

Cognitive Neuroscience 2018: Brain insulin resistance: Targeting PI3K/AKT/GSK3- β pathway in intracerebroventricular-streptozocin induced rat model of Alzheimer's disease - Ansab Akhtar - Panjab University

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Alzheimer's disease featuring dementia, cognitive deficits and behavioral alterations is one of the most common prevalent neurodegenerative diseases affecting majorly elderly people termed as sporadic AD. Universal prevalence of AD is sharply increasing, expected to affect almost 115 million people by 2050. Down-regulation of insulin signaling pathway of PI3K/AKT plays a significant role in the pathophysiology of AD. Intracerebroventricular streptozocin is used for the model of sporadic Alzheimer's disease has been established. Animals are divided into various groups comprising normal control, sham control, diseased and drug treated groups. Protocol lasts for up to 21 days sacrificing animals on 22nd day followed by the isolation of serum and dissection of cortex and hippocampus, preserving the same for further analysis. Behavioral studies show the biochemical estimations and molecular techniques are done for evaluating several parameters of control, diseased and treated groups of animals. observable studies like Morris water maze, novel object recognition and actophotometer are performed for cognition, memory and locomotor activity. Biochemical estimations for antioxidant activity are performed as glutathione reductase assay, catalase assay, glutathione S-transferase assay, lipid peroxidation assay, superoxide dismutase assay and protein carbonylation assay. Protein concentrations are determined by biuret method. Cholinergic activity is determined by acetylcholinesterase assay. Inflammatory cytokines like TNF- α , IL-6 is determined by ELISA method. Mitochondrial dysfunction is evaluated estimating mitochondrial enzyme complex 1, 2, 3 and 4. Histopathology is done. Molecular techniques like western blotting for Akt protein and RT-PCR for PI3-K, AKT, p-AKT, NF- κ B and GSK 3- β is performed for gene expression analysis.

Introduction:

Alzheimer's disease (AD) is neuro-pathologically characterized by the damage of neurons and synapses as well as the formation of senile plaques from amyloids and neurofibrillary tangles (NFTs) composed of hyper-phosphorylated Tau, which is the most prevalent cause of age-related dementia. Soluble amyloid species including oligomers may alter hippocampal synaptic plasticity and impair memory. The hyper-phosphorylated Tau is the principal component of helical filaments in intracellular NFTs. Amyloid-beta (A β) deposition occurs prior to the accumulation of the hyper-phosphorylated Tau in the AD brain. Soluble A β oligomers are isolated from the brain extract of patients with Alzheimer's disease (AD) accelerate Tau hyper-phosphorylation. In spite of the observations representing the

pathophysiological roles of soluble A β species in Alzheimer's disease (AD) pathogenesis. How A β induces the hyper-phosphorylation of Tau in AD brains remains an unanswered question. Although research efforts have provided insights into the biology of Alzheimer's disease (AD) the underlying routes mediating the progressive decline in cognitive function are still poorly understood. The precise molecular events that control the death of neuronal cells along with A β are unclear.

The characterization of full-length Tau has shown that the Tau protein can undergo many transitional conformations, and each of the conformations may represent a potentially toxic object. Accumulation of the misfolded Tau intermediates in the human brain causes tauopathies the most common form of Alzheimer's disease (AD). The PI3K/AKT/GSK-3 β pathway appears to be crucial for Alzheimer's disease (AD) because it promotes protein hyper-phosphorylation in Tau. In particular, glycogen synthase kinase-3 β (GSK-3 β) plays a key role in the neuronal response to stress by phosphorylating and compromising the transcriptional activity of the cAMP response element binding which regulates the transcription of the brain-derived neurotrophic factor (BDNF) and other neuropeptides that are important in the regulation of long-term memory and in the maintenance of synaptic plasticity. Thereby contributing to the pathology of neuronal degeneration. Furthermore GSK-3 β is probably the most documented kinase implicated in the abnormal hyper-phosphorylation of Tau protein.

Several potential preventive factors against Alzheimer's disease (AD) have been suggested by epidemiological research, including modifiable lifestyle factors such as diet. Researchers have demonstrated that dietary choices can play an important role in the neuroprotection of Alzheimer's disease (AD). Because many factors in life influence brain function, several interventions might be promising in the prevention of brain dysfunction in Alzheimer's disease (AD). The main objective of this article is to review the studies linking potential protective factors to pathogenesis of Alzheimer's disease (AD) focusing particularly on the roles of the PI3K/AKT/GSK-3 β pathway.

Results:

Initially we explore the effects of different concentrations of glutamate on the proliferation of SH-SY5Y cells and PC12 cells using MTT test. The result indicated that the inhibition rate of 7.5 mM glutamate-treated PC12 cells reached $48.2 \pm 1.5\%$ (< 0.001) and 15 mM glutamate-treated SH-SY5Y cells led to $39.12 \pm 2.1\%$ decreases the cell viability. Thus the glutamate at

7.5 mM and 10 mM was selected as model concentration. To investigate the impact of biatractylolide on cell damage induced by glutamate, we evaluated cell viability using the MTT approach. The cells were treated with various concentrations of biatractylolide for 30 min before glutamate treatment for 24 h. The result showed that the biatractylolide led to a dose-dependent increase on PC12 and SH-SY5Y cells proliferation.

Acridine Orange:

We have observed the morphological characteristic of apoptotic cells using the AO/EB staining test. And the morphological changes including chromatic agglutination, karyopyknosis and nuclear fragmentation could be observed in glutamate model group by fluorescence microscopy. Compared with the model group. The addition of different concentrations of biatractylolide can markedly inhibit cell damage and improve cell morphology in a concentration-dependent manner.

Determination of LDH Activity:

We next determined the effects of biatractylolide on LDH activity in PC12 cells and SH-SY5Y cells. We found that model group has an increase of LDH release being $280.3 \pm 0.3\%$ and $318.22 \pm 0.1\%$ as compared to control cells. However preincubation with biatractylolide at the concentration of 15 M and 20 M significantly obstructed LDH release in the PC12 cells. Which was decreased from $180.5 \pm 0.9\%$ to about $195.3 \pm 2.1\%$ as compared to model group. In addition to this biatractylolide (10 M, 15 M, and 20 M) distinctly decreased LDH activity of SH-SY5Y cells, being $174.2 \pm 0.4\%$ (\circ), $243.6 \pm 0.1\%$ (\circ), and $272.1 \pm 2.9\%$ as compared to model group.