Committee for Aid and Education in Neurochemistry (CAEN)
CATEGORY 1B: Research supplies for use in the applicant's home laboratory,
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REPORT:
Statins as Neuroprotective Agents Against Memory Loss in Alzheimer's-induced dementia in rat

BACKGROUND

Alzheimer's disease (AD) is a prevalent cause of dementia especially in aging populations. It is estimated that around 5.2 million Americans of all ages have AD in 2014 ("2014 Alzheimer's disease facts and figures," 2014). AD is a progressive neurodegenerative brain disorder, with associated onset of dementia and impairment of other cognitive abilities (Ropper & Samuels, 2009). Hypercholesterolemia is one of the proposed mechanisms for development of AD (Martins et al., 2009). Lipid-lowering drugs have been, therefore, investigated for their potential preventive and therapeutic effect in AD (Buxbaum, Geoghagen, & Friedhoff, 2001). Data from epidemiological studies have supported the evidence suggested by experimental data with results showing low incidence of AD among statin users (a cholesterol-lowering agent acting on the 3-hydroxy-3-methylglutrayl-coenzyme A (HMG-CoA) reductase (Evans et al., 2009; Fonseca, Resende, Oliveira, & Pereira, 2010; Isingrini, Desmidt, Belzung, & Camus, 2009). A second proposed theory for AD is the insulin-resistance theory with recent evidence suggesting that AD might be the brain-form of diabetes mellitus (Akter et al., 2011; de la Monte & Wands, 2008).

SPECIFIC AIMS AND HYPOTHESIS

The current proposal investigated the potential protective effect of Ezetimibe against the development of AD-induced dementia in Streptozotocin-rat models based on behavioral, laboratory and pathological outcomes. We compared the proposed neuroprotective effect of Ezetimibe to that of statins. Our hypotheses are Ezetimibe, might provide a neuroprotection in STZ-rat models of dementia. We postulated that Ezetimibe would work through lowering both serum and CSF cholesterol level, along with improving insulin utilization in brain.

WORK PLAN

Animals: Fifty Westar female rats weighing 250gm purchased from a local vendor. The age of the rats will be between 12-20 months to represent 40-60 age period in humans during which preventive drugs will be used (To, Friendly, & Sengupta, 2013). Animals were randomly allocated into seven groups (n=8) as follow: Group A: Control: DW; B: CMC control: CMC; C: STZ control: STZ; D: ACSF control: ACSF; E: Treated1: Simvastatin +STZ; F: Treated2: Ezetimibe + STZ; G: Treated3: Simvastatin + Ezetimibe + STZ. All animals housed in a room with a temperature around 24 °C under a 12-h light/12-h dark cycle. Rats maintained in groups no greater than four per cage. Menoufia Medical School IRB ethical committee approved all procedures.

Preparing Rat Model of Alzheimer's disease: We worked on a non-transgenic rat model, with STZ-rat as the model of choice for this project. After its injection intra-cerebroventricularly, STZ can

provide many features found in SAD: cognitive impairment, Amyloid beta protein deposition, oxidative stress and a brain form of insulin resistance (Salkovic-Petrisic, Knezovic, Hoyer, & Riederer, 2013). Using stereotaxic apparatus, bilateral ICV injection of STZ (after anesthesia with intraperitoneal Ketamine to provide sufficient time for the operation) at a dose of 3 mg/kg on day 1 and 3 will be done to Group E, F, and C with a 0.4 mm external diameter hypodermic needle covered with a polypropylene tube except for 3 mm of the tip region and attached to a 10 µl Hamilton microliter syringe. Bilateral injection was chosen rather than unilateral injection to ensure the distribution of the dose over both sides instead of its concentration on one side. Rat brain atlas used to identify the exact site of injection and appropriate post-operative care will follow this procedure to ensure the best outcomes.

Dosage and duration: Treated groups received a daily dose (10 mg/kg P.O.) of Ezetimibe (Ezetrol®) (using mouth gavage) for 15 days after STZ first injection to test its preventive effects (Dalla et al., 2009). Group B received 0.5% w/v Carboxy Methyl Cellulose (CMC, 1 0 ml/kg P.O.) daily for 15 days. Simvastatin (Zocor®) was given in a dose of 10 mg/kg/day P.O. using an oral tube for 15 days after 1st ICV-STZ injection (Dalla, Singh, Singh Jaggi, & Singh, 2010).

Behavioral assessment: Cognitive and memory tests were carried out at the start of the experiment to ensure that groups are comparable. After the 15th day, groups were subjected to **Morris Water Maze test (MWM)** to assess spatial learning and memory. MWM was conducted as previously explained (Bromley-Brits, Deng, & Song, 2011). To test the short-term memory in rats, we used the **Novel Object Recognition Test**.

Laboratory Investigations: Serum lipid profile (Cholesterol, TG, LDL, HDL) measured and correlated to CSF cholesterol.

Pathological, Biochemical and Immunohistochemical analysis: After 21 days of first STZ injection, rats were sacrificed by perfusion through heart with ice-cold normal saline after treatment under ether anesthesia and brains will be removed to prepare: 1.Congo-red stained sections of hippocampus will be used to evaluate Amyloid beta plaques (the pathological hallmark of AD), 2.Silver stained sections of hippocampus to evaluate the neuroprotective effect of Ezetimibe, 3.Sections from hippocampus stained with antibodies against insulin receptors (Anti-IR) as an indicator of brain insulin signaling pathway; and thus brain insulin resistance, 4.After being homogenized and centrifuged, the supernatant was subjected to biochemical assays to assess the oxidative stress through measurement of reduced glutathione levels.

Statistical Analysis: The number of animals to be used was based on power analysis. We did all our best to keep the numbers of animals per treatment at a minimum but sufficient to ensure statistical power. We used 125% to compensate for accidental animal loss.

Results: Simvastatin treated group showed better performance in MWM and novel object recognition tests. Both drugs showed potential protective effects through reducing amyloid plaques and tau proteins. IGF-1 receptors showed up regulation in hippocampus and frontal cortex with both drugs.

Conclusion: Both Simvastatin and Ezetimibe could prevent AD, evidenced by reducing amyloid and tau proteins. A key mechanism for this is through up regulation of IGF-1 receptors in the hippocampus and cerebral cortex that needs further evaluation.

Future plan: we plan to extend our results understand the underlying biological mechanisms. We plan to use 2VO animal model to produce cerebral hypoperfusion/ischaemia as a model of neurodegeneration especially AD. We will examine the possibility of using L-Carnitine as a neuroprotective agent VS statins and examine the results behaviorally, biochemically and histopathologically.

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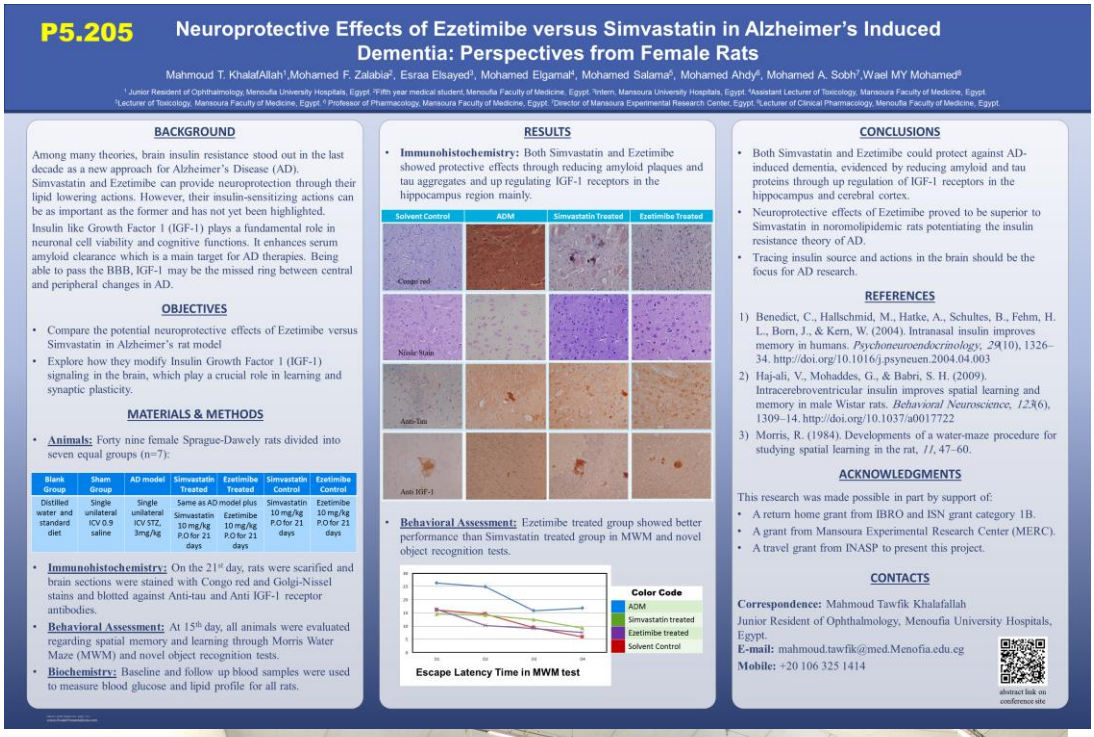
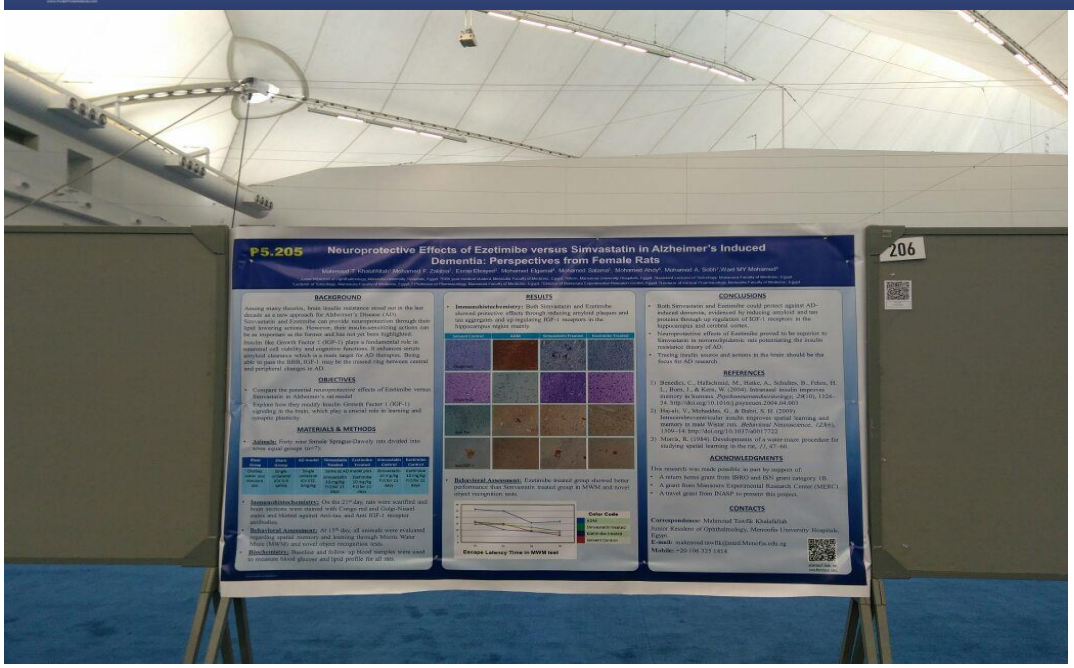
Conferences/ Presentations

Our results were presented in 2016 annual meeting of AAN, Vancouver, Canada 15-21 April. It was presented as invited talk & received Young Scientist Travel Award.





Publications

Neuroprotective effects of Ezetimibe versus Simvastatin in Alzheimer's induced Dementia: perspective from female rats (Manuscript in preparation).

Budget

The fund was spent on the obtaining the chemicals and glassware's, behavioral apparatus, micro pipettes, commercial kits for biochemical assays, rats, rat food, high fat diet etc..