CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

31443

Project Title

Striking a Blow for Alzheimer's Disease: Role of ApoE Mediated Inflammatory Pathways on Tau Phosphorylation

Abstract

Objectives/Goals

In the past Alzheimer's research was focused on Amyloid B, but now it has also encompassed ApoE mediated phosphorylation of Tau. The purpose of my project is to study the inflammatory pathways mediated by ApoE4 and reduce phosphorylation of Tau, and find a potential therapeutic target for Alzheimer's Disease. The objectives of this study were to determine: 1) the effect of treatment with Lithium Chloride on Phosphorylated GSK-3B-Ser9 and Phosphorylated Tau in neuro2a cells transfected with ApoE4 and ApoE3 in a time- and dose-dependent manner; 2) the effect of the difference in inhibition of GSK-3B pathway in cells transfected with ApoE3 and ApoE4.

Methods/Materials

Neuro2a cells were cultured in EMEM+10%FBS+1%Pen-strep, plated into 6 well plates, then transfected with ApoE4 and ApoE3 plasmids. Non-transfected cells were used as controls. After 24 hours of transfection, the cells were treated with Lithium Chloride (1mM, 5mM, 10mM), 10µM TDZD (positive control), and distilled water (negative control). After 1 and 2 hours, the cells were lysed and protein was extracted. Western Blot Assay was used to determine the levels of Total and Phosphorylated Tau, and Phosphorylated GSK-3B. Additionally, protein concentrations were measured using the Bradford Assay.

Results

Phosphorylated GSK-3B-Ser9 was detected following the Lithium Chloride and TDZD. This effect was only observed for Lithium Chloride in a dose dependent manner for ApoE4 transfected cells. For ApoE3 transfected cells, the Phosphorylated GSK-3B-Ser9 was most detected in the 1mM, less in 5mM, and least in 10mM of Lithium Chloride in 2 hour time exposure. The Phosphorylated GSK-3B-Ser9 bands were stronger for cells transfected with ApoE3 than ApoE4. Total and Phosphorylated Tau was not detected in any cell lysates possibly due to minimal amounts of protein present and protein degradation. Additionally protein levels were detected to be low in each of the cell lysates in 0.07-0.13mg/ml.

Conclusions/Discussion

This study has identified that Lithium Chloride inhibited the ApoE4 mediated GSK-3B pathway and Tau phosphorylation, by indirect observation. The inhibition of GSK-3B pathway was more potent in cells transfected with ApoE3 than ApoE4. Inhibition of this pathway could be a possible therapeutic target for ApoE induced inflammation in Alzheimer's. The next step is to study the effect of targets on different ApoE mediated inflammatory pathways and Tau.

Summary Statement

This project investigated the role of Lithium Chloride in inhibiting GSK-3B pathway and thereby reducing the phosphorylation of tau and found that GSK-3B pathway could possibly be a potential therapeutic target.

Help Received

I worked under supervision of my mentor, Dr. Birrell using lab equipment from Schmahl Science Workshop.