ANTI-INFLAMMATORY EFFECT OF PIOGLITAZONE MARKED BY REDUCTION OF C - REACTIVE PROTEIN IN HIGH FAT FED RATS

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ABSTRACT

Background: C- reactive protein is among the most commonly studied inflammatory markers and it is one of risk factors in cardiovascular diseases. Pioglitazone has shown anti-inflammatory effect in some studies.

Objectives To assess anti-inflammatory effect of pioglitazone by measuring C- reactive protein level in high fat fed non-diabetic rats.

Methods: A comparative animal study was conducted at the Post Graduate Medical Institute, Lahore in which 27, adult healthy male albino rats were divided into three groups. Hyperlipidemia was induced in all three groups by giving hyperlipidemic diet containing cholesterol 1.5%, coconut oil 8.0% and sodium cholate 1.0%.After four weeks, Group A (control) was given 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 four weeks. C- reactive protein (CRP) was estimated at zero, 4th and 8th week.

Results: There was significant increase in the level of CRP after giving high lipid diet from 0 to 4^{th} week in all groups. Multiple comparisons by ANOVA revealed significant difference between groups at 8^{th} week only. *Post hoc* analysis disclosed that CRP level was significantly low in pioglitazone treated group compared to control and gemfibrozil groups ($p<0.001$), while difference between control and gemfibrozil was not significant.

Conclusion: Pioglitazone decreased CRP levels in diet induced hyperlipidemic non- diabetic rats.

Keywords: Hyperlipidemia, Pioglitazone, Gemfibrozil, Lipid profile, CRP

INTRODUCTION

Inflammation plays an integral role in the pathogenesis of cardiovascular events[1]. One of the potential inflammatory markers is C-reactive protein (CRP), an acute phase substance that shows extent of inflammation and has proved to be an independent as well as a combined risk factor in a number of cardiovascular diseases[2]. CRP induces endothelial cells to produce monocyte chemotactic protein -1 leading to expression of intercellular adhesion molecule-1 and vascular adhesion molecule-1 by them. Furthermore, CRP stimulates the monocyte release of pro-inflammatory cytokines that contribute the pathogenesis of the cardiovascular disease[3]. Multiple clinical trials and medical investigations involve measurement of CRP in cardiac and vascular diseases. Various studies have proposed the role of hs-CRP in determining serum lipid profile fluctuations in response to dietary interventions, suggesting that these changes depend on the individual's baseline CRP concentration. For example, men with high baseline serum CRP concentration had increased fasting Cholesterol, triglycerides, LDL cholesterol level after consuming a high fat diet. Similarly, low baseline serum CRP concentration has opposite effect[4].

Among the pharmaceutical interventions, Statins are the best choice for cardiovascular diseases so far. Apart from their hypolipidemic action, they also exert anti-inflammatory actions[5] by stimulating the expression of PPAR-γ [6].

Pioglitazone is a member of the thiazolidinediones (TZDs) family of antidiabetic agent that stimulates the peroxisome proliferator- activated receptor-γ and α. In addition to its beneficial effects on glucose and lipid metabolism, this gene has multiple effects including anti-inflammatory properties[7]. Clinical studies have shown that it is able to reduce inflammatory markers such as CRP in diabetic as well as non-diabetic patients [8,9]. Activation of PPAR-γ by pioglitazone as well as simvastatin reduce the inflammatory markers in patients of ischemic heart disease with metabolic syndrome [10].

We have previously shown that pioglitazone improves serum lipid profile in male albino hyperlipidemic rats[11]. However, the regularity effect of pioglitazone on inflammatory markers such as CRP has not been described. Hence the present study was designed to evaluate the effect of pioglitazone on the expression of CRP in male albino rats fed on high fat diet.

METHODOLOGY

This experimental animal study was started after taking approval from ethical committee of Post Graduate Medical Institute (PGMI), Lahore. A total of 27 adult healthy male albino rats,7-8 week of age weighing between 230-275g were purchased from National Institute of Health, Islamabad and were kept in animal house of PGMI, Lahore in iron cages under hygienic conditions. The room temperature was maintained at 25 ±2 ^{O}C under a day/night cycle. After one week of acclimatization rats were divided into three groups and fed hyperlipidemic diet throughout study period of 8 weeks. Hyperlipidemic diet contained cholesterol 1.5% coconut oil 8.0% and sodium cholate 1.0%[11]. After 4 weeks group A (control) rats were given 2 ml/Kg body weight of distilled water, group B (experimental) rats were given 10 mg/2 ml/Kg body weight of pioglitazone and group C (experimental) rats were given 10 mg/2 ml/Kg body weight of gemfibrozil daily as a single morning dose. Both drugs were of pharmaceutical grade, dissolved in distilled water daily and given by oral route.

Body weight of each rat was measured weekly and doses were adjusted accordingly. At baseline, after 4 weeks of high fat feeding and at end of experiment (4 weeks of high fat feeding and treatment with pioglitazone or gemfibrozil), blood sample was taken, after overnight fasting of 12 hours, by cardiac puncture and put in vacutainer. After clotting and centrifugation, serum was separated and stored at 4 $^{\circ}$C for 1 hour until analyzed for C - reactive protein. CRP in each sample was assayed by an immunoturbidimetric method by using a standard assay kit. Blood sugar level was estimated by oxidase method at 4th week to exclude diabetes.

Data was analyzed by SPSS 21. After checking normal distribution by Shapiro Wilk test, it was expressed as mean ± SD. Comparison between groups was made by ANOVA followed by *post hoc* Tukey's test. Mauchley's test of sphericity was applied to validate repeat measure ANOVA which was followed by *post hoc* analysis with Bonferroni adjustment.

RESULTS

Level of CRP in all groups was similar at 0 week. After high fat diet it increased in all groups at 4th week. After treatment with pioglitazone it decreased at 8th week but continued to increase in control and gemfibrozil treated groups (Table1).

Multiple comparisons by ANOVA revealed significant difference between groups at 8th week only. *Post hoc* analysis disclosed that CRP level was significantly low is pioglitazone treated group compared to control and gemfibrozil groups. While difference between control and gemfibrozil was not significant (Table 2).

When repeat measure ANOVA was applied p value was <0.001 in each group (Table1). *Post hoc* analysis of each group revealed significant increase in CRP level from 0-4 and 0-8 weeks in all groups. From 4-8 week there was significant decrease in pioglitazone treated group, while significant increase in control and gemfibrozil treated group (Table3).

DISCUSSION

Silent inflammation causes a significant contribution to the pathogenesis as well as complications of atherosclerosis[12] and poses an enormous burden on public health system. There are several markers to detect this inflammation out of which the role of CRP cannot be denied. It has a long half-life of 19 hour, due to which it is a stable marker for cardiovascular status monitoring. It plays an important role in detecting subclinical diseases and assessing the wellbeing of average population[13].

Objective of this study was to investigate the effect of pioglitazone in comparison to gemfibrozil, on CRP level of diet induced nondiabetic hyperlipidemic albino rats. For this purpose, 27 male albino rats were divided into three groups. All rats were fed high fat and cholesterol diet and developed hyperlipidemia at 4th week of study period without developing diabetes. In this study young male albino rats were used because they are prone to develop hyperlipidemia as compared to their female counter parts. High lipid diet promotes atherosclerosis by generation of various inflammatory and pro oxidant elements[14].After 4th week rats in three groups were administered distilled water, pioglitazone and gemfibrozil respectively. The basic reason for comparison between pioglitazone and gemfibrozil was that both are agonist of peroxisome proliferator activated receptor PPAR. Both improved lipid profile in our previous study[11].

CRP level was significantly increased in all groups at 4th week as compared to baseline. After treatment with pioglitazone it significantly decreased at 8th week, although not up to baseline. Numerically level was slightly higher than baseline, but statistically the difference was significant. Reason may be continued administration of atherogenic diet. Secondly it may be age related factor. Had another group of normal rats been added, it would have been interpreted better. A study conducted on hyperlipidemic rabbits demonstrated significantly lower level of CRP in pioglitazone treated animals as compared to control group after 18 weeks of treatment with p value of < 0.01. In our study CRP was lower in pioglitazone treated group as compared to control group with p value of < 0.001 after 8 weeks of treatment [15]. Clinical studies have also demonstrated anti-inflammatory property of pioglitazone checked by measuring serum CRP level. In type 2 diabetes mellitus patients, pioglitazone treatment for 12 weeks decreased serum CRP level with p value < 0.001 [16]. In another study on diabetic patients effect of pioglitazone was more than metformin with p value 0.04 after 16 weeks of treatment [8]. Even non diabetic patients have shown significant decrease (p value 0.03) in CRP level after 24 weeks treatment with pioglitazone [9].

In this study gemfibrozil failed to decrease the level of C-reactive protein as compared to pioglitazone. This might be because pioglitazone is an agonist of both PPAR-α and PPAR-γ while gemfibrozil is PPAR- α agonist only. Other possibility may be because by acting through PPAR-γ pioglitazone also raises the level of plasma adiponectin[17]. Adiponectin is a protein involved in glucose and fatty acid metabolism. Its biosynthesis is disturbed in obesity, type 2 diabetes mellitus, metabolic syndrome and dyslipidemia which may contribute to atherosclerosis[18]. There is evidence that adiponectin has anti-inflammatory effects, improves endothelial function and thereby decreases atherosclerosis of vascular wall[19]. Pioglitazone raises plasma level of adiponectin which by an anti-inflammatory mechanism decreased level of CRP. Apart from that pioglitazone has also shown to upregulate antioxidant stress genes in healthy volunteers [17].

CONCLUSION

Results of this study demonstrate probable anti-inflammatory role of pioglitazone, evidenced by significant decrease in CRP level in non-diabetic hyperlipidemic rats in presence of continued high fat feeding.

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TABLES

Table 1: Effect of Pioglitazone and Gemfibrozil on CRP level (Mean ± SD) of non-diabetic hyperlipidemic rats (n=9)

Weeks	Group A (Control)	Group B (Pioglitazone)	Group C (Gemfibrozil)	p value by ANOVA
0	2.59 ± 0.28	2.63 ± 0.32	2.67±0.23	0.828
4	3.55 ± 0.44	3.59 ± 0.34	3.6±0.32	0.951
8	4.42 ± 0.30	2.93 ± 0.33	4.28±0.39	< 0.001
p value by repeat measure ANOVA	< 0.001	< 0.001	< 0.001	

Table 2: Multiple comparison of CRP level between groups at 8th weeks by post hoc Tukey's test

Group		Mean difference	Significance
A (Control)	B (Pioglitazone)	1.49	<0.001
	C (Gemfibrozil)	0.14	0.665
B (Pioglitazone)	C (Gemfibrozil)	-1.35	<0.001

Table 3: Multiple comparison of CRP level between study times within groups

		Group A (Control)		Group B (Pioglitazone)		Group C (Gemfibrozil)	
Time (weeks)		Mean difference	Significance	Mean difference	Significance	Mean difference	Significance
0	4	-0.953	0.002	-0.962	<0.001	-0.921	<0.001
	8	-1.828	<0.001	-0.294	0.009	-1.603	<0.001
4	8	-0.874	0.005	0.668	0.002	-0.682	0.048

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