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Research Article

COMPARATIVE EFFICACY OF PIOGLITAZONE VERSUS ROSIGLITAZONE ON LIPID PROFILE AND OXIDATIVE STRESS IN HYPERCHOLESTEREMIC RATS

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ABSTRACT
This study was performed to compare efficacy of Pioglitazone (PIO) versus Rosiglitazone (ROS) on lipid profile and oxidative stress in hypercholesteremic rats. Hypercholesteremic condition in normal rats was induced by including 0.75 gm% cholesterol and 1.5 gm% bile salt powder in normal diet. PIO and ROS were administered as 30 mg/kg/day and 5mg/kg/day dose levels, respectively, to the hypercholesteremic rats. Plasma lipid profile parameters and antioxidant properties were estimated by using standard methods. Statistical analysis was done by one way analysis of variance (ANOVA). Treatment with PIO resulted in significant decreases in serum TG and VLDL and significant increase in serum HDL, but no significant decrease in cholesterol and LDL. On the other hand ROS did not improve any lipid profile parameter. PIO increased activities of catalase enzyme and concentration of malondialdehyde significantly, but there were no significant changes in the superoxide dismutase activity and ascorbic acid concentration in this group while no change in antioxidant activity parameters in ROS treated group. The present study demonstrated that treatment with only PIO improves the plasma lipid profile and also reduces oxidative stress in hypercholesteremic animals. Therefore, PIO would be better choice than ROS in diabetic patients associated with hypercholesteremia.

Key Words: *Antioxidant, Hypercholesteremia, Pioglitazone, Rosiglitazone.*

INTRODUCTION
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose level caused by insulin deficiency and often combined with insulin resistance. DM, if not treated, ultimately lead to specific long-term complications affecting the vital organs like nervous system, heart, eye and kidney (Pandey, 2011) Diabetic patients have an increased prevalence of hyperlipidemia, which increases their overall rate of developing cardiovascular disease. The lipid profile abnormalities associated with DM include elevated triglyceride levels, decreased high-density lipoproteins (HDL) cholesterol levels and a qualitative change to small, dense low density lipoproteins (LDL) particles. Collectively, this abnormality in lipid profile leads itself to a greater risk of atherosclerosis and other cardiovascular related diseases (Mazumder, 2009). Free radical production has also been reported to be increased in diabetic patients and these free radicals deplete the activities of anti-oxidative defense systems with modification of activities of antioxidant enzymes. Thus, increase in oxidative stress and/or changes in antioxidant capacity play an important role in complications of diabetes (Shanmugam, 2011).
To summarize, most of the complications of diabetic patients are associated with increased lipid levels and oxidative stress. Therefore, an ideal oral treatment for diabetes would be a drug that not only controls hyperglycemia, but also prevents other complications of diabetes by its lipid lowering and antioxidant properties. Unfortunately, among the currently available anti-diabetic drugs, the choice is very limited.
The Thiazolidinediones (TZD) or Glitazones, a category of novel anti-diabetic medication, have emerged as an effective treatment option to control hyperglycemia in patients with type-2 DM. TZD are synthetic

ligands for *peroxisome proliferator-activated receptor-γ* (PPAR-γ) that act as insulin sensitizers in metabolic organs like skeletal muscle, liver and adipose tissue. The first generation drugs, ciglitazone and troglitazone, were withdrawn due to episodes of severe hepatotoxicity, but there are rare reports of hepatotoxicity with second-generation drugs i.e. Pioglitazone (PIO) and Rosiglitazone (ROS) (Biswas, 2012).

The objective of the present study was to compare efficacy of PIO versus ROS on lipid profile parameters and oxidative stress in hypercholesteremic rats.

METHODS

Animals

Healthy male adult albino rats of Wistar strain weighing 200-250 gm were used for this study. They were kept on standard balanced diet and water *ad libitum* in a well-ventilated animal unit. The care and procedures, adopted for the present investigation, were in accordance with the approval of Institutional Animal Ethics Committee.

Drugs

Powdered salt forms of PIO and ROS were obtained as gift samples from Dr. Reddy's laboratories Ltd., India. Cholesterol and bile salt were purchased in pure and edible powder form from Yucca Enterprises, Wadala (E) Mumbai, India. All other chemicals and reagents used in the investigations of present study were of analytical grade.

Study design

Study was conducted as follows: After ten days adaptation period, 24 animals were divided into four groups, each group containing six animals **(n=6).** The groups were treated as follows for **four weeks**:

Group I: Control group (Only standard diet is given).

Group II: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt of the weight of the total diet to induce hypercholesteremia (Visavadiya, 2005)

Group III: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with PIO (30mg/kg/day p.o.) as a suspension (Biswas, 2012).

Group IV: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with ROS (5mg/kg/day p.o.) as a suspension (Potenza, 2009).

Collection of blood samples

On 30^{th} day, after overnight fasting, blood was collected directly from heart of rat anaesthetized with ether. Abdomen was opened by taking a midline incision. Blood was sent to biochemistry laboratory in plain bulb; plasma was separated by centrifugation. Liver was excised and, both plasma and liver were kept frozen until analyzed.

Biochemical analysis

Plasma lipid profile was assessed by following parameters by standard methods: serum total cholesterol by Modified Roeschlau`s Method (Roeschlau, 1974), serum total triglycerides (TG) by method of Wako, modified by McGowan and Fossati (McGowan, 1983), serum total HDL by Phosphotungstic Acid method (Klaus Loreniz, 1979), serum total LDL and serum total very low density lipoproteins (VLDL) by Friedewald formula (Chatterji, 2007).

Antioxidant potential was assessed by following parameters: Hepatic ascorbic acid by Schaffert RR et al method (Schaffert, 1955), catalase activity in liver by Cohen G et al method (Cohen, 1970), serum malondialdehyde (MDA) by Pasha and Sadasivadu method (Pasha, 1984), serum superoxide dismutase activity (SOD) by Marklund and Marklund method (Marklund, 1974).

Statistical Evaluation

The results are expressed as means ± SD (standard deviation). Significant differences among groups were determined by one way Analysis of variance (ANOVA). *Post hoc analysis* was done by using software StatPac. Differences were considered significant if $P < 0.05$.

RESULTS

Plasma lipid profile

PIO as 30mg/kg/day treatment to hypercholesteremic rats resulted in no significant decrease in total serum cholesterol and serum LDL-C as well, but serum HDL-C level increased significantly ($P < 0.01$) in this group. There were no significant changes in all these parameters in ROS treated group (Table 1).

Table1: Effects of Pioglitazone and Rosiglitazone on serum total cholesterol, serum LDL and serum HDL level in rats.

Groups (n=6)	Treatment given	Serum TC (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)
Group I	Control	127.12 ± 6.51	50.04 ± 5.41	66.78 ± 2.24
Group II	HC	303.52 ± 10.35	250 ± 11.27	42.65 ±1.94
Group III	HC+30P	304.48 ± 9.36 ^{NS}	242.27 ± 9.59 ^{NS}	53.12 ± 3.93*
Group IV	HC+5R	306.44 ± 10.56 ^{NS}	256.19 ± 14.87 ^{NS}	40.23 ± 3.22 ^{NS}

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC+30P = Hypercholesteremic+ 30 mg/kg/day Pioglitazone, HC + 5R = Hypercholesteremic+5mg/kg/day Rosiglitazone, TC = Total Cholesterol, LDL = low density lipoproteins, HDL = high density lipoproteins, NS – Non significant compared to Group II, * P < 0.01 compared to Group II and Group IV (one way ANOVA).*

Table 2: Effects of Pioglitazone and Rosiglitazone on serum TG and VLDL level in rats.

Groups (n=6)	Treatment given	Serum TG (mg/dl)	Serum VLDL (mg/dl)
Group I	Control	51.53 ± 2.75	10.31 ± 0.55
Group II	HC	54.29 ±3.28	10.86 ± 0.66
Group III	HC+30P	48.48 ± 4.26*	9.70 ± 0.85*
Group IV	HC+5R	57.22 ± 4.88 ^{NS}	11.44 ± 1.08 ^{NS}

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC+30P = Hypercholesteremic+ 30 mg/kg/day Pioglitazone, HC + 5R = Hypercholesteremic+5mg/kg/day Rosiglitazone, TG = Total triglycerides, VLDL = very low density lipoproteins, *P < 0.05 compared to Group II and Group IV, NS – Non-significant compared to Group II (one way ANOVA).*

The values were decreased from 54.29±3.28 mg% to 48.48±4.26 mg% and from 10.86±0.66 mg% to 9.70 ±0.85 mg% in case of triglyceride and VLDL, respectively, in 30mg/kg PIO treated group (Table 2). There were significant decrease in serum triglyceride ($P < 0.05$) and serum VLDL ($P < 0.05$) level with treatment of 30mg/kg/day PIO treated group, but not with 5mg/kg ROS treated group.

Antioxidant activities:

There was no significant increase in total ascorbic acid in liver in PIO and ROS treated groups ($P= 0.35$ and $P= 0.68$, respectively). Catalase activity in liver is increased significantly ($P < 0.05$) only in 30mg/kg PIO treated group (Table 3).

Table 3: Effects of Pioglitazone and Rosiglitazone on total ascorbic acid and activities of catalase in liver of rats.

Groups (n=6)	Treatment given	Total ascorbic acid (µ/g)	Catalase nm H_2O_2 decomposed/sec/gm
Group I	Control	56.53 ± 2.75	20.31 ± 0.55
Group II	HC	44.29 ±3.28	13.86 ± 0.66
Group III	HC+30P	46.48 ± 4.36 ^{NS}	15.34 ±0.75*
Group IV	HC+5R	43.92 ± 4.01 ^{NS}	14.08 ±1.01 ^{NS}

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC+30P = Hypercholesteremic+ 30 mg/kg/day Pioglitazone, HC + 5R = Hypercholesteremic+5mg/kg/day Rosiglitazone, NS – Non-significant compared to Group II, * P < 0.05 compared to Group II (one way ANOVA).*

Table 4: Effects of Pioglitazone and Rosiglitazone on serum MDA and SOD level in rats.

Groups (n=6)	Treatment given	Serum MDA (nmol/ml)	Serum SOD (U/ml)
Group I	Control	1.41 ± 0.27	11.93 ± 0.64
Group II	HC	3.47 ±0.40	5.78 ± 0.73
Group III	HC+30P	2.10 ±0.51*	6.37 ± 0.54 ^{NS}
Group IV	HC+5R	3.92 ± 0.95 ^{NS}	5.22 ± 0.88 ^{NS}

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC+30P = Hypercholesteremic + 30 mg/kg/day Pioglitazone, HC + 5R = Hypercholesteremic + 5mg/kg/day Rosiglitazone, MDA = Malondialdehyde, SOD = Superoxide dismutase, *P < 0.05 compared to Group II and Group IV, NS – Non-significant compared to Group II (one way ANOVA).*

Serum MDA was decreased in PIO and ROS treated groups as compared to hypercholesteremic group (i.e. from 3.47 ± 0.40 nmol/ml to 2.10 ± 0.51 nmol/ml and from 3.47±0.40 nmol/ml to 3.92±0.95 nmol/ml), respectively Table 4. But the reduction of only PIO treated group was significant ($P < 0.05$). The activity of SOD changed in both experimental PIO and ROS treated groups as compared to hypercholesteremic group i.e. from 5.78±0.73 U/ml to 6.37±0.54 U/ml and from 5.78±0.73 U/ml to 5.22±0.88 U/ml, respectively, in group III and IV. (Table No. 4). These changes in SOD activity were not statistically significant ($P > 0.05$).

DISCUSSION

Insulin resistance is an important characteristic feature of type-2 diabetes and is commonly related with increased levels of lipid profile parameters i.e. cholesterol, triglycerides and low density lipoproteins that are considered to be important risk factor for atherosclerosis and other cardiovascular related diseases. Normalization of these lipid profile parameters may reduce the accelerated atherosclerosis and the related complications in diabetic patients (Mazumder, 2009). Also certain complications related to diabetes are

associated with increased oxidative stress, induced by the generation of free radicals (Shanmugam, 2011). In short, treatment compounds, which are having both hypolipidemic and antioxidant properties, would be useful as anti-diabetic agents.

The present study was conducted to compare efficacy of PIO versus ROS on different lipid profile parameters and oxidative stress in hypercholesteremic rats. Effects of PIO in hypercholesteremic rats, demonstrated in the present study, were related primarily to a decreased levels of total serum triglycerides and VLDL-cholesterol while increase in HDL-cholesterol level. There were no significant decreases in serum cholesterol as well as LDL-cholesterol in PIO treated group (Group III). Also ROS did not improve any lipid profile parameter, but adversely affected serum triglyceride level.

Lipoprotein disorder is among the most common metabolic disease occurring in human which may lead to coronary artery disease. Excessive levels of blood cholesterol accelerate the atherogenesis and lowering of high blood cholesterol reduces the incidence of coronary heart disease. Also knowledge about the levels of cholesterol sub-fractions is more meaningful than simple plasma cholesterol level. Higher the level of LDL-cholesterol and lower HDL-cholesterol, greater is the risk of atherosclerotic heart disease and vice a versa (Ajayi, 2009). Thus, increase in levels of serum cholesterol, serum-triglycerides and decreased values of HDL-C adversely affect the process of atherosclerosis, which ultimately increases the risk of coronary artery disease (Durrington, 2003 and Assmann, 2003). This particular fact that PIO did not produce deleterious effect on lipid profile parameter definitely indicates some beneficial role of PIO in diabetes with hyperlipidemia and seems to be advantageous over ROS which affected the lipid profile adversely.

Radicals and other reactive oxygen species (ROS) are formed constantly in the human body and are removed by the enzymatic and non-enzymatic antioxidant defense systems. ROS includes free radicals, non free radicals and various forms of activated oxygen. They are involved in pathogenesis of various serious diseases such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis and inflammation (Singh, 2008). Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing oxidative stress related complications of such type of diseases.

Presently noted decreased levels of MDA, a product of lipid per-oxidation, and increased levels of catalase enzyme activities in PIO treated group (Group III) indicate the possible role of PIO as an antioxidant. Antioxidant activity of PIO is reported to be mediated by blocking the vicious cycle of ROS production, improves insulin sensitivity and halts the pro-inflammatory signaling transduction (Hsiao, 2008). In past, many experimental studies were conducted with PIO to confirm its antioxidant activity. In one study, PIO retrieved hepatic antioxidant DNA repair in a mice model of high fat diet (Hsiao, 2008) and in another study, PIO inhibited the nicotinamide-streptozotocin induced sperm abnormalities in type-2 diabetic Wistar rats (Rabbani, 2010). Hasegawa T et al showed antioxidant properties of PIO in cardiac allotransplantation (Hasegawa, 2011).

In present study, ROS did not improve any antioxidant parameter. In one past study, by Maria A. Potenza et al (Potenza, 2009), ROS treatment of spontaneously hypertensive rats ameliorated cardiovascular patho-physiology via its antioxidant mechanisms in the vasculature. Authors concluded that ROS therapy in these rats increases SOD activity and decreases p22phox, a catalytic subunit of NADPH oxidase, expression in the vasculature to reduce oxidative stress.

Taken together, these observations of present study indicate that PIO administration to hypercholesteremic animals can reduce serum TG and increase serum HDL-C levels and also improve antioxidant enzyme activities while ROS did not improve any lipid profile parameter and also there was no improvement in any antioxidant parameter. Therefore, PIO could be a better option than ROS in diabetic patients associated with hypercholesteremia.

There were certain drawbacks of this experiment. Sample size was small; duration of therapy was also short. We investigated antioxidant activities of PIO and ROS in hypercholesteremic condition, instead of

diabetic condition in which these drugs are mainly used. All biochemical tests of antioxidant property were not performed due to unavailability of agents.

Conclusion

Thus, we conclude that PIO could improve lipid profile and decrease oxidative stress in hypercholesteremic conditions while ROS did not improve any lipid profile parameter and also no improvement in any antioxidant parameter which suggest that PIO may reduce cardiovascular risk by its hypolipidemic and antioxidant actions in patients with type 2 diabetes.

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