ORIGINAL ARTICLE

Pioglitazone improves serum lipid profile in diet induced hyperlipidaemic non diabetic rats

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Abstract

Objective: To evaluate the anti-dyslipidaemic effects of pioglitazone in diet-induced non-diabetic hyperlipidaemic rats and to compare them with gemfibrozil.

Methods: This comparative animal study was conducted at the Postgraduate Medical Institute, Lahore, Pakistan, from July to September 2011, and comprised Sprague Dawley albino rats divided into three equal groups. Initially all three groups were given high-lipid diet containing cholesterol 1.5g, coconut oil 8ml and sodium cholate 1.0g per 100g of rat chow to induce hyperlipidaemia. From 4th to 8th week, Group A (control) was given 0.5ml of distilled water, Group B was given pioglitazone 10mg/kg body weight, and Group C was given gemfibrozil 10mg/kg body weight as single morning dose by oral route for a period of 04 weeks in addition to hyperlipidaemic diet. Serum lipid levels were estimated at zero, 4th and 8th week. Blood sugar level was estimated at 4th week to exclude diabetic rats.SPSS 17 was used for data analysis.

Results: Of the 27 rats, each group had 9(33.33%) rats. At the start of the study, the mean weight was 254.44±14.67g in Group A, 255.11±14.66g in Group B and 252.22±14.18g in Group C. It was 352.22±16.79g, 332.22±17.19 and 328.11± 12.92 at the 8th week. The mean total cholesterol at 0 week was 71.4±4.88 mg/dl in Group A, 71.9±7.03 in Group B and 73.4±5.27 in Group C. At the 8th week, the values were 161.8±9.2 mg/dl, 100.8±7.0 and 95.0±6.6. The mean low-density lipoproteincholesterol levels in the respective groups were 30.2±4.9mg/dl, 32.2±7.0 and 33.6±6.0 at 0 week; 77.8±8.4, 85.1± 15.3 and 86.9± 6.3 at the 4th week and 113.9± 10.1, 60.4± 9.2 ($p \leq 0.001$) and 54.8± 6.6 ($p \leq 0.001$) at the 8th week.The mean serum high-density lipoprotein cholesterol at the 8th week was 11.4± 1.7 mg/dl, 19.7± 2.4 ($p \leq 0.001$) and 19.2± 2.5 ($p \leq 0.001$) in the three groups, respectively.

Conclusion: Treatment with pioglitazone improved serum lipid profile of non-diabetic hyperlipidaemic rats equivalent to that of gemfibrozil.

Keywords: Pioglitazone, Hyperlipidaemia, Gemfibrozil, Lipid profile. (JPMA 66: 1286; 2016)

Introduction

Cardiovascular diseases (CVD) due to atherosclerosis are currently the leading cause of death in the West as well as in developing countries. Dyslipidaemia is the most important risk factor for the development of atherosclerosis.[1] Both genetic disorder and lifestyle having sedentary behaviour and diet high in calories, saturated fat and cholesterol contribute to dyslipidaemia. Across the globe, the incidence of death from cardiovascular and circulatory diseases has risen by one-third between 1990 and 2010.[2]

Dyslipidaemia is characterised by elevated low-density lipoprotein (LDL) cholesterol, high triglycerides (TG) and low high-density lipoprotein (HDL) cholesterol level. Epidemiological, clinical, genetic and experimental studies indicate that high serum levels of TG, LDL cholesterol and low level of HDL cholesterol are associated with atherosclerosis causing increased risk of vascular strokes and coronary heart disease (CHD).[3] Diabetic patients are more prone to developing atherosclerosis and type 2 diabetics are affected more often than type 1 diabetics.[4]

Thiazolidinediones (TZD), pioglitazone and rosiglitazone are used for the treatment of type 2 diabetes. Pioglitazone is less potent agonist of the peroxisome proliferator-activated receptor gamma (PPAR-γ) than rosiglitazone, but is effective in reducing both fasting blood glucose and glycatedhaemoglobin (HbA1c) level. Pioglitazone, widely used as antidiabetic agent, not only has an impact on glycaemic control but it has also been shown to increase HDL and decrease LDL and TG in diabetics.[5] All anti-diabetic drugs have varying effect on lipid profile but overall pioglitazone has shown more favourable lipid-lowering effect in comparison to other antidiabetics[6,7] including rosiglitazone.[7,8] Cardiovascular benefits of pioglitazone are found to be better than those of

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rosiglitazone and glimipride in type 2 diabetic patients.[9,10] In non-diabetic patients at high cardiovascular risk, co-administration of pioglitazone with atorvastatin had additional beneficial effect on lipid profile when compared to atorvastatin alone.[11] In addition to PPAR-γ pioglitazone has mild PPAR alpha (PPAR-α) agonist action which may be responsible for better lipid and cardiovascular profile.[12]

Pioglitazone by acting on PPAR-γ improves serum lipid profile in diabetics by improving insulin sensitivity and decreasing insulin resistance. Anti-dyslipidaemic effects of pioglitazone are mainly mediated through activation of PPAR-α.[12] In the past decade a number of new targets for treatment of dyslipidaemia were identified. One idea is combined activation of PPAR-α and PPAR-γ receptors. This will add the effects of PPAR-α and PPAR-γ receptors on lipid metabolism and their use will not be restricted to type 2 diabetes.[13] It is reasonable to study the effect of pioglitazone on lipid profile of non-diabetics and compare it with that of gemfibrozil, a fibric acid derivative, which shows anti-hyperlipidaemic effect by acting on PPAR-α. So this study was designed to compare the effect of pioglitazone and gemfibrozil on serum lipid profile of non-diabetic hyperlipidaemic rats.

Materials and Methods

This comparative animal study was conducted at the Postgraduate Medical Institute (PGMI), Lahore, Pakistan, from July to September 2011, and comprised Sprague-Dawley rats. Approval was obtained from the institutional ethical committee. Rats weighing between 230-275 grams were purchased from the National Institute of Health (NIH), Islamabad, and were kept in animal house at the PGMI in iron cages under hygienic conditions. The room temperature was maintained at 25±2 degrees Celsius under a natural day/night cycle. They were kept for acclimatisation for one week with free access to food and water. After one week, they were fed hyperlipidaemic diet containing cholesterol (1.5g), coconut oil (08ml) and sodium cholate (1.0g per 100g of rat chow).[14] All the ingredients were mixed thoroughly to make a homogenous mixture. Diet was prepared fresh at 2 weeks interval and stored at 2-8°C in a closed container. Diet containers were protected from light and moisture.

The rats were divided into three equal groups. They were fed hyperlipidaemic diet throughout the study period of 8 weeks. After first 4 weeks, Group A was given 0.5ml of distilled water, Group B was given pioglitazone 10mg/kg body weight[15] dissolved in distilled water and Group C was given gemfibrozil 10mg/kg body weight[16] dissolved in distilled water. All treatments were given by oral route as morning daily dose for a period of 4 weeks.

Body weight of each rat was measured at the beginning of the study, 4th week and then at weekly interval up to 08 weeks in order to adjust dose. At zero, 4th and 8th week, 02ml of blood was collected by cardiac puncture under light anaesthesia using 03ml disposable syringe with 27-gauge needle after overnight fasting of 12 hours. Blood was transferred to a labelled centrifuge tube and allowed to clot at room temperature for one hour and centrifuged at 3,000 revolutions/minute (rpm) for ten minutes. Serum was separated and stored at -20°C until analysed for lipid profile. During overnight fasting animals were kept deprived of food but had free access to water. Lipid profile estimation was done by enzymatic end-point method using commercially available kits (Randox) on spectrophotometer. Parameters measured were total cholesterol, triglycerides, HDL and LDL cholesterol. LDL/HDL ratio was calculated. Fasting blood glucose (FBG) level was estimated at 4th week to exclude diabetic rats.

SPSS 17 was used for data analysis. The numeric data was presented as descriptive statistics i.e. mean ± standard deviation (SD). To see the significance of outcome in 3 study groups repeated measurement analysis of variance (ANOVA) was applied. To see multiple comparisons in 3 study groups Tukey's test was applied. $P \leq 0.05$ was considered significant.

Results

Of the 27 rats, each group comprised 9(33.33%) rats. At the start of the study, the mean weight was 254.44±14.67g in Group A, 255.11±14.66g in Group B and 252.22±14.18g in Group C. This increased to 314.44±20.83g, 324.11±16.31g and 323.56±13.31g by the 4th week. At the 8th week, the mean weight in Group A stood at 352.22±16.79g compared to 332.22±17.19 in Group B (p=0.033) and 328.11± 12.92 in Group C (p=0.009).The difference between Group B and C was not significant (p=0.846).

At the 4th week, the mean FBG level was 73.56±8.78mg/dl, 76.78±11.10mg/dl and 73.11±11.08mg/dl in Groups A, B and C, respectively.

After 4 weeks, all the rats developed hyperlipidaemia. The mean total cholesterol at 0 week was 71.4±4.88mg/dl in Group A, 71.9±7.03 in Group B and 73.4±5.27 in Group C. The respective values stood at 122.7±7.2, 126.1±7.7 and 132.3±5.1 at the 4th week. At the 8th week, however, Group A had mean cholesterol of 161.8±9.2 mg/dl compared to 100.8±7.0 in Group B ($p \leq 0.00$) and 95.0±6.6 in Group C ($p \leq 0.001$). Similarly, the mean TG values in Group A, B and C were 88.4±5.1mg/dl, 88.8±5.1 and

Table: Effect of pioglitazone and gemfibrozil on body weight and lipid profile of hyperlipidemic rats. Data represents mean±SD (n=9).

Parameter	Week	Group A Control	Group B Pioglitazone	Group C Gemfibrozil
Body weight (g)	0	254.44± 14.67	255.11± 14.66	252.22± 14.18
	4	314.44± 20.83	324.11± 16.31	323.56± 13.31
	8	352.22± 16.79	332.22±17.19*	328.11± 12.92**
Total cholesterol (mg/dl)	0	71.4± 4.88	71.9± 7.03	73.4± 5.27
	4	122.7±7.2	126.1± 7.7	132.3± 5.1
	8	161.8± 9.2	100.8± 7.0***	95.0± 6.6***
Triglycerides (mg/dl)	0	88.4± 5.1	88.8± 5.1	86.9± 6.2
	4	141.1± 6.2	143.8± 8.3	139.9± 7.1
	8	181.3± 5.5	101.3± 6.3***	103.3± 5.5***
LDL- cholesterol (mg/dl)	0	30.2± 4.9	32.2± 7.0	33.6± 6.0
	4	77.8± 8.4	85.1± 15.3	86.9± 6.3
	8	113.9± 10.1	60.4± 9.2***	54.8± 6.6***
HDL- cholesterol (mg/dl)	0	23.2± 3.1	21.6± 2.4	22.3± 2.2
	4	16.3± 2.2	15.3± 2.9	17.0± 2.2
	8	11.4± 1.7	19.7± 2.4***	19.2± 2.5***
LDL/HDL ratio	0	1.34± 0.37	1.52±0.42	1.53±0.42
	4	4.87± 1.02	5.82± 1.88	5.23± 1.10
	8	10.19±1.99	3.16±0.86***	2.90± 0.56***

*p-value ? 0.05, ** p-value ? 0.01, *** p-value ? 0.001 vs Group A.
LDL: Low-density lipoprotein.
HDL: High-density lipoprotein.
SD: Standard deviation.

86.9±6.2 at 0 week; 141.1±6.2, 143.8±8.3 and 139.9±7.1 at the 4th week; and 181.3±5.5, 101.3±6.3 ($p\leq 0.001$) and 103.3±5.5 ($p\leq 0.001$) at the 8th week. The mean LDL-cholesterol levels in the respective groups were 30.2±4.9mg/dl, 32.2±7.0 and 33.6±6.0 at the beginning; 77.8± 8.4, 85.1± 15.3 and 86.9± 6.3 at the 4th week and 113.9± 10.1, 60.4± 9.2 ($p\leq 0.001$) and 54.8± 6.6 ($p\leq 0.001$) at the 8th week.

The difference in serum total cholesterol, TG and LDL cholesterol levels in Group B and C at 8th week were not significant (p=0.846, p=0.746 and p=0.369, respectively).

The mean serum HDL cholesterol at the 8th week was 11.4± 1.7 mg/dl, 19.7± 2.4 ($p\leq 0.001$) and 19.2± 2.5 ($p\leq 0.001$) in Group A, B and C. The difference of serum HDL cholesterol in Group B and C was not significant (p=0.907).

Serum LDL/ HDL ratio at 0 week was 1.34±0.37 in Group A, 1.52±0.42 in Group B and 1.53±0.42 in Group C, whereas it was 4.87±1.02, 5.82±1.88 and 5.23±1.10 at the 4th week. At the 8th week, the ratio was 10.19±1.99 in Group A, 3.16±0.86 in Group B ($p\leq 0.001$) and 2.90± 0.56 in Group C ($p\leq 0.001$).The difference of LDL/ HDL ratio in Group B and C at the 8th week was not significant (p=0.908) (Table).

Discussion

This study was conducted on non-diabetic hyperlipidaemic rats to observe the effect of pioglitazone on lipid profile and compare it with that of gemfibrozil, a known PPAR-α agonist. Different types of diets are used in animal models to induce hyperlipidaemia like animal fat and vegetable oil. The main objective of this study was to determine anti-hyperlipidaemic effect of drugs on non-diabetic hyperlipidaemic rats, so animal fat was not used in this study because animal fat, in addition to increasing serum lipid profile, also leads to development of diabetes.[17] Coconut oil, which contains almost 90% saturated fats, was used to induce hyperlipidaemia. Long-chain saturated fatty acids have been considered a risk factor for insulin resistance and diabetes mellitus. Coconut oil contains medium- and short-chain fatty acids.[18] In this study, no experimental animal developed diabetes and this was ruled out by doing fasting blood glucose level at 4 weeks of study.

During the current study, hyperlipidaemic diet containing coconut oil, cholesterol and sodium cholate was given to animals of all groups. This diet caused significant increase in body weight of animals and dyslipidaemia after 4 weeks. Pioglitazone and gemfibrozil were given to experimental groups for the next 4 weeks. At the end of the study, body weight of animals was low and lipid profile had markedly improved in both study groups as

compared to the control group.

Weight gain was significantly less in the pioglitazone group as compared to the control group with p-value < 0.05 and insignificantly more as compared to gemfibrozil group. Paradoxically, other studies on non-diabetic rats have demonstrated more gain in body weight of animals receiving pioglitazone verses high fat diet control,[19,20] although the difference was insignificant in one study.[20] The reason for gain in weight was perhaps fluid retaining property of pioglitazone.[21]

Lipid profile showed decrease in triglycerides, total cholesterol, LDL-cholesterol and LDL/HDL ratio while increase in HDL-cholesterol levels; all with p-value < 0.001 as compared to high-fat diet controls. Consistent with this, a study on diet-induced obese rats demonstrated decrease in triglycerides and free fatty acids both with pioglitazone and ragaglitazar with p-value < 0.001; but the effect was greater than that of bezafibrate.[19] In our study, the effect of pioglitazone was equivalent to that of gemfibrozil. It was maybe due to weaker effect of bezafibrate in rodents.[22] Similarly, pioglitazone significantly reduced triglycerides, total cholesterol and LDL-cholesterol and increased HDL-cholesterol in normoglycemic, high-cholesterol-fed rats with p-value <0.01.[20] In another study on high-fat-fed rats, pioglitazone decreased triglycerides, total cholesterol, LDL-cholesterol and LDL/HDL ratio but failed to improve HDL-cholesterol level.[23] Clark et al.[24] studied the effect of pioglitazone on lipid profile of obese cats demonstrating decrease in triglycerides and total cholesterol, supporting the results of our study.

This study was done on non-diabetic hyperlipidaemic rats in order to determine the PPAR-α effect of pioglitazone on lipid profile. In the past very limited studies were done on pioglitazone to observe PPAR-α effect. Orasanu et al.[25] demonstrate that pioglitazone could also regulate PPAR-α target gene indirectly, for example lipoprotein lipase (LPL), a positively regulated PPAR-γ target gene can generate PPAR-α ligands through very low-density lipoprotein (VLDL) hydrolysis. From equivalent effect of pioglitazone and gemfibrozil in the present study, it seems that pioglitazone is a strong PPAR-α agonist, but high-cholesterol diet leads to insulin resistance without hyperglycaemia.[17]

One of the limitations of our study was that neither another insulin sensitiser like metformin was used for comparison nor serum insulin level was determined to access insulin resistance. Had that been done, contribution of PPAR-γ and PPAR-α effect would have become clear.

Conclusion

Feeding rats with high-fat and high-cholesterol diet induced marked dyslipidaemia in 4 weeks, which significantly improved after treatment with pioglitazone and gemfibrozil, with insignificant difference between both treatments. Although both treatments failed to bring lipid profile to the baseline, it can be considered as anti-hyperlipidaemic drug in non-diabetic patients who do not tolerate other anti-dyslipidaemic drugs.

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Conflict of Interest: None.

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