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Research Article

Hypolipidemic and antioxidant activities of pioglitazone in hyperlipidemic rats

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ABSTRACT

Background: Diabetes mellitus (DM) is an endocrine disorder characterized by abnormal carbohydrate, lipid and protein metabolism along with specific long-term complications which are associated with hyperlipidemia and oxidative stress. Hence, it is important to find hypoglycemic drug that improves lipid profile and reduces oxidative stress in diabetic patient. This study, therefore, was performed to investigate hypolipidemic and antioxidant potential of Pioglitazone (PIO) in hyperlipidemic rats.
Methods: Hyperlipidemia was induced in normal rats by including 0.75 gm% cholesterol and 1.5 gm% bile salt in normal diet and these rats were used for the experiments. PIO hydrochloride was administered as 10 mg/kg and 30 mg/kg dose levels to the hyperlipidemic rats. Hypolipidemic activity was estimated by plasma lipid profile parameters while antioxidant potential was estimated by ascorbic acid, catalase activity, malondialdehyde and superoxide dismutase activity using standard methods. Statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnett's test.
Results: Treatment with 10 mg/kg and 30 mg/kg dose levels of PIO hydrochloride resulted in a significant decrease in serum TG and VLDL only in 30 mg/kg PIO treated group and significant increase in serum HDL in both groups, but no significant decrease in cholesterol and LDL in both PIO treated groups. PIO increased activities of catalase enzyme and concentration of malondialdehyde significantly in only 30 mg/kg PIO treated group. But there were no significant changes in the superoxide dismutase activity and ascorbic acid concentration in both PIO treated groups.
Conclusions: The present study demonstrated that treatment with 10 mg/kg and 30 mg/kg dose levels of PIO hydrochloride improves the plasma lipid profile and also reduces oxidative stress in hyperlipidemic animals.

Keywords: Antioxidant, Diabetes, Hyperlipidemia, Pioglitazone

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the vital organs like nervous system, eye and kidney.[1] Hyperglycemia in diabetic patients is associated with increase in lipid level and ultimately an increase in the atherogenic index. Therefore, DM is recognized as a major risk factor for cardiovascular diseases such as atherosclerosis and heart attack.[2] As diabetic patients have adverse changes related to their plasma lipid profile, it becomes very important to find an anti-diabetic agent that could improve lipid profile.

Free radical production has been reported to be increased in diabetic patients and increased glucose level appears to be the most important contributing factor for the generation of reactive oxygen species. These radicals deplete the activities of antioxidative defense systems with modification of activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Increase in oxidative stress and changes in antioxidant capacity, induced by a high glucose, play a central role in complications of diabetes.[3] Therefore, it is important to find hypoglycemic drug that reduces

oxidative stress in diabetic patient. In short, an ideal oral treatment for diabetes would be a drug that not only controls the glucose level but also prevents the hyperlipidemia and other complications of diabetes by its antioxidant mechanism. Unfortunately, among the currently available anti-diabetic drugs, the choices are very limited.

The Thiazolidinediones drugs, a category of anti-diabetic medication, have emerged as an effective treatment option for improving glycemic control in patients with type-2 diabetes mellitus. This novel class of drugs acts as agonists for nuclear transcription factor peroxisome proliferators-activated receptor gamma (PPAR-γ) and primarily improve insulin sensitivity by enhancing the transcription of several insulin responsive genes. These drugs have been reported to improve parameters like plasma lipid profile and blood pressure which are very beneficial for the diabetic patients associated with cardiovascular diseases. The first generation drug, troglitazone, was withdrawn due to episodes of severe liver injury while the second-generation rosiglitazone and pioglitazone (PIO) showed no increased risk of hepatotoxicity.[4]

In past studies, PIO has inconsistent effects on lipid profile parameters.[4-7] The objective of the present study was, therefore, to investigate exact effect of PIO on different lipid profile parameters and also its antioxidant effect in hyperlipidemic rats.

METHODS

Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC). Male Albino rats weighing 200-250 gm were used for the experiment. They were kept on balanced diet and water ad libitum in a well-ventilated animal unit.

Drugs

Pioglitazone hydrochloride was obtained as a gift sample from Dr. Reddy's laboratories Ltd, India. Cholesterol and bile salt were purchased in pure powder form from Yucca Enterprises, Wadala (E) Mumbai, India. 0.75 gm% cholesterol and 1.5 gm% bile salt of weight of total diet were used to produce hyperlipidemia in normal male albino rats.[8] All other chemicals and reagents used in the present study were of analytical grade.

Study design

Study was conducted as follows:
After 10 days adaptation period, 24 animals were divided into four groups, each containing six animals (n=6). The groups were treated as follows for four weeks:

Group I: Control group (Only standard diet was 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 hyperlipidemia.

Group III: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hyperlipidemia, along with Pioglitazone (10mg/kg/day p.o.)[4] as suspension.

Group IV: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hyperlipidemia along with Pioglitazone (30mg/kg/day p.o.)[4] as suspension.

Collection of blood samples

On 30th 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,[9] serum total triglycerides (TG) by method of Wako, modified by McGowan and Fossati,[10] serum total high density lipoproteins (HDL) by Phosphotungstic Acid method,[11] serum total low density lipoproteins (LDL) and serum total very low density lipoproteins (VLDL) by Friedewald formula.[12]

Antioxidant potential was assessed by following parameters: Hepatic ascorbic acid by Schaffert RR et al method,[13] catalase activity in liver by Cohen G et al method,[14] serum malondialdehyde (MDA) by Pasha and Sadasivadu method,[15] serum superoxide dismutase activity (SOD) by Marklund and Marklund method.[16]

Statistical Evaluation

The results are expressed as means ± SD (standard deviation). Significant differences among groups were determined by one way Analysis of variance (ANOVA). Dunnett's test was used for post hoc test analysis. Differences were considered significant if $p < 0.05$.[17]

RESULTS

Plasma lipid profile

PIO as 10mg/kg and 30mg/kg treatment to hyperlipidemic rats resulted in no significant decrease in total serum cholesterol and serum LDL-C as well. Serum

HDL-C level increased significantly ($p< 0.01$) in both PIO treated groups (Table 1). These changes in HDL-C were dose dependent.

Table 1: Effect of pioglitazone on serum total cholesterol, serum LDL and serum HDL level in male albino rats.

Groups (n=6)	Treatment given	Sr. TC (mg/dl)	Sr. LDL (mg/dl)	Sr. HDL (mg/dl)
Group I	Control	127.12 ± 6.51	50.04 ± 5.41	66.78 ± 2.24
Group II	HL	303.52 ± 10.35	250 ± 11.27	42.65 ±1.94
Group III	HL+10P	307.12 ± 10.25 NS	248.19 ± 15.82 NS	48.23 ± 3.07*
Group IV	HL+30P	304.48 ± 9.36 NS	242.27 ± 9.59 NS	53.12 ± 3.93*#

(All values are Mean ± Standard Deviation). HL = Hyperlipidemic group, HL + 10P = Hyperlipidemic + 10mg/kg Pioglitazone, HL+30P = Hyperlipidemic + 30 mg/kg Pioglitazone, 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, # $p < 0.05$ compared to Group III (ANOVA followed by Dunnett's test).

Table 2: Effect of pioglitazone on serum TG and serum VLDL level in male albino rats.

Groups (n=6)	Treatment given	Sr. TG (mg/dl)	Sr. VLDL (mg/dl)
Group I	Control	51.53 ± 2.75	10.31 ± 0.55
Group II	HL	54.29 ±3.28	10.86 ± 0.66
Group III	HL+10P	50.33 ± 3.99 NS	10.06 ± 0.78 NS
Group IV	HL+30P	48.48 ± 4.26*	9.70 ± 0.85*

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10P = Hyperlipidemic+ 10mg/kg Pioglitazone, HL+30P = Hyperlipidemic+ 30 mg/kg Pioglitazone, TG = Total triglycerides, VLDL = very low density lipoproteins, NS – Non-significant compared to Group II, * $p < 0.05$ compared to Group II (ANOVA followed by Dunnett's test).

Table 3: Effect of pioglitazone on total ascorbic acid and activities of catalase in liver of male albino 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	HL	44.29 ±3.28	13.86 ± 0.66
Group III	HL+10P	44.93 ± 3.89 NS	14.47 ±0.68 NS
Group IV	HL+30P	46.48 ± 4.36 NS	15.34 ±0.75*

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10P = Hyperlipidemic+ 10mg/kg Pioglitazone, HL+30P = Hyperlipidemic+ 30 mg/kg Pioglitazone, NS – Non-significant compared to Group II, * P < 0.05 compared to Group II (ANOVA followed by Dunnett's test).

There were significant decrease in serum triglyceride (p<0.05) and serum VLDL (p<0.05) level with treatment of 30mg/kg PIO treated group, but not with 10mg/kg PIO treated group (p=0.101). 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).

Antioxidant activities

There was no significant increase in total ascorbic acid in liver in both PIO treated groups (p= 0.78 and p= 0.35) i.e. group III and IV, respectively. Catalase activity in liver is increased significantly (p<0.05) only in 30mg/kg PIO treated group (Table 3).

Table 4: Effect of pioglitazone on serum MDA and serum SOD level in male albino rats.

Groups (*n*=6)	Treatment given	Sr. MDA (nmol/ml)	Sr. SOD (U/ml)
Group I	Control	1.41 ± 0.27	11.93 ± 0.64
Group II	HL	3.47 ±0.40	5.78 ± 0.73
Group III	HL+10P	3.23 ± 0.31 NS	6.67 ± 0.79 NS
Group IV	HL+30P	2.10 ±0.51*	6.37 ± 0.54 NS

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10P = Hyperlipidemic+ 10mg/kg Pioglitazone, HL+30P = Hyperlipidemic+ 30 mg/kg Pioglitazone, MDA = Malondialdehyde, SOD = Superoxide dismutase. NS = Non significant compared to Group II, * $p < 0.05$ compared to Group II (ANOVA followed by Dunnett's test).

The lipid peroxidation product, malondialdehyde, in serum decreased in PIO treated groups as compared to hyperlipidemic group (i.e. from 3.47±0.40 nmol/ml to 3.23±0.31 nmol/ml and from 3.47±0.40 nmol/ml to 2.10±0.51 nmol/ml) in 10mg/kg and 30mg/kg PIO treated groups, respectively (Table 4). But the reduction of only 30mg/kg PIO treated group was significant (p<0.05).

The activity of superoxide dismutase increased in both experimental PIO treated groups as compared to hyperlipidemic group i.e. from 5.78±0.73 U/ml to 6.67±0.79 U/ml and from 5.78±0.73 U/ml to 6.37±0.54 U/ml, respectively, in group III and IV (Table 4). These increases in superoxide dismutase activity were not statistically significant (p=0.1115).

DISCUSSION

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia, leading to disturbance in carbohydrate, lipid and protein metabolism.[2] Insulin resistance is an important characteristic feature of type-2 diabetes and is commonly associated with the abnormalities in circulating lipids and lipoproteins that are considered to be important risk factors for atherosclerosis in diabetic individuals. Correction of these abnormalities may reduce the accelerated atherosclerosis and the related complications in diabetic patients. There is also increasing evidence that most of the complications related to diabetes are associated with oxidative stress, induced by the generation of free radicals.[3] Therefore, treatment compounds with both lipid lowering and antioxidant properties would be useful as anti-diabetic agents.

Therefore, the present study was conducted to assess the hypolipidemic and antioxidant activities of PIO, one of the novel anti-diabetic medications, in hyperlipidemic rats. The lipid-lowering effects of PIO in hyperlipidemic rats, demonstrated in the present investigation, were related primarily to a decreased levels of total serum triglycerides and VLDL-cholesterol while increase in HDL-cholesterol level. There was no significant decrease in serum cholesterol as well as LDL-cholesterol in both PIO treated groups (Group III and IV).

The impact of PIO on the lipid profile in diabetic patients is not clearly consistent.[4-7] PIO increases adipocyte differentiation and stimulates the distribution of new adipose tissue to both retroperitoneal and subcutaneous sites by activation of PPARγ receptors.[4] Most studies show that PIO have an overall beneficial effect since PIO was found to increase HDL-C and lowered plasma TG and VLDL. Rosenblatt et al[6] studied the impact of pioglitazone on glycemic control and atherogenic dyslipidemia in patients with type 2 diabetes mellitus. The study demonstrated that there were significant changes in triglycerides and HDL whereas the levels of total cholesterol and LDL were found to be non-significant. However, negative effects with PIO therapy such as increase in cholesterol and LDL levels have also been reported. In one study, by Hirose H et al,[7] TG and HDL remained unaffected and cholesterol and LDL levels increased significantly by administration of PIO. Because of these inconsistent effects of PIO on lipid profile, this study was undertaken.

Elevated levels of triglycerides are associated with atherosclerosis, even in the absence of hypercholesterolemia, and predispose to cardiovascular disease.[18] High level of HDL-C is associated with fewer problems with cardiovascular diseases and vice versa. It is also well known that an increase in HDL-C level could potentially contribute to reversal of atherogenesis. This is because high level of HDL-C protects endothelial cells from the cytotoxic effects of oxidized LDL.[19] In the present study, a significant lowering of plasma TG level with simultaneous increase in plasma HDL-C definitely indicate the beneficial role of PIO administration to hyperlipidemic animals.

The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. ROS includes free radicals, non free radicals and various forms of activated oxygen. They are involved in various physicochemical processes and pathogenesis of various

serious diseases such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation.[20] Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing complications of such type of diseases.

Presently noted lower levels of MDA and higher levels of catalase enzyme activities in PIO treated groups (mainly Group IV) indicate the possible role of PIO as antioxidants. Antioxidant activity of pioglitazone in diabetic patients is reported to be mediated by blocking the vicious cycle of ROS production, improve insulin sensitivity and halt the pro-inflammatory signaling transduction.[21] In past, many studies were conducted with PIO to confirm its antioxidant activity.[4,21-23] Taken together, these observations indicate that PIO administration to hyperlipidemic animals can reduce serum TG and increase serum HDL-C levels and also improve antioxidant enzyme activities.

CONCLUSION

Thus, we conclude that PIO could improve lipid profile and decrease oxidative stress in hyperlipidemic conditions which suggest that PIO may reduce cardiovascular risk by its hypolipidemic and antioxidant actions in patients with type 2 diabetes, especially when it is associated with increased triglyceride level.

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