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THE EFFECT OF NEBIVOLOL AS A BENEFICIAL AGENT IN THE TREATMENT OF RENAL ISCHEMIA/REPERFUSION INJURY IN DIABETIC RATS

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ABSTRACT

Present study was designed to evaluate effect of Nobivolol as a beneficial agent in the treatment of renal ischemia/reperfusion injury in diabetic rats. Diabetic rats manifest abnormal renal hemodynamic responses, with persistent renal vasodilation at reduced renal perfusion pressures. 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. 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, Nobivolol (2 mg/kg/day, p.o) was administered for 15 days. On the 16th day, ischemia was induced in contra lateral kidney for 45 min, followed by reperfusion for 24 hr. Renal function marker and oxidative parameter were estimated at the end of 24 hr reperfusion. At the end of experimental period the level of malondialdehyde formation/ lipid peroxidation (LPO) in kidney tissue and serum marker Creatinine, Urea and Uric acids were significantly increased. Whereas, the activity of biomarkers of oxidative stress such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were found to be decreased significantly compared to control rats. Nobivolol improved the renal dysfunction and oxidative stress after renal ischemia/reperfusion injury in diabetic rats. In conclusion, Nobivolol as a beneficial agent in the treatment of renal ischemia/reperfusion injury in diabetic rats

KEYWORDS: Nobivolol; Ischemia reperfusion injury; Renal Marker; Type 2 diabetes

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 (Manuela, 2003), Renal ischemia/reperfusion (I/R) injury is a major cause of acute renal failure (ARF) (Radhakrishnan, 1997), 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 (Thadhani, 1996; Paller, 1998). 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 (Maxwell, 1997; Adam, 2000).

The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin I, which is converted to angiotensin II with the help of angiotensin-converting

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enzyme (ACE) (Gavras, 1996). Angiotensin II is an important mediator in kidney injury. Accumulating evidence suggests that angiotensin II stimulates intracellular formation of reactive oxygen species (ROS) such as the superoxide anion and hydrogen peroxide that leads to kidney damage (Sachse, 2007).

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 (Michael, 2008). It is reported to possess antihypertensive, anti-oxidant activity, and also reduces renal fibrosis and prevents endothelial dysfunction (Groot, 2004; Pires, 2005).

The present study, we investigated the protective effect of Nobivolol on renal I/R injury in diabetic rats and other word effect of Nobivolol on renal marker and oxidative stress of kidney tissues in I/R induced renal damage in diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals

Nobivolol hydrochloride was obtained as a gift sample from Torrent 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*. The animal experiment was approved by Animal Ethical Committee of the Institute (1163/a/08/CPCSEA).

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 (Masiello, 1998). Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

Experimental Protocol

The rats were divided into three groups each consisting of six animals:

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

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, 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.

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 seven day, 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. Serum creatinine and urea levels were measured by assay kits (SPAN Diagnostics Pvt. India) and Serum Uric acid levels were measured by assay kits (Crest Biosystems Ltd. India).

Preparation of Tissue Homogenate

After sacrificing the animals, their kidneys were quickly removed, perfused immediately with ice cold hypertonic saline solution, weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 0°C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of following antioxidant parameters. The levels of Lipid peroxidation (LPO) formation and the activities of endogenous antioxidant enzymes such as catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by the method of Slater and Sawyer (Slater, 1971) Hugo Aebi as given by Hugo (Huge, 1984) Moron *et al* (Moron, 1979) and Mishra and Fridovich (Mishra, 1992).

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

Characterization of Type 2 Diabetes

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 (Table 1).

Table 1. Effect of Streptozotocin (65mg/kg/day, p.o) and Nicotinamide (110 mg/kg/day, p.o) on serum glucose and HbA1c changes level in rats.

Groups	Glucose	HbA1c
CON	101.8 ± 6.799	5.455 ± 0.3729
STZ + NIC	332.8 ± 9.167***	9.900 ± 0.6323***

Values are expressed as mean ± SEM for six animals in the group. ***P<0.001 considered statistically significant as compared to respective Control group.

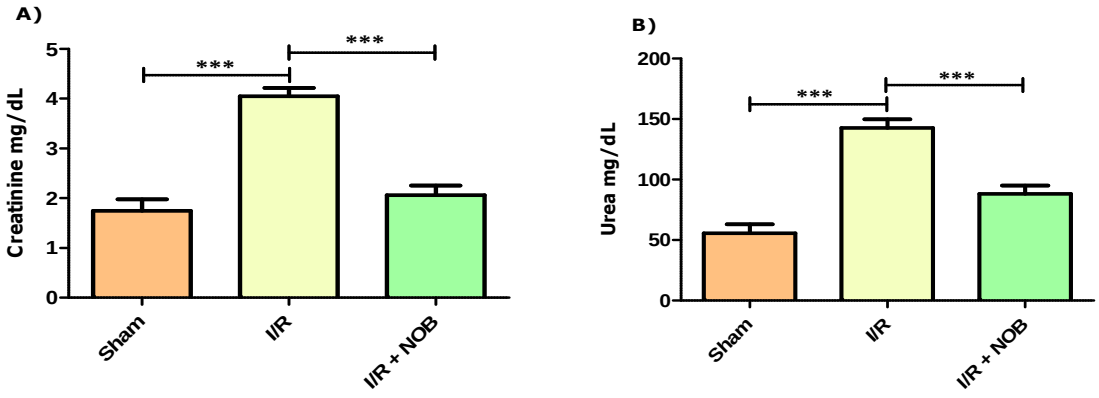
Effect of NOB on kidney function marker

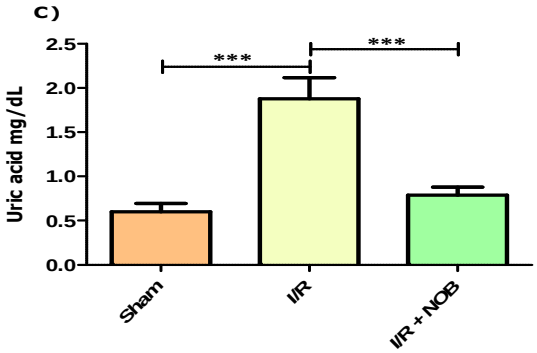
The six rats which underwent renal I/R exhibited a significant increase in the serum concentrations of creatinine ($P < 0.001$), urea ($P < 0.001$), and uric acid ($P < 0.001$) compared with the sham control animals, suggesting a significant degree of glomerular dysfunction mediated by renal I/R. In 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.1).

Effect of NOB on antioxidant activity

Renal I/R group of diabetic rats showed significantly decreased enzymatic activity of superoxide dismutage (P<0.001), catalase (P<0.001), and reduced glutathione (P<0.001) when compared with the sham control rats. These declining trends were significantly (P<0.001) decreased in the group treated with NOB compared with those in the I/R-only group (Fig. 2). Renal I/R produced a significant (P<0.001) increase in MDA levels in comparison with the sham operation in the rats. Treatment with NOB before renal I/R was associated with a significantly (P<0.001) lower MDA level than that in the rats that underwent only renal I/R (Fig. 2).

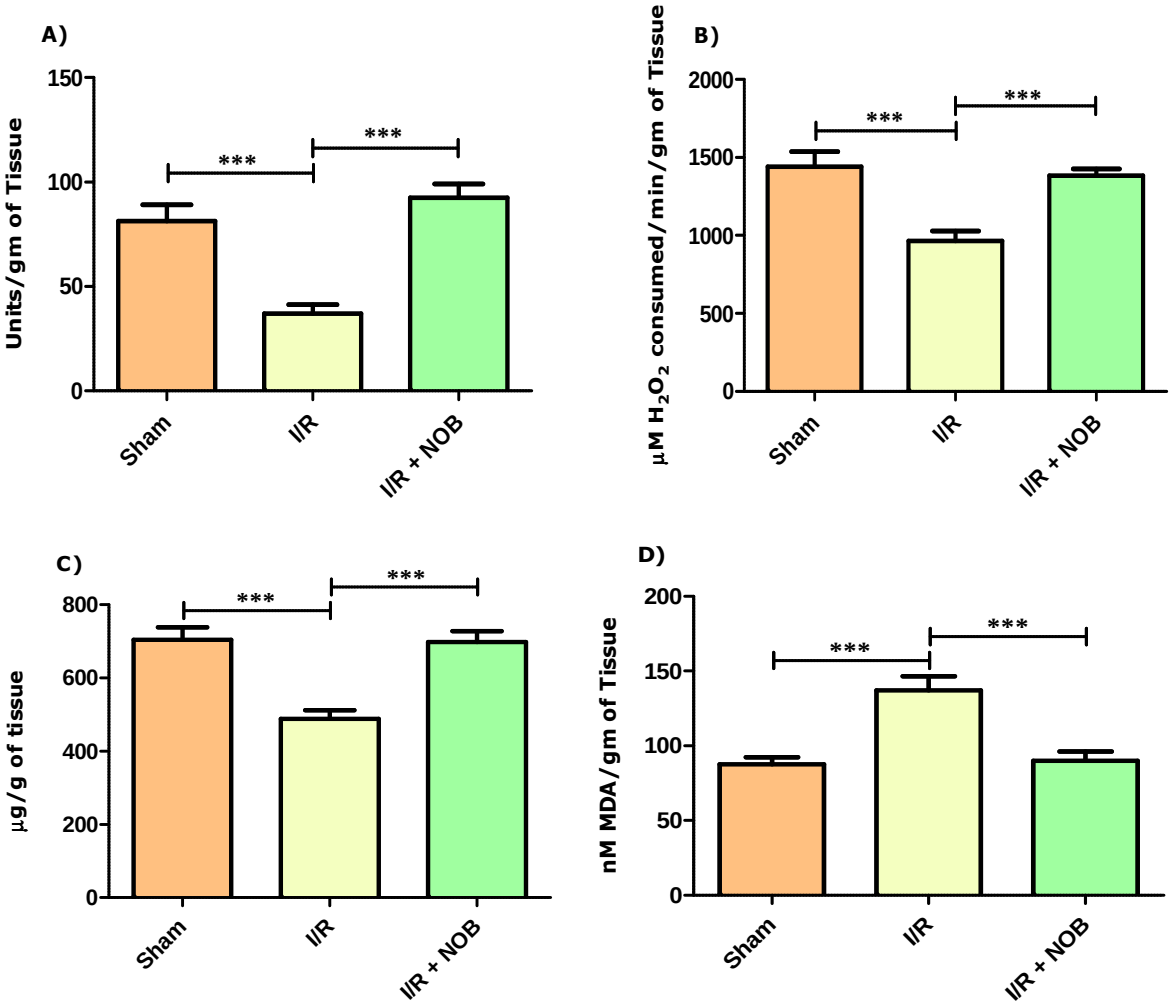
Figure 1. Effect of Nobivolol (2 mg/kg/day, p.o) on serum Creatinine (A), Urea (B), and Uric acid (C) in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.





Values are expressed as mean ± SEM for six 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 Nobivolol (2 mg/kg/day, p.o) on Superoxide dismutase (A), Catalase(B), Reduced glutathione (C) and lipid peroxidation (D) in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



Values are expressed as mean ± SEM for six 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 evaluate Nobivolol on renal complication in I/R 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 (Thadhani, 1996; Paller, 1998). 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.

In renal I/R injury, ROS are capable of reacting with lipids leading to lipid peroxidation of biological membranes, which in turn impacts enzymatic processes, such as ion pump activity, inhibiting transcription and repair of DNA. If lipid peroxidation remains unchecked, it will ultimately result in cell death (Chatterjee, 2000; Singh, 2004).

In our study, animals subjected to renal I/R demonstrated an increase in the renal MDA and at tenuated antioxidant enzymes pool. Lipid peroxidation and antioxidant enzymes are important indexes of oxidant injury (Muller, 2000). Demonstrations of lipid peroxidation as indexes for oxidative damage may help us better understand the effects of ROS on the cellular components (Ozyurt, 2001). Renal I/R-induced oxidative stress was associated with impaired kidney function, leading to a marked increase in serum creatinine, urea, and uric acid levels.

Pretreatment with Nobivolol prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of antioxidant enzymes activity in rats exposed to the renal I/R. Furthermore, the impaired kidney function was significantly improved by Nobivolol.

The rennin-angiotensin system plays a pivotal role in regulation of blood pressure. Renin acts on angiotensinogen to form angiotensin-I, which is converted to angiotensin-II with the help of angiotensin-converting enzyme.6 Accumulating evidence suggests that angiotensin-II stimulates intracellular formation of ROS such as superoxide anion and hydrogen peroxide that leads to kidney damage (Sachse, 2007). Generation of ROS has been postulated as one of the major factors contributing to this reperfusion injury. Oxidative stress can result from increased ROS production, and/or from decreased ROS scavenging capability. The ROS attach to the polyunsaturated fatty acids in the membrane lipids and result in peroxidation, which may lead to disorganization of cell structure and function. After reperfusion and reoxygenation, the imbalance between restoration of oxygen supply and mitochondrial respiratory function results in massive generation of superoxide anion in mitochondria (Muller, 2000; Ozyurt, 2001). Under these conditions, the defensive system, which is known as antioxidant or antioxidant enzymes, cannot prevent the escape of ROS, especially in mitochondria, and their effects on other intracellular sites. This cascade of events is known as reperfusion injury (Ozyurt, 2001).

Previous studies have shown that Serum Creatinine, Uric acid and Urea level increases greater than 3-fold in the Kidney disease (Myers, 1916; Baumann, 1919; Folin, 1915; Krauss, 1922; Hitzenberger, 1921). In the present work, there was a significant reduction in the levels of Serum Creatinine, Uric acid and Urea in the nebivolol-treated I/R group as compared to the I/R control.

In this study, renal I/R increased oxidative stress products including tissue MDA and depleted the antioxidant enzymes pool, as is evident from the declined activity of superoxide dismutage, catalase, and reduced glutathione. It can be speculated that pretreatment with Nobivolol prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe increasing of ROS products and depletion of superoxide dismutage and reduced glutathione in rats exposed to the renal I/R.

CONCLUSIONS

It is important to inhibit oxidative stress and improve renal marker to prevent renal I/R injury in diabetic condition. Our data support a role for Nobivolol as a beneficial agent in the treatment of renal ischemia/reperfusion injury in diabetic rats.

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