PREVENTION OF DEVELOPMENT OF TYPE 2 DIABETES MELLITUS BY LOSARTAN IN COMPARISON WITH PIOGLITAZONE IN A RAT MODEL

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ABSTRACT

Background: Due to the increased incidence and prevalence of type 2 diabetes mellitus, there is increased need for the search of drugs that may be useful in preventing/delaying the onset of this disease.

Objective: To compare the preventive effects of losartan and pioglitazone on the development of type 2 diabetes mellitus in a rat model fed on a high fat diet.

Methods: Study was conducted on 45 Sprague-Dawley rats divided into three randomized groups. All the rats were fed a high fat diet meant for inducing type 2 diabetes. Pioglitazone or losartan was given to the rats in group B and C respectively. Fasting blood glucose levels and body weight were determined weekly. At the end of the 12 week study period, Serum Insulin and CRP (C-Reactive Protein) levels were also determined.

Results: Rats in groups B and C gained less weight and had lower fasting BSL than those in control group A. Results show that the mean serum insulin and CRP levels were significantly low in group B and C as compared to those in group A while difference between group B and C was statistically insignificant. The number of diabetic rats in group B and C was significantly lower as compared to group A at the end of study. Number of diabetic rats remained constant in pioglitazone treated rats after 10th week of study, while it kept on increasing in losartan treated group.

Conclusion: Pioglitazone delays and prevents development of type 2 diabetes while losartan only has a delaying effect.

Key Words: Losartan, Pioglitazone, Prevention of Type 2 Diabetes, Serum Insulin, CRP

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].

There is now substantial evidence that progression to type 2 diabetes can be prevented or delayed in high risk patients by both behavioral interventions like promoting weight loss, increased physical activity , dietary modification and pharmacological interventions including metformin, glitazones, acarbose and orlistat[2].However, these pharmacological interventions are not without their side effects such as gastrointestinal distress with the use of metformin, acarbose and orlistatand weight gain and edema associated with thiazolidinones. These adverse effects result in poor compliance, a problem faced in the DPP and DREAM trials where many subjects failed to fully adhere to the drug regimendue to poor tolerability to drugs like metformin[3] and rosiglitazone[4]. Hence the search for safe and effective alternate drugs including ACE inhibitors and ARBs continues.

The renin angiotensin aldosterone system (RAAS) is the hormonal cascade that functions in the homeostatic control of blood pressure, tissue perfusion and extracellular volume. Any disturbance in this system plays an important role in the pathogenesis of cardiovascular and renal disorders. The RAAS is initiated by the regulated secretion of renin by the juxtaglomerular cells of the kidneys.Renin catalyzes the initial, rate limiting step of the RAAS cascade by the hydrolysis of angiotensinogen to angiotensin 1, which is then hydrolyzed by angiotensin converting enzyme(ACE) to angiotensin 2[5]. Angiotensin 2 exerts most of its physiological actions after binding to and stimulating angiotensin2 type 1 (AT1) receptors. The frequent association of diabetes mellitus with hypertension, retinopathy, nephropathy and cardiovascular disease has implicated the RAAS in the initiation and progression of all these disorders. Elevated levels of aldosterone are directly involved in the pathogenesis of insulin resistance[6]. It has also been shown that angiotensin 2 can cause insulin resistance by interfering with the insulin stimulated increase in insulin receptor substrate 1 (IRS-1) associated phosphatidyl inositol kinase activity, which is the

molecular signal transduction mechanism of insulin involved in glucose transport and metabolic effects, whichis decreased in diabetics[7]. Angiotensin 2 impairs insulin biosynthesis and promotes beta cell apoptosis possibly due to long term vasoconstriction induced restricted blood flow to pancreas[8].

Currently many studies are being conducted to clarify the extent to which inhibition of the RAAS can reduce the incidence of diabetes in high risk individuals. One noticeable study was conducted on genetically induced diabetic mice to determine the effect of an ARB, losartan on beta cell function and glucose intolerance. Losartan improved glucose induced insulin release and insulin synthesis from pancreas. It also delayed the onset of diabetes and improved glucose tolerance but had no effect on insulin sensitivity of peripheral tissues[9].

MATERIALS AND METHODS

45 Sprague-Dawley rats, aged 4 weeks were purchased from the University of Veterinary Animal Sciences, Lahore. Rats were kept in the animal house of Post graduate Medical Institute, Lahore in iron cages under hygienic conditions. Room temperature was maintained at 23±2°C under natural day/night cycle. Rat chow and water was provided *ad libitum*. Animals were allowed one week to acclimatize and were divided into three groups of 15 rats each, using balloting method.

Induction of Obesity and Type 2 Diabetes Mellitus: After one week of acclimatization the rats were fed on high fat and sucrose diet consisting of (by weight)

Beef fat 30%
Sucrose 10%
Normal rat chow[9] 60%

Sodium cholate (10 g/kg) was added to increase the intestinal absorption of fat.

Drugs: pioglitazone and losartan were obtained in pure generic form from Mass Pharmaceuticals and administered to the rats through a gastric tube.100 mg of both pioglitazone and losartan were dissolved in 10 ml of distilled water so 1 ml contained 10 mg of drug.

Group A:0.5 ml of distilled water by gavage was given as a single morning dose for 12 weeks.

Group B: pioglitazone in dose of 10mg/kg body weight[9] by gavage daily as a single morning dose for 12 weeks.

Group C: losartan was given in dose of 10 mg/kg body weight[10] by gavage daily as a single morning dose for 12 weeks.

Parameters:

Body Weight of Rats: Each rat was weighed at the start of study and weekly thereafter.

Blood Glucose Level: Fasting blood glucose level was measured every week using a glucometer (AccuChek®) using a drop of blood obtained from the tail vein[11].

Serum Insulin and C- Reactive Protein:

At the end of the study, rats were kept on 12 hour fast[12] and blood was collected by cardiac puncture[13]. Samples were then centrifuged at room temperature at 3000-4000 rpm for 5 minutes. Serum was stored at -20°Cuntil being analyzed. Seruminsulin was estimated using insulin ELISA (enzyme-linked immunosorbent assay) kit (Nova Tec Immundiagnostica GmbH). Serum C-Reactive protein (CRP) was estimated using a CRP slide test (Analyticon Biotechnologies AG).

Statistical Analysis: The data was analyzed using SPSS 17.0.Mean ± SD were calculated for body weight, fasting BSL, insulin and CRP. Graphs were plotted and one way ANOVA was applied to compare the above variables among groups. *Post hoc*Tukey's test was applied to observe which groupmean differs. Chi square test was applied to compare the number of rats developing diabetes. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Body Weight: Mean weight of animals at the start of study was around 80 grams. It was increased in all groups over the 12 week study period and the mean body weight at the end of study was 382± 48,345± 45,342 ±38 grams in group A, B and C respectively. Rats in group Band C showed significantly lower weight gain in comparison to the rats in group A(37g and 40g with p-values 0.047 and 0.029 respectively). Difference in weight gain between groups Band C was insignificant (Fig 1).

Fasting Blood Glucose Level: Mean fasting blood glucose level of rats at the start of study was around 90 mg/dl. At the end of study period i.e. at 12 week mean fasting blood glucose level was152± 12,123± 17,132± 17mg/dl in groups A, B and C respectively. Fasting blood glucose level at the end of study in groups B and C was significantly lower than that of group A(p-values 0.001 and 0.004 respectively).Difference between groups Band C was insignificant.

When expressed as percentage mean blood glucose level of group Band C was 19% and 13% less than that of group A.

Serum Insulin: Serum insulin level in all the groups was measured at the end of study period i.e.12 weeks and it was observed that the mean serum insulin level in group B and C was low as compared to that of group A. The difference between group A and B was significant

with p-value less than 0.001 and between A and C withp-value 0.004,whereas the difference between group B and C was insignificant with p-value 0.71 (table 1).

C-Reactive Protein: in all the study groups CRP level was measured at the end of 12 week and it was observed that the mean CRP level in group B and C was low as compared to that of group A. The difference between group A and B was significant with p-value 0.001 and between A and C with p- value 0.035, whereas the difference between group Band C was insignificant with p-value 0.439 (table 1).

Pattern of Development of Diabetes over 12 Week Period:

With cut off value of fasting BSL ≥126 mg/dl, diabetes started appearing in group A in the 7th week with one rat showing 126 mg/dland the number increased to 14 rats by the end of 12 weeks. On the other hand, in group B and C diabetes appeared in8th week affecting 1 rat in group B and 3 rats in group C. In group B, number of diabetic rats increased to 4 till the end of study period. In group C this number increased to 8 by the end of study period (table 2).

Number of Diabetic Rats at the End of Study by Different Parameters:

The number of diabetic rats in group B and C was significantly lower as compared to group A when measured by different parameters. Table 3 shows the number of diabetic rats at the end of study by different parameters.

Tables & Figures:

Table 1: Effect of pioglitazone and losartan administration on serum insulin and C-reactive protein levels (mean ± SD) at end of 12 week study period in rats (n=15) fed on high fat diet.

Parameter	Group		
	Positive control (A)	Pioglitazone (B)	Losartan (C)
Serum insulin µ IU/ml	23.20 ± 5.52	12.07 ± 6.82 ***	14.13 ± 8.83 **
C-reactive protein mg/l	9.46 ± 1.78	6.43 ± 2.22 ***	7.41 ± 2.45 *

*** p-value ≤ 0.001 B vs. A, **p-value ≤ 0.01 C vs. A, * p-value ≤ 0.05 C vs. A

Table 2: Number of rats with fasting blood sugar level ≥ 126 mg/dl in positive control, pioglitazone and losartan treated groups of high fat fed rats (n=15) over 12 week study period.

Week	**Group**		
	Positive control (A)	**Pioglitazone (B)**	**Losartan (C)**
0	0	0	0
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	1	0	0
8	5	1	3
9	11	3	5
10	14	4	6
11	14	4	6
12	14	4	8

Table 3: Number of diabetic rats at end of 12 week study period inpositive control, pioglitazone and losartan treated groups of high fat fed rats (n=15)confirmed by different parameters.

Parameter	Group		
	Positive control (A)	Pioglitazone (B)	Losartan (C)
Fasting BSL ≥126mg/dl	14	4***	8**
Fasting BSL ≥144mg/dl	14	4***	6***
Serum insulin ≥11 µ IU/ml	14	4***	6***
CRP ≥ 6 mg/l	14	4***	6***

*** p-value ≤ 0.001 B and C vs. A, **p-value ≤ 0.01 C vs. A

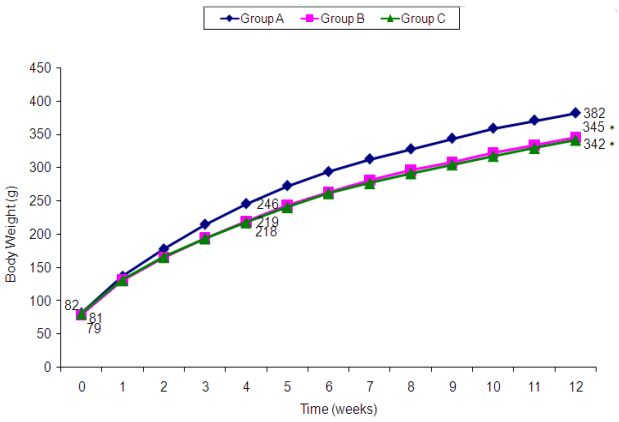
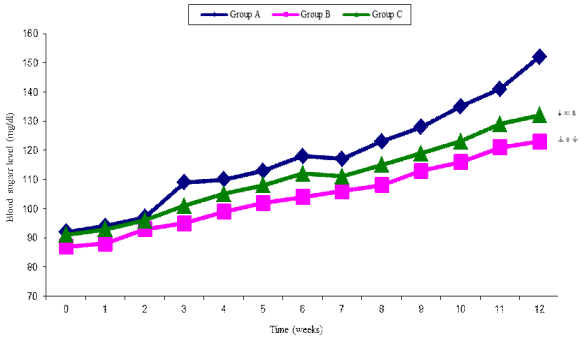


Fig 1: Effect of pioglitazone and losartan on mean body weight of rats (n=15) fed on high fat diet over 12 week study period.
* p-value ≤ 0.05 B and C vs. A

Group A: Positive control, Group B : Pioglitazone, Group C : Losartan

Fig 2: Effect of pioglitazone and losartan on mean fasting blood sugar level of rats (n=15) fed on high fat diet over 12 week study period.



*** p-value ≤ 0.001 B and C vs. A
Group A: Positive control, Group B : Pioglitazone, Group C : Losartan

DISCUSSION

Type 2 diabetes and other non-communicable diseases (NCD) are agrowing public health challenge globally. Multiple factors including genetic predisposition, insulin resistance, increased insulin secretory demand, glucotoxicity, lipotoxicity, impaired incretin release/action, amylin accumulation, and decreased beta cell massplay a causative role in progressive beta cell dysfunction that is characteristic of prediabetes. Interventions preventing progression to type 2 diabetes should therefore delay or prevent beta cell failure[14].

It has been estimated that at the time of clinical diagnosis of type 2 diabetes only 50 - 60% of the pancreatic beta cell capacity remains because of the fact that the disease process has already existed for more than 10 years. The complications of diabetes like retinopathy, nephropathy, peripheral vascular disease, nephropathy and the socioeconomic costs emphasize the need for immediate actions for the prevention of disease. Therefore, it is rational to reduce the increased burden of type 2 diabetes by either preventing or delaying its onset[15].

The present study was conducted to determine the preventive effect, if any, of an ARB losartan on the development of type 2 diabetes mellitus in a rat model and to compare it with one of the thiazolidinedione, pioglitazone which is already recommended for use in diabetics.

Rats were fed on high fat and sucrose diet. Such a rat model closely resembles type 2 diabetes, in contrast to diabetes induced by drugs like streptozocin and alloxan and such a model is also considered as the best model to study the human metabolic syndrome[16].

The parameters that were used to confirm the results in this study were body weight, fasting blood glucose level, fasting insulin level, CRP and number of rats that developed diabetes during the study period.All the parameters in controlgroup (A) were high as compared to experimental groups (B and C). Increasedserum insulin and CRP levels are indicators of type 2 diabetes[17].

When number of diabetic rats was compared, it was observed that both pioglitazone and losartan delayed the onset of diabetes. Number of diabetic rats in pioglitazone group remained same for rest of study period, while in losartan group the number kept on increasing.

Various studies have been carried out to investigatethe reduction in the incidence of diabetes with use of thiazolidinedione[18]; in the DREAM study rosiglitazone reduced the incidence of diabetes by 60%. However, use of rosiglitazone is now being restricted due to concern about its cardiovascular safety. The ACT NOW study on pioglitazone showed a significant 72% decrease in incidence of diabetes. However pioglitazone was associated with significant weight gain and edema in this study[19].

The beneficial effects of ARBs and ACE Inhibitors in the prevention of micro vascular and macro vascular complications of diabetes mellitus are well established. Treatment with ARBs has a vasculoprotective effect as shown in the LIFE study in which losartan was associated with significant reductions in MI, stroke and cardiovascular mortality as compared to atenolol in diabetic patients. The LIFE study also showed that the risk of new-onset diabetes was reduced by 26% with losartan compared to atenolol in hypertensive non-diabetic patients[20]. Mortality and Morbidity study (CHARM) showed that ARB therapy was associated with an overall 22% reduction in development of diabetes as compared to placebo in patients with heart failure[21]. Data from the ONTARGET trial conducted on patients with cardiovascular disease showed similar rates of incident diabetes with ACE Inhibitor or ARB therapy[22]. The NAVIGATOR study showed that administration of a single daily dose of valsartan reduced the risk of diabetes but not of cardiovascular events in patients with impaired glucose tolerance and established cardiovascular disease or risk factors. The relative reduction of 14% in the risk of diabetes in the valsartan translates to 38 fewer case of diabetes per 1000 patients treated for five years[23].

In these studies prevention of diabetes was not the primary end point. There is marked variation in human population and apart from pharmacological interventions other measures were also taken to improve glycemic control. The incidence of diabetes was the primary outcome of the present study, individual variation was kept to a minimum and technique involved the earliest possible intervention strategy in the prevention of type 2 diabetes. The results of the present study showed a 66.6% reduction in the incidence of type 2 diabetes in the pioglitazone fed rats and a 53% reduction in the incidence of type 2 diabetes in the losartan fed rats. The reduced incidence of diabetes in the pioglitazone fed rats was comparable with human studies that showed a 50-70% reduction in incidence of diabetes. However, the present study showed a higher reduction in the incidence of diabetes in the ARB (losartan) fed rats as compared to human studies involving ARBs for the prevention of type 2 diabetes. This study was stopped at a point when number of diabetic rats was still increasing in losartan group, had it been continued further the picture would have been more elucidating.

The peripheral vasodilatory actions of ACE inhibitors and ARBs lead to an improvement in skeletal muscle blood flow, the primary target for insulin action and an important determinant of glucose uptake. This increases the surface area for glucose exchange between the vascular bed and skeletal muscles, which has been reported to increase insulin sensitivity. Thesedrugs also increase blood flow to the pancreatic islet cells, thus increasing insulin secretion.

Protection against new onset diabetes may also be related to adipocyte function. High levels of angiotensin II are associated with reduced differentiation of pre-adipocytes into mature adipocytes. This impairs cells' ability to store fat, which in turn shunts fat to skeletal muscles and liver, worsening insulin resistance. Reducing levels of angiotensin II or blocking angiotensin II receptors by ARBs will rectify this effect. In addition redistribution of lipids from peripheral tissues will increase insulin sensitivity[25].

CONCLUSION

These results show that pioglitazone has delaying as well as preventive effect in development of type 2 diabetes while losartan has only delaying effect. Had the study been continued the preventive role of losartan, if any, would have been observed.

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