COMPARATIVE STUDY OF TELMISARTAN WITH PIOGLITAZONE ON INSULIN RESISTANCE IN TYPE 2 DIABETIC MICE

Asma Khan, Bushra Tayyaba Khan*, Aisha Qayyum

Fazaia Medical College Islamabad Pakistan,*Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To evaluate and compare the effects of telmisartan and pioglitazone on peripheral insulin resistance in diabetic mice.

Study Design: Randomized control trail.

Place and Duration of Study: National Institute of Health, Islamabad and pharmacology dept, Army Medical College, from 17th March to 17th June 2014.

Material and Methods: Twenty four BALB/c mice, both male and female, of 35 to 40 grams were used for this study. Animals were randomly divided into four groups. Two were taken as control groups, one was normal control and the other was diabetic control. Two were taken as interventional groups and received either pioglitazone or telmisartanfor four weeks after induction of diabetes.

Results: After treatment, pioglitazone reduced all the biochemical parameters significantly when compared with diabetic control. Negative correlation between glucose and insulin was changed into positive correlation (r-value, 0.92) with significant *p*-value (0.015) in pioglitazone treated group, while telmisartan only managed to convert a negative correlation between insulin and glucose into statistically non-significant positive.

Conclusion: Telmisartan although reduces glucose levels and improves beta cell mass but the effect is statistically non-significant as compared to pioglitazone. In hypertensive type 2 diabetics a combination of these two drugs may help in reducing the dose of pioglitazone and consequently the cardiovascular adverse effects of pioglitazone.

Keywords: Diabetes mellitus, Peroxisome proliferator activated receptor gamma (PPAR gamma), Pioglitazone, Telmisartan, Renin angiotensin system (RAS).

INTRODUCTION

Globally over the past two decades, type 2 diabetes has become more prevalent than before. Sedentary life style and obesity could be one of the attributing factors as obesity has a strong link to endanger insulin resistance. Raised peripheral insulin resistance along with insulin deficiency can lead to diabetes[1]. Insulin resistance also has strong impact on other chronic ailments including hypertension, hyperlipidaemia, and atherosclerosis. Data reveals that 70% of diabetics develop hypertension during the course of disease and hypertension is twice more common in diabetics as compared to non-diabetics vice versa. Recently it has been suggested that renin angiotensin system (RAS) is implicated in the development of type 2 diabetes and interruption of RAS may improve the glycaemic control in type 2 diabetics[2]. This reduction in insulin resistance with better long term course of the disease is through complex mechanisms. These mechanism include both haemodynamic and non-haemodynamic elements. Telmisartan, an angiotensin type 1 (ATI) receptor blocker, reportedly has beneficial effects on glycemic control more than other ATI receptor blockers and this unique feature of telmisartan can be due to its peroxisome proliferator activated receptors (PPARγ) agonistic activity[3].

PPAR are group of nuclear hormone receptors with ligand binding transcription factors. They play an essential role in the metabolism of fats and lipids on activation. They

Correspondence: Dr Asma Khan, Senior Lecture Fazaia Medical College House No. 181 Lane No 4 Askari 9, Chaklala Rawalpindi Pakistan (*Email: asmazeb26@yahoo.com)*

Received: 25 Jan 2016; revised received: 18 Mar 2016; accepted: 16 May 2016

also determine sensitivity of insulin in peripheral tissues especially those which are being involved in the glucose homeostasis like adipose tissue and skeletal muscles[4].

Thiazolidinedione are PPARγ agonists and by their action they improve glycaemic control in type 2 Diabetes mellitus (DM). Pioglitazone is one of the thiazolidinedione and it reduces insulin resistance by acting on peripheral tissues including liver, skeletal muscles and adipose tissues and increase uptake of glucose through activation of PPARγ[5]. Though thiazolidinediones provide good glycaemic control in type 2 diabetics, there is association of cardiovascular complications along with their administration due to fluid retention and adipogenesis[6]. This study was designed to evaluate the properties of telmisartan as partial PPARγ agonist, its effect on insulin resistance and to compare its anti-diabetic effect with pioglitazone.

MATERIAL AND METHODS

It was a randomized control trail. The study was performed using facilities of animal house of National Institute of Health, Islamabad. Biochemical parameters were estimated in the department of chemical pathology, Army Medical College Rawalpindi. Conventional guidelines for animal experimentation (NIH Publication No. 85-23, revised 1996) were followed for all procedures.

Ethics committee of centre for research in experimental and applied medicine (CREAM) Army Medical College, Rawalpindi gave approval for this study. Total duration of this study was of twelve weeks after initial acclimatization of one week. Streptozocin used in this study was purchased from Sigma Chemicals USA. Telmisartan and pioglitazone were gifted generously from werrick pharmaceuticals Pakistan. Standard laboratory conditions were maintained in animal house of National Institute of Health, Islamabad.

Twenty four BALB/c mice, both male and female, of 35 to 40 grams were used for this study. Animals were selected through nonprobability convenience sampling and then divided by lottery method into four groups. A model resembling type 2 diabetes in humans with a combination of insulin resistance and insulin deficiency was developed with administration of high fat diet consisted of 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal[7] for two weeks prior to streptozocin injection. They were weighed initially and also after two weeks of high fat diet and non-significant rise in weight was observed.After initial 2 weeks of dietary manipulation, all mice except for group-1 (normal control), received five injections of low dose of STZ (35 mg/ kg) intra-peritoneally, on

Table-I: Baseline and pretreatment biochemical parameters of all groups.

Para-meters	Normal control(GroupI) (Mean ±SD)			Diabetic control (Group II)(Mean ±SD)			Diabetic with Pioglitazne(Group III)(Mean ±SD)			Diabetic with Telmisartan (Group IV)(Mean ±SD)		
	Baseline	Pre.T	*p*-value	Baseline	Pre.T	*p*-value	Baseline	Pre.T	*p*-value	Baseline	Pre.T	*p*-value
Glucose (mg/dl)	114.83 ± 6.67	108.33 ± 9	0.23	116.33 ± 8.4	298.33 ± 25.12	< 0.001	114.67 ± 6.24	299 ± 21.31	< 0.001	115.83 ± 6.8	293.33 ± 23.18	< 0.001
Insulin (µIU/ml)	0.16 ± 0.02	0.15 ± 0.02	0.73	0.16 ± 0.04	0.17 ± 0.03	0.697	0.16 ± 0.04	0.17 ± 0.03	0.55	0.14 ± 0.02	0.17 ± 0.03	0.07
Homa IR	0.04 ± 0.005	0.04 ± 0.008	0.6	0.05 ± 0.02	0.12 ± 0.014	0.001	0.04 ± 0.015	0.12 ± 0.02	< 0.001	0.04 ± 0.009	0.12 ± 0.013	< 0.001
Homa β (%)	108.04 ± 6.07	122.26 ± 12.33	0.08	105.03 ± 11.15	26.18 ± 7.9	< 0.001	109.3 ± 15.7	25.98 ± 7.9	< 0.001	94.08 ± 6.47	26.51 ± 5.6	< 0.001

- *p*-value is significant < 0.05*,*p* value is highly significant < 0.01** and calculated with baseline levels. All value are expressed as means ± SD (Standard deviation)
- Pre treatment = Pre.T

five consecutive days[8]. Fasting glucose levels started rising after one week of fifth streptozocin injection and mice with fasting blood glucose levels more than 250 mg/dl were considered as diabetic[9]. Once they were declared diabetic, they were separated and divided into three groups with six mice in each group. From this point on, all groups received normal pallet diet and water ad *libitum* for the rest of study. One group of these diabetic mice, group II was taken as diabetic control and did not receive any drug for the rest of the study. Group III received pioglitazone (15.4 mg/kg)[10] for four weeks through oral gavage after suspending it in vegetable oil. Group IV received telmisartan (8 mg/kg)[11] suspended in vegetable oil once daily through oral gavage for four weeks. This dose was selected for telmisartan because previous studies showed that at this dose glucose utilization in response to insulin was improved and also insulin secretion was decreased which indicated better sensitization to the insulin in peripheral tissues[11]. Both control groups also received the vehicle. Baseline blood samples were collected initially after one week of acclimatization of animals through tail vein in un-anaesthetized state. After diabetic model was prepared, blood sample was again collected for all parameters. Terminal cardiac sampling was done after four weeks of drug intervention.

For estimation of fasting blood glucose levels, samples were collected after a fast of six hours. Fasting blood glucose levels were estimated by GOD-PAP enzymatic method using auto analyser.

For analysis of fasting insulin levels, samples were collected at six hours fast and stored at -80°C. Plasma insulin levels were measured by ELISA method using Rat Insulin Elisa Kit[12].

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin concentration using the formula; Fasting blood glucose (mg/dl) × fasting insulin (µIU/ml) /405[13].

Percentage of functional beta cell mass (HOMA-β)[14] was measured by using equation: 360×Insulin / Glucose −63.

The results of the serum analysis were expressed as mean±standard error of mean (SEM). Arithmetic means and S.E.M were calculated using SPSS version 22. Level of significance between baseline, pretreatment and post treatment levels was determined by applying paired sample "t" test and for multiple comparisons between groups, one way ANOVA followed by Post hoc Tukey Test was applied. Pearson correlation was also determined between fasting glucose and insulin levels. A *p*value <0.05 was taken as significant.

Table-II: Post treatment biochemical parameters of all groups.

Parameters	Normal Control (group I) ± S.E.M	Diabetic Control (group II) ± S.E.M	Diabetic with pioglitazone (groupIII) ± S.E.M	Diabetic with telmisartan (group IV) ± S.E.M
Glucose (mg/dl)	108.33±1.84 (1)	301.33±8.39(0.36)	182.67±5.54(< 0.001**)	289.17±5.26(0.74)
Insulin (µIU/ ml)	0.15±0.005 (0.9)	0.16±0.007(0.4)	0.14±0.006(0.04*)	0.15±0.012(0.18)
Homa IR	0.04±0.002 (1)	0.12±0.003(0.4)	0.06±0.005(< 0.001**)	0.11±0.012(0.2)
Homa β (%)	122.01±1.64 (0.97)	24.24±1.94(0.36)	41.68±0.83(0.006**)	24.15±1.08(0.3)

p-value is significant < 0.05*, *p*-value is highly significant < 0.01** and calculated with pretreatment levels. All value are expressed as means ± S.E.M.

RESULTS

When we compared twenty four mice at baseline, there was non-significant difference among the groups regarding all parameters and the correlation between glucose and insulin was positive and significant (*r* value 0.851, *p* value 0.032). After diabetic model was developed, we compared the baseline parameters of each group with its pretreatment levels, it was found that

there was non-significant difference in group I while highly significant difference in other three groups as shown in table-I. Similarly there was highly significant difference between the three diabetic groups and group I (normal control) regarding all parameters (*p* value <0.001), except for insulin levels which showed non-significant difference with group I (normal control) and that could be explained with the relative deficiency of insulin due to destruction of pancreatic β cells induced by streptozotocin. At this stage, table-II and fig-1. Correlation between glucose and insulin in this group changed from strong negative to strong positive (*r* value 0.92, *p* value 0.02). Telmisartan though reduced glucose and insulin resistance and increased pancreatic β cell mass but statistically it was not significant. Correlational analysis in this group revealed a change from strong negative to non-significantly positive correlation (*r* value 0.43, *p*-value 0.16) as shown in fig-2. In this fig-2 the upward fit line showed that the values of insulin and glucose

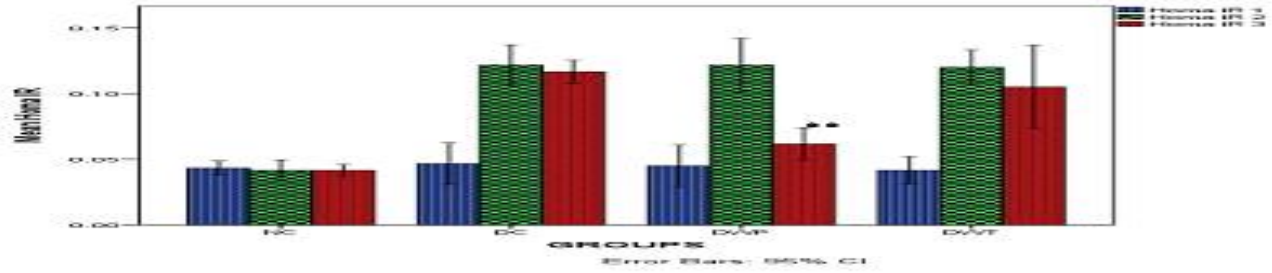

Figure-1: Comparison of Homa IR levels in all groups.

Homa IR 1=baseline levels, Homa IR 2=pre-treatment levels in all diabetic groups, Homa IR 3=post treatment levels. NC=Normal control, DC=Diabetic control, DWP=Diabetic with pioglitazone, DWT=Diabetic with telmisartan,(*p*-value is significant <0.05*, *p*-value is highly significant <0.01**).

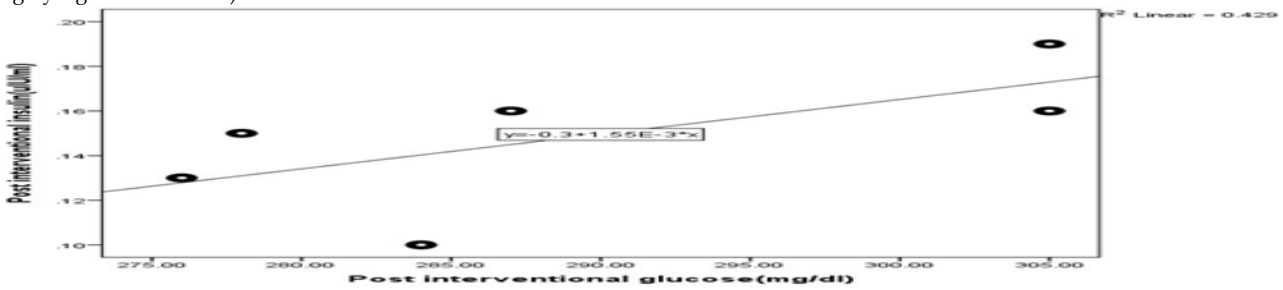

Figure-2: Scatter diagrams for Pearson correlation between glucose and insulin levels in Telmisartan treated group (Group IV).

R^2 Linear describes the % variability in insulin values with unit change of glucose levels.

correlation between glucose and insulin became negative with significant *p* value in all diabetic groups (*r* value -0.905, *p* value 0.013). After administering pioglitazone and telmisartan to group III and IV (diabetic groups) for four weeks respectively, the parameters were compared among both pre and post treatment groups. It was found that with the treatment of pioglitazone, all parameters had changed significantly with *p* values < 0.001 for fasting glucose and Homa IR while 0.006 and 0.04 for homa b and insulin respectively as shown in had become directly proportional to each other after administering telmisartan for four weeks which meant that with unit rise in glucose, there was proportional rise in secretion of insulin with positive *r* value.In diabetic control group and in the telmisrtan treated group, prior to intervention, *r* value was significantly negative and the conversion to positive *r* value supports the anti-diabetic properties of telmisartan.

Multiple comparisons between groups after intervention with pioglitazone and telmisartan,

showed that there was highly significant difference with regard to plasma glucose levels, insulin resistance and percentage of functional pancreatic β cell mass with *p* values less than 0.001 while non-significant difference between group II and group IV (*p* value 0.458, *p* value 0.633, *p*value 1) which showed weak anti diabetic properties of telmisartan.

DISCUSSION

Hyperinsulinemia and hypertension are the two components of cardio metabolic syndrome that integrate closely and have a frequent co-occurrence in genetically predisposed individuals. Insulin resistant states can predispose to hypertension through effects of insulin on tissues and vasculature, and also through its actions on sympathetic nervous system[15]. It has emerged as a consensus over the past few years that overstimulation of RAS is linked to the development of diabetes. Besides RAS also plays a central role in the pathogenesis of essential hypertension. Angiotensin receptor blockers (ARBs) are considered as an important class of antihypertensive drugs. In this study we evaluated the anti-diabetic properties of telmisartan and compared it with pre-treatment levels and also with pioglitazone which is a known insulin sensitizer.

Telmisartan was selected because of its partial PPARγ agonist properties and was considered superior amongst ARBs to improve peripheral insulin resistance[16] as shown in the study done by Li L and his collegues. Telmisartan can improve glucose intolerance even through mechanisms independent of its PPAR γ agonistic properties as show by the work done by Shiota and his collegues[17]. After treatment, it was revealed that pioglitazone can effectively reduce blood glucose levels and reduce insulin resistance in peripheral tissues significantly and it was consistent with the work done by Rosenblatt and his fellows[18]. There was significant difference between its pretreatment levels and also with the control diabetic group. Also it had changed the correlation between glucose and insulin from negative to positive significantly which means that the relative deficiency of insulin with increased levels of glucose had been compared with its administration. Telmisartan reduced fasting plasma glucose levels and reduced peripheral resistance as shown by Chu and his colleagues[19], but it was insignificant when compared with control diabetic and pioglitazone treated groups. However the ability of telmisartan to change negative correlation between glucose and insulin into a positive correlation reflected positively on its ability as an anti-diabetic agent.

CONCLUSION

Telmisartan although reduces glucose levels and improves beta cell mass but the effect is statistically non-significant as compared to pioglitazone.In hypertensive Type 2 diabetics a combination of these two drugs may help in reducing the dose of pioglitazone and consequently the cardiovascular adverse effects of pioglitazone.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Leung PS, Gasparo M. Involvement of the Pancreatic Renin-angiotensin system in insulin resistance and the metabolic syndrome. J Cardiometab Syndr. 2006;1(3):197-203.
2. Putnam K, Shoemaker R, Yiannikouris F, Cassis LA. The renin-angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. Am J Physiol Heart Circ Physiol. 2012;302(6):H1219-30.
3. Deppe S, Böger RH, Weiss J, Benndorf RA. Telmisartan: a review of its pharmacodynamic and pharmacokinetic properties. Expert Opin Drug Metab Toxicol. 2010;6(7):863-71.
4. Boulanger H, Mansouri R, Gautier JF, Glotz D. Are peroxisome proliferator-activated receptors new therapeutic targets in diabetic and non-diabetic nephropathies? Nephrol Dial Transpl. 2006; 21(10): 2696-702.
5. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. Diabetes Care. 2000;23(11):1605-11.
6. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, et al. Thiazolidinedione use, fluid retention, and congestive heart failure a consensus statement from the american heart association and american diabetes association. Circulation. 2003; 108(23): 2941-8.

7. Gilbert ER, Fu Z, Liu D. Development of a nongenetic mouse model of type 2 diabetes. Exp Diabetes Res. 2011;2011:416254
8. Wang HJ, Jin YX, Shen W, Neng J, Wu T, Li YJ, et al. Low dose streptozotocin (STZ) combined with high energy intake can effectively induce type 2 diabetes through altering the related gene expression. Asia Pac J Clin Nutr. 2007;16(Suppl 1):412-7.
9. Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52(4):313-20.
10. Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. Combining a dipeptidyl peptidase-4 inhibitor, alogliptin, with pioglitazone improves glycaemic control, lipid profiles and beta-cell function in db/db mice. Brit J Pharmacol. 2009; 157(3): 415-26.
11. Miesel A, Muller-Fielitz H, Johren O, Vogt FM, Raasch W. Double blockade of angiotensin II (AT(1))-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments in obese rats. Brit J Pharmacol. 2012;165(8):2721-35.
12. Collino M, Aragno M, Castiglia S, Miglio G, Tomasinelli C, Boccuzzi G, et al. Pioglitazone improves lipid and insulin levels in overweight rats on a high cholesterol and fructose diet by decreasing hepatic inflammation. Brit J Pharmacol. 2010;160(8):1892-902.
13. Akagiri S, Naito Y, Ichikawa H, Mizushima K, Takagi T, Handa O, et al. A mouse model of metabolic syndrome; Increase in visceral adipose tissue precedes the development of fatty liver and insulin resistance in high-fat diet-fed male KK/Ta Mice. J Clin Biochem Nutr. 2008;42(2):150-7.
14. Pfutzner A, Derwahl M, Jacob S, Hohberg C, Blumner E, Lehmann U, et al. Limitations of the HOMA-B score for assessment of beta-cell functionality in interventional trials-results from the PIOglim study. Diabetes Technol. Ther. 2010; 12(8): 599-604.
15. El-Atat FA, Stas SN, McFarlane SI, Sowers JR. The relationship between hyperinsulinemia, hypertension and progressive renal disease. J Am Soc Nephrol. 2004;15(11):2816-27.
16. Li L, Luo Z, Yu H, Feng X, Wang P, Chen J, et al. Telmisartan improves insulin resistance of skeletal muscle through peroxisome proliferator-activated receptor-delta activation. Diabetes. 2013; 62(3): 762-74.
17. Shiota A, Shimabukuro M, Fukuda D, Soeki T, Sato H, Uematsu E, et al. Telmisartan ameliorates insulin sensitivity by activating the AMPK/SIRT1 pathway in skeletal muscle of obese db/db mice. Cardiovascular Diabetology.2012; 11(1): 139.
18. Rosenblatt S, Miskin B, Glazer NB, Prince MJ, Robertson KE, Group PS. The impact of pioglitazone on glycemic control and atherogenic dyslipidemia in patients with type 2 diabetes mellitus. Coronary Artery Dis. 2001;12(5):413-23.
19. Chu KY, Lau T, Carlsson P-O, Leung PS. Angiotensin II type 1 receptor blockade iproves β-cell function and glucose tolerance in a mouse model of type 2 diabetes. Diabetes. 2006;55(2):367-74.

.....