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ORIGINAL ARTICLE

Comparison of Effect of Losartan with Pioglitazone on β-Cell Function in a Rat Model of Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To find out the effect of losartan in comparison with pioglitazone on β-Cell function in a type 2 diabetic rat model.

Patients and Methods: This was a randomized control trial study and was carried out at Postgraduate Medical Institute (PGMI), Lahore from June to August 2011. In this study 45 Sprague-Dawley rats of 5 weeks of age were randomized into three groups. All the rats were fed with a high fat and sucrose diet. Pioglitazone or losartan were given along with this diet to the rats in-group HFD-PIO and HFD-LOS respectively, while group HFD was kept under control. Body weight and fasting blood glucose levels were determined weekly. At the end of 12 weeks, blood glucose and serum insulin levels were determined. A marker of β-cell function, HOMA-β, was also calculated.

Results: At the end of study period, body weight, fasting blood glucose, serum insulin, and HOMA-β levels were significantly lower in the HFD-PIO and HFD-LOS groups as compared to the control HFD group. The difference in these parameters between the HFD-PIO and HFD-LOS groups was not significant.

Conclusion: Losartan is significantly comparable to Pioglitazone in improving β-cell function.

Key words: β-Cell function, HOMA-β, Losartan, Pioglitazone.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing
[2] Interpretation and discussion
[3] Data analysis, interpretation and manuscript writing, [4, 5] Active participation in data collection

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Article info.
Received: April 25, 2017
Accepted: August 17, 2017

Cite this article. *Shad MN, Aslam H, Shamim MO, Chiragh S, Ahmad Z. Comparison of Effect of Losartan with Pioglitazone on β-Cell Function in a Rat Model of Type 2 Diabetes Mellitus.JIMDC;6(3):157-160.*

Funding Source: *Nil*
Conflict of Interest: *Nil*

Introduction

Diabetes mellitus, one of the most common non-communicable diseases has become a worldwide epidemic. This disease is the fourth most common cause of death globally and is expected to affect 450 million people by the year 2030.[1] Insulin resistance and β-cell dysfunction plays a major role in the pathogenesis of type 2 diabetes mellitus. Defects in insulin secretion predisposes to type 2 diabetes mellitus. Studies have shown that individuals genetically predisposed to develop type 2 diabetes mellitus, when exposed to chronically increased levels of free fatty acids (for example in obesity or due to high-fat diet) may be prone to lipotoxicity that results in decreased insulin secretion through beta cell apoptosis. The resulting hyperglycemia and decreased insulin leads to glucotoxicity (glucose toxicity) that further decreases insulin secretion and predisposes to insulin resistance. Lipid-induced beta-cell dysfunction may precede the decrease in beta cell mass.[2]

β-Cell dysfunction is a key feature of development of type 2 diabetes mellitus.[3] Drug research has focused on developing drugs that improve β-Cell function, including insulin secretagogues like the sulfonylureas and glinides, incretin analogs like DPP-4 inhibitors and GLP-1 analogs, as well as insulin itself.[4] Thiazolidinediones (Glitazones) are a group of drugs used for treatment of type 2 diabetes which have shown to preserve beta cell function by protecting the beta-cell from lipotoxicity. β-cell stress is reduced with thiazolidinedione therapy, but enhanced with sulfonylurea therapy.[5] Thiazolidinediones mediate their actions through PPAR-γ receptors. PPAR-γ activation acts to maintain β cell function.[6] Renin angiotensin aldosterone system (RAAS) is also implicated in the development of β-Cell dysfunction. Evidence suggests that the RAAS affects insulin secretion. Angiotensin II impairs insulin biosynthesis and promotes beta cell apoptosis possibly due to long-term vasoconstriction-induced restricted blood flow to the pancreas.[7]

Hence, pharmacologic inhibition of this system would be useful in preserving beta cell function. From the present research point of view and the most studied among inhibitors of RAAS are the selective AT1 receptor blockers (ARBs). Development of 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 effect of PPAR-γ agonist activity on improving insulin secretion has been mentioned. This provides a strategic rationale and pharmacological platform for the study of dual ARB/PPAR-γ agonist losartan on β-cell function in a rat model of β cell dysfunction.

Patients and Methods

This was a randomized control trial and was carried out at Postgraduate Medical Institute (PGMI), Lahore. Sprague-Dawley rats of 4 weeks of age were purchased from the University of Veterinary & 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 with 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. The second group was given pioglitazone in dose of 10mg/kg body weight daily orally as a single morning dose for 12 weeks and labeled as HFD-PIO group.[11] Third group was given losartan in dose of 10mg/kg body weight[1] daily orally as a single morning dose for 12 weeks and labeled as HFD-LOS group.[12]

Follow up

Body Weight of Rats: Each rat was weighed initially and after every week.

Measurement of Fasting Blood Glucose: Fasting blood glucose level was measured every week using a glucometer (AccuChek) using a drop of blood obtained from the tail vein.

Blood Sampling: After 12 weeks, rats were kept on 12 hours 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 serum insulin determination.

Biochemical Methods / Measurement of Serum Insulin: Serum insulin was estimated using insulin ELISA (enzyme-linked immunosorbent assay) kit (NovaTecImmundiagnostica GmbH).

Calculation of Index of β Cell Function: Homestatic Model Assessment of β-Cell function (HOMA- β) is a surrogate marker of β-cell function. Many investigators have demonstrated strong relationships between this surrogate marker and responses measured with clamp procedure. HOMA- β was calculated as follows.[13]

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin}}{\text{Glucose} - 63}$$

Drugs: Pioglitazone and losartan were obtained from Mass Pharmaceuticals

The data were entered and analyzed using SPSS 17.0. Mean ± S.D was calculated for quantitative variables like fasting blood glucose levels, fasting insulin levels and

HOMA- β values. One-way ANOVA was applied to compare the above variables among the groups. Bartlett's test was applied to see whether variances were significantly different.

Results

Mean body weight at beginning of study was 82±8, 79±7 and 81±5 g in group HFD, HFD-PIO and HFD-LOS 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. As shown in table 1, the difference between HFD-PIO and HFD-LOS group was not significant according to the

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 weeks, 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).

Serum insulin level was measured at end of 12-week study period and it was observed that 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. Barlett's test was applied to assess for statistical significance of these results (Table 1).

HOMA-β calculated at end of study revealed significantly lower values in HFD-PIO and HFD-LOS group as compared to that of HFD control group with p-value < 0.01 and < 0.05 respectively. (Table 1).

Discussion

In the present study, the role of losartan in improving β-cell function was evaluated in high fat fed diabetic rats in comparison with pioglitazone. For this purpose, 45 Sprague-Dawley rats of 5 weeks of age were randomized into three groups. All the rats were fed with 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-groups 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, serum insulin levels were determined. A marker of β-cell function, homeostatic model assessment of β-cell function (HOMA-β), was also calculated at the end.

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 the increase in body weight is associated with type 2 diabetes, both groups treated with drugs along with a high-fat diet showed significantly less increase in body weight. A similar effect on body weight of rats was observed in one 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 also demonstrated a decrease in blood glucose level with losartan in a dose-dependent manner in a genetic diabetic mice model.[12] Serum insulin levels were measured at the end of the study period (12 weeks) and they were found to be significantly raised in the HFD group as compared to HFD-PIO and HFD-LOS groups. Hyperinsulinemia with fasting and basal hyperglycemia have been observed in some models of type 2 diabetes due to high fat diet.[10] HOMA-β is an index of β-cell function insulin.[13] Decrease in HOMA-β by losartan, an angiotensin receptor blocker, in the present study is supported by other studies on

Table 1: Body weight and metabolic characteristics of HFD fed rats at end of 12-weeks study period

Group	Body Weight (g) mean ± SD	Blood Glucose (mg/dl) mean ± SD	Serum Insulin (µIU/ml) mean ± SD	HOMA-β mean ± SD
HFD	382 ± 48	152 ± 12	23.20 ± 5.52	89.83 ± 3.948
HFD-PIO	345 ± 45*	123 ± 17***	12.07 ± 6.82***	72.47 ± 3.617**
HFD-LOS	342 ± 38*	132 ± 17***	14.13 ± 8.83**	66.942 ± 6.943*

**p-value ≤ 0.05, **p- value ≤ 0.01, *** p value ≤ 0.001 as compared to group HFD Bartlett's test applied*

diabetes. HOMA-β decreased in a human study with the use of losartan during 24 weeks follow up in hypertensive.[17] Various other angiotensin receptor blockers also showed the same results. In one study, twenty-six weeks of treatment with valsartan increased glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with impaired glucose metabolism.[18]

Possible mechanisms of improvement of β-cell function by losartan include that a decrease in activity of the renin-angiotensin aldosterone system by ARBs has been observed to cause significant reductions in reactive oxygen species, protein kinase C and NADPH oxidase activities in pancreatic islet cells. This has led to enhanced β cell survival. Increase in β cell mass also results from a protective effect by ARBs against harmful effects of inflammatory and oxidative stress. Enhanced β cell survival & an increase in β cell mass has seen to increase insulin secretion and improve glucose tolerance.[19] The primary target for insulin action & an important determinant of glucose uptake is skeletal muscle blood flow. ACE inhibitors & ARBs cause vasodilation in peripheral blood vessels, which leads to an increase in skeletal muscle blood flow. This is by increasing the surface area for glucose exchange between the vascular bed & skeletal muscles. This action also facilitates an increase in blood flow to the pancreatic islet cells, thus further increasing insulin secretion.[19]

Conclusion

The results of present study indicate that losartan improves β-cell function, which is significantly comparable to that of pioglitazone.

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