Chapter 7

Summary
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Insulin resistance is one of the salient features of type 2 diabetes, hypertension, polycystic ovarian syndrome and other human diseases. However, the molecular basis of insulin resistance in these subjects is not understood completely. Recent studies have linked the role of oxidative stress and redox sensitive serine kinase pathways (NF-κB, JNK and p38 MAPK) in insulin resistance. Nevertheless, the molecular mechanism(s) by which oxidative stress and redox sensitive serine kinase pathways lead to insulin resistance is not known. Even though the beneficial effect of antioxidants are reported in insulin resistance and diabetes, the mechanism by which antioxidants improve insulin sensitivity is unclear. In view of the above, the present study was designed to investigate the role of oxidative stress and redox sensitive serine kinase pathways in insulin resistant subjects (type 2 diabetes and rheumatoid arthritis), invitro (rat L6 muscle cells) and animal models (high fat fed rats) of insulin resistance.

In the invitro study, we investigated the effect of prolonged low grade oxidative stress and antioxidants on insulin action and redox sensitive serine kinase pathways in cultured rat L6 myotubes. Rat L6 myoblasts were cultured in DMEM at 37°C and 5 % CO₂ to differentiate into myotubes. Myotubes were pretreated with antioxidants for 18 hours and exposed to oxidative stress with a H₂O₂ generating system (glucose/glucose oxidase) for 12 hours. Effect of oxidative stress and antioxidants on insulin sensitivity was measured by 2-deoxy¹⁴C- glucose uptake. Intracellular redox balance was evaluated in cell lysates by estimating the reduced glutathione concentration and total antioxidant capacity. Insulin signaling and redox sensitive serine kinase pathways were studied by western blotting and immunoprecipitation analysis using specific antibodies. Our results showed, treatment of L6 myotubes with H₂O₂ decreased the insulin stimulated IRS-1
tyrosine phosphorylation and glucose uptake. H$_2$O$_2$ treatment impaired the redox balance, activated the redox sensitive serine kinase NF-kB and JNK pathways and increased IRS-1 serine phosphorylation. Antioxidant treatment restored insulin action by inhibiting the NF-kB and JNK pathways through preserving intracellular redox balance in H$_2$O$_2$ treated cells.

In the animal study, we have investigated the role of oxidative stress and redox sensitive serine kinase pathways on insulin action in high fat diet (HFD) induced insulin resistance. Male Wistar rats were divided into four groups: the control group – received a rodent chow, control + antioxidant group – fed with rodent chow supplemented with 0.2 % (w/w) vitamin E, 0.3 % (w/w) vitamin C and 0.5 % (w/w) α-lipoic acid, high fat diet group – received high fat diet and high fat + antioxidant group – fed with high fat diet supplemented with above antioxidants. Animals were provided with specific diets and water ad libitum for 9 weeks. At the end of 9 week experimental period, fasting plasma insulin, intraperitoneal glucose and insulin tolerance tests were performed to evaluate insulin sensitivity. Oxidative stress parameters such as plasma MDA, erythrocyte reduced glutathione and catalase activity was estimated. Reverse-transcriptase polymerase chain reaction (RT-PCR) was performed with skeletal muscle RNA to quantify the expression of catalase and glutathione peroxidase. Insulin signaling and redox sensitive serine kinase pathways were studied in skeletal muscle homogenate by western blotting and immunoprecipitation analysis using specific antibodies. We found that fat feeding to rats for nine weeks impaired insulin sensitivity, redox balance and insulin stimulated IRS-1 tyrosine phosphorylation. HFD feeding significantly increased the IRS-1 serine phosphorylation and activated the redox sensitive NF-kB and JNK pathways. Antioxidant
supplementation along with HFD diet restored the redox balance, inhibited NF-kB and JNK pathways and improved insulin action.

In the human study, we assessed the insulin resistance, oxidative stress and lymphocyte redox sensitive serine kinase pathways (NF-kB, p38MAPK and JNK) in newly diagnosed type 2 diabetes (type 2 DM) and rheumatoid arthritis (RA) subjects. Twenty newly diagnosed type 2 DM (10 males and 10 females) and 20 newly diagnosed RA patients (12 females and 8 males) in the age group of 30-50 years were included in this study. As control, 20 age matched healthy volunteers (11 females and 9 males) were recruited. After 12 hours of fasting, blood samples were collected from the subjects and lymphocytes, plasma and RBC were separated using Ficoll hypaque. HOMA-IR was calculated to asses the insulin resistance from the fasting plasma glucose and insulin values. Oxidative stress parameters such as plasma total antioxidant capacity, MDA, RBC and lymphocyte reduced glutathione and RBC catalase activity were estimated. Redox sensitive serine kinase pathways were studied in lymphocytes by western blotting analysis. We found insulin resistance and oxidative stress in both type 2 DM and RA subjects. The NF-kB and JNK pathways were activated in lymphocytes of type 2 DM subjects whereas activation of only NF-kB pathway was found in RA subjects.

Our results from humans (type 2 diabetes and RA), invitro (rat L6 muscle cells) and animal models (high fat fed rats) of insulin resistance, clearly demonstrates the oxidative stress induced activation of redox sensitive serine kinase pathways. Invitro studies in rat L6 muscle cells and animal studies in fat fed rats revealed IRS-1 as the potential target for these activated redox sensitive kinase pathways. Activation of these redox sensitive serine kinases phosphorylate the IRS-1 and thereby increased the IRS-1 phosphoserine content. Increased IRS-1 serine phosphorylation impairs the interaction of
IRS-1 with insulin receptor and thereby decreases the insulin stimulated IRS-1 tyrosine phosphorylation. This results in insulin resistance in oxidative stress. In support of this, antioxidant treatment restored the redox balance, inhibited the activation of redox sensitive serine kinase pathways, decreased the IRS-1 serine phosphorylation and retained insulin action. We hope the outcome of the present study has the potential to add to our understanding of the molecular basis of insulin resistance and the beneficial effect of antioxidant therapy in insulin resistance.