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Research Article

Effects of Pioglitazone, Glimepiride, Nobivolol, Valsartan and Hesperidine on Serum Diabetic Marker and Lipid Metabolizing Enzymes in Isoproterenol Induced Myocardial Infarction in Normal and Diabetic Rats

Jagdish Kakadiya*, Nehal Shah [1]

Pharmacology Department, Dharmaj Degree Pharmacy College, Petlad -Khambhat Road, Dharmaj, Anand-388430, Gujarat, INDIA.

ABSTRACT

Present study was designed to evaluate effect some synthetic drugs and some herbal compound on Glucose, HbA1c and Lipid Metabolizing Enzymes in isoproterenol induced myocardial infarction in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Pioglitazone (10 mg/kg, p.o), Glimepiride (0.5 mg/kg, p.o), Nobivolol (2 mg/kg, p.o), Valsartan (8 mg/kg, p.o) and Hesperidin (100 mg/kg, p.o) were administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and Nicotinamide (12mg/kg, i.p, NIC) and after isoproterenol (200 mg/kg, s.c.) induced myocardial infarction in rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) serum and heart tissues sample were collected, and glucose, HbA1c and cholesterol ester synthetase (CES), lecithin Cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL) were find out. Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose, glycosylated hemoglobin (HbA1c), significant ($p<0.001$) increased in the level of heart tissues CES and significant ($p<0.001$, $p<0.01$) decreased LCAT and LPL as compared to respective control groups. Treatment with Pioglitazone, Glimepiride significantly ($P<0.001$) decreased and Hesperidin significant ($P<0.05$) decreased HbA1c, glucose and CES level but treatment with nobivolol significant ($p<0.05$) decreased CES level without change on glucose and HbA1c. Treatment with Pioglitazone and Glimepiride significantly ($P<0.001$) increased and Hesperidin and Nobivolol ($P<0.01$) increased LCAT and LPL as compared to diabetic control. Treatment with Valsartan shows no change on Glucose, HbA1c and lipid metabolizing enzyme in diabetic rats. This study concluded that Pioglitazone and Glimepiride may show reduced diabetes marker, CES and protect LCAT and LPL on experimentally induced myocardial infarction in type 2 diabetic rats.

Keywords: Lipid metabolizing enzymes; Glucose; HbA1c; cardioprotective.

INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance (Kahn 1990, Leibowitz 1990). Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor (Uemura 2003), due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (Senses 2001).

Reactive oxygen species may contribute to the events of atherogenesis and leading to the progression of atherogenic lesions by promoting oxidation of low density lipoproteins (Benelli 2005). Isoproterenol has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of heart muscles. It also increases the level of serum and myocardial lipids (Prince 2005) and also increases the level of cholesterol in the tissues which in turn leads to coronary heart disease (Sathish 2003).

Recently, a protective effect of Pioglitazone against oxidative stress in liver and kidney of diabetic rabbits (Gumieniczek 2003) has been reported. Pioglitazone (PIO) hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. Glimepiride (GLI) an oral blood glucose lowering drug of the sulfonylurea class is reported to have pancreatic and extra pancrea-

* Corresponding Author
Email: jagdishkakadiya@yahoo.co.in
Contact: +91-9825882922 Fax: 91-2697245808
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tic effects as well. The blockages of K_{ATP} channels of pancreatic cells by sulphonylurea are critical in the regulation of glucose regulated insulin secretion. Nebivolol (NEB) is a β1-adrenoceptor blocking drug that possesses certain unusual pharmacological properties by which it differs from conventional β1-blockers. Recent evidence suggest that blockade of the renin-angiotensin system ameliorates diabetes induced cardiac dysfunction. Because, angiotensin receptor blockers Valsartan (VAL) blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscles and the adrenal gland. Hesperidin (HES) is an abundant and inexpensive byproduct of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae). It is reported to have anti-allergic, radio protective, immunomodulator, anti-hypertensive and anti-oxidant properties. When Hesperidin is administered orally, it is hydrolyzed by intestinal micro flora to yield a major active metabolite Hesperidin.

So far the effect of PIO, GLI, NOB, VAL and HES on lipid metabolizing enzymes of experimentally induced myocardial infarction in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to investigate the effect of PIO, GLI, NOB, VAL and HES treatment on serum diabetic marker and lipid metabolizing enzymes in Isoproterenol Induced myocardial infarction in type 2 diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Nobivolol was obtained as a gift sample from Torrent Pharmaceuticals Pvt. Ltd., Ahmadabad, India. Hesperidin was obtained from ACROS Lab, US. Pioglitazone hydrochloride and Valsartan was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (210 ± 15 g) were housed in group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitium*.

Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose (Masiello 1998). Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study. Drugs were administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and carried out for further estimations.

EXPERIMENTAL PROTOCOL

Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (**ND-CON**)] and normal saline subcutaneously on 29th and 30th day.

Group 2: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (**D-CON**)] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 3: Non-diabetic control treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-PIO)**] and normal saline subcutaneously on 29th and 30th day.

Group 4: STZ-NIC diabetic rats treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-PIO)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 5: Non-diabetic control treated with GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-GLI)**] and normal saline subcutaneously on 29th and 30th day.

Group 6: STZ-NIC diabetic rats treated with GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-GLI)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 7: Non-diabetic control treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-VAL)**] and normal saline subcutaneously on 29th and 30th day.

Group 8: STZ-NIC diabetic rats treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-VAL)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 9: Non-diabetic control treated with NEB (2 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC

for 4 weeks (**ND-NOB)**] and normal saline subcutaneously on 29th and 30th day.

Group 10: STZ-NIC diabetic rats treated with NOB (2 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-NOB)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 11: Non-diabetic control treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-HES)**] and normal saline subcutaneously on 29th and 30th day.

Group 12: STZ-NIC diabetic rats treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-HES)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

BIOCHEMICAL ESTIMATIONS

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of Diabetic Markers and Lipid Metabolizing Enzymes

On 4th week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose and HbA1c were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). The activities of lipid metabolizing enzymes such as cholesterol ester synthetase (CES), lecithin: Cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL) were determined from the heart tissues sample as suggested by Kothari et al (Kothari 1973), Hitz et al (Hitz 1983) and Slater et al (Slater 1996).

Statistical Analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of synthetic drugs and herbal on diabetic marker

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals. There was a significant ($p<0.001$) increase in Glucose and HbA1c after myocardial infarction in D-CON group as compared to ND-CON group. Treatment of PIO, GLI and HES in STZ-NIC diabetic rats significant ($p<0.001$, $p<0.05$) decreased levels of serum Glucose and HbA1c compared to respective diabetic control (Table 1).

Table 1: Changes in Glucose and HbA1c in rats

Groups	Glucose	HbA1c
ND-CON	101.8 ± 6.799	5.455 ± 0.3729
D-CON	332.8 ± 9.167***	9.900 ± 0.6323***
ND-PIO	95.67 ± 7.654	4.937 ± 0.4211
D-PIO	189.3 ± 8.3530***	6.618 ± 0.3421***
ND-GLI	97.67 ± 8.429	4.825 ± 0.4115
D-GLI	162.0 ± 11.72***	6.133 ± 0.3325***
ND-NOB	96.17 ± 6.954	4.820 ± 0.3265
D-NOB	302.3 ± 9.622	9.413 ± 0.4993
ND-VAL	93.00 ± 7.967	4.715 ± 0.3950
D-VAL	301.8 ± 11.48	9.363 ± 0.4487
ND-HES	98.17 ± 6.650	4.865 ± 0.3047
D-HES	294.0 ± 12.94*	7.872 ± 0.425*

Values are expressed as mean ± SEM for six animals in the group. * P<0.05, *** P<0.001 compared to respective control group.

Effect of NEB on Lipid Metabolizing Enzymes enzyme

There was a significant ($p<0.001$) increase in CES after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 1). Treatment of PIO and GLI in STZ-NIC diabetic rats (D-PIO, D-GLI) significant ($p<0.001$) decrease levels of CES as compared to diabetic control. Treatment of HES and NOB in diabetic rats significant ($p<0.05$) decrease levels of CES but treatment of VAL in diabetic rats no change level of CES as compared to diabetic rats. There was a significant ($p<0.001$, $p<0.001$) increase in LCAT and LPL after myocardial infarction in D-CON group as compared to respective ND-CON group (Fig. 1). Treatment of PIO and GLI in STZ-NIC diabetic rats significant ($p<0.01$) decrease levels of LCAT and LPL as compared to respective diabetic control. Treatment of HES and NOB in diabetic rats significant ($p<0.05$) decrease levels of LCAT and LPL but treatment of VAL in diabetic rats no change level of LCAT and LPL as compared to diabetic rats.

DISCUSSION

The present study was under taken with the objective of exploring the Pioglitazone, Glimepiride, Nobivolol, Valsartan and Hesperidin on diabetic marker and Lipid Metabolizing Enzymes experimentally induced myocardial infarction in diabetic rats.

Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Patients with diabetes show an increased mortality concerning cardiovascular events. They more often suffer from myocardial infarction as non-diabetics mostly with a more serious course. Moreover, the post-infarction course is affected with a worse prognosis as in non-diabetics (Abel 2005).

Table 2: Effect of Pioglitazone (10 mg/kg/day, p.o), Glimepiride (0.5 mg/kg/day, p.o), Nobivolol (2 mg/kg/day, p.o), Valsartan (8 mg/kg/day, p.o) and Hesperidin (100 mg/kg/day, p.o) on Cholesterol ester synthetase, Cholesterol ester synthetase, Lipoprotein lipase in experimentally induced myocardial infarction in normal & diabetic rats

Groups	Cholesterol ester synthetase (CES) (nanomoles of cholesterol esterified /hr/100 mg tissue)			Lecithin Cholesterol acyl transferase (LCAT) (mmoles of cholesterol esterified /hr/100 mg tissues)			Lipoprotein lipase (LPL) (micromoles of free fatty acids librated/100mg tissues)		
ND-CON	9.050	±	0.32	22.63	±	0.97	19.31	±	0.76
D-CON	13.56	±	0.63***	17.56	±	0.83**	13.57	±	0.64***
ND-PIO	10.29	±	0.53	20.01	±	0.76	19.42	±	0.92
D-PIO	9.932	±	0.61***	22.66	±	1.06**	18.48	±	0.91**
ND-GLI	9.797	±	0.45	19.68	±	0.88	19.04	±	0.7267
D-GLI	9.697	±	0.71***	22.67	±	0.99**	18.44	±	1.019**
ND-NOB	7.935	±	0.40	19.68	±	0.52	18.98	±	0.99
D-NOB	11.37	±	0.44*	20.99	±	0.71*	17.46	±	1.13*
ND-VAL	8.003	±	0.55	22.18	±	1.00	18.98	±	0.99
D-VAL	13.79	±	0.32	17.54	±	0.56	17.46	±	1.13
ND-HES	10.17	±	0.52	20.36	±	0.78	19.26	±	1.03
D-HES	10.96	±	0.65*	21.26	±	0.96*	17.16	±	0.88*

In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. STZ causes diabetes by the rapid depletion of β-cells and thereby brings about a reduction in insulin release. HbA1c level has been reported to be increased in patients with diabetes mellitus (Paulsen 1993). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (Koening 1976). The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes (Gabbay 1976). Elevated levels of HbA1c observed in our study reveal that diabetes animals had prior high blood glucose level. Treatment of PIO, HES and GLI decreased glucose and HbA1c in diabetic rats as compared to diabetic control. Treatment of VAL and NOB no significantly change as compared to diabetic rats.

Lipids play an important role in cardiovascular disease, by modifying the composition, structure and stability of cell membranes. Altered lipid metabolism is considered to accelerate the development of atherosclerosis, a major risk factor in myocardial infarction. High levels of circulating cholesterol and its accumulation in heart tissue is well associated with cardiovascular damage (Slater 1976). In the present study there was a significant decrease in cardiac LCAT and LPL activity whereas a significant increase in the activity of CES was found in ISO intoxicated rats. HDL is the main substrate for LCAT for cholesterol esterification and incorporation (Nestel 1983).

A significant increase in the level of LCAT in diabetic rats treated with PIO, GLI and HES. In the present study hypertriglyceridemia observed in ISO intoxicated rats is due to decrease activity of LPL in the myocardium tissues. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage (Upaganlawar 2009). PIO and GLI alter the activities of LCAT, LPL and CES near to the normal, indicating the potential lipid lowering effects of PIO and GLI.

Pioglitazone and Glimepiride treatment reduced glucose, HbA1c and improve Lipid Metabolizing Enzymes in diabetic rats which suggest cardioprotective activity and control diabetes. Hesperidin treatment also reduced Lipid Metabolizing Enzymes and diabetic marker so cardioprotective and hypoglycemic activity. But Nobivolol treatment improved Lipid Metabolizing Enzymes as compared to diabetic control so cardioprotective without change of glucose but Valsartan treatment no change Lipid Metabolizing Enzymes and diabetic marker as compared to diabetic control. This study concluded that PIO at 10 mg/kg and GLI at 0.5 mg/kg may show improved LCAT, CES and LPL which suggest both drug better effects on cardiac complication in diabetic rats.

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Author's Statements

Competing interests: The authors declare no conflict of interest.

Animal Rights

The institutional and (inter) national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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