EFFECT OF THIAZOLIDINEDIONES ON THE SERUM BIOMARKER LEVELS IN THE NICOTINAMIDE-STREPTOZOTOCIN INDUCED TYPE-2 DIABETES

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Summary

Thaizolidinediones (TZDs), a PPAR-γ ligands are used to ameliorate the insulin resistance in the type-2 diabetes (T2DM). Recent clinical data suggest that the chronic therapy of TZDs increases the cardiovascular risk like congestive heart failure. Pioglitazone (PIO) and rosiglitazone (RSG) were tested in three doses viz., 1, 10 and 100 mg/kg to study their influence on the biomarker levels in nicotinamide (230 mg/kg) and STZ (65 mg/kg) induced T2DM. Administration of PIO and RSG for 4 weeks significantly altered the serum glutamate oxaloacetate (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH) and creatinine kinase (CK-MB) levels in the diabetic animals. A dose-dependent decrease in the level of SGOT was observed after the administration of PIO and the peak reduction ($P<0.001$) was observed at 10 and 100 mg/kg compared to the diabetic animals. In addition, PIO reduced ($P<0.001$) the SGPT and LDH levels at the tested doses in the diabetic condition. On the other hand, RSG at 10 and 100 mg/kg reduced ($P<0.001$) the SGOT and SGPT levels, while the lower dose of RSG (1 mg/kg) decreased ($P<0.05$) only the SGOT level compared to the T2DM. At 100 mg/kg, RSG was also found to enhance ($P<0.001$) the level of LDH in the diabetic animals. Further, both PIO and RSG at 100 mg/kg increased the CK-MB level compared to the diabetic rats. The results indicated that PIO and RSG suppressed the NA-STZ mediated cytolytic damage in T2DM however, both drugs at 100 mg/kg increased the CK-MB levels suggesting the cardiac risk.

Key words: Pioglitazone, rosiglitazone, type-2 diabetes, biomarker levels.

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Introduction

Thaizolidinediones (TZDs) are novel insulin-sensitizing agents that are being widely used as first- and second-line drugs in patients with type-2 diabetes mellitus (T2DM). These agents exert their action by binding to the nuclear receptors called peroxisome proliferator-activated receptors gamma (PPAR-γ) [1]. PPARs are the transcription factors that heterodimerize with the retinoid X receptors and activates the gene transcription. There are two TZDs currently available for clinical use i.e., pioglitazone (PIO) and rosiglitazone (RSG) [2]. Apart from anti-diabetic effect, TZDs are also found to be useful in cancer, inflammatory disorders and polycystic ovary syndrome [2-5].

In the cardiovascular (CV) studies, TZDs have been shown to inhibit cardiac hypertrophy and reduce infarct size after coronary artery ligation [6]. Administration of RSG to the STZ induced diabetic rats has been reported to limit the post ischemic injury and improved the functioning of isolated hearts [7]. In addition, TZDs are reported to exhibit anti-hypertensive and anti-dyslipidemic effect in the high fat diet induced obesity models [8]. TZDs are also been reported to attenuate the hyperglycemia mediated increase in the angiotensin-II responses in insulin-resistant diabetic models [9]. These data suggests that TZDs is likely to have expanded therapeutic role, particularly in the growing number of cardiac patients with established chronic heart failure and impaired insulin sensitivity [10].

However, the recent data from the clinical studies indicated that TZDs increases the cardiovascular complications especially in the patients with the history of pre-existing heart ailments [11]. In a retrospective analysis, it was observed that patients receiving TZDs had 12.4% risk of developing heart failure compared to 8.4% in the control group, after 36 months of follow-up. The fluid retention property after the TZDs therapy has been attributed to the exacerbation of heart failure [12]. Although mechanistic basis for fluid retention remains elusive, some evidence suggests that it could be related to the increased cell permeability along with interference in the renal hemodynamics [13]. Further, epidemiological evidences from the previous studies indicate that abnormality in the glucose metabolism is the leading cause for the CV diseases. The severity of this problem can be illustrated from the data available which suggests that the mortality rate due to CV diseases is higher in diabetic patients than in the general population [14]. In spite of the gravity of this complication, there is paucity of information relating to the diabetes, TZDs therapy and CV defects. Hence, the present study was designed with an objective to evaluate the effect of PIO and RSG on the serum biomarker levels in the normal as well as nicotinamide-streptozotocin induced T2DM in Wistar rats.

Materials and methods

Chemical

The gift samples of pioglitazone (PIO) and rosiglitazone (RSG) were obtained from Biocon (India) Ltd, Bangalore. The kits for the biomarker estimation were purchased from Ranbaxy Fine Chemicals Ltd., Baddi India. The reagents and other chemicals used in this study were of analytical grade and procured from the regular suppliers.

Animals

Eight week-old healthy, laboratory bred, male Wistar rats weighing 180 ± 10 gm were maintained under standard laboratory conditions such as temperature 22 ± 2^{o} C, 12 hour light / dark cycle and provided water and pellet food *ad libitum*. The experiments were conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals, Chennai, India) approved animal house after obtaining the prior approval from the 'Institutional Animal Ethics Committee' (Ref.No: AACP/IAEC/P-31/2005).

Induction of diabetes [15]

Experimental T2DM was developed in adult rats by administering streptozotocin (STZ) and nicotinamide (NA). The animals received intraperitoneal administration of NA - 230 mg/kg (SD Fine-Chem Ltd, Mumbai, India) dissolved in saline 15 min before an administration of STZ – 65 mg/kg, ip (Sigma Aldrich, USA) dissolved in 0.1 M citrated buffer (pH 4.5) immediately before use. Blood glucose was estimated after 2 days and the animals with glucose level ≈ 180 ± 8 mg/dl are only selected for the study.

Dosage, treatment and sampling

The animals were divided mainly in to three groups ie., control, diabetic and treatment. The treatment group received three doses of PIO and RSG (1, 10 and 100 mg/kg) orally per day for 4 weeks after the induction of diabetes. Three doses of TZDs were selected as per the OECD guidelines for testing the acute toxicity of compounds in rodents [16]. Accordingly, a ten fold lower and higher dose than the therapeutic were selected, 10 mg/kg being the reported therapeutic concentration of PIO [17] and RSG [18] in rats. The control and diabetic animals were administered saline (0.5 ml/kg) daily through out the treatment period. Before the administration, PIO and RSG were suspended in 1% w/v carboxy methyl cellulose (CMC).

Estimation of serum biomarker levels

Blood samples were collected from the retro-orbital plexus under light ether anesthesia. The serum was separated by centrifugation (1000 rpm) and immediately analyzed to determine the antioxidant enzyme activity.

a. Serum glutamate oxaloacetate transaminase (SGOT) / Aspartate transferase (AST) [19]

The principle depends on the reaction between L-aspartate and α-ketoglutarate in the presence of SGOT to form oxaloacetate and L-glutamate. Oxaloacetate then reacts with NADH with the help of malate dehydrogenase to form L-malate and NAD which can be measured at 340

nm. The rate of decrease in absorbance due to the oxidation of NADH to NAD^+ is proportional to SGOT activity.

b. Serum glutamate pyruvate transaminase (SGPT) / **Alanine transferase** (ALT) [20]

In this method, the reaction between L-alanine and α-ketoglutarate in the presence of SGPT results in the formation of pyruvate and L-glutamate Pyruvate further reacts with NADH to form L-lactate and NAD^+ in the presence of lactate dehydrogenase. The rate of decreased in absorbance at 340 nm due to the oxidation of NADH to NAD will indicate the SGPT activity.

c. Lactate dehydrogenase (LDH) [21]

Lactate dehydrogenase catalyses the conversion of pyruvate to lactate with simultaneous oxidation of reduced NADH to NAD^+. The rate of decrease in absorbance due to the formation of NAD is measured at 340 nm and is proportional to the enzyme activity in the sample.

d. Creatine kinase-MB (CK-MB) [22]

The procedure involves the measurement of CK activity in the presence of an antibody to the CK-M monomer. The formation of NADPH from the reaction of glucose-6-phosphate and $NADPH^+$ in the presence of glucose-6-phosphate dehydrogenase can be measured at 340 nm. The rate of increase in absorbance is used to measure the activity of CK-MB.

Statistics

The statistical analysis of the results were done by Student‘t’ test followed by One-way Anova. $P<0.05$ is considered to indicate the significance.

Results

A. Effect of Pioglitazone on the serum biomarker levels in the NA-STZ induced T2DM

T2DM after the administration of NA and STZ produced a significant ($P<0.001$) increase in the level of SGOT, SGPT, LDH and CK-MB compared to the normal animals. Three doses of PIO (1, 10 and 100 mg/kg) were tested in diabetic animals to study their influence on the biomarker levels. Administration of PIO at lower dose (1 mg/kg) significantly reduced the SGOT ($P<0.05$), SGPT ($P<0.001$) and LDH ($P<0.01$) level without affecting the CK-MB level compared to the diabetic animals. At 10 mg/kg, PIO further reduced ($P<0.001$) the SGOT, SGPT and LDH level but did not alter the CK-MB level in the diabetic rats. Similarly, when PIO at 100 mg/kg was tested, a significant ($P<0.001$) reduction in the serum SGOT, SGPT and LDH levels was observed, however PIO at this dose (100 mg/kg) significantly ($P<0.001$) increased the CK-MB level compared to the T2DM. Further, administration of PIO at 100 mg/kg did not induce any significant change in the tested biomarker levels in the normal animals (Table-1).

Table-1: Effect of Pioglitazone on serum biomarker levels in NA-STZ induced type-2 diabetes

Treatment and dose (mg/kg)	Serum Biomarkers level			
	AST / SGOT (IU/L)	**ALT / SGPT** (IU/L)	**LDH** (IU/L)	**CK-MB** (IU/L)
Control (Saline- 1 ml / 500 gm)	51.84 ± 4.24	30.91 ± 4.22	87.01 ± 3.65	589.2 ± 4.23
Pioglitazone (100 mg/kg)	53.89 ± 3.08	32.77 ± 2.94	90.26 ± 4.39	591.59 ± 5.62
Nicotinamide (230) + **STZ** (65)	172.53 ± 6.45 †	158.36 ± 3.18 †	134.21 ± 4.63 †	623.75 ± 7.04 †
NA-STZ + Pioglitazone (1 mg/kg)	161.43 ± 5.85 † *	128.49 ± 6.52 † ***	124.94 ± 5.43† **	619.88 ± 5.57 †
NA-STZ + Pioglitazone (10 mg/kg)	149.53 ± 5.76 † **	128.32 ± 4.64 † ***	120.64 ± 4.66† ***	628.21 ± 4.49 †
NA-STZ + Pioglitazone (100 mg/kg)	138.64 ± 4.65 † ***	120.73 ± 5.90 † ***	110.75 ± 6.69†***	635.04 ± 4.87† ***

Values are expressed as Mean ± SD, STZ - Streptozotocin, NA – Nicotinamide; *Statistics: Student 't' test followed by One way Anova.* †*P<0.001 compared with control group*; *P<0.05, **P<0.01, ***P<0.001 *compared with the diabetic group*

B. Effect of Rosiglitazone on the serum biomarker level in NA-STZ induced T2DM

The NA-STZ induced T2DM enhanced (P<0.001) the level of SGOT, SGPT, LDH and CK-MB compared to the normal group. Dose-dependent alterations in the biomarker levels were observed when RSG was tested in three doses (1, 10 and 100 mg/kg). RSG at 1 mg/kg significantly decreased (P<0.05) the SGOT level without affecting the concentration of SGPT, LDH and CK-MB in diabetic condition. At 10 mg/kg, RSG produced a significant (P<0.001) decrease in the level of SGOT and SGPT but did not alter the LDH and CK-MB levels compared to the diabetic group. Further, the highest tested dose of RSG (100 mg/kg) produced a significant decrease (P<0.001) in the SGOT and SGPT levels, but enhanced the serum level of LDH

(P<0.05) and CK-MB (P<0.01) compared to the diabetic animals. However, RSG (100 mg/kg) in the normal animals did not induce any significant effect on the biomarker levels (Table-2).

Table-2: Effect of Rosiglitazone on serum biomarker levels in NA-STZ induced type-2 diabetes

Treatment and dose (mg/kg)	Serum Biomarkers level			
	AST / SGOT (IU/L)	**ALT / SGPT** (IU/L)	**LDH** (IU/L)	**CK-MB** (IU/L)
Control (Saline- 1 ml / 500 gm)	51.84 ± 4.24	30.91 ± 4.22	87.01 ± 3.65	589.2 ± 4.23
Rosiglitazone (100 mg/kg)	50.98 ± 6.66	33.52 ± 3.98	90.62 ± 6.91	593.23 ± 7.69
Nicotinamide (230) + **STZ** (65)	172.53 ± 6.45†	158.36 ± 3.18 †	134.21 ± 4.63 †	623.75 ± 3.04 †
NA-STZ + Rosiglitazone (1 mg/kg)	164.28 ± 4.95 † *	152.04 ± 7.60 † *	135.86 ± 6.41†	622.23 ± 4.59 †
NA-STZ + Rosiglitazone (10 mg/kg)	140.08 ± 6.47† ***	124.78 ± 5.74 † ***	137.54 ± 7.66 †	626.42 ±5.98 †
NA-STZ + Rosiglitazone (100 mg/kg)	132.54 ± 5.82 † ***	112.87 ± 5.37 † ***	140.22 ± 4.58 † *	631.5 ± 3.54 † **

Values are expressed as Mean ± SD, STZ - Streptozotocin, NA - Nicotinamide
Statistics: Student 't' test followed by One way Anova.
†*P<0.001 compared with control group*
*P<0.05, **P<0.01, ***P<0.001 *compared with the diabetic group*

Discussion

The present study indicated that administration of NA and STZ significantly increased the serum levels of SGOT, SGPT, LDH and CK-MB (Table-1 and 2). In the earlier study, Punitha et al (2006) has reported that the administration of NA and STZ increases the biomarker levels in T2DM [23]. The administration of NA prior to STZ has an advantage that NA limits the cytolytic damages induced by the STZ. The combination of NA-STZ produces sustained hyperglycemia which has been reported to mimic the clinical T2DM. Besides, the NA-STZ induced diabetic condition can be maintained for longer duration (4-6 weeks) which could be useful to study the influence of chronic hyperglycemia on the host cell physiology [15].

Our study indicated that administration of PIO and RSG reduced the serum levels of SGOT and SGPT, PIO in addition also decreased the LDH levels in the diabetic animals (Table-1 and 2). SGOT and SGPT though not the specific cardiac enzymes but their levels are found to be elevated in myocardial injury, hence, their estimation is reported to suggest the time and severity of cardiac damage. The decrease in the level of SGOT and SGPT indicated that PIO and RSG minimized the NA-STZ mediated cytolytic damages including cardiac cells in T2DM. Further, the non-significant alteration in the biomarker levels when PIO and RSG were tested at 100 mg/kg in normal animals (Table-1 and 2) supports our finding that these agents are not only safe but also posses the ability to reduce the cytolytic damages. This property of PIO and RSG can be attributed to the antioxidant effect, since the earlier studies indicated that compounds exhibiting the antioxidant activity can suppress the cytolytic damage caused by STZ-induced T2DM [24]. The antioxidant property of TZDs is already established in the literature. The mechanism suggested for the antioxidant effect include, modulation in the expression of different NAD(P)H oxidase subunits, increase in the expression of (cu, zn) superoxide dismutase and inhibition in the mitogen-activated protein kinases (MAPKs) – NF-kB signaling pathways responsible for the generation of reactive oxygen species [25,26,27]. The antioxidant related beneficial effects of TZDs have been reported, where the administration of these agents has improved the functioning of left ventricle after the ischemic injury [7,9,10]. Likewise, other studies also reported that administration of TZDs can augment the cardiac performance by enhancing the systolic and diastolic actions and suppressing the cardiac hypertrophies [6,8].

Another important finding of this study is that PIO and RSG at 100 mg/kg increased the serum levels of CK-MB and RSG at this dose also enhanced the LDH level in the diabetic animals (Table-1 and 2). CK-MB is a cardiac enzyme and its level in the serum indicates the extent of damage to the cardiac cells. Similarly, estimating the LDH levels also suggests the extent of myocardial injury [28]. The elevation in the level of CK-MB by PIO and RSG, and LDH by RSG indicate that these agents might potentate the myocardial lesions in the T2DM. Although, a direct relationship between cardiac damage and TZDs therapy could not be established from the reported animals studies. However, the clinical findings indicate that the damage to heart is related to the fluid retention [12]. The mechanism suggested for this include, increased fluid reabsorption in kidney, decreased arteriolar resistance or increase in the insulin sensitivity as insulin itself can cause edema by vasodilation or by increasing the endothelial permeability. In addition, PPAR-activation is reported to stimulate renin-angiotensin system causing the release of endothelin-1 and nitric oxide [29].

Considering the above information, it can be suggested that TZDs therapy at higher dosage and for longer duration should be carefully monitored and further studies are essential to establish the exact role of TZDs on the cardiac functioning in T2DM.

Conclusion

T2DM after the administration of NA-STZ significantly increased the level of SGOT, SGPT, LDH and CK-MB. Administration of PIO reduced the SGOT, SGPT and LDH level while RSG reduced the SGOT and SGPT levels but enhanced the LDH at highest tested dose (100 mg/kg) in the diabetic animals. However, both the drugs at 100 mg/kg elevated the serum level of CK-MB in the diabetic condition. The results indicated that though PIO and RSG prevented the cytolytic damage but could possess the potential to increase the cardiac damage in the NA-STZ induced diabetic condition.

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