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Effects of pioglitazone on atherogenic risk predictor indices of alloxan-induced diabetic rabbits

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Abstract

The effects of pioglitazone alone and in combination with either sulphonylurea or metformin on the atherogenic risk predictor indices of alloxan-induced diabetic rabbits were studied using five groups (A, B, C, D and E) of rabbits. Results of the mean values of plasma glucose, lipoproteins and atherogenic risk predictor indices determined after 4 and 8 weeks of drug administration showed that the plasma glucose, total cholesterol, LDL-cholesterol, triglycerides and VLDL levels significantly ($p<0.05$) decreased while HDL-cholesterol significantly ($p<0.05$) increased in the treated groups C, D and E when compared to group B, the diabetic control, after 4 weeks of drug administration. A greater positive effect was recorded after 8 weeks. The mean values of atherogenic risk predictor indices-- LDL-C/HDL-C and log (TG/HDL-C) after 4 and 8 weeks of drug administration were found to be significantly ($p<0.05$) decreased in the treated groups C, D and E when compared to group B (diabetic control) with a greater decrease also occurring after 8 weeks. The findings also showed positive synergistic effects of co-administered antidiabetic drugs like sulphonylurea and metformin on the atherogenic risk predictor indices.

Key words: Pioglitazone, metformin, sulphonylurea, atherogenic risk predictor indices.

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INTRODUCTION

Pioglitazone is a thiazolidine antidiabetic agent that depends on the presence of insulin for its mechanism of action. It decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. It also enhances cellular responsiveness to insulin, improves hepatic sensitivity to insulin and improves dysfunctional glucose homeostasis. In patients with type 2 diabetes, the decreased insulin resistance produced by pioglitazone results in lower blood glucose concentration, lower plasma insulin levels and also lower HbA values[1].

Patients with type 2 diabetes are at high risk for and have higher mortality from coronary heart disease (CHD)[2]. A cluster of cardiovascular risk factors associated with insulin resistance, a core defect in this disease, contributes to this increased risk for CHD[3]. Dyslipidaemia, very common in patients with type 2 diabetes, is one of these risk factors[4] and it is generally characterized by increased plasma triglyceride (TG) and decreased high-density lipoprotein-cholesterol (HDL-C) concentrations, preponderance of small, dense low-density lipoprotein (LDL) as well as increased apolipoprotein B concentration. Although the major focus on the connection between lipids and CHD is on low-density lipoprotein - cholesterol (LDL-C), the important roles of HDL-C and TG have been recognized and their combined effect called atherogenic dyslipidaemia[5].

Dobiasova and Frohlich[6] proposed the term atherogenic risk predictor indices of plasma (ARPI) and defined it as the ratio LDL-C/HDL-C or Log (TG/HDL-CH) on the bases that people with high ARPI have a higher risk for CHD than those with low ARPI; ARPI is positively correlated with the fractional esterification rate of HDL (FER$_{HDL}$), and ARPI is inversely correlated with LDL particle size. Because FER$_{HDL}$ predicts particle size in HDL and LDL, which in turn predicts CHD risk, the simultaneous use of TGs, LDL-C and HDL-C (all readily available in a plasma lipoprotein profile) as ARPI may be useful in predicting plasma atherogenicity. Correspondingly, insulin resistance (decreased insulin sensitivity), is often accompanied by increased CHD risk and is also often associated with increased LDL-C and TG levels as well as decreased HDL-C concentration and a predominance of small, dense LDL particles.

The mechanism of action of pioglitazone, basically, is its ability to activate peroxisome proliferators-activated receptor- gamma, (PPAR-γ). PPAR-γ receptor is a group of intracellular receptors inside the cell nucleus found in tissues important for insulin action such as adipose tissue, skeletal muscle and liver. Activation of PPAR- γ nuclear receptors by pioglitazone helps to modulate the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism. The transcribed genes then help to promote cell differentiation[7], decrease lipolysis and cause free fatty acid release. PPAR- γ, when activated improves production of mature adipocytes that have improved insulin sensitivity and are capable of storing much free fatty acids. All these help to reduce atherogens in the plasma and effect glycaemic control as well.

It is therefore logical to speculate that reducing insulin resistance (enhancing insulin sensitivity) can potentially correct dyslipidaemia and in so doing, improve ARPI. This study was designed to test whether improvements in insulin sensitivity by pioglitazone alone and in combination with metformin or with sulphonylurea, were associated with improvements in atherogenic risk predictor indices of alloxan-induced diabetic rabbits.

MATERIALS AND METHODS

Animals
Thirty (30) adult male rabbits (2.5-2.8kg) were received from the Animal Science Unit of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and allowed to acclimatise for a week at the Animal House of Imo State University, Owerri, The animals were randomly assigned into five (5) groups of six (6)

rabbits each and kept in a cage of five separate divisions.

Induction of diabetes and preparation of drugs

Diabetes was induced in four groups of animals (B, C, D and E) by intra-peritoneal injection of alloxan solution with concentration of 40mg/ml at the doses of 80mg/kg body weight of the rabbit[8]. Pioglitazone hydrochloride (45mg), an antidiabetic drug and a member of the thiazolidinedione class of drugs with a brand name of Actos (Eli Lilly and Co., Indianapolis, U.S.A.); Glibenclamide (daonil as the brand name) from Aventis Pharma Ltd., Ankleshwar, India and Metformin hydrochloride (glucophage as the brand name) from Merck Marker (Pvt) Ltd., Quetta, Pakistan were used in the experiment.

Pioglitazone was prepared by dissolving one tablet (containing 45mg of pioglitazone) in 45ml of water. Glibenclamide, (daonil), was prepared by dissolving one tablet (containing 5mg) in 50ml of water. Metformin hydrochloride, (Glucophage) was prepared by dissolving three tablets (containing 1500mg of glucophage) in 10ml of water.

Experimental design

The animals were separated into five groups of 6 rabbits each and arranged as shown:

Group A (Healthy control): Healthy rabbits neither induced with diabetes nor treated with any drug; *Group B* (Diabetic control): These were alloxan induced diabetic rabbits that were not treated throughout the study; *Group C*: Pioglitazone monotherapy test group. They were administered pioglitazone hydrochloride solution with concentration of 1mg/ml at the dose of 0.8mg/kg body weight: *Group D*: Pioglitazone + Metformin combination therapy test group. They were administered pioglitazone hydrochloride solution with concentration of 1mg/ml at the dose of 0.8mg/kg body weight and metformin solution with concentration of 150mg/ml at the dose of 62.5mg/kg body weight and *Group E*: Pioglitazone + Glibenclamide combination therapy test group. They were administered pioglitazone hydrochloride solution with concentration of 1mg/ml at the

dose of 0.8mg/kg body weight and glibenclamide solution with concentration of 0.1mg/ml at the dose of 0.08mg/kg body weight. The drugs were administered early in the morning after meal once daily for 8 weeks.

Laboratory assays

Using a 10ml syringe in each case, 6ml of fasting venous blood samples were collected from the ear lobe of all the rabbits after 4 and 8 weeks of drug administration and immediately transferred into EDTA bottles. The anticoagulated blood was then centrifuged at 3000 rpm using Wisperfuge model 1384 centrifuge (Tamson, Holland) for 10 min and the resulting plasma was used for plasma glucose and lipid estimations. Triglyceride was measured using the extraction method[9]; Total cholesterol was measured using the colorimetric method[10], while HDL-cholesterol was measured using the extraction before colorimetric determination method[10]. LDL-cholesterol was calculated using the Friedwald formula. The atherogenic risk predictor indices were calculated using the formulae of Dobiasova and Frohlich[6].

Statistical analysis

Significant differences between the mean values obtained from test animals and the control were statistically assessed using Duncan multiple range test.

RESULTS AND DISCUSSION

The results of the mean values of plasma glucose and lipoprotein levels of the five groups of animals are shown in Table 1. The plasma glucose, triglycerides, total cholesterol, LDL-cholesterol and VLDL levels significantly ($p < 0.05$) decreased, while HDL-cholesterol significantly increased ($p < 0.05$) in the treated groups of C, D and E when compared to group B, (diabetic control), after 4 weeks of drug administration. The same trend was observed but with a greater positive effect when drug administration lasted for 8 weeks showing a time-related positive effect of the drugs on the parameters. Table 2 shows the mean values of atherogenic risk predictor indices {HDL-CH/T-CH, LDL-CH/HDL-Ch and Log (TG/HDL-CH}

Table 1: Mean values of plasma lipoproteins and glucose levels after 4 and 8 weeks of treatment.

PARAMETERS	Group A (Healthy control)	Group B (Diabetic control)	Group C Pioglitazone Alone	Group D Pioglitazone+ Metformin	Group E Pioglitazone + sulphonylurea.
TOTAL-CH (mg/dl)					
4 weeks	115.3±2.5*	131.7±3.1	108.3±1.0	106.8±2.2	105.1±3.0*
8 weeks	117.4±2.1*	130.3±3.6	101.0±0.8**	99.8±2.1**	98.1±2.2**
TG (mg/dl)					
4 weeks	109.5±2.5*	117.9±1.3	104.4±1.6*	103.1±1.5*	102.0±1.2*
8 weeks	106.6±1.9*	120.1±1.14	97.6±1.4**	96.1±1.0**	95.2±1.1**
HDL-CH (mg/dl)					
4 weeks	47.9±2.3*	31.0±1.3	48.2±1.5*	49.3±1.3*	50.2±1.8*
8 weeks	46.2±2.0*	28.1±1.05	52.2±1.9**	53.4±1.5**	54.1±1.8**
LDL-CH (mg/dl)					
4 weeks	45.3±4.0*	77.2±4.3	39.4±1.2*	36.9±1.9*	34.6±1.7*
8 weeks	49.9±2.7*	78.1±5.2	29.7±1.7**	27.2±2.0**	24.9±1.2**
VLDL (mg/dl)					
4 weeks	21.9±0.5*	23.6±0.3	20.9±0.9*	20.6±0.3*	20.4±0.3*
8 weeks	21.3.3±0.4*	24.02±0.2	19.5±5.0	19.0±0.2	19.0±0.3*
FPG (mg/dl)					
4 weeks	100.2±4.0*	164.8±3.5	98.4±4.1*	90.0±2.7*	85.2±2.5*
8 weeks	103.4±4.2*	164.8±3.5	92.0±3.7**	88.5±4.0**	76.2±2.9**

*Significantly different from diabetic control (group B); **Significantly different from diabetic control and test groups after 4 weeks. FPG=Fasting Plasma Glucose; HDL-CH = High Density Lipoprotein – Cholesterol; LDL-CH = Low Density Lipoprotein – Cholesterol; T-CH =Total – Cholesterol; TG = Triglycerides*

Table 2: The mean values of plasma Atherogenic Risk Predictor Indices recorded after 4 and 8 weeks of treatment.

INDEX	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
HDL-CH/ T-CH					
4 weeks	0.42±0.02*	0.24±0.01	0.45±0.03*	0.46±0.01*	0.48±0.00
8 weeks	0.39±0.01*	0.22±0.01	0.51±0.02*	0.54±0.03*	0.55±0.02*
LDL-CH/ HDL-CH					
4 weeks	0.95±0.25*	2.49±0.30	0.82±0.40*	0.75±0.20*	0.69±0.10*
8 weeks	1.08±0.20*	2.78±0.35	0.57±0.38	0.51±0.25*	0.46±0.15*
Log (TG/HDL-CH)					
4 weeks	0.36±0.02*	0.58±0.01*	0.34±0.03*	0.32±0.01*	0.31±0.02*
8 weeks	0.36±0.01*	0.63±0.02*	0.26±0.02*	0.26±0.01*	0.25±0.02*

Significantly different from diabetic control, (group B); Values of HDL-CH/TOTAL-CH ratio < 0.3 are atherogenic and undesirable; Values of LDL-CH/HDL-CH ratio > 2.3 are atherogenic and undesirable

of the five groups of rabbits. The indices were found to be significantly (p<0.05) improved thus indicating that the drugs were antiatherogenic and so desirable in the treated groups C, D and E, (p<0.05) when compared to group B (diabetic control). From the results, it is evident that an additive effect was exhibited when pioglitazone was combined with other antidiabetic drugs like sulphonylurea and metformin.

This study further confirms the antiatherogenic efficacy of pioglitazone as it can significantly reduce the plasma atherogens, LDL-CH, total-CH, and triglycerides and the calculated

atherogenic indices of the three treated groups (C, D, and E). This work confirms and extends the findings[11] that pioglitazone considerably reduced the atherogenic indices of plasma within a little period of time.

Atherogenesis increases with increase in LDL-CH, triglycerides, total-cholesterol and a decrease in HDL-CH. These often occur in diabetes. Pioglitazone acts by decreasing the atherogens (LDL-C, total-C and triglycerides) and increasing the antiatherogen HDL-C. Increase in HDL-C reduces (and so improves) other indices due to its ability to transport cholesterol from the body cells to the liver for excretion. So, it enhances cholesterol removal from the body, thereby reducing the rate of occlusion of blood vessels and so also reduces atheroma formation. LDL-C, on the other hand, transports cholesterol to the cells and increases the potential of atheroma formation[12].

This work also showed that pioglitazone is a powerful antidiabetic drug due to its ability to reduce significantly ($p<0.05$) the plasma glucose of diabetic rabbits within a period of four weeks with even a more significant reduction when the drug administration was extended to 8 weeks. This result justifies the use of pioglitazone[13] to improve glycaemic control and also to treat patients with type II diabetes in U.S.A. It also supports the claim that pioglitazone can be used to effectively treat type II diabetes[14].

This study also showed that the combination of pioglitazone with other classes of antidiabetic drugs (like sulphonylurea and metformin) improves its antidiabetic and antiatherosclerotic activity with a more helpful result obtained with sulphonylurea (as seen in group E) than with metformin (group D). This finding may be because sulphonylurea instigates insulin production and pioglitazone has been proved to work more effectively in the presence of insulin[15]. Since pioglitazone has been found to be effective in preventing the occurrence of atherosclerosis even under diabetic conditions and since also its ability to improve the atherogenic risk predictor indices as well as reduce blood glucose level is increased by its combination with sulphonylurea, its use in

monotherapy and in more efficacious combinations may therefore be encouraged for both diabetic and coronary heart disease patients.

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