Mechanisms of Lithium Chloride Neuroprotection in Alzheimer's Disease

Department of Anesthesiology and Critical Care, University of Pennsylvania

Lauren St. Louis* (CAS'24), Roland Zhang* (CAS'24), Grace Liang, Robert Vera, Huafeng Wei

Problem

Alzheimer's Disease (AD) is a major health problem affecting nearly 6 million Americans age 65 and older. Alzheimer's is the most common neurodegenerative disease, with 95% of patients having sporadic AD (SAD). Symptoms of AD severely deteriorate an individual's quality of life. Though our understanding of AD has advanced, no disease-modifying therapy is currently available.

Background

Calcium dysregulation in AD

- N-methyl-D-aspartate (NMDA) glutamate receptor (NMDARs) over activation leading to excessive Ca^{2+} influx and associated excitotoxicity has been considered a major upstream AD pathology. ApoE4, a widely considered primary SAD risk factor is capable of aggravating NMDARs over activations. Soluble amyloid 42 oligomers (Aβo) are also considered a risk factor of SAD, and can affect NMDARs activity and pathologically worsen Ca^{2+} dysregulation in AD.
- Calcium dysregulation in AD is increasingly considered a primary upper stream pathologly, leading to multiple down stream pathologies, such as mitochondrial Ca^{2+} overloading and dysfunction, generation of pathological reactive oxygen species (ROS) and reduction of ATP production, neurodegeneration and impaired neurogenesis
- Lithium's potential to inhibit mitochondrial dysfunction, neurodegeneration/impaired neurogenesis, synaptogenesis, and cognitive dysfunction in AD makes it a promising treatment for AD (Fig. 1).

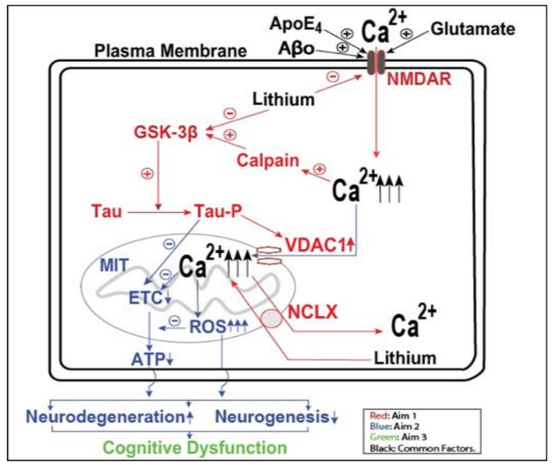

Figure 1. Mechanisms of lithium neuroprotection in Alzheimer's Disease (AD)

Hypothesis

The objective of our studies is to study mechanisms of lithium neuroprotection in AD. We hypothesized that lithium provides neuroprotection by amelioration of Ca^{2+} dysregulation in AD and associated downstream mitochondria dysfunction and impairment of neurogenesis.

Methods

Cell Culture

iPSC's

Induced Pluripotent Stem Cells (iPSC's) are a more modern approach of cell culture with a unique set of advantages. Taken from the skin fibroblasts of individuals, the cells are reprogrammed into stem cells with the ability to differentiate into multiple different types of cells.Ren Our research involves iPSC's obtained from a SAD patient with parent AopE4/E4 gene and the gene edited isogenic ApoE3/E3. This will allow us to study the role of ApoE4 on calcium dysregulation in AD and mitochondria dysfunction and impairment of neurogenesis. The iPSCs were cultured according to the protocol we have described in detail before (Wang et al., Anesthesiology, 2020).

Human brain neuroblastoma cells

A Human brain neuroblastoma cell line (SH-SY5Y) was maintained with standard protocol we described before (Gao et al, Current Alzheimer Research, 2020). This cell line is a common model for neurodegenerative disorders because the cells can be easily differentiated into to neurons by the addition of specific compounds. The SH-SY5Y cells we utilized featured AD knock-ed in presenilin-1 point mutation (M146V) and corresponding WT PS1 control cells.

ReNcell CX cells

ReNcell CX cells, an immortalized human NPC line, were obtained from human fetal cortex (Millipore, USA) and cultured following the manufacturer's protocol as we described previously (Qiao et al., Anesthesiology, 2017). ReNcell CX cells can differentiate into neurons.

Aquaporin Cytosolic Calcium Measurements

Four groups of cells were cultured with standard protocol. The ApoE3 line and the ApoE4 line each had a group of control cells with 0 treatments and a group of lithium chloride pretreated cells at 1mM, treated daily during medium exchange for a week prior. Cells were plated on circular glass slides designed for our Aquaporin machine used to measure cytosolic Ca^{2+}. Cells reached approximately 80 % confluence prior to Ca^{2+} measurements.

Cells were transfected using lipofectamine 3000 for an hour prior to running experiment. Cytosolic Ca^{2+} concentrations measurements were recorded. A baseline for approximately one minute was measured, before glutamate was added allowing for an influx of Ca^{2+} into the cytosol (as seen in Fig. 2 A). Cytosol Ca^{2+} concentrations were measured for the duration until up to 1000 seconds.

Mitochondria Oxygen Consumption Measurements

The effects of the SAD risk factor, glutamate, on mitochondrial oxygen consumption rate (OCR) were determined. Mitochondrial OCR was recorded via a Seahorse Mito Stress Test setup. Cells were seeded at 100,00 per well.

Results

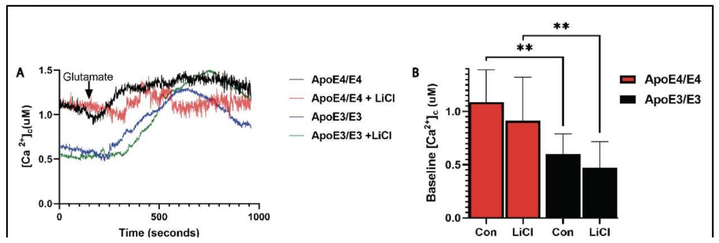

Figure 2. Induced pluripotent stem Cells (iPSCs) from a sporadic Alzheimer disease (SAD) patient with parent ApoE4/E4 had significantly increased baseline level of cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$), compared to its isogenic ApoE3/E3 iPSCs. Cells were pretreated with lithium chloride (1 mM) for 24-72 hours and challenged with glutamate (15 mM) (**A**). Control (Con) received no treatments. **P<0.01 (**B**), N=4-7 from 4 separate experiments. Two-way ANOVA by Prism 9.

Obtained results were collected from Aquaporin data. Graph 2A. demonstrates the average cytosolic Ca^{2+} concentration throughout the duration of the experiments over all the trials run. Graph 2B. includes the statistically significant (**=P<.01) data that SAD patients modeled by iPSC's have a higher baseline concentration than their control counterparts.

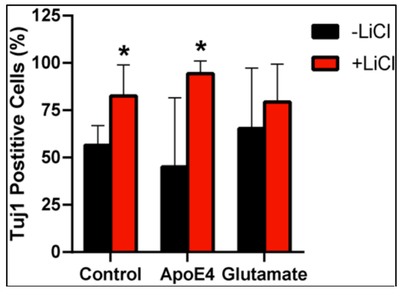

Figure 3. Lithium pretreatment significantly ameliorated ApoE4/glutamate-mediated impairment of neurogenesis. The presence of lithium treatment significantly increased average percentage of Tuj1 positive cells in both control and cells treated with ApoE4 or glutamate in ReNcell CX cells. Also, lithium pretreatment significantly inhibiteded impairment of neurogenesis. * P<0.05 compared to control without lithium pretreatment. N=6, student t test by Prism 7

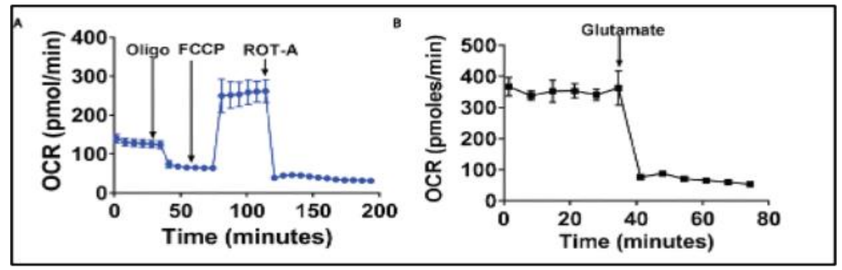

Figure 4. Glutamate significantly impaired mitochondria oxygen consumption rate (OCR) in human neuroblastoma cells (SH-SY5Y).

Conclusions

Based on collected data in Figure 2, cells carrying the APoE4 gene or cells from AD patient do show higher baseline cytosolic Ca^{2+} concentration, which supports the hypothesis of Ca^{2+} dysregulation in AD. Lithium inhibited ApoE4/glutamate mediated impairment of neurogenesis as partial mechanisms as a therapeutic drug in AD, and need further studies.

Future Research

Repeated trials at different concentrations can strengthen our methodology and help support our hypothesis

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* PURM 2022 Recipients

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