ORIGINAL ARTICLE

Effect of Losartan in Comparison with Pioglitazone on Weights of Liver &Visceral Adipose Tissue on a Rat Model of Type 2 Diabetes Mellitus

MUHAMMAD NAUMAN SHAD[1], AJAZ FATIMA[2], MUHAMMAD SAIR[3], *SADIA CHIRAGH[4], ZEESHAN AHMAD[5]*

ABSTRACT

Background: There is a strong link between type 2 diabetes mellitus (TD2M) and non-alcohol fatty liver disease (NAFLD). The common pathophysiological factor between them is increased insulin resistance. Other features of NAFLD include increased mass of liver and visceral adipose tissue. Objective of this study was toevaluate the beneficial role, if any,of losartan in comparison with pioglitazone onpreventing development of these features of NAFLD.

Methods: 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. 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. Body weight and fasting blood glucose levels were determined weekly. At the end of 12 weeks, serum insulin levels were determined.

Results: At the end of study periodmean body weight, fasting blood glucose, serum insulin and weight of liver had significantly lower levels in both experimental groups as compared to group HFD. Difference between group HFD-PIO and HFD-LOS was statistically insignificant for all the above parameters. However, mean weight of visceral fat had insignificantly lower levels in both the experimental groups as compared with the control group and difference between the experimental groups was also insignificant.

Conclusion: Both losartan and pioglitazone may reduce the progression towards insulin resistance and NAFLD.

Keywords: Losartan, Pioglitazone, NAFLD, TD2M

INTRODUCTION

Diabetes mellitus is one of the most common and deadliest chronic diseases. The number of deaths attributed worldwide to diabetes is increasing yearly especially in the higher age group. Diabetes accounted for 12.8% of global all-cause mortality among people aged 20–79 in 2015 and a global prevalence of 642 million is expected by 2040[1].

Type 2 diabetes mellitus accounts for 80-90% cases of diabetes worldwide. Obesity, certain type of diets as well as a sedentary life style play secondary roles in the development of type 2 diabetes mellitus[2]. Diminished insulin secretion and impaired insulin action complete the pathogenesis of type 2 diabetes mellitus.

Numerous studies have shown that a high fat, Diet, especially a diet rich in saturated fatty acids increases the risk of type 2 diabetes mellitus[3]. 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 and various other features of the metabolic syndrome.

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that includes fatty liver caused by other than alcohol. It is histologically and clinically different than alcohol related fatty liver disease. NAFLD may also progress to liver cirrhosis and in a few cases to hepatocellular cancer[4].

Insulin resistance has been described as the crucial pathophysiological factor in the development of NAFLD. Thus it is not surprising that there is a high incidence of NAFLD in patients with TD2M, that there is a strong correlation between NAFLD and consumption of high fat diet & between NAFLD and the metabolic syndrome.

Therefore owing to the prevalence and incidence of the TD2M, its complications and mortality rate & with it chances of developing NAFLD along with its complications, the primary aim should be to prevent or delay the onset of TD2M as well as

[1]*Associate Professor of Pharmacology, Sahara Medical College, Narowal*
[2]*Associate Professor of Pharmacology, Lahore Medical & Dental College, Lahore*
[3]*Assistant Professor of Pharmacology, Margalla Institute of Health Sciences, Rawalpindi*
[4]*Retired Professor of Department of Pharmacology, PGMI, Lahore*
[5]*PhD Research Scholar, Pharmacology, Faculty of Pharmacy, University of Sargodha, Sargodha*
Correspondence to Dr. Muhammad Nauman Shad
Email: mnauman2002saj@yahoo.com

NAFLD. Many strategies in the form of lifestyle modifications and/or medications are now being employed in patients who have a high risk of developing TD2M.

However many individuals are not able to accept life style changes and achieve the desired targets (like weight loss), so other interventions become necessary. Therefore pharmacological intervention for the prevention of type 2 diabetes is now generally recommended as a secondary intervention to follow or to be used in conjunction with modifications in lifestyle. Drugs that are currently being used for this purpose include the insulin sensitizers, metformin (a biguanide) and pioglitazone (a glitazone)[5]. Insulin sensitizers like pioglitazone have been studied for treatment of NAFLD[6].

Recently the renin angiotensin aldosterone system (RAAS) has also been found to be involved in pathogenesis of TD2M through increased insulin resistance[7]. This lead to the study of inhibitors of RAAS, including ACE Inhibitors and Angiotensin receptor blockers (ARBs), for the prevention and treatment of TD2M.

The present study was carried out to study the effect, if any, of losartan in comparison with pioglitazone on some parameters of NAFLD including weights of liver &visceraladipose tissue on a rat model of TD2M.

MATERIALS AND METHODS

The Diabetic Model: 45 Sprague-Dawley rats of 4 weeks of age were bought from the University of Veterinary & Animal Sciences in Lahore.They were kept under hygienic conditions in iron cases in the animal house of PGMI, Lahore. The experimental animals were given free access to rat chow and water. Under a natural day and night cycle, the room temperature was maintained at 25±2°C. One week was given for the animals to acclimatize. The rats were fed on high fat diet containing 30% beef fat and 10% sucrose[8] at 5 weeks of age.

Study Design: Animals were randomly divided into 3 groups of 15 rats each. Throughout the study period of 12 weeks, all three groups of rats were fed high fat and sucrose. The first group, labeled asHFD (high fat diet) group was given distilled water daily orally as a single morning dose. Every morning of the same study period, a second group was given pioglitazone in dose of 10mg/kg body weight[9]orally. This second group was labeled as HFD-PIO group. Similarly, every morning for 12 weeks, a third group labeled HFD-LOS, was given a second drug, losartan in dose of 10mg/kg body weight[10] daily.

Body Weight of Animals: Body weight of each rat was taken initially and after every week.

Fasting Blood Glucose: Using a drop of blood obtained from the tail vein, fasting blood glucose levels were measured every week using a glucometer (AccuChek).

Serum Insulin: At the end of 12 weeks, blood was collected by cardiac puncture[12] after keeping the rats on a 12 hour fast[11]. Blood samples were then centrifuged for 5 minutes at 3000-4000 rpm at room temperature. Finally, until serum could be analyzed for insulin, it was stored at –20°C [13]. The ELISA kit (NovaTecImmundiagnostica GmbH) was used to estimate fasting serum insulin levels.

Weight of Liver and Visceral Adipose Tissue: The animals were dissected after being sacrificed and their livers and visceral adipose tissues were separated and weighed individually.

Drugs: Pioglitazone and losartan were obtained from Mass Pharmaceuticals

Statistical Analysis: SPSS 20 was used to analyze the date after entering it. Mean ± S.D. values were determined for quantitative variables like body weight &blood glucose level. It was also calculated for insulin tolerance test as well as fasting serum insulin levels. To compare the above variables among the groups, one-way ANOVA was applied. Lastly, to observe which group means differed, the Post hoc Tukey's test was applied.

RESULTS

Body weight: The mean body weights of the rats at the beginning of the study were82±8, 79±7 and 81±5 g in group HFD, HFD-PIO and HFD-LOS respectively. The body weight increased in all groups over the 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).

Fasting Blood Glucose: The mean fasting blood glucose levels of the rats at the start of study were 92±9, 87±7 and 91±7 mg/dl in groups HFD, HFD-PIO and HFD-LOS respectively. Fasting blood glucose levels increased in all groups over the study period. At 12 weeks fasting blood glucose levels were significantly less in HFD-PIO and HFD-LOS groups 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: Serum insulin levels were measured at the end of 12 week study period. It was observed that levels were significantly lower in HFD-PIO and HFD-LOS groups as compared to that of HFD group with p-value 0.001 and 0.004 respectively. Difference

between HFD-PIO and HFD-LOS groups was not significant (Table: 1).

Weight of Liver and Visceral Adipose Tissue: Mean weight of livers was significantly lower in both experimental groups as compared to that of control.Difference between HFD-LOS and HFD-PIO groups was not significant. However mean weight of visceral adipose tissue showed no significant difference with the control group and with each other for the HFD-PIO and HFD-LOS groups. There was a decrease in the mean weight of visceral adipose tissue in both HFD-PIO as well as the HFD-LOS groups as compared to the control group, but it 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 Glucosemg/dl	Serum Insulin µlU/ml	Weight of Liver	Weight of Abdominal fat
HFD	382±48	152±12	23.20±5.52	14.55 ± 0.571	13.01 ± 0.438
HFD-PIO	345±45*	123±17***	12.07±6.82***	13.533 ± 0.847**	12.57 ± 0.745
HFD-LOS	342±38*	132±17***	14.13±8.83**	13.707 ± 0.911*	12.75 ± 0.897

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

DISCUSSION

In the current study, role of pioglitazone and losartan in preventing development of features of NAFLD, including weight of liver and abdominal fat in rats fed on a high fat diet, was studied. 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[15]. 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, serum insulin was determined by ELISA method.

At the start of the study, the mean body weight of the rats 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 TD2M, 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[16].

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[10].

Serum insulin level was measured at the end of study period (12 week) and it was found to be significantly raised in HFD group as compared to HFD-PIO and HFD-LOS groups. Fasting serum insulin levelswere raised that indicated insulin resistance. This is in line with an early stage of type 2 diabetes[17]. In many models of type 2 diabetes due to high fat diet, hyperinsulinemia with fasting and basal hyperglycemia is a common feature[18]. 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[19, 20].

Mean weight of livers was significantly low in both experimental groups as compared to that of control.Difference between HFD-LOS and HFD-PIO was not significant. However mean weight of visceral adipose tissue showed no significant difference with the control group and with each other for the HFD-PIO and HFD-LOS groups. There was a decrease in the mean weights in both HFD-PIO as well as the HFD-LOS groups as compared to the control group, but it was not significant.

This study thus indicates the development and or progression towards NAFLD, as shown by the rise in mean body weight, blood glucose, serum insulin as well as weight of the liver and visceral adipose tissue of the rats in the control group fed on a high fat diet only. This rise was significantly reduced for all these parameters, except mean weight of visceral adipose tissue, with both pioglitazone and losartan fed rats.

A possible significant reduction in the mean weight of visceral adipose tissue may have occurred in a longer study. This was a preventive study with most rats in the control developing diabetes mellitus with less number developing diabetes in the HFD-PIO and HFD-LOS groups[21].

The liver is a major organ involved in glucose metabolism and thus contributes tremendously to the development of insulin resistance and type 2 diabetes mellitus (T2DM). The mechanisms include accumulation of liver fat, alteration of energy metabolism and inflammatory signals derived from

various cell types including immune cells. Lipotoxins, mitochondrial function, cytokines and adipokinesproduced by the adipose tissue, including adiponectin and leptin, have been proposed to play a major part in the development of both NAFLD and T2DM. Commonly, patients with NAFLD are resistant to insulin[22].

Probable mechanisms of improvement of insulin resistance by losartan may be decrease in adiponectin levels as suggested by studies conducted on human subjects[20,23].

The vasodilation produced by of ACE inhibitors and ARBs improves skeletal muscle blood flow. Consequently insulin and glucose delivery to the skeletal muscles increases. The surface area for glucose exchange between the vascular beds and skeletal muscles is also improved. It may be important mechanism by which inhibition of the RAAS improves glucose uptake and metabolism in insulin-sensitive tissues[17, 24].

ARBs induce the expression of the glucose transporter GLUT4, thus increasingglucose uptake and decreasing insulin resistance in skeletal muscle tissue[25, 26].

Another possible mechanism is through activation of PPAR-γ activation. Losartan[27] and telmisartan[28]have shown to increase PPAR-γ expression and PPAR-γ improvesinsulin sensitivity by translocating GLUT 4 to the plasma membrane in the skeletal muscle.

CONCLUSION

Both losartan and pioglitazone may reduce the progression towards insulin resistance and NAFLD.

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