffer and substrate mix powder were dissolved in 11.4 mL of distilled pure water. Next, 50 µL of media was removed from each well by careful aspiration, as to not displace any seeded cells, and was placed into a new 96 well plate. 30 µL of the LDH substrate solution was then dispensed into each well containing only treatment media. After the LDH assay, the seeded cells were used for duplicate MTT assays for the respective treatment duration. After a half-hour of incubation at room temperature and out of direct light, the resulting solution was then analyzed spectrophotometrically using the BioTek microplate reader at a wavelength of 495 nm for the actual colorimetric reading and 650 nm to assess background noise in the reading. The measured absorbance values were used relatively to compare released LDH levels from the treated cells in culture.

Oil Red Staining: Old media was removed from the plate and 1,000 µL of 5% formalin was added into each well. After, cell plates were covered for 10 minutes and fixatives were removed, Oil Red O staining solution was made. 3 parts of Oil Red to 2 parts of dH_2O was made by adding 4,500 µL of oil red to a tube and 3,000 µL of dH_2O to the oil red. Undissolved oil red material was filtered out by using a syringe and filter. After 10 minutes, the fixative was removed from plates and 60% isopropanol was added to the plates. After 1,000 µL of working solution was added to each well, cells were stained for 30 minutes 1,000 µL of distilled water was used to clean wells.

Data analysis: The percent survival of the treatments were conducted on Microsoft Excel version 16.26. One-way ANOVA test was used to determine statistical significance for MTT Assays. MTT Assay and LDH Assay data was transferred from the BioTek microplate reader program to Microsoft Excel version 16.26. Cell imaging and cell count was taken using the ImageJ program.

Molecular Docking of M40, TLR4, and Somatostatin on BACE1 Enzyme

Saving BACE1 Protein Data Bank (PDB) File: In order to molecularly dock M40, TLR4, and somatostatin to the BACE1 enzyme, certain softwares were downloaded. These softwares include; MGL Tools, Cygwin, Discovery Studio Visualizer, and Autodock 4.2. After being downloaded, the 2ZHV Beta-secretase 1 enzyme crystal structure at pH of 7.0 PDB file was obtained from the RCSB PDB database. This specific BACE1 enzyme was utilized due to BACE1 being located in the neurons in the brain in which the brain has a similar pH of 7.0. Next, Discovery Studio Visualizer was used to open the BACE1 PDB file. Once the file has been opened, the BACE1 hetatm was removed and then saved as a PDB file once again.

Saving Ligand Protein Data Bank File: In order to retrieve the PDB files for M40, TLR4, and somatostatin, the PubChem database was used. Each of the ligands were saved as 3D Spatial Data Files (SDF) and opened in Discovery Studio Visualizer. The 3D image of the ligand was then saved as a PDB file.

Saving BACE1 as a PDBQT File: Autodock 4.2 was opened in order to open the BACE1 PDB file. The BACE1 PDB file was then edited to add only the polar hydrogens and Kollman charges. Once added, the BACE1 enzyme was selected as the macromolecule and target to be saved as a PDBQT file.

Saving Ligand as a PDBQT File: Similar to saving BACE1 as a PDBQT file, Autodock 4.2 was opened to access the ligand PDB file. Each ligand was opened and had the saved format converted from PDBQT to PDB. The root of the ligand was detected and the number of torsions were set to 3 active torsions. Aromatic carbons were viewed at an aromaticity criterion of 7.5 degrees for each angle and the ligand was then saved as a PDBQT file.

Creating a Grid Parameter File (GPF): A grid box was formed for each ligand with dimensions of 60×60×60 as the default setting and then saved as a GPF format.

Creating a Docking Parameter File (DPF): BACE1 was selected as the macromolecule in which each of the ligands were docked on. A genetic algorithm with default parameters, a docking parameter with its default settings, and LamarkianGA algorithms were applied to BACE1. This file was saved in a DPF file format.

Molecular Docking with Cygwin Software: After opening the Cygwin software, coding commands were inserted. The beginning commands follow as: (cd), (ls), (cd 1), (autogrid4.exe-p a.gpf-l a.glg &), (tail-f a.glg &), (autodock4.exe-p a.dpf-l a.dlg &), (tail-f a.dlg &). After successfully completing these commands, the BACE1 PDB file was copied into a new command. The following commands were inputted after the BACE1 PDB: (grep '^DOCKED' a.dlg | cut-c9->a.pdbqt), (cut-c66 a.pdbqt>a.pdb), (catTarget.pdb a.pdb | grep-v '^END ' | grep-v '^END$'>complex.pdb) respectively.

Molecular Docking Image of Ligand and BACE1 complex: Autodock was opened in order to analyze the a.dlg file. Each protein conformation was observed and the data of binding energy and ligand efficiency was recorded to be displayed through a table. The 3D images of the ligands and BACE1 enzymes were captured and depicted as figures.

The molecular docking method was adapted by Danish Rizvi SM et al.[19].

Results and Discussion

Figure 1: First, an MTT assay was conducted in order to determine cell metabolic activity of both undifferentiated and differentiated cells. In **Figure 1.** CLA had the greatest percent survival indicating that there would be a significant decrease in undifferentiated cells due to the high percentage of CLA cells present. CLA also ameliorated the percent survival of galanin and M40 treatment which reconfirms how CLA had an increased percent survival compared to the other treatments.

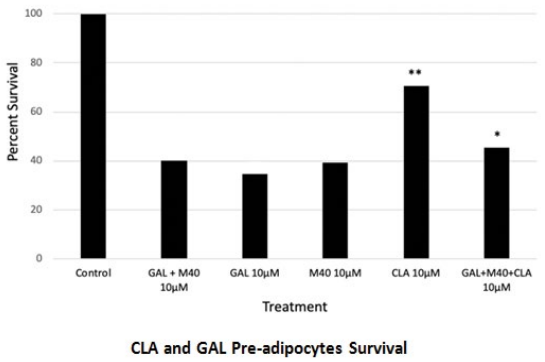

Figure 1 MTT Assay on 3T3-L1 preadipocytes and adipocytes in the presence of CLA (10 μM) and GAL (10 μM). (A) CLA promoted pre-adipocyte metabolic activity when compared to GAL+M40+CLA in 3T3 undifferentiated cells. *=p<0.01, **=p<0.001.

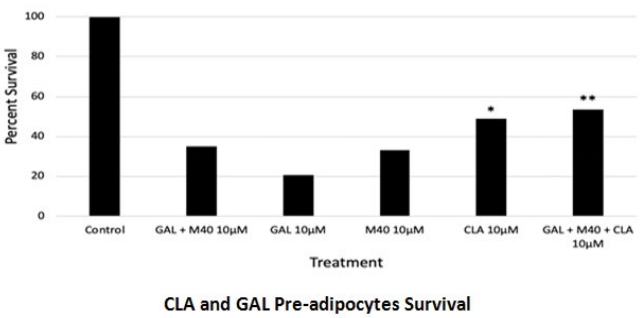

Figure 2 MTT Assay on 3T3-L1 adipocytes in the presence of CLA (10 μM) and GAL (10 μM). GAL+M40+CLA promoted adipocyte metabolic activity when compared to GAL alone in 3T3 undifferentiated cells. *=p<0.001, **=p<0.0001.

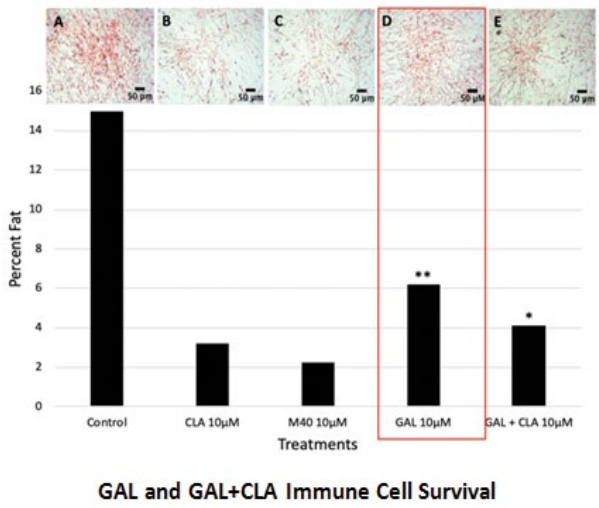

Figure 3 Percent fat of treatments with Oil Red staining on RAW 264.7 immune cells under x10 magnification. *=p<0.01, **=p<0.001.

MTT assay was conducted in order to determine cell metabolic activity of the 3T3 differentiated cells **Figure 2.** with the same treatments in 3T3 differentiated cells, CLA, and GAL+M40+CLA were both significant however in the differentiated cells the combined treatments had a greater percent survival than CLA independently indicating that CLA as a weight-loss dietary supplement is more efficient in decreasing cell count in preadipocytes.

Next, oil red staining was conducted in order to enhance cell imaging of fats and oils in the immune cells. In **Figure 3.** galanin had the greatest percent fat other than the control indicating that an increase in fat due to the positive feedback loop can cause cell proliferation and aggregation of fat in immune cells as seen in image D. Also the combination of galanin and CLA indicated how CLA is able to also provide its function as decreasing percent fat not only in adipocytes cells but also immune cells.

In **Figure 4.** another MTT assay was conducted in order to determine if LPS, had the same response in differentiated cells which would aid in the linking between adipocytes in obese individuals and inflammation of amyloid-beta in Alzheimer's disease patients. The results indicated that LPS had the lowest cell proliferation and that there was no immune cell response since LPS had the lowest percent survival out of the other treatments. Results also confirmed previous studies indicating that galanin was able to increase the percent survival of the differentiated cells due to its function in the body.

Since LPS had a low percent survival indicated in **Figure 4.** an LDH assay to determine cell cytotoxicity of the differentiated cells was conducted to determine if amyloid-beta plaque formation would occur. The results indicated that there was no amyloid-beta aggregation due to the lack of inflammatory response from the differentiated cells in **Figure 5.** This explains why TPA had

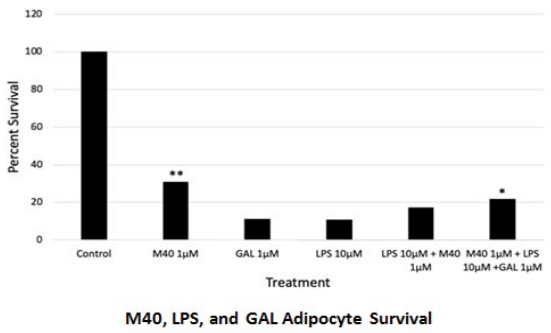

Figure 4 Percent survival of treatments with 1 µM and 10 µM concentrations compared to control in 3T3 differentiated cells in MTT Assay. *=p<0.001, **=p<0.0005.

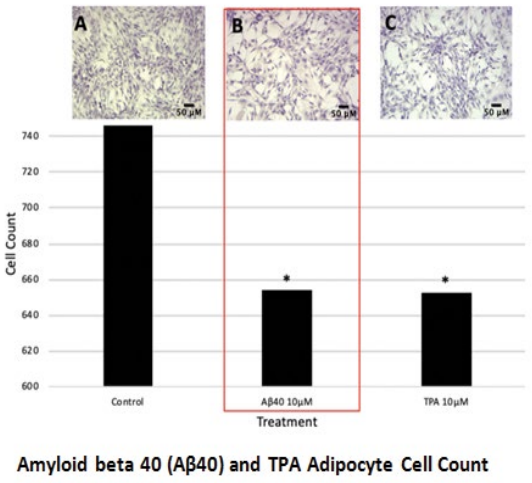

Figure 5 Effect of Aβ40 and TPA under x4 magnification on 3T3 differentiated cells in LDH Assay.*=p<0.05.

relatively the same amount of cells because it was not able to induce the PKC pathway due to the lack of amyloid-beta 40 cells present.

After molecularly docking the three ligands, it was discovered that somatostatin was the best ligand to bind to BACE1. Somatostatin had the lowest binding energy value and highest ligand efficiency indicating that this ligand was able to bind to BACE1 the greatest. This data supports the hypothesis previously mentioned as it demonstrates how due to somatostatin being a direct influence to causing fat inflammation and emulating obesity, the binding to the enzyme responsible for the major biomarker of Alzheimer's disease, was the greatest. This data signified how the binding of the other ligands played less of a significant role in amyloid beta-peptide formation and the overall diagnosis for Alzheimer's disease.

The 3D image results from **Figure 6.** demonstrated how the default dimensions of the grid box presented a possible limitation to the study as it potentially did not accurately depict the binding

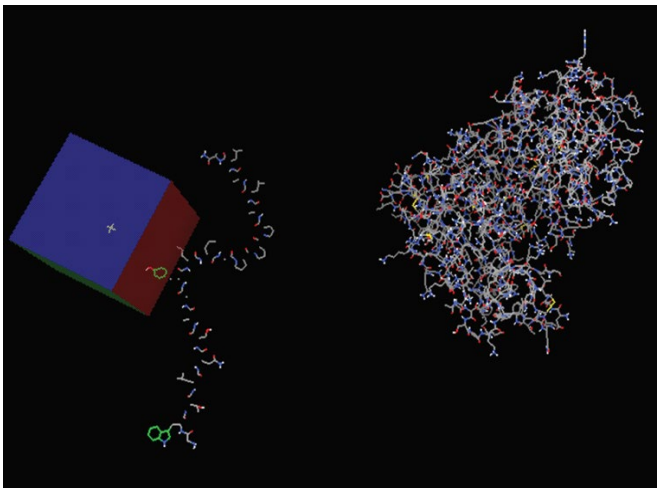

Figure 6 The 3D molecular structures of M40 with a grid box and BACE1 enzyme. The grid box of M40 was not wide enough and potentially caused the inaccurate confirmation of M40.

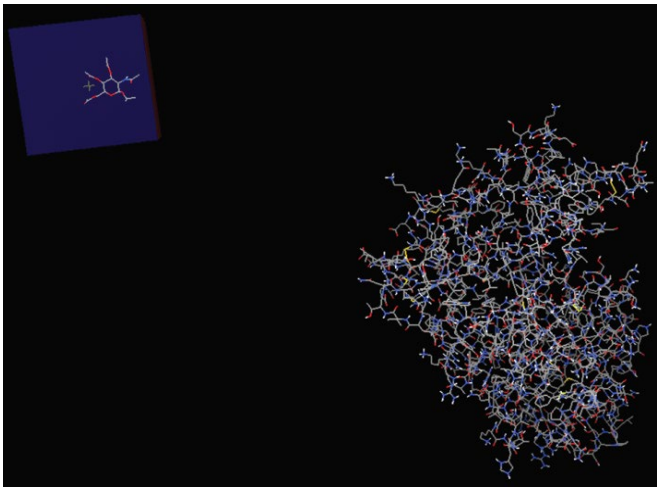

Figure 7 The 3D molecular structures of TLR4 with a grid box and BACE1 enzyme. The grid box of TLR4 was too wide and potentially caused inaccuracy of TLR4 conformations.

energy and ligand efficiency values of the M40 ligand [20]. The structure of the box grid in comparison to the 3D M40 structure was not properly oriented and therefore could have impacted the resulting values. However, although a possible source of error has been highlighted, the results continued to support part of the hypothesis. Due to the drastic difference between binding energy and ligand efficiency values of M40 compared to the most successful binding ligand, somatostatin, it could be presumed that because of M40 inhibiting galanin production, this ligand was not to be the most successful.

The 3D image results from **Figure 7.** exemplified how the grid box defaulting dimensions did not systematically evaluate the binding and ligand efficiency values in a proper manner. In this figure the structure of the box grid was much larger in comparison to the

Table 1: Binding energy and ligand efficiency values for each ligand. Somatostatin was the most optimal ligand to bind BACE1 and had the greatest ligand efficiency compared to M40 and TLR4.

Ligand	Binding Energy (ΔG)	Ligand Efficiency
M40	-5.7226	-0.19
TLR4	-1.6431	-0.24
Somatostatin	-14.9986	-0.13

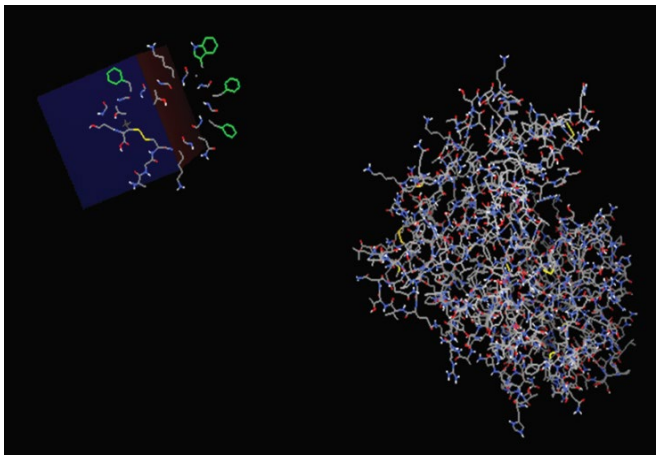

Figure 8 The 3D molecular structures of somatostatin with a grid box and BACE1 enzyme. The grid box of somatostatin had the proper default dimensions for somatostatin conformations compared to the other ligands.

TLR4 molecular structure, indicating that there were multiple insignificant binding positions featured. Similar to **Figure 6.** the data values in **Table 1**. did support part of the hypothesis. TLR4 was not hypothesized to result in having the most optimal binding status with BACE1, which is supported through the high binding energy and low ligand efficiency values reported in **Table 1.**

Figure 8: The 3D image results from **Figure 8.** symbolize what the correct default dimensions of the grid box are supposed to illustrate. The grid box was properly orientated and thus represented an accurate number of somatostatin conformations. The values obtained from this study presented in **Table 1.** supported the hypothesis that somatostatin would be the favorable ligand compared to the other ligands when binding to BACE1. The direct impact of somatostatin's function in relating to obesity as it inhibits the release of insulin and results in fat accumulation, is proposed as an explanation to why this compound was the most successful in binding to the BACE1 enzyme.

Conclusions and Future Investigation

A cure for Alzheimer's disease is still yet to be discovered. However, after this study it is indicated that there are several ways to minimize risk of obtaining the neurodegenerative disease when older and that certain ligands have more significant binding properties with the BACE1 enzyme as signified by the low binding energy and the high ligand efficiency values. In **Figure 1. and Figure 2.** CLA induced increased fat reduction in preadipocyte cells when compared to adipocytes. GAL was also discovered to be more significant in stimulating fat production in preadipocytes than adipocyte cells. **Figure 3.** GAL induced fat inflammation increases the risk of Alzheimer's disease hallmarks. In **Figure 4.** M40 supported an increased cell survival in differentiated adipocytes. **Figure 5.** TPA did not induce plaque formation in adipocyte cells due to lack of inflammatory response. **Table 1.** suggested that somatostatin was the most significant ligand with contributing factors to obesity since it had the greatest potential to bind with BACE1 and the pathways in which it is completed. **Figure 6.** Exemplified that M40 had an indirect impact when binding to BACE1 because of its function as a galanin antagonist and therefore prevented the positive feedback loop from occurring and a lack in fat accumulation. The lack of fat inflammation did not mimic obesity, and therefore resulted in high binding energy and low ligand efficiency. However, M40 was hypothesized to have the least binding with BACE1, and not TLR4, there was an error in this study. TLR4 was not hypothesized to have the least binding potential with BACE1 as it is indirectly involved in inflammation, similar to that observed in obese individuals. A potential limitation to this study that caused an error was the defaulting of the grid box size. The docking space of a ligand is critical in which a space that is not wide enough will result in an inaccurate number of conformations, while a docking space that is too wide will subsequently have non-pertinent binding positions included. It is crucial to have the optimal amount of grid box space in order for the ligand to successfully dock with the BACE1 enzyme [20]. This research can be used further to determine the link between obesity and Alzheimer's disease. Future research would include testing other treatments on differentiated and undifferentiated cells such as ginseng and berberine. Ginseng berry extract reduces glucose levels and increases energy expenditure [21]. Berberine is a compound typically found in Chinese herbal plants that leads to decreased plasma cholesterol and triglyceride levels in hypercholesterolemic patients [22]. Recent studies have indicated how both treatments have been linked to Alzheimer's disease, however, further research needs to be conducted with the cells. Additionally, polychlorinated biphenyls would be tested on amyloid-beta 40. Polychlorinated biphenyls are environmental contaminants that can also affect the human body when ingested, similar to a high-fat diet that induces galanin in production. Due to somatostatin having the lowest binding energy with BACE1 from proper orientation and accuracy of the grid box, further research would be conducted in three-fold for molecular docking. First, other biological agents that have similar functions in producing fat accumulation to emulate obesity would be investigated. These biological agents would assist in identifying if there is a correlation present between factors contributing to obesity as a ligand and the binding to BACE1. Second, associating which compounds have a strong potential for binding with BACE1, would aid in the elucidation of a treatment in which the inhibitors of such compounds can be tested as a preventive method for BACE1

function. Finally, testing other procedures instead of defaulting to grid box measurements would be conducted. Multiple grid arrangements, as a procedure, has been denoted in a recent study to have increased the rate of docking and the binding of ligands to unknown sites of certain proteins [23].

Conflicts of Interests

The other authors have no conflicts of interest to disclose.

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References

1 Alzheimer's Association, Alzheimer's disease facts and figures (2020) Alzheimer's and Dementia 16(3): 391-460.

2 Lis I, Pelczyńska M, Miller-Kasprzak E, Kujawska-Łuczak M, Mądry E, et al. (2020) Association of serum omentin concentration with anthropometric, physiological, and biochemical parameters in obese individuals. Nutrition 79-80: 110866.

3 Cholerton B, Baker LD, Craft S (2013) Insulin, cognition, and dementia. Eur J Pharmacol 719(1-3): 170-179.

4 Leibowitz SF (2007) Overconsumption of dietary fat and alcohol: Mechanisms involving lipids and hypothalamic peptides. Physiol Behav 91(5): 513-521.

5 Sandoval-Alzate HF, Agudelo-Zapata Y, González-Clavijo AM, Natalia EP, Espinel-Pachón CF (2016) Serum galanin levels in young healthy lean and obese non-diabetic men during an oral glucose tolerance test. Sci Rep 6: 31661.

6 Guo L, Shi M, Zhang L, Li G, Zhang L, et al. (2011) Galanin antagonist increases insulin resistance by reducing glucose transporter 4 effect in adipocytes of rats. Gen Comp Endocrinol 173(1): 159-163.

7 Bartfai T, Langel U, Andell S, Land T, Gregersen S, et al. (1993) Galanin-receptor ligand M40 peptide distinguishes between putative galanin-receptor subtypes. Proc Natl Acad Sci 90(23): 11287-11291.

8 Koba K, Yanagita T (2014) Health benefits of conjugated linoleic acid (CLA). Obes Res Clin Pract 8(6): 525-532.

9 Lehnen TE, Ramos da Silva M, Camocho A, Marcadenti A, Lehnen AM (2015) A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. J Int Soc Sports Nut 12(36): 1-11.

10 O'Toole TJ, Sharma S (2020) Physiology Somatostatin. StatPearls.

11 Rorsman P, Huising MO (2018) The somatostatin-secreting pancreatic δ-cell in health and disease. Nat Rev Endocrinol 14(7): 404-414.

12 Vaure C, Liu Y (2014) A comparative review of toll-like receptor 4 expression and functionality in different animal species. Front Immunol 5: 316.

13 Ngkelo A, Koremu M, Mike Y, Ian A, Paul AK (2012) LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Giα dependent PI-3kinase signalling. J Inflamm 9(1): 1.

14 Zhan X, Boryana S, Frank RS (2018) Lipopolysaccharide associates with amyloid plaques, neurons and oligodendrocytes in Alzheimer's disease brain: A review. Front Aging Neurosci 10: 42.

15 NCI drug dictionary. NIH. National Cancer Institute. Patient Education Publications.

16 Zhu G, Chen Y, Zhang X (2017) 12-O-Tetradecanoylphorbol-13-acetate (TPA) is an anti-tumorigenic in liver cancer cells via inhibiting YAP through AMOT. Sci Rep 7: 44940.

17 Chung YW, Kim HK (2011) Dual function of protein kinase C (PKC) in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced manganese superoxide dismutase (MnSOD) expression: activation of CREB and FOXO3a by PKC-alpha phosphorylation and by PKC-mediated inactivation of Akt, respectively. J Biol Chem 286(34): 29681-29690.

18 Norins LC (2019) Predicted economic damage from a quick, simple Alzheimer's disease cure. Med Hypotheses 133: 109398.

19 Danish Rizvi SM, Shakil S, Haneef M (2013) A simple click by click protocol to perform docking: AutoDock 4.2 made easy for non-bioinformatics. EXCLI J 12: 831-857.

20 Feinstein WP, Brylinski M (2015) Calculating an optimal box size for ligand docking and virtual screening against experimental and predicted binding pockets. J Cheminform 7: (18).

21 Li Z, Kim HJ, Park MS, Ji GE (2018) Effects of fermented ginseng root and ginseng berry on obesity and lipid metabolism in mice fed a high-fat diet. J Ginseng Res 42(3): 312-319.

22 Hao M, Yan L, Lixian L, Xiao Y, Ying G, et al. (2017) The design and synthesis of a novel compound of berberine and baicalein that inhibits the efficacy of lipid accumulation in 3T3-L1 adipocytes. Bioorg Med Chem 25(20): 5506-5512.

23 Ban T, Ohue M, Akiyama Y (2018) Multiple grid arrangement improves ligand docking with unknown binding sites: Application to the inverse docking problem. Comput Biol Chem 73: 139-146.