Exploring the Link between Insulin Resistance and Neurodegenerative Disorders and its Possible Mechanism(s)

SYNOPSIS

Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

by

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BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

2017

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

CERTIFICATE

This is to certify that the thesis entitled **Exploring the Link between Insulin Resistance and Neurodegenerative Disorders and its Possible Mechanism(s)** and submitted by **Sorabh Sharma** ID No **2013PHXF0007P** for award of Ph.D. degree of the Institute embodies original work done by him under my supervision.

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1. Introduction

Type 2 diabetes mellitus (T2DM) and other metabolic disorders, such as obesity and atherosclerosis, are well-recognized epidemiological and clinical risk factors for cardiovascular complications. Recently, studies have also suggested an increased risk for developing dementia of Alzheimer's type in T2DM patients. The risk of T2DM for other neurodegenerative conditions, including Parkinson's disease (PD), has been inconsistently reported. However, the exact role and mechanisms by which T2DM exacerbate neurodegenerative diseases are still elusive. In the recent past, numerous studies have demonstrated that insulin resistance increases the risk of developing neurodegenerative diseases including Alzheimer's disease (AD) and PD. During insulin resistance, the insulin receptor signaling may be impaired primarily at the level of insulin receptor substrate (IRS)-1/2. Furthermore, the loss of function of insulin receptor signalling may lead to stimulate protein aggregation. Thus, it is predicted that reduced insulin receptor expression in the brain and dysregulated activity of the insulin receptor signaling pathway may lead to increased amyloid-β (Aβ) accumulation. Similar is the case for PD, where impaired insulin receptor signalling has been reported to be associated with α-synuclein alterations.

Activated insulin receptor phosphorylates the intracellular substrate, IRS-1/2, which associate downstream with p85 subunit, growth factor receptor binding protein 2, and the Syp protein tyrosine phosphatase leading to activate the phosphoinositide 3-kinase (PI3kinase)-Akt pathway to affect many downstream cellular functions. However, during insulin resistance condition, this signaling pathway gets impaired leading to aberrant activation of serine/threonine kinase, glycogen synthase kinsae-3β (GSK-3β), which is the major kinase involved in tau hyperphosphoryation. Numerous studies have reported up-regulation in expression as well as increased activity of GSK-3β in the respective brain regions of AD and PD patients. Moreover, actions of GSK-3β affecting nuclear functions, specifically gene expression, include indirect effects via its regulation of many transcription factors, and more direct effects resulting from the regulation of recently identified epigenetic mechanisms. The term 'epigenetics' is widely used to refer to changes in gene expression that are not caused by changes in the DNA sequence and that involve classical epigenetic mechanisms, namely, DNA methylation and histone modifications. Among these, histone acetylation/deacetylation has been widely studied and is carried out by opposing activities of two enzymes histone acetyltransferases (HATs) and histone deacetylases (HDACs). Recent reports indicate that HDAC-2 bind with IRS-1 in liver cells of the db/db mouse and results in decreased

acetylation and reduced insulin receptor-mediated tyrosine phosphorylation of IRS-1. In addition, GSK-3β has also been reported to interact and phosphorylate several HDACs. Conversely, several HDAC inhibitors have been reported to increase the inhibitory serine-phosphorylation of GSK-3β. Moreover, it has been demonstrated that diabetes can induce changes in HDAC levels in the brain, which, in turn, trigger a detrimental molecular cascade that leads to compromised neuronal structure integrity and synaptic plasticity. Thus, there is a huge possibility that epigenetic mechanisms involving HDACs and GSK3β could be involved in insulin resistance induced neurodegeneration.

2. Objectives

1. To standardize in-vivo animal model of insulin resistance and insulin resistance induced neurodegeneration.

2. To examine the association between insulin resistance and neurodegenerative diseases including, AD and PD.

3. To explore the epigenetic mechanism(s), primarily histone acetylation/deacetylation, involved in insulin resistance mediated AD and PD pathogenesis.

4. To evaluate the effect of GSK-3β and HDAC inhibitors alone/or in combination in AD and PD associated with insulin resistance.

3. Experimental Design

1. Development of insulin resistance by high fat diet feeding.

2. Development and standardization of animal models for insulin resistance induced AD and PD pathology.

3. Screening of GSK-3β inhibitors and HDAC inhibitors to investigate the plausible mechanism(s) of insulin resistance induced AD and PD pathology.

4. Results and Discussion

4.1. Time course for development of insulin resistance and insulin resistance induced cognitive deficits and neurodegeneration

For induction of insulin resistance, the Swiss albino mice were fed with high fat diet (HFD). The level of serum glucose, insulin, triglycerides, total cholesterol and LDL-cholesterol were measured after every 2 weeks interval. We observed that HFD feeding results in significantly increased body weight, serum glucose and insulin level as early as 4 week after diet feeding. These changes in circulating glucose and insulin levels were further reflected in a significant increase in the HOMA-IR index, a quantitative measure of insulin resistance, which appeared

at 4 weeks after diet feeding. Interestingly, increased level of serum triglyceride, total cholesterol and LDL-cholesterol were observed in HFD fed mice as compared to NPD fed mice. Although we observed hyperglycemia and hyperinsulinemia at 4 weeks after diet feeding, however we continued to feed the mice with HFD until we got persistant increase in serum insulin and glucose levels, i.e upto 8 weeks.

Further, we determine the relationship between insulin resistance and neurobehavioral alterations. In order to achieve this, the animals fed with HFD were subjected to behavioral tasks to evaluate their cognitive functions and neuronal health. Initially, to examine the effect of HFD feeding on locomotor activity of animals we performed spontaneous locomotor activity test and found no significant effect of HFD feeding on locomotor activity at any time point. Then, we used MWM task to evaluate the cognitive performance of HFD and NPD fed mice. We found that the mice fed with HFD performed poorly in MWM task. These mice showed higher escape latency than NPD fed mice as early as 4 weeks after diet feeding and their performance worsen over 6 to 8 weeks after diet feeding. Further, we performed the histopathological study to determine the effect of HFD feeding on neuronal health. Although neuronal damage in some of the structures of hippocampus was observed after 4 weeks of diet feeding, however, significant neuronal damage in both DG and CA1 regions of hippocampus was evident after 6 to 8 week of HFD feeding. These structural changes found in the hippocampus may arise from peripheral insulin resistance as well as alterations in brain insulin signaling. Thus, in our following studies to explore the molecular mechanism(s) involved in insulin resistance induced neurodegeneration, we used 8 weeks of HFD diet feeding as an animal model.

4.2. Plausible mechanism(s) of neuroprotective action of GSK3β inhibitor and HDAC inhibitors in insulin resistance induced AD pathology

The HFD fed mice treated with selective GSK-3β inhibitors (Indirubin-3'-monoxime and AR-A014418) and pan HDAC inhibitors, Suberoylanilide hydroxamic acid (SAHA) and sodium butyrate, attenuated the insulin resistance condition along with significant improvement of cognitive functions as assessed by battery of behavioral tests including, morris water maze, passive avoidance task and novel object recognition task. Moreover, the mice treated with GSK3β inhibitors and HDAC inhibitors showed significant reduction in oxidative stress (MDA, nitrite) and pro-inflammatory marker (TNF-α) and ameliorate antioxidant enzyme levels (reduced glutathione) as compared to alone HFD fed mice. In addition, these compounds attenuated the elevated levels of $A\beta_{(1-42)}$ and tau hyper-

phosphorylation (p-tau) (markers of AD pathology) in HFD fed animals. Further, treatment with GSK3β and HDAC inhibitors results in significant amelioration of cAMP response element binding protein (CREB) and brain derived neurotrophic factor (BDNF) levels in HFD fed animals. Neuroprotective effects of these compounds were also confirmed by histopathological analysis, where it was observed that treatment with GSK-3β inhibitors and HDAC inhibitors results in reduction of percentage of damaged neurons in dentate gyrus and CA1 regions of hippocampus when compared with alone HFD fed animals. These results clearly indicate an alteration in GSK-3β activity and epigenetic mechanisms regulating gene expression in brain may play a key role in the etiology and progression of neurodegenerative diseases.

In fact, from a therapeutic point of view, HDAC inhibitors have emerged as promising compounds that might help manage neurodegenerative processes. However, the toxicity associated with HDAC inhibitors has limited the clinical data available. In order to reduce side effects while maintaining benefits, one strategy would be to develop isoform-selective HDAC inhibitors. Moreover, since the side effects reported for HDAC inhibitors in humans are dose dependent, the use of lower doses of pan-HDAC inhibitors to reduce their toxicity could represent another solution. In this dissertation work, we tested both these approaches.

In the first approach, to evaluate the therapeutic potential of low dose combination of GSK-3β inhibitor and HDAC inhibitor in cognitive decline associated with HFD induced insulin resistance, we selected Indirubin-3'-monoxime as GSK-3β inhibitor and SAHA as HDAC inhibitor, respectively. Interestingly, we found that low dose combination of these compounds was more effective as compared to either drug alone in ameliorating cognitive decline as assessed by battery of behavioral tests. Also, we observed that low dose combination of these drugs was more effective in attenuating $A\beta_{(1-42)}$, tau phosphorylation, oxidative stress markers, pro-inflammatory marker (TNF-α) and GSK-3β level. Further, significant amelioration of reduced histone H3 acetylation, CREB and BDNF levels was observed with low dose combination of these drugs when compared to either drug alone. Moreover, reduction in percentage damaged neuronal count in histological studies further elucidates the neuroprotective effect of this low dose combination.

In addition to HFD induced insulin resistance model, we also explored the potential of low dose combination of GSK-3β inhibitor and HDAC inhibitor in brain specific insulin resistance model induced by Intracerebroventricular Streptozotocin (ICV-STZ)

administration. For this purpose, we used lithium chloride as GSK-3β inhibitor and valproate as HDAC inhibitor. Although both these drugs are non-selective inhibitors of GSK-3β and HDACs, respectively, but these were selected because these drugs are clinically safe and used for mood disorders. The low dose combination of both these drugs was found to ameliorate ICV-STZ induced cognitive decline, oxidative stress, pro-inflammatory marker and GSK-3β level in a synergistic manner. Moreover, the combination of both these drugs improved the CREB, BDNF levels and increased neuronal count in hippocampus of ICV-STZ treated rats, in a synergistic manner as compared with either drug alone.

In second approach, we identify the involvement of class selective HDACs in insulin resistance induced neurodegeneration. For this purpose, we used class selective HDAC inhibitors, where Class I inhibitors (HDACi 4b and CI-994) and Class II selective inhibitor (MC-1568) and Class III inhibitor (Sirtinol) were used. We found that Class II selective HDAC inhibitor (MC-1568) was more effective in attenuating cognitive deficits and neuronal loss as compared to Class I selective inhibitors (HDACi 4b and CI-994) and Class III inhibitor, Sirtinol.

4.3. Plausible mechanism(s) of neuroprotective action of GSK3β inhibitor and HDAC inhibitors in insulin resistance induced PD pathology

Although some of the clinical reports suggest that insulin resistance could also be a risk factor for PD development, however experimental data is scarce. Some studies have demonstrated an increased GSK-3β activity in brain tissues of PD patients along with increased serine phosphorylation of IRS-2, indicating the possibility of involvement of insulin resistance in PD. It seems that modulation of GSK-3β activity could be an important factor for PD development, especially in insulin resistance condition. Moreover, epigenetic modifications could also modulate insulin signaling and its downstream targets. It has been observed that histone acetylation regulates IRS-1 tyrosine phosphorylation and facilitates insulin-stimulated signal transduction. Thus, in the last part of this work, we evaluated whether insulin resistance is linked to PD pathology or not. For this purpose, we first standardized an animal model which could mimic the co-morbid insulin resistance and PD condition. For development of insulin resistance, we fed the male Wistar rats with HFD for eight weeks, followed by 6-hydroxydopamine (6-OHDA) administration in medial forebrain bundle of rats, a toxin used widely for PD induction in animals. As expected, the 6-OHDA treatment produced nigral dopaminergic degeneration as evidenced by the loss of striatal dopamine level. The dopamine loss was correlated with impaired performance in behavioral

tasks such as rotarod, narrow beam walk test and locomotor activity. Interestingly, we found that prior exposure to HFD exacerbated the effects of 6-OHDA on striatal dopamine loss and behavioral observations in rats, indicating that HFD-induced insulin resistance is associated with a reduced capacity of nigral dopaminergic terminals to cope with 6-OHDA-induced neurotoxicity. Thus, insulin resistance may be an important modifiable risk factor for PD.

Further, to our surprise, we found a significant increase in GSK-3β activity and reduced histone H3 acetylation in HFD+6-OHDA treated rats as compared to alone HFD rats. We administered GSK-3β inhibitor (Indirubin-3-monoxime) and HDAC inhibitor (SAHA) in these animals and found that these compounds significantly ameliorate the HFD+6-OHDA induced exacerbated motor deficits, restore dopamine levels, CREB and increased neurotrophic factor, BDNF level.

5. Conclusions

Altogether, the findings of the present work strongly suggest that insulin resistance may induce epigenetic modifications affecting neurological mechanisms in the brain leading to increased susceptibility to insults associated with neurodegeneration. The molecular mechanisms involved in neurodegenerative processes associated with insulin resistance induced AD and PD may include elevated GSK-3β activity, reduced histone H3 acetylation, increased oxidative stress and neuro-inflammation. Moreover, these changes occurred concurrently with reduced CREB and BDNF levels. Based upon our results, we suggest that either a low dose combination of HDAC inhibitor and GSK-3β inhibitor could be used for long term therapy of cognitive deficits related with insulin resistance conditions or isoform specific Class II HDAC inhibitors could be used. In line with these, we also demonstrated the beneficial effects of GSK-3β inhibitors and HDAC inhibitors in ameliorating insulin resistance induced exacerbated PD pathology. Thus, our study provides, for the first time, an epigenetic explanation for the increased risk of neurodegeneration associated with insulin resistance and also suggests that epigenetic modulation might be used along with GSK3β inhibitors to provide therapeutic effects under such disease conditions.

6. Future Scope of the work

- Exploring the expression of each isoform of Class II HDACs in brain regions during insulin resistance condition will further open the new avenues.

- Combining low dose combinations of HDAC inhibitors with other drugs already showing potential in these conditions.
- Targeted drug delivery formulations of these combinations can also been performed (This work also already been approved by DST, India).
- Studies investigating safety and toxicity profile of the tested drugs during long term usage.
- Experiments identifying other epigenetic mechanisms such as histone methylation, phosphorylation in insulin resistance condition can be performed.