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ORIGINAL ARTICLE

Effect of Losartan in Comparison with Pioglitazone on Lipid Profile in a Rat Model of Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To evaluate the effect of losartan in comparison with pioglitazone on lipid profile in a type 2 diabetic rat mode.

Materials and Methods: This case control study was conducted in Postgraduate Medical Institute (PGMI), Lahore from June to August 2011. Forty-five Sprague-Dawley rats of 5 weeks of age were randomized into three groups. All the rats were fed a high fat and sucrose diet. Pioglitazone or Losartan were given along with this diet to the rats in groups HFD-PIO and HFD-LOS respectively, while group HFD was kept as control. At the end of 12 weeks, serum samples were obtained from all the animals and total cholesterol, HDL-cholesterol and triglyceride levels were obtained using kit method. LDL-cholesterol was determined using the Friedewald formula.

Results: At the end of study period, lipid profile parameters were statistically improved between HFD-PIO and the control HFD group. The difference in the lipid profile parameters between the HFD-LOS and the control HFD group as well as between the HFD-PIO and HFD-LOS groups was not significant.

Conclusion: The ARB losartan has a small but insignificant effect on lipid profile.

Key words: Lipid Profile, Losartan, Pioglitazone, Type-2 Diabetes Mellitus

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

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Introduction

Diabetes mellitus is one of the leading chronic diseases in almost every country[1], is characterized by increased blood glucose levels. Its associated complications including dyslipidemia lead to increased morbidity and mortality. Patients with adverse lipid profile including hypertriglycedemia, high levels of low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL) are at high risk of cardiovascular disease.[2] Many patients with type 2 diabetes have an abnormal lipid profile characterized by increased triglycerides and decreased high density lipoproteins HDL-C.[3] Studies have shown that a high fat plus high carbohydrate diet play a major role in the pathogenesis of type 2 diabetes mellitus and dyslipidemia.[4] A diet that contains a high percentage of saturated fatty acids and refined carbohydrates in *combination* is an unhealthy diet that may lead to insulin resistance, type 2 diabetes mellitus, dyslipidemia and various other features of the metabolic syndrome. Such

diets have been used to induce diabetes and dyslipidemia in animal models.[5]

It is well known that cholesterol homeostasis is fundamental for appropriate insulin secretory function of β cells. Excessive cholesterol accumulation in β cells, when exposed to chronically increased levels of free fatty acids (for example in obesity or due to high fat diet), may cause lipotoxicity and reduce insulin secretion, causing β cell dysfunction & decreased β cell mass.[6]

Dyslipidemia has been seen to be associated with dysfunction of pancreatic β cells and this is particularly evident in people with elevated total cholesterol (TC) and LDL-C levels.[7] Thiazolidinediones (Glitazones) are a group of drugs used for treatment of type 2 diabetes which have shown to preserve beta cell function by protecting beta-cell from lipotoxicity.[8] Glitazones are selective agonists of peroxisome proliferator activated receptor gamma (PPARγ), a nuclear receptor which is most highly expressed in adipose tissue and controls the transcription and translation of a variety of genes involved in glucose and lipid metabolism.[9] PPAR-γ is currently regarded as a therapeutic target in the metabolic syndrome.[10] Pioglitazone, one of the major glitazones being used in the treatment in T2DM has shown to improve lipid profile including decrease in triglycerides and low-density lipoprotein (LDL), increase in high-density lipoprotein (HDL) and decrease in serum fatty acids.[11]

Renin-angiotensin-aldosterone system has been linked with obesity-related hypertension and it is also involved in the association among obesity, metabolic syndrome, dyslipidemia, insulin resistance, chronic kidney disease, and hypertension.[12] ACE Inhibitors have shown positive effects on the lipid profiles in children with the metabolic syndrome. Results in the study showed statistically significant decrease in LDL & triglyceride levels and significant increase in HDL levels.[13] Now several ARBs (Angiotensin Receptor Blockers) including Telmisartan, Irbesartan and Losartan have shown to possess PPAR-γ agonist activity. Beneficial effects of PPAR-γ agonist activity on improving insulin sensitivity have been mentioned. This provides a strategic rationale and pharmacological platform for the use of dual ARB/PPAR-γ agonists to target the metabolic syndrome and its cardiovascular sequelae.[10]

This provides the basis for the present study to observe effects, if any, of a dual ARB/PPAR-γ agonist losartan on lipid profile in a rat model of type 2 diabetes mellitus.

Materials and Methods

This randomized control trial was conducted at Postgraduate Medical Institute (PGMI), Lahore from June 2018 to August 2018. The sample size was estimated by using 5% level of significance and 80% power of test with expected frequency of diabetes mellitus; 36, 57 and 94 percent in glitazones, angiotensin receptor blockers and control groups respectively.[15,16] The estimated sample size was 13 in each group (total 39). It was increased to 15 in each group (total 45) to increase the accuracy of the results and to decrease the margin of error. 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 $\pm$ 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.[14] 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 10mg/kg body weigh daily orally as a single morning dose for 12 weeks and labeled as HFD-PIO group.[15] Third group was given losartan in dose of 10mg/kg body weight daily orally as a single morning dose for 12 weeks and labeled as HFD-LOS group.[1] Drugs Pioglitazone and losartan were obtained from Mass Pharmaceuticals**.** Each rat was weighed initially and after every week. Fasting blood glucose level was measured every week using a glucometer (AccuChek) using a drop of blood obtained from the tail vein. 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 analyzed for lipid profile. Total cholesterol, HDL-cholesterol and triglyceride levels were obtained using

calorimetric kit methods. LDL-cholesterol was determined using the Friedewald formula:[17]

LDL = Total Cholesterol – HDL Cholesterol – Triglycerides/5.

The data was entered and analyzed using SPSS 17.0. Mean ± SD was analyzed for quantitative variables like body weight, fasting blood glucose levels and lipid profile. One-way ANOVA was applied to compare the variables among the groups. Bonferroni's post-test was applied to see whether variances were significantly different.

Results

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 respectively. 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. Difference between HFD-PIO and HFD-LOS group was not significant (Table 1). At 12 weeks, mean serum cholesterol, LDL and triglyceride levels were higher and mean HDL level was lower in the control HFD group as compared to the HFD-PIO and HFD-LOS groups (Table 2). The mean serum cholesterol, LDL and triglyceride levels were higher and mean HDL level was lower in the HFD-LOS group as compared to the HFD-PIO group (Table 2). Total cholesterol and LDL levels were found to be significantly less in the HFD-PIO group as compared to that of HFD group. Triglyceride levels were also significantly less. HDL levels showed no significance between HFD-PIO and the control HFD group (Table 2). There was no statistical difference in any of the lipid parameters between HFD-LOS and the control HFD group as well as between the HFD-PIO and HFD-LOS groups (Table 2).

Discussion

In the present study, the effect of losartan on lipid profile was evaluated on rats fed a high fat plus sucrose diet, in comparison with pioglitazone. For this purpose, 45 Sprague-Dawley rats of 5 weeks of age were randomized into three groups. All the rats in each group 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 developing the metabolic syndrome characterized by dyslipidemia, impaired glucose tolerance, insulin resistance and hypertension.[5]

Pioglitazone and losartan were given along with this diet to the rats in group HFD-PIO and HFD-LOS respectively, while group HFD was kept as control. At the end of 12 weeks, serum samples were obtained from all the animals and total cholesterol, HDL-cholesterol and triglyceride levels were obtained using kit method. LDL-cholesterol was determined using the Friedewald formula. At 12 weeks, mean serum cholesterol, LDL and triglyceride levels were higher and mean HDL level was lower in the control HFD group as compared to the HFD-PIO and HFD-LOS groups. Total cholesterol, triglyceride and LDL levels were found to be significantly less in the group of rats fed pioglitazone along with the high fat diet (HFD-PIO), as compared to the rats fed high fat diet alone (HFD).The rise in serum cholesterol, LDL & triglyceride levels and decrease in HDL cholesterol in rats fed on a high fat diet are consistent with effects of development of the metabolic syndrome in such animals.[5] Similarly, findings are also seen in humans fed a high fed diet.[18] The decrease in triglycerides, total cholesterol and LDL cholesterol by pioglitazone is also consistent with several studies on this drug. Thiazolidinediones, especially pioglitazone, have shown to have favorable effects on plasma lipids. Pioglitazone has shown in several studies to reduce triglyceride levels, total cholesterol and LDL cholesterol.[19]

Increase in HDL cholesterol however was not significant in the present study. The mechanisms by which pioglitazone produces these effects are highly complex and may involve anti-oxidant, anti-thrombotic, anti-inflammatory, anti-apoptotic and anti-infective properties, as well as effects on endothelial function and repair. These may be associated with their effects on peroxisome proliferator-activated receptor-gamma (PPARγ) receptors.[20] The present study showed a small but insignificant decrease in triglycerides, total cholesterol and LDL in rats fed a high fat diet plus losartan as compared to the rats fed on a high fat diet alone. HDL cholesterol also showed no significant change. Studies have shown variation in effects by different ARBs on lipid profile.[21] It may also have been because the present

study was a preventive study in which some of the rats developed diabetes and some did not when fed on a high fat diet alone (HFD) or with pioglitazone (HFD-PIO) or losartan (HFD-LOS).

Table 1: Body weight, fasting blood glucose and insulin levels of HFD fed rats at end of 12 week study period (n=45)

Groups	Body Weight (g)	p-value*	Fasting Blood Glucose (mg/dl)	p-value*	Serum Insulin (µIU/ml)	p-value*
HFD (mean±SD)	382 ± 48	---	152 ± 12	----	23.20 ± 5.52	----
HFD-PIO (mean±SD)	345 ± 45	≤ 0.05	123 ± 17	≤ 0.001	12.07 ± 6.82	≤ 0.001
HFD-LOS (mean±SD)	342 ± 38	≤ 0.05	132±17	≤ 0.001	14.13 ± 8.83	≤ 0.01

Table 2: Lipid profile of HFD fed rats at end of 12 week study period (n=45)

Groups	Total Cholesterol	p-value*	Triglycerides	p-value*	LDL	p-value*	HDL	p-value*
HFD (mean ± SD)	138.5±7.97	___	169.2±9.93	____	84.1 ± 7.17	____	20.6 ± 3.22	_____
HFD-PIO (mean ± SD)	125.1±14.25	< 0.01	157.1 ± 16.35	<0.05	69.3 ± 13.94	< 0.01	24.4 ± 3.60	> 0.05
HFD-LOS (mean ± SD)	131.3±15.18	> 0.05	162.3 ± 17.44	>0.05	76.3 ± 14.59	> 0.05	22.6 ± 3.94	>0.05

There was also no significant difference in effects on lipid profile parameters between rats fed on a high fat diet plus losartan and rats fed a high diet plus pioglitazone.

Conclusion

The topical Nifedipine is more effective in relieving the pain and healing as compared to oral form in CAF. Further research work on large scale is recommended to evaluate the oral and topical forms of Nifedipine for the treatment of CAF.

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