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ORIGINAL ARTICLE

COMPARATIVE EFFECTS OF LOSARTAN AND PIOGLITAZONE ON INSULIN RESISTANCE IN RATS

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ABSTRACT

Background: Insulin resistance is a key feature of type 2 diabetes mellitus. Peroxisome Proliferator activated receptor – gamma (PPAR-γ) agonists are known to decrease insulin resistance. The renin angiotensin aldosterone system (RAAS) is also implicated in the development of insulin resistance; drugs acting on this system are expected to improve it. Objective of this study was to evaluate the beneficial role, of losartan in comparison with pioglitazone on insulin resistance in a type 2 diabetic rat model fed on high fat and sucrose diet.

Methods: A total of 45 Sprague – Dawley rats of 5 weeks of age were randomized into three groups. All the rats were fed a high fat (HFD) and sucrose diet. Pioglitazone (PIO) and losartan (LOS) was given along with this diet to the rats in group HFD–PIO and HFD–LOS respectively, while group HFD was kept as control. Body weight and fasting blood glucose levels were determined weekly. At the end of 12 weeks, insulin tolerance test (ITT) was performed in all groups. Serum insulin and C-reactive protein (CRP) levels were also determined. Markers of insulin sensitivity, Homeostatic Model assessment of insulin resistance (HOMA–IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.

Results: At the end of study period body weight, fasting blood glucose, serum insulin, C-reactive protein and HOMA–IR had significantly lower values and QUICKI had a significantly higher value in both experimental groups as compared to group HFD. Insulin tolerance test gave significantly lower blood glucose levels at all reading times in both experimental groups as compared to group HFD. Difference between group HFD–PIO and HFD–LOS was statistically insignificant for all parameters. Relationship between CRP and HOMA–IR was positive and relationship between CRP and QUICKI was negative.

Conclusion: Losartan is as effective in improving insulin resistance as pioglitazone. This effect might be mediated through an anti-inflammatory mechanism.

Key Words: Metabolic Syndrome, Insulin Resistance, CRP, HOMA-IR, QUICKI, Losartan, Pioglitazone.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease due to multiple etiologic factors characterized by abnormally high blood glucose levels caused by disturbances in carbohydrate, protein and fat metabolism; primarily resulting from a defect in insulin secretion, insulin action or both.[1]

Impaired insulin action implies insulin resistance which is defined as a state of reduced responsiveness to normal circulating levels of Insulin. It connotes resistance to the effects of insulin on glucose uptake, metabolism or storage. It develops due to defects in insulin signaling including decreased insulin receptor tyrosine kinase activity, defects in IRS_1 (insulin receptor $substrate_1$) and PI_3 kinase (phosphatidyl$_3$ kinase) activities that lead to a profound defect in glucose transport and glycogen synthesis.[2]

Since insulin resistance is a key feature of type 2 diabetes mellitus, therefore drug development has focussed on developing drugs that increase insulin sensitivity, otherwise known as insulin sensitizers. The thiazolidinediones are selective ligands for peroxisomal proliferator activated receptor – gamma (PPAR-γ). When activated by a ligand such as the glitazones, PPAR-γ binds to the Retinoid X Receptor (RXR) to form a heterodimer. This binds to DNA to regulate the transcription and translation of a different of proteins involved in glucose and lipid metabolism.[3] PPAR-γ is the master regulator of adipogenesis, stimulating the production of small insulin-sensitive adipocytes, which are more insulin sensitive than large adipocytes.[4] Glitazones increase levels of adiponectin and decrease expression of resistin. Glitazones stimulates fatty acid storage in subcutaneous adipocytes and decrease hepatic triglycerides and thus improve insulin sensitivity in the liver.[5]

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Renin angiotensin aldosterone system (RAAS) is also implicated in the development of insulin resistance. Elevated levels of aldosterone take part directly in the pathogenesis of insulin resistance. Aldosterone increases the expression of adipokines that causes reduced expression of insulin receptors leading to impaired insulin – induced glucose uptake. Overweight and obesity favor the adrenal secretion of aldosterone; free fatty acids as well as adipokines stimulate the production of aldosterone. In turn, increased levels of aldosterone may lead to insulin resistance.[6] Angiotensin II also decreases circulating adiponectin[7] and being a potent vasoconstrictor opposes the vasodilator effect of NO, contributing to insulin resistance. Hence agents that inhibit or block this system would be useful in improving insulin sensitivity. From the present research point of view and the most studied among inhibitors of RAAS; development of selective AT1 receptor blockers (ARBs) began in 1990 with the synthesis of losartan, an orally active, non-peptide angiotensin II receptor antagonist. Since then several others have been synthesized including valsartan, irbesartan, telmisartan, candesartan, eprosartan and olmesartan.[8] ARBs including telmisartan, irbesartan and losartan have shown to possess PPAR-γ agonist activity.[9] Beneficial effects of PPAR-γ agonist activity on improving insulin sensitivity have been mentioned. This provides a strategic rationale and pharmacological platform for the study of dual ARB / PPAR-γ agonist losartan on a rat model of insulin resistance.

METHODS

Sprague – Dawley rats of 4 weeks of age were purchased from the University of Veterinary and Animal Sciences, Lahore and kept in the animal house of PGMI in iron cages under hygienic conditions. Room temperature was maintained at 25 ± 2°C under natural day / night cycle with free access to rat chow and water. They were allowed one week to acclimatize. From 5 weeks of age rats were fed on high fat diet containing 30% beef fat and 10% sucrose.[10]

Animals were divided randomly into 3 groups of 15 animals each. All three groups were fed high fat and sucrose diet throughout study period of 12 weeks. First group was given distilled water daily orally as a single morning dose and labeled as HFD (high fat diet) group. Second group was given pioglitazone in dose of 10 mg/kg body weight[11] daily orally as a single morning dose for 12 weeks and labeled as HFD – PIO group. Third group was given losartan in dose of 10 mg/kg body weight[12] daily orally as a single morning dose for 12 weeks and labeled as HFD – LOS group. Pioglitazone and losartan were obtained from Mass Pharmaceuticals.

Body weight and fasting blood glucose level were measured initially and after every week. Fasting blood glucose level was measured with a glucometer (Accu Chek) using a drop of blood obtained from the tail vein. At 12 week insulin tolerance test (ITT) was performed. Rats were kept on 6 hour fast and then injected 1 unit/kg regular insulin intraperitoneally. Blood glucose levels were determined prior to injection and 30, 60, 120 minutes after injection by glucometer. Blood samples were collected by tail bleed.

After 12 weeks, rats were kept on 12 hour fast and blood was collected by cardiac puncture. Samples were then centrifuged at room temperature at 3000 – 4000 rpm for 5 minutes. Serum was stored at –20°C until being analyzed for insulin and CRP determination. Serum insulin was estimated using insulin ELISA kit (NovaTecImmundiagnostica GmbH). Serum C-reactive protein was estimated using a CRP slide test (Analyticon Biotechnologies AG). Homeostatic model assessment of insulin resistance (HOMA – IR) and quantitative insulin sensitivity check index (QUICKI) are markers of insulin sensitivity. Many investigators have demonstrated strong relationships between these surrogate markers and insulin responses measured with clamp procedure. They were calculated as follows:[13]

HOMA IR = Fasting Insulin (μIU/ml) × Fasting Glucose (mg/dl)/405

QUICKI = 1 / [log (fasting insulin μIU/ml) + log (fasting glucose mg/dl)]

Statistical Analysis

The data was entered and analyzed using SPSS 17.0. Mean ± S.D. was given for quantitative variables like body weight, blood glucose level and insulin tolerance test as well as insulin level, C-reactive protein level, HOMA – IR and QUICKI values. One – way ANOVA was applied to compare the above variables among the groups. Post hoc Tukey's test was applied to observe which group mean differs. Pearson's correlation coefficients were calculated to evaluate relationship of CRP with HOMA – IR and QUICKI.

RESULTS

Mean body weight at beginning of study in group HFD, HFD – PIO and HFD – LOS was 82 ± 8, 79 ± 7 and 81 ± 5 g respectively. The body weight increased in all groups over 12 week study period but weight gain in rats of HFD – PIO and HFD – LOS group was significantly less as compared to those of HFD group with p-value < 0.05. Difference between HFD-PIO and HFD – LOS group was not significant (Table 1).

Mean fasting blood glucose level of animals at the start of study was 92 ± 9, 87 ± 7 and 91 ± 7 mg/dl in group HFD, HFD – PIO and HFD – LOS. Fasting blood glucose level increased in all groups over the study period. At 12 week fasting blood glucose level was significantly less in HFD – PIO and HFD – LOS group as compared to that of HFD group with p-value < 0.001. Difference between HFD – PIO and HFD – LOS group was not significant (Table 1).

Table 1: *Body weight and metabolic characteristics of HFD fed rats at end of 12 week study period. Data represents mean ± SD of 15 samples.*

Group	*Body Weight (g)*	*Blood Glucose mg/dl*	*Serum Insulin µIU/ml*	*HOMA – IR*	*QUICKI*	*CRP mg/l*
HFD	382 ± 48	152 ± 12	23.20 ± 5.52	9.00 ± 2.49	0.28 ± 0.02	9.46 ± 1.78
HFD – PIO	345 ± 45*	123 ± 17**	12.07 ± 6.82***	3.92 ± 3.03***	0.32 ± 0.03***	6.43 ± 2.22***
HFD – LOS	342 ± 38*	132 ± 17***	14.13 ± 8.83**	4.99 ± 3.71**	0.32 ± 0.03**	7.41 ± 2.45*

*p-value ≤ 0.05, **p- value ≤ 0.01, ***p value ≤ 0.001 as compared to group HFD

ITT performed at 12 week study period revealed that blood glucose level was significantly lower in HFD – PIO and HFD – LOS group as compared to that of HFD group at all reading times while difference between HFD-PIO and HFD – LOS group was not significant (Fig. 1).

At end of 12 week study period serum insulin level was significantly lower in HFD – PIO and HFD – LOS group as compared to that of HFD group with p-value 0.001 and 0.004 respectively. Difference between HFD – PIO and HFD – LOS group was not significant (Table: 1). CRP level was significantly lower in HFD – PIO and HFD – LOS group as compared to that of HFD group with p-value 0.001 and 0.035 respectively. Difference between HFD– PIO and HFD – LOS group was not significant (Table 1). HOMA – IR revealed significantly lower value in both experimental groups as compared to that of control (Table 1), while QUICKI revealed significantly higher value in both experimental groups as compared to that of control (Table 1).

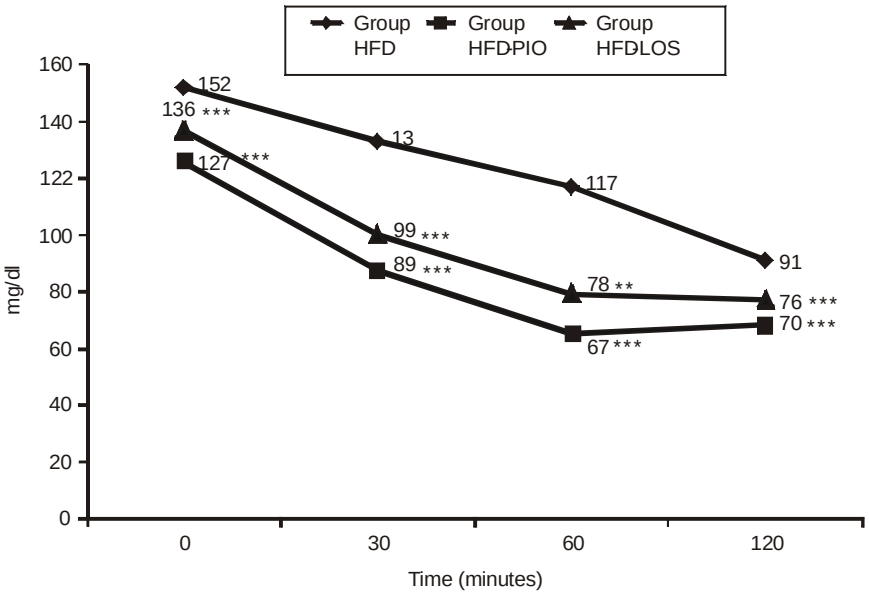

Fig. 1: *Changes in blood glucose level (mg/dl) during intraperitoneal insulin tolerance test in the three groups of rats at 0, 30, 60 and 120 minutes.*
**p-value ≤ 0.01 for Group HFD-LOS versus Group HFD at time 60 minutes
***p-value ≤ 0.001 for Group HFD-LOS versus Group HFD at times 0, 30 and 120 minutes and for Group HFD-PIO versus Group HFD at all time.

There was positive relationship between CRP level and HOMA – IR with p-value 0.181, 0.001 and 0.000 in group HFD, HFD – PIO and HFD – LOS respectively. There was negative relationship between CRP level and QUICKI with p-value 0.062, 0.002 and 0.000 in group HFD, HFD – PIO and HFD – LOS respectively.

DISCUSSION

In the present study role of losartan in improving insulin resistance was evaluated and compared with pioglitazone in hyperglycemic rates fed on high fat and sucrose diet. For this purpose 45 Sprague – Dawley rats of 5 weeks of age were randomized into three groups. All the rats were fed a high fat and sucrose diet. Such an animal model is the best model to study the human metabolic syndrome. Numerous studies have shown that a diet rich in saturated fatty acids and refined carbohydrates increases the risk of diabetes.[14] Pioglitazone and losartan was given along with this diet to the rats in group HFD – PIO and HFD – LOS respectively, while group HFD was kept as control. Body weight and fasting blood glucose levels were determined weekly. At the end of 12 weeks, insulin tolerance test (ITT) was performed in all groups. Serum insulin and C-reactive protein (CRP) levels were also determined. Markers of insulin sensitivity, homeostatic model assessment of insulin resistance (HOMA – IR) and quantitative insulin sensitivity check index (QUICKI) were calculated.

Mean body weight of animals at the start of study was around 80 grams which increased steadily in all study groups during the study period but increase was more in HFD group as compared to HFD – LOS and HFD – PIO groups. As increase in body weight is associated with type 2 diabetes, both groups treated with drugs along with high fat diet showed significant less

increase in body weight. Similar effect on body weight of rats was observed in a study using telmisartan and candesartan.[15]

Mean fasting blood glucose level was significantly low in both experimental groups as compared to that of control. Difference between HFD – LOS and HFD – PIO was not significant. Chu *et al*. (2006) also demonstrated decrease in blood glucose level with losartan in a dose dependent manner in genetic diabetic mice model.[12]

Insulin tolerance test was performed at the end of 12 weeks and it was observed that losartan improved insulin tolerance comparable to pioglitazone while Chu *et al* (2006) did not find improvement in ITT after intraperitoneal injection of insulin to genetically diabetic mice treated with losartan.[12]

Serum insulin level was found to be significantly raised in HFD group as compared to HFD – PIO and HFD – LOS groups. Raised fasting serum insulin level indicate insulin resistance that is characteristic of early stage of type 2 diabetes.[16] Hyperinsulinemia with fasting and basal hyperglycemia is seen in some models of type 2 diabetes due to high fat diet.[17] The results of present study correlate with the results of human studies carried out by other workers, which also show decrease in insulin levels with losartan compared with control group.[18,19]

C reactive protein is a peptide that is elevated in a variety of inflammatory conditions and inflammation is a key attribute of diabetes.[20] Results of present study show decreased CRP levels in experimental groups. Similar effect on CRP level was observed in a human study.[21]

HOMA – IR is an index of insulin resistance.[13] Decrease in HOMA – IR by losartan, an angiotensin receptor blocker, in the present study is supported by other studies on diabetes. HOMA – IR decreased in human studies with use of losartan during 6 month follow up in type 2 diabetics[19] and in patients with chronic heart failure.[22] Various other angiotensin receptor blockers also showed the same results. Improvement in HOMA – IR was observed in a human study in which telmisartan was used in patients of type 2 diabetes and hypertension.[23] In other studies olmesartan improved peripheral insulin sensitivity in human subjects after 6 months[24] and 12 months of treatment.[25]

QUICKI is an index of insulin sensitivity[13], it showed improvement with losartan in this study comparable to that of pioglitazone. All the results support the hypothesis of present study.

Probable mechanism of improvement of insulin resistance by angiotensin receptor blockers may be increase in adiponectin level as suggested by studies conducted on human subjects.[23,26]

The peripheral vasodilatory actions of ACE inhibitors and ARBs lead to an improvement in skeletal muscle blood flow. This improves insulin and glucose delivery as well as increases the surface area for glucose exchange between the vascular bed and skeletal muscles. It may be important mechanism by which inhibition of the RAAS improves glucose uptake and metabolism in insulin – sensitive tissues.[16,27]

ARBs induce the expression of the glucose transporter $GLUT_4$, thus increasing glucose uptake and decreasing insulin resistance in skeletal muscle tissue.[28,29]

Another possible mechanism is through activation of PPAR-γ activation. Losartan[9] and telmisartan[31] have shown to increase PPAR-γ expression and PPAR-γ improves insulin sensitivity by translocating GLUT 4 to the plasma membrane in the skeletal muscle.

Studies indicate that ARBs reduce levels of inflammatory markers like CRP[21], TNF α and IL-6.[22] In present study CRP level was low in both experimental groups as compared to that of HFD group.

It is ***concluded*** that the results of present study indicate that losartan improves insulin resistance comparable to that of pioglitazone. Positive correlation of CRP with HOMA – IR and negative correlation with QUICKI indicates that improvement in insulin sensitivity might be mediated through an anti-inflammatory mechanism.

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