EFFECT OF PIOGLITAZONE, GLIMEPIRIDE AND NEBIVOLOL ON RENAL MARKER IN RENAL REPERFUSION INDUCED RENAL DAMAGE IN TYPE 2 DIABETIC RATS

Jagdish Kakadiya[1], Nehal Shah[2]

[1]Pharmacy Department, Jodhpur National University, Narnadi, Jhanwar road, Jodhpur, Rajasthan, India

[2]Pharmacology Department, Dharmaj Degree Pharmacy College, Petlad -Khambhat Road, Dharmaj, Anand-388430, Gujarat, India.

SUMMARY

Present study was designed to evaluate effect some synthetic drugs on Renal Marker in renal Ischemia/Reperfusion (I/R) induced renal damage in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Ischemia/reperfusion injury, which is commonly seen in the field of renal surgery or transplantation in diabetic condition, is a major cause of acute renal failure. Diabetic rats manifest abnormal renal hemodynamic responses, with persistent renal vasodilation at reduced renal perfusion pressures. 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. After right nephrectomy, Pioglitazone (10 mg/kg, p.o), Glimepiride (0.5 mg/kg, p.o) and Nobivolol (2 mg/kg, p.o) were administered for 15 days. On the 16th day, ischemia was induced in contra lateral kidney for 45 min, followed by reperfusion for 24 hr. Creatinine was estimated at the end of 24 hr reperfusion. Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose and glycosylated hemoglobin (HbA1c). At the end of experimental period the level of serum creatinine was significantly increased. Treatment with Pioglitazone in diabetic rats was significantly ($P<0.05$) decreased Creatinine but treatment with Nobivolol in diabetic rats was significantly ($P<0.001$) decreased and treatment with Glimepiride no change. This study concluded that Nebivolol may better reduce renal complication in Ischemia/Reperfusion induced renal damage in type 2 diabetic rats.

KEYWORDS: Streptozotocin, Nicotinamide, Renal reperfusion, Creatinine

INTRODUCTION

Ischemia/reperfusion (I/R) is an important cause of organ dysfunction, often causing high mortality Ischemic cell injury in the kidney occurs during cardiovascular surgery, renal transplantation, as well as the early allograft rejection subsequent to renal transplantation (1), Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF) (2), which is faced in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery, angioplasty, aortic aneurysm surgery, and elective urological operations. In these conditions, I/R injury initiates a complex and interrelated sequence of events, resulting in injury to and the eventual death of renal cells (3, 4). Several factors have been implicated in the pathophysiological changes occurring while renal I/R injury including vascular or microvascular injury, endothelial dysfunction, accelerated cell necrosis, granulocyte activation, and modulation of nitric oxide/angiotensin II axis (5, 6).

Hyperglycaemia is most probably a contributing factor in the development of ischemic an ARF in many patients. Both clinical and experimental data suggest that hyperglycaemia increases the risk of ARF (7-9). Hyperglycaemia also worsens the outcome in renal transplantation (10). Conversely, I/R combined with hyperglycaemia could also be important in the development of diabetic nephropathy.

Organ injury as a consequence of ischemia followed by reperfusion is a major clinical problem. I/R injury is the most common cause of acute renal failure as seen after renal transplantation, major abdominal and vascular surgery, coronary bypass surgery, and in trauma and sepsis (11).

Recently, a protective effect of Pioglitazone against oxidative stress in liver and kidney of diabetic rabbits (12) has been reported. Pioglitazone (PIO) hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. PIO showed dose dependant beneficial effects in many of the pathological conditions including reduction in blood glucose, lowering blood pressure and restoring endothelial functions in animals (13). Pioglitazone lowers blood pressure and restores blunted endothelium-dependent vasodilatation in fructose-fed rats (14), insulin-resistant rheus monkey (15), SHR (16) and sucrosefed SHR (17).

Nebivolol 1-(6-fluorochroman-2-yl)-2-[[2-(6-fluorochroman- 2-yl)-2-hydroxy-ethyl] amino] ethanol is a third generation β-blocker having highly selective β1 adrenergic receptor

blockade (18). It is reported to possess antihypertensive, anti-oxidant activity, and also reduces renal fibrosis and prevents endothelial dysfunction (19, 20).

Glimepiride (GLI) an oral blood glucose lowering drug of the sulfonylurea class is reported to have pancreatic and extra pancreatic effects as well. The blockages of K_{ATP} channels of pancreatic cells by sulphonylurea are critical in the regulation of glucose regulated insulin secretion.

So far the effect of PIO, GLI and NOB on renal marker in experimentally induced renal damage in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to investigate the effect of PIO, GLI and NOB on serum creatinine in I/R induced renal damage in diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Nobivolol was obtained as a gift sample from Torrent Pharmaceuticals Pvt. Ltd., Ahmadabad, India. Pioglitazone hydrochloride and Glimepiride 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 or mice 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 (21). Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

Experimental Protocol

Animals were divided into following groups, each group containing 10 animals and the treatment period for whole study was 4 weeks.

Group 1: Animals served as sham-operated (underwent all surgical procedures without ischemia reperfusion, **Sham**).

Group 2: After right nephrectomy on day 1, vehicle (0.5 % sodium CMC) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24 hr (**I/R** control).

Group 3: After right nephrectomy on day 1, Pioglitazone (10 mg/kg/day, p.o.) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24 hr (**I/R + PIO**).

Group 4: After right nephrectomy on day 1, Glimepiride (0.5 mg/kg/day, p.o.) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24 hr (**I/R + GLI**).

Group 5: After right nephrectomy on day 1, Nobivolol (2 mg/kg/day, p.o.) was administered for 15 days; on day 16, ischemia was produced in the left kidney for 45 min, followed by reperfusion of 24 hr (**I/R + NOB**).

Surgical Procedure

The progress of the experiment	
Day 1	Unilateral right nephrectomy
Day 15	Treatment
Day 16	45 minutes ischemia (left kidney)
Day 17	24 hr reperfusion

Right nephrectomy was performed through a right flank incision (2 cm) under general anesthesia, ketamine (100 mg/kg, i.p.). After right nephrectomy, several treatments were given as mentioned previously for 15 days. On day 16, ischemia was produced in the left kidney by performing a left flank incision and dissecting the left renal pedicle to expose the renal vessels. Non traumatic vascular clamps were used to stop blood flow (in artery and vein) for 45 min.

Reperfusion was established by removing the clamp after 45 min ischemia. The abdominal wall (muscular layer and skin) was closed with 4.0 mononylon suture. At the end of reperfusion period (after 24 hr), blood samples were collected and used for the estimation of renal function (BUN and creatinine). The abdomen was opened, and the kidneys were harvested for the biomarkers of oxidative stress.

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 Kidney function marker

Blood was collected from the rats by retro-orbital puncture at the time of sacrify and was allowed to clot for 10 minutes at room temperature. Clots were centrifuged at 2500 rpm for 10 minutes to separate the serum. A serum creatinine level was measured by assay kits (SPAN Diagnostics Pvt. India).

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

Single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia in 70 to 80 % the animals (Table 1).

Effect of drug on Creatinine

The six rats which underwent renal I/R exhibited a significant increase in the serum concentrations of creatinine ($P < 0.001$) compared with the sham control animals, suggesting a significant degree of glomerular dysfunction mediated by renal I/R. In I/R+PIO treated diabetic rats, serum creatinine level was significantly ($p< 0.05$, $n = 6$) higher as compared to I/R control

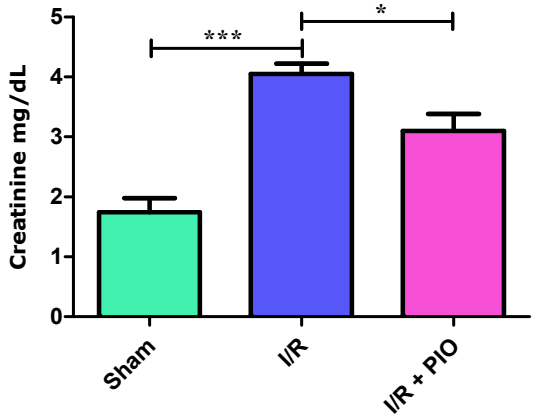
group alone (Fig.1), but In I/R+GLI treated diabetic rats, serum creatinine level was no significantly change as compared to I/R control group alone (Fig.2). In I/R + NOB treated diabetic rats, serum creatinine, urea and uric acid levels were significantly ($p< 0.001$, $n = 6$) higher as compared to I/R control group alone (Fig.3).

Table 1. Effect of Streptozotocin and Nicotinamide on Glucose in rats.

Groups	Glucose (gm)				
	Before Treatment	1 weeks	2 weeks	3 weeks	4 weeks
ND	102.7±5.43	103.3±6.16	103.3±5.34	102.5±6.22	101.8± 6.799
D	371.5±9.54***	375.7±7.25***	370.8±10.33***	340.6±8.41***	332.8± 9.16***

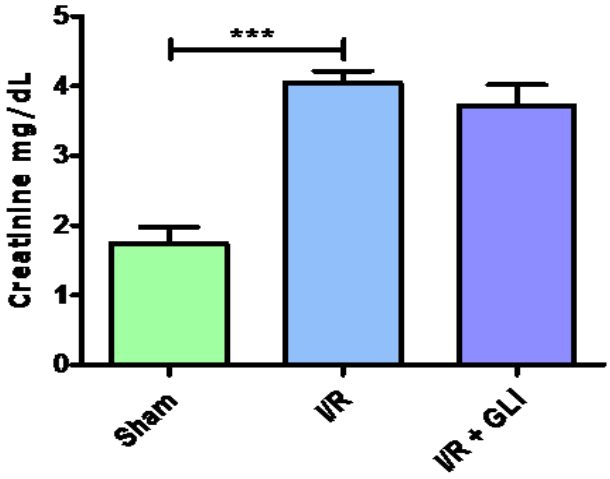
Values are expressed as mean ± SEM (n=10). ***P<0.001 compared to ND group. ND = nondiabetic, D = Diabetic

Figure 1. Effect of Pioglitazone (10 mg/kg/day, p.o) on serum creatinine in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



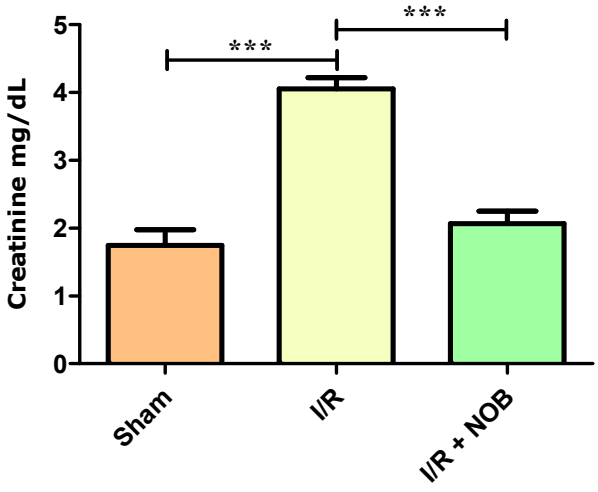
Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Figure 2. Effect of Glimepiride (0.5 mg/kg/day, p.o) on serum creatinine in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Figure 3. Effect of Nebivolol (2 mg/kg/day, p.o) on serum creatinine in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



Values are expressed as mean ± SEM for ten animals in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

DISCUSSION

The present study was under taken with the objective of exploring the Pioglitazone, Glimepiride and Nobivolol on Serum Creatinine in experimentally induced renal damage in diabetic rats.

The transient discontinuation of renal blood supply is encountered in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, and elective urological operations. This transient discontinuation causes renal I/R injury which results in decreased glomerular filtration and renal blood flow and increased urine output characterized by natriuresis and impaired concentrating ability. Much of this tubular and glomerular dysfunction has been postulated to occur during the reperfusion period following anoxia, and generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury.

Acute Tubular Necrosis (ATN) and the ensuing renal failure induced by ischemia and reperfusion injury or sepsis remains a major cause of morbidity and mortality among patients in the intensive care unit (22). Ischemia-induced ATN has an attendant 30% mortality rate, and many survivors require dialysis (22). Indeed, this common clinical entity occurs during cardiopulmonary bypass (23), kidney transplantation (24-26), aortic bypass surgery (27), accidental or iatrogenic trauma (28), sepsis (29), hydronephrosis (30), and elective urological operations (31).

Previous studies have shown that Serum Creatinine increases greater than 3-fold in the Kidney disease (32-36). In the present work, there was a significant reduction in the levels of Serum Creatinine in the nebivolol-treated I/R group as compared to the I/R control.

Pioglitazone and Nebivolol treatment reduced Serum creatinine in experimentally induced renal damaged in diabetic rats which suggest renoprotective activity and Sham control diabetes, but not significantly change in Glimepiride treated diabetic rats. This study concluded that PIO at 10 mg/kg may show reduced Creatinine which better effects on renal complication in diabetic rats and NOB at 2 mg/kg may show more reduced Creatinine same as sham control, so Nebivolol may reduced renal complication in Ischemia/Reperfusion induced renal damage in type 2 diabetic rats.

ACKNOWLEDGEMENT

The authors would like to thank the principal of Dharmaj Degree Pharmacy College of Pharmacy for providing facilities for work.

AUTHOR'S STATEMENTS

Competing interests. The authors declare no conflict of interest.

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ADDRESS FOR CORRESPONDENCE

Mr. Jagdish L. Kakadiya

Pharmacology department,

Dharmaj Degree Pharmacy College,

Petlad-Khambhat Road, Dharmaj,

Anand-388430, Gujarat, India.

jagdishkakadiya@yahoo.co.in

M: 09825882922, Fax No.:91-2697245808.