ABSTRACT

Type 2 diabetes mellitus (T2DM) is reaching epidemic proportions worldwide with an increasing percentage of the adult population being affected. The pathogenesis of T2DM results due to insulin resistance in target tissues with an impairment in insulin secretion. Among the various factors, hyperinsulinemia plays a crucial role in the development of insulin resistance. Hyperinsulinemia condition develops into the two pathophysiological disorders T2DM and obesity. Although, there are various pharmacological approaches for the management of insulin resistance, they have been reported to exhibit several side effects. As an alternative to the existing allopathic treatments, natural therapeutic approaches are being investigated due to their limited side effects. The present study investigates the role of hyperinsulinemia in impaired insulin signaling and explores an herbal based therapeutic approach for reverting hyperinsulinemia induced insulin resistance in 3T3-L1 adipocytes.

Chronic hyperinsulinemia treatment in in vitro was studied by incubating differentiated 3T3-L1 adipocytes with logarithmic (0.01, 0.1 and 1 μM) doses of insulin (referred to as hyperinsulinemia) for different time periods (4, 8 and 12 h). Hyperinsulinemia was found to impair insulin stimulated glucose uptake in a dose and time dependent manner in 3T3-L1 adipocytes with a complete inhibition observed at 12 h. A significant inhibition of insulin downstream signaling IRS1(Y-989), Akt(S-473) and GLUT4 at 12 h of hyperinsulinemia
treatment was observed. Downregulation of pPTEN(S-380) at 12 h clearly showed that PTEN (non-phospho) might be responsible for the impairment of PI3K/Akt signaling by dephosphorylating PIP3. Moreover, suppression of pGSK3β(S-9) and p70S6K(T-389) further confirms Akt inhibition during hyperinsulinemia.

Correlating with the hyperinsulinemia impaired insulin signaling, an increased level of reactive oxygen species (ROS) and stress response signals like pERK1/2(T-202/Y-204) and pJNK(Y-185) was observed. A well-known antioxidant N-acetylcysteine (NAC) was used to study the relation between ROS and insulin resistance during hyperinsulinemia. NAC showed significant suppression of ROS and stress response pERK1/2(T-202/Y-204) and pJNK(Y-185) expression with no effect on restoration of insulin sensitivity. These results clearly suggest that ROS does not play any role in hyperinsulinemia induced insulin resistance.

The tumor suppressor p53 impairs insulin signaling at multiple sites. Our study observed impaired insulin signaling and downregulation of pPTEN(S-380). Since it is well known that p53 impairs PI3K/Akt through PTEN, the role of p53 was evaluated during hyperinsulinemia. Interestingly, during hyperinsulinemia, an enhanced expression of p53 level was observed with a downregulation of pMDM2(S-166) expression in a time dependent manner with no cytotoxicity. Transient knockdown studies performed to study the link between p53 and hyperinsulinemia showed that p53 knockdown improved insulin sensitivity in hyperinsulinemia induced insulin resistant 3T3-L1 adipocytes. Inhibition of p53 by gene silencing improved insulin stimulated glucose uptake
and insulin downstream signaling IRS1(Y-989), Akt(S-473) in insulin resistant 3T3-L1 adipocytes, thus revealing a novel link between p53 and insulin signaling in adipocytes.

Since p53 is activated by various signals arising from DNA damage, telomere shortening and oxidative stress, we assessed the effect of hyperinsulinemia on telomere length. Hyperinsulinemia treated adipocytes exhibited critically shortened telomere length in comparison to untreated adipocytes. Telomere shortening and genotoxic stress activated p53 is known to increase systemic inflammation through the activation of NFκB and TNFα. Likewise adipocytes exposed to hyperinsulinemia showed increased levels of NFκB and TNFα indicating that hyperinsulinemia induces inflammation in 3T3-L1 adipocytes.

To restore insulin sensitivity in hyperinsulinemia induced insulin resistant 3T3-L1 adipocytes, herbal compounds as a therapeutic option was investigated. Natural products Aloe emodin glycoside (AEG), Chlorogenic acid (CGA) and Methyl tetracosanoate (MT) have been observed to exhibit anti-diabetic activity by our group and hence were chosen for the present study. Both pre-treatment and post-treatment of AEG (10 pg/ml), CGA (10 ng/ml) and MT (100 ng/ml) restored insulin sensitivity in hyperinsulinemia induced insulin resistant 3T3-L1 adipocytes. But CGA showed much more promising increase in glucose uptake in both pre and post treated state on par with the positive control (Insulin 0.1 µM/30 mins). Hence, CGA was chosen for further studies to understand its role in restoring insulin sensitivity during hyperinsulinemia.
Hyperinsulinemia impaired insulin signaling was significantly recovered by CGA. Post treatment of CGA during hyperinsulinemia significantly upregulated IRβ, IRS-1(Y-989), Akt(S-473) and GLUT4 expression compared to control and positive control. However, CGA pre-treatment showed an upregulation of IRβ and GLUT4 levels, but could not restore Akt (S-473) expression which was inhibited by hyperinsulinemia. Since CGA pre-treatment could not restore Akt phosphorylation, we decided to unravel its role in PI3K independent pathway. Interestingly, CGA pre-treatment was found to exhibit significant upregulation of CAP expression. These results suggested that CGA pre-treatment and post-treatment restores insulin stimulated glucose uptake in hyperinsulinemia state through PI3K independent and dependent pathways respectively.

Further, we extended our study to assess the protective effect of CGA on hyperinsulinemia induced stress. We observed significant protection by CGA against hyperinsulinemia induced telomere attrition. Similarly, the expression of p53, NFκB and TNFα was observed to be decreased by CGA in both pre and post treated conditions during hyperinsulinemia.

In summary, p53 silencing improves insulin sensitivity in 3T3-L1 adipocytes exposed to hyperinsulinemia revealing a novel link between p53 and insulin signaling in adipocytes. The study also identified CGA as a potential therapeutic compound aiding in restoration of insulin sensitivity in hyperinsulinemia induced insulin resistant 3T3-L1 adipocytes.