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Effect of Valsartan on Renal Marker, Nitrite and Histopathology of Kidney in Ischemia/Reperfusion Induced Renal Damage in Diabetic Rats

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Keywords

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Renal ischemia/reperfusion injury
Nitrite
Type 2 diabetes

Abstract

Present study was designed to evaluate the effect of Valsartan on renal marker, nitrite and histopathology of kidney in Ischemia/reperfusion induced renal damage in diabetic 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. 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, Valsartan (8 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 histopathology were estimated at the end of 24 hr reperfusion. At the end of experimental period the level of nitrite in kidney tissue, serum marker Albumin and Blood urea nitrogen were significantly changed. Valsartan improved the renal dysfunction and nitrite after renal ischemia/reperfusion injury in diabetic rats. Light microscopic evaluation of the kidneys of the diabetic rats with I/R only showed tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis, whereas, Valsartan improve tubular dilation, loss of interstitial hemorrhage, and glomerular atrophy. In conclusion, Valsartan as a beneficial agent on renal marker, nitrite and histopathology of kidney in Ischemia/reperfusion induced renal damage in diabetic rats.

1. Introduction

Ischemic injury to brain, heart, and kidney is associated with high morbidity and mortality. Improving the ability of these organs to tolerate ischemic injury would have important implications. Ischemic insults are often recurrent in diabetic patients. In the setting of loss of renal blood flow autoregulation that characterizes the post-ischemic kidney [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].

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 (ACE) [7]. 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 [8].

Recent studies clearly indicate that nitric oxide is fundamentally involved in the regulation of renal hemodynamics and homeostasis [9]. Moreover, it has been shown that reactive oxygen species (ROS) increase in the areas of ischemia and reperfusion [10]. ROS avidly react with nitric oxide (NO) and produce highly reactive nitrogen species (peroxynitrite), which can lead to functional NO deficiency. Peroxynitrite is an important agent that causes lipid peroxidation of vascular membranes [11, 12]. Studies proved that functional deficiency of NO leads to increased renal sympathetic nerve activity and this sympathetic activation increases β receptor activities in the body [13]. In addition, studies have shown that the β adrenoceptor present in kidney were exclusively of the β 1 type [14].

Recent evidence suggest that blockade of the rennin-angiotensin system ameliorates diabetes induced renal dysfunction. Because activation Valsartan (VAL) - Angiotensin II receptor (AT 1) blocker 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. Angiotensin receptor antagonists are widely used as antihypertensive in diabetic and non diabetic patients. Valsartan is reported for its renoprotective activity in diabetic rats.

The present study, we investigated Valsartan on renal marker and histopathology of kidney in Ischemia/reperfusion induced renal damage in diabetic rats, and other word effect of Valsartan on renal complication in I/R induced renal damage in diabetic rats.

2. Materials and Methods

Drugs and Chemicals

Valsartan hydrochloride 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*. 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 [15]. 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, Valsartan (8 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 + VAL).

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. Blood urea nitrogen level was measured by assay kits (SPAN Diagnostics Pvt. India) and Serum Albumin levels were measured by assay kits (Crest Biosystems Ltd. India).

Estimation of kidney Tissue Nitrite Levels

The level of nitrite level was estimated by the method of Lepoivre et al. [16]. To 0.5 mL of tissue homogenate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

Histopathology

For light microscopic evaluation, kidneys were fixed in 10% phosphate buffered formalin. Paraffin-embedded specimens were cut into 6 mm-thick sections and stained with hematoxylin and eosin (H&E). The kidneys were examined under a light microscope (Olympus Bioxl) for the presence of tubular changes and interstitial inflammatory cell infiltration by an observer blinded to the animal treatment group.

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 $p < 0.05$.

3. 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 VAL on kidney function marker

The six rats which underwent renal I/R exhibited a significant increase in the serum concentrations of Albumin (1.950 ± 0.1746 mg/dL tissue, $p<0.001$, $n = 6$) and blood urea nitrogen (66.55 ± 3.32 mg/dL tissue, $p<0.001$, $n = 6$) compared with the sham control animals (4.133 ± 0.1532, 25.96 ± 3.396 mg/dL tissue, respective Sham control, $p<0.001$, $n = 6$), suggesting a significant degree of glomerular dysfunction mediated by renal I/R. In I/R+VAL treated diabetic rats, serum Albumin and blood urea nitrogen levels were significantly (3.094 ± 0.3227, 25.96 ± 3.396 mg/dL tissue, respective Sham control $p<0.01$, $p<0.01$, Respective Sham control, $n = 6$) higher as compared to I/R control group alone (Fig.1).

Effect of VAL on kidney Tissue Nitrite Levels

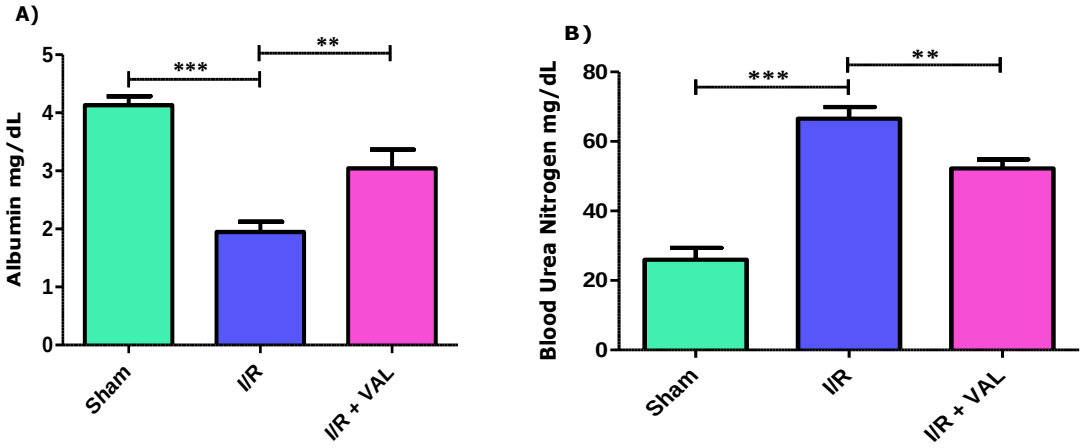
Renal I/R resulted in a significant decrease in the tissue levels of nitrite (127.5±7.68 nmol/gm tissue, $p<0.05$, $n = 6$) as compared to values obtained from the tissue of sham-operated animals (156.5 ± 9.68, $n = 6$). In I/R+VAL treated rats, a significant increase in the tissue levels of nitrite as compared to I/R control group alone (from 127.5±7.68 to 170.6±9.72 nmol/gm tissue, $p<0.05$, $n = 6$) (Fig.2).

Histopathological Analysis

Light microscopic evaluation of the sham-operated groups revealed a regular morphology of renal parenchyma with well-designated glomeruli and tubuli (Fig. 3). The sham control group of rats did not show any morphological changes. By contrast, the kidneys of the diabetic rats with I/R only showed tubular cell swelling, interstitial edema,

tubular dilatation, and moderate to severe necrosis, whereas, Valsartan Reduced tubular dilation, loss of interstitial hemorrhage, and glomerular atrophy were the regenerated features (Fig. 3).

Figure 1. Effect of Valsartan (8 mg/kg/day, p.o) on serum Albumin (A) and Blood Urea Nitrogen (B) in the diabetic rats exposed to renal ischemia/reperfusion (I/R) injury.



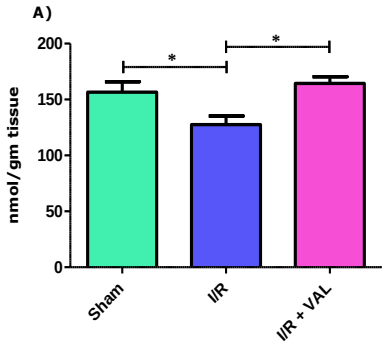
Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group. **A)*****P<0.001 sham vs. I/R control, **P<0.01 I/R vs. I/R+VAL, **B)** ***P<0.001 sham vs. I/R control, **P<0.01 I/R vs. I/R+VAL.

4. Discussion

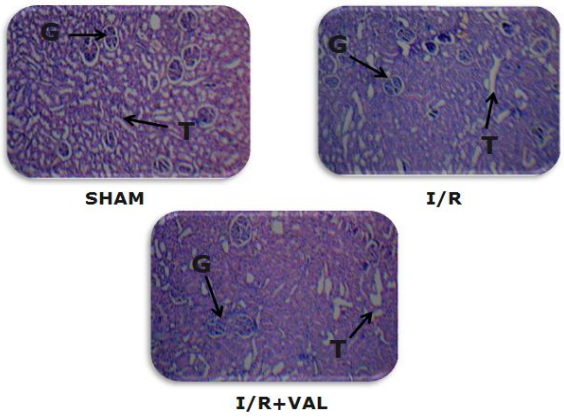
The present study was under taken with the objective of exploring evaluate the effect of Valsartan as a beneficial agent in the treatment of Ischemia/reperfusion induced renal damage in diabetic rats. The transient discontinuation of renal blood supply is encountered in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, and elective urological operations [3, 4]. 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. Acute renal failure produced by ischemia and reflow is histopathologically characterized by extensive tubular damage, tubular cell necrosis, glomerular injury, and signs of tubular obstruction with cell debris [17, 18].

Figure 3. Morphological Changes Assessed by Histopathological Examination of Kidneys of diabetic Rats Exposed to Ischemia/Reperfusion (I/R) Injury With and Without Preceded Treatment with Valsartan (VAL) and Sham Operation. **Sham:** kidney section of a rat in the sham operation group shows normal glomeruli and tubuli, **I/R**: Kidney section of a rat exposed to bilateral renal ischemia/reperfusion shows severe interstitial hemorrhage surrounding the glomeruli and tubuli. Tubular epithelial degeneration is apparent, **I/R+VAL**: Kidney section of the rats with ischemia/reperfusion injury treated with Valsartan, in which slight degeneration of tubuli and glomeruli are seen. **G** = Glomerul, **T** = Tubuli.

Figure 2. Effect of Valsartan (8 mg/kg/day, p.o) on Tissue nitrate level in kidney tissue in 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. *P<0.05 sham vs. I/R control, *P<0.05 I/R vs. I/R+VAL.



In the clinical settings, renal I/R are a consequence of systemic hypoperfusion with subsequent circulatory resuscitation, such as following aortic cross-clamping or renal transplantation [19]. There is good evidence from both in vivo and in vitro studies that the formation of NO plays an important role in I/R [20]. One of the important mechanisms for I/R injury is excessive ROS, which scavenges NO and cumulates in reduced NO bioavailability [21]. Endothelial cells produce less bioactive NO in the presence of higher oxidative stress [22, 23].

In the present study, there was a significant decrease NO in I/R control group in comparison to the sham-operated group, A increase in NO level in the Valsartan-treated I/R+VAL group showed significant improvement in renal function as compared to the I/R control and I/R+VAL groups.

Previous studies have shown that peroxynitrite level in the heart increases greater than 10-fold in the first minute of reperfusion [24]. In this study, there was a significant decrease in tissue nitrite levels in kidney of I/R group animals as compared to sham-operated group. Moreover, peroxynitrite could initiate lipid peroxidation, which damages the proximal tubular cells, nitration of tyrosine residues (nitro tyrosine), and nitration of cellular proteins, with a subsequent loss of protein structure resulting in reduction of the kidney function [25, 26]. Similar results were obtained in this study as well. There was a significant increase (3 fold) in the levels of serum BUN and significant decrease (3 fold) in the levels of serum albumin in I/R control group, whereas animals treated with VAL improve of renal function in comparison to I/R control group.

5. Conclusions

It is important to improve renal marker, nitrite and histopathology parameter to prevent renal I/R injury in diabetic condition. Our data support effect of Valsartan as a beneficial agent in renal complication in diabetic rats.

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