Mechanisms of Lithium Chloride Neuroprotection in Alzheimer’s Disease

Background
Calcium dysregulation in AD
- N-methyl-D-aspartate (NMDA) glutamate receptor (NMDARs) over activation leading to excessive Ca2+ 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 Ca2+ dysregulation in AD.
- Calcium dysregulation in AD is increasingly considered a primary upper stream pathology, leading to multiple down stream pathologies, such as mitochondrial Ca2+ 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).

Methods

Hypothesis
The objective of our studies is to study mechanisms of lithium neuroprotection in AD. We hypothesized that lithium provides neuroprotection by amelioration of Ca2+ dysregulation in AD and associated downstream mitochondrial dysfunction and impairment of neurogenesis.

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.

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 neurons by the addition of specific compounds. The SH-SY5Y cells we utilized featured AD knock-in presenilin 1 point mutation (M146V) and corresponding WT PS1 control cells.

ReNcll CX cells
ReNcll 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). ReNcll CX cells can differentiate into neurons.

Aquaporin Cytosolic Calcium Measurements
Four groups of cells were cultured with standard protocol. The ApoE4 line and the ApoE4 line each had a group of control cells with 6 treatments and a group of lithium chloride pretreated cells at 1mM, treated daily during medium exchange for a week. Cells were plated on circular glass slides designed for our Aquaporin machine used to measure cytosolic Ca2+. Cells reached approximately 80% confluence prior to Ca2+ measurements. Cells were transfected using lipofectamine 3000 for an hour prior to running experiment. Cytosolic Ca2+ concentrations measurements were recorded. A baseline for approximately one minute was measured, before glutamate was added allowing for an influx of Ca2+ into the cytosol (as seen in Fig. 2). Cytosol Ca2+ 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 Mini Stress Test setup. Cells were seeded at 100,000 per well.

Results
Obtained results were collected from Aquaporin data. Graph 2A, demonstrates the average cytosolic Ca2+ concentration throughout the duration of the experiments over all the trials run.

Figure 3. Lithium pretreatment significantly ameliorated ApoE4/glutamate-mediated impairment of neurogenesis. The presence of lithium treatment significantly increased average percentage of Tuji+ positive cells in both control and cells treated with ApoE4 or glutamate in ReNcll CX cells. Also, lithium pretreatment significantly inhibited 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 Ca2+ concentration, which supports the hypothesis of Ca2+ dysregulation in AD. Lithium inhibited ApoE4/glutamate mediated impairment of neurogenesis as partial mechanism 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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References
Wei H, Jia S, Qiao H, Wei H. Intramembrane Amyloidogenic Ameliorated Impaired Neurogenesis and Synaptogenesis in Induced Pluripotent Stem Cells Derived From Patients with Alzheimer’s Disease. Anesthesiology. 2020; 133(2): 562-70.

Graph 2. Induced pluripotent stem cells (iPSCs) from a sporadic Alzheimer disease (SAD) patient with parent ApoE4/E4 had significantly increased baseline level of cytosolic Ca2+ concentration (in mM), 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.