Comparative effects of metformin and pioglitazone on lipid profile of rabbits

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Abstract: This is the initial part of study in which the effects of two oral hypoglycemic drugs metformin and pioglitazone were studied on lipid profile of rabbits. White rabbits of both sexes were equally divided in to three groups each comprising of seven animals. Control group was given distilled water 2m1/kg, animals of group II were given metformin in the dose of 22mg/kg and animals of group III received pioglitazone in the dose of 0.5mg/kg. Serum concentration of cholesterol, very low-density lipoprotein (VLDL), triglycerides (TGs), low density lipoprotein (LDL) and high density lipoprotein (HDL) were measured after 8 week of oral dosing. Results shows that after 8 weeks animals received metformin did not reveal any significant change in lipid profile, but animals received pioglitazone showed significant ($P<0.05$) decrease in lipid profile, the decrease in cholesterol, LDL, VLDL and triglycerides is favorable however decrease in HDL is troublesome and warrant further investigations.

Keywords: Metformin; pioglitazone; diabetes mellitus; cholesterol; lipoproteins.

INTRODUCTION

Type II diabetes mellitus is one of the major risk factors that can predispose diabetics to cardiovascular diseases. Even the pre-diabetic condition with impaired glucose tolerance has been associated with an increased risk of cardiovascular events and an increased mortality risk (Petersen and McGuire, 2005). The risk increases even more in the presence of abnormal lipid profile called dyslipidemia characterized as decrease in HDL, increase in TGs, VLDL and LDL cholesterol. The reason behind dyslipidemia in diabetes is mainly related with insulin resistance, which results in increased lipolysis, more release of fatty acids from adipose tissues and increased secretion of chylomicrons. Therefore if the insulin resistance is lowered in the treatment of type II diabetes, lipid profile may improve and the risk of cardiovascular diseases may reduce.

Insulin sensitizers are those hypoglycemic drugs, which increase insulin sensitivity or decrease insulin resistance. Two main classes of drugs in this category are biguanides and thiazolidinediones. Pioglitazone is the member of thiazolidinedione family, which acts by activation of a specific nuclear receptor, the peroxisome proliferator-activated receptor gamma (PPAR-gamma). When used as monotherapy the effect of pioglitazone supersedes metformin in decreasing TGs and increasing HDL levels (Betteridge, 2007; Quinn *et al.,* 2008). Pioglitazone has recently been investigated to possess activity against endothelial dysfunction, which is induced by ischemia and reperfusion (Yuka *et al*., 2014). Another use of this oral hypoglycemic drug is in the treatment of polycystic ovary syndrome (PCO) where it produces comparable effects with metformin (Sangeeta, 2012).

Another insulin sensitizer metformin reduces glucose output by acting on liver and, by augmentation of glucose uptake by the peripheral tissues, especially muscle. These effects are mediated by the activation of liver kinase B1 (LKB-1), which regulates the adenosine monophosphatase protein kinase (AMPK). AMPK further phosphorylates and inactivates a transcriptional co-activator, transducer of regulated CREB protein 2 (TORC2), as a result there is decrease in the transcription of enzymes involved in gluconeogenesis (Shaw *et al*., 2005).

Besides hypoglycemic activity metformin also improves endothelial dysfunction, decreases oxidative stress and improves lipid profile resulting in lessening of cardiovascular mortality and morbidity (Rojas and Gomes. 2013).

Recently researches have elucidated the role of metformin in decreasing insulin resistance, which explains the free radical scavenging properties of metformin. They attribute this effect of metformin with the regulation of LYR motif-containing 1 (LYRM1), which is thought to be involved in adipose tissues homeostasis and induction of mitochondrial reactive oxygen species (ROS) contributing to obesity related insulin resistance (Zhang *et al*., 2014; Qin *et al.*, 2014)

Experimental Section

The study was carried out after the approval from Board of advanced studies and research, University of Karachi and ethical issues pertaining to experimental studies on animals were monitored by departmental research committee.

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Animals

Rabbits of either sex 1.2 to 1.5kg were purchased from the local animal supplier and kept for a conditioning period about a week in the animal house, Department of Pharmacology. The animals were maintained on standard feed and water ad libitum, at an ambient temperature between 22-25°C, with a 12h light and dark cycle. Animals were randomly divided into 3 groups with 7 rabbits in each group.

Group I served as control and was given 2ml/kg distilled water. The other two groups were administered metformin (22mg/kg) and pioglitazone (0.5mg/kg) after dissolving in distilled water for a period of 60. All drugs were given orally.

Laboratory Assays

After an overnight fast on day 60, blood samples were collected through cardiac puncture in plain tubes, kept at room temperature for 6 h and then centrifuged to get clear serum. Serum concentrations of total cholesterol, VLDL, LDL, HDL and TGs were measured by enzymatic colorimetric assays.

STATISTICAL ANALYSIS

Data was analyzed by SPSS version 13.0 and t-test was used for independent samples test. $P \leq 0.05$ was considered significant and $P \leq 0.01$ was considered highly significant.

RESULTS

Table I shows comparative effect of metformin and pioglitazone on lipid profile of animals after the completion of eight weeks of dosing. Metformin showed no significant change in lipid profile when compared to control, however pioglitazone showed significant decrease in cholesterol (5.8±2.6) and LDL (2.2±0.45) while there was highly significant decrease in TGs (16.2±3.1), HDL (5.2±0.45) and VLDL (3±0.71) as compared to Control.

DISCUSSION

Diabetes was once simply thought as a disease in which the main issue was to avoid eating sugar or rice, however with the increase in health awareness everyone gets frightened even by a glimpse of hyperglycemia, glucose intolerance or increase in glycosylated hemoglobin (HbA1c), since it maybe an indication of pre-diabetes which may develop in to diabetes, if proper measures are not taken to prevent it (Zhang *et al*., 2009). Hence a person may face life time challenges to avoid complications like poor perfusion of tissues, weight gain hyperlipidemia and associated heart risks. Good glycemic control, life style changes and the decrease in insulin resistance really helps in reducing diabetes associated cardiovascular risks.

In the present study two commonly used insulin sensitizers as initial therapy for type II diabetes and even in the pre-diabetic state were compared to investigate their beneficial effects on the lipid profile, which may other wise cause atherosclerosis, hypertension and other heart diseases.

The effects of metformin and pioglitazone on lipid profile were mostly in accordance to the earlier studies with the exception of one contradictory highly significant result. Metformin is thought to maintain lipid profile and it did the same in present study, since caused no significant change in the levels of lipid profile. On the other hand pioglitazone decreased the cholesterol ($P<0.05$), VLDL ($P<0.01$), LDL ($P<0.05$), Triglycerides ($P<0.01$) and HDL ($P<0.01$). The decrease in HDL was not the usual effect observed in earlier studies, rather it appeared to improve the cardiovascular risk factors and used safely as first line treatment in diabetes type II (Erem *et al*., 2014). Similarly another study showed that pioglitazone causes suppression of atherosclerosis by increasing HDL (Davidson *et al*., 2008)

These results suggest that changes in lipid profile may be dose dependent, while other possibility would be the difference in enzymes involved in regulation of lipid metabolism, their transcription factors, up regulation and down regulation mechanisms, or maybe there is some resistance in drugs efflux mechanism. However further work is needed to understand HDL decrease by pioglitazone because normally with the decrease in TGs

Table 1: Comparison of Lipid Profile in Pioglitazone and Metformin groups after 8 weeks of treatment

Parameters	Serum Concentration (mg/dl)		
	Control	Metformin	Pioglitazone
Cholesterol	26±7	22.8±7.2	*5.8±2.6
Triglycerides	74±34	62.4±11.9	**16.2±3.1
High density lipoprotein	12.2±2.2	12.2±5.6	**5.2±0.45
Low density lipoprotein	11.2±5.3	9.6±6.8	*2.2±0.45
Very low density lipoprotein	14.6±6.5	12.2±2.3	**3±0.71

n=7 Values are mean ± SD * $P \leq 0.05$ was considered significant as compared to control ** $P \leq 0.01$was considered highly significant as compared to control

there is increase in HDL as observed in insulin resistance.

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