GLIMEPIRIDE REDUCES ON EXPERIMENTALLY INDUCED ISCHEMIA/REPERFUSION IN DIABETIC RATS

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ABSTRACT: Hyperglycaemia is most probably a contributing factor in the development of ischaemic acute renal failure (ARF) in many patients. Both clinical and experimental data suggest that hyperglycaemia increases the risk of ARF. Present study was designed to evaluate in Glimepiride reduced on experimentally induced ischemia/reperfusion in diabetic 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. After right nephrectomy, Glimepiride (0.5 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. Glimepiride improved the Serum Uric acid and tissues parameter LPO after renal ischemia/reperfusion injury in diabetic rats. In conclusion, Glimepiride shows low may improve experimentally induced ischemia/reperfusion in type 2 diabetic rats.

KEYWORDS: Glimepiride; Ischemia reperfusion injury; oxidative stress; type 2 diabetes

INTRODUCTION

Hyperglycaemia is most probably a contributing factor in the development of ischaemic acute renal failure (ARF) in many patients. Both clinical and experimental data suggest that hyperglycaemia increases the risk of ARF (Wald, 1990; Van, 2001; Goor, 1996). Hyperglycaemia also worsens the outcome in renal transplantation (Thomas, 2001; Thomas, 2000). Conversely, ischaemia/reperfusion (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. Renal ischemia/reperfusion (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 (Thadhani, 1996).

In the setting of loss of renal blood flow autoregulation that characterizes the post-ischemic kidney (Conger, 1997), Renal I/R injury is a major cause of acute renal failure (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 (Adam, 2000; Maxwell, 1997).

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) (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).

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. It has been demonstrated to be effective in the treatment of non-insulin dependent diabetes mellitus with insulin resistance. GLI is pharmacologically distinct from glibenclamide because of differences in receptor-binding properties which could result in a reduced binding to cardiomyocyte K_{ATP} channels. Some have reported the antioxidative properties of GLI. Moreover, GLI is pharmacologically different than other sulfonylurea drugs for its cardiovascular effects.

The present study, we investigated the protective effect of Glimepiride on renal marker and oxidative stress of kidney in diabetic rats and other word effect of Glimepiride on reduced on experimentally induced ischemia/reperfusion induced renal damage in diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals

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*. 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, 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).

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 0ºC 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 (Hugo, 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.

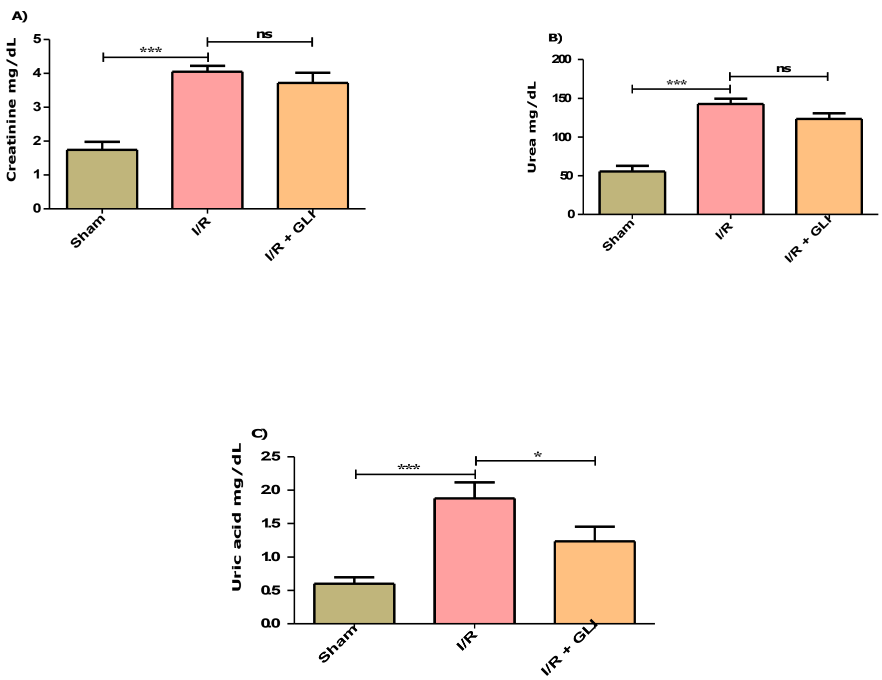
Groups	Glucose	HbA1c
CON	101.8 ± 6.799	5.455 ± 0.3729
STZ + NIC	332.8 ± 9.167***	9.900 ± 0.6323***

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 GLI 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. Treatment with Glimepiride significantly (P<0.05) reduced Serum uric acid in I/R + GLI group compared to Sham group (Fig. 1). but, In I/R+GLI treated diabetic rats, serum creatinine and urea levels were significantly no change as compared to I/R control group alone (Fig.1).

Figure 1. Effect of Glimepiride (0.5 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.

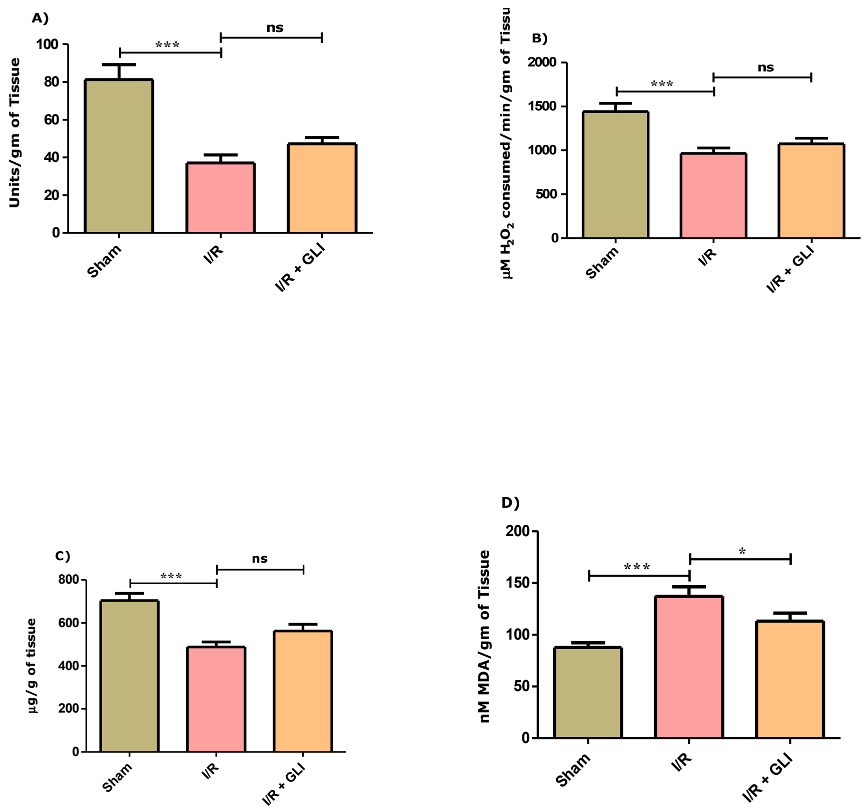


Values are expressed as mean ± SEM for six animals in the group. ns = Non Significant, *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Sham group.

Effect of GLI 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 no significantly, respectively Sham control Group treated with GLI 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 Glimepiride significantly (P<0.05) increased MDA in I/R + GLI group compared to Sham group (Fig. 2).

Figure 2. Effect of Glimepiride (0.5 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. ns = Non Significant, *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 Glimepiride on renal marker 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 (Paller, 1998; Thadhani, 1996). 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.

Intracellular oxidative stress, due to an increased production of superoxide by the electrontransport chain in the mitochondria, has been proposed as a unifying explanation for most metabolic alterations in diabetes (Brownlee, 2001). I/R are also a state where oxidative stress has been implied (Chien, 2001).

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, 1992). The finding that hyperglycaemia increases renal I/R injury may have implications for the understanding of diabetic nephropathy as well. Ischaemia has been suggested in the development of diabetic nephropathy (Ziyadeh, 1996).

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 (Singh, 2004). Demonstrations of lipid peroxidation as indexes for oxidative damage may help us better understand the effects of ROS on the cellular components (Muller, 2000).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 Glimepiride prevented renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of only one parameter antioxidant enzymes activity in rats exposed to the renal I/R. Furthermore, the low impaired kidney function was significantly improved by Glimepiride.

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. 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). 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 Glimepiride 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 very low change oxidative stress and renal marker so, that concluded very low prevent renal I/R injury in diabetic condition. Our data support a role for Glimepiride shows low may improve experimentally induced ischemia/reperfusion in type 2 diabetic rats.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

REFERENCES

Adam A, Raij L, 2000. Nitric oxide--angiotensin II axis in renal and cardiovascular injury. J Nephrol., 13:211-20.

Brownlee M, 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813–820.

Chatterjee PK, Cuzzocrea S, Brown PA, et al, 2000. Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. Kidney Int., 58:658-73.

Chien CT, Lee PH, Chen CF, Ma MC, Lai MK, Hsu SM., 2001. De novo demonstration and co-localization of free-radical production and apoptosis formation in rat kidney subjected to ischemiaureperfusion. J Am Soc Nephrol, 2: 973–982.

Conger, J. Adv, 1997. Renal Replace. Ther. 4, Suppl., 1: 25–37.

Gavras HP, Salerno CM, 1996. The angiotensin II type 1 receptor blocker losartan in clinical practice: a review. Clin Ther., 18:1058-67.

Goor Y, Peer G, Iaina A et al., 1996. Nitric oxide in ischaemic acute renal failure of streptozotocin diabetic rats. Diabetologia, 39: 1036–1040

Hugo EB, 1984. Oxidoreductases acting on groups other than CHOH: catalase. In: Colowick SP, Kaplan NO, Packer L, editors. Methods in Enzymology, vol. 105. London 7 Academic Press, 121– 5.

Maxwell SR, Lip GY, 1997. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol., 58:95-117.

Mishra HP, Fridovich I, 1992. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biochem, 247:3170– 5.

Moron MS, Depierre JW, Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta, 582:67– 78.

Muller DN, Dechend R, Mervaala EM, et al 2000. NF-kappaB inhibition ameliorates angiotensin II-induced inflammatory damage in rats. Hypertension, 35:193-201.

Ozyurt H, Irmak MK, Akyol O, Sogut S., 2001. Caffeic acid phenethyl ester changes the indices of oxidative stress in serum of rats with renal ischaemia-reperfusion injury. Cell Biochem Funct., 19:259-63.

Paller MS, 1998. Acute renal failure: controversies, clinical trials, and future directions. Semin Nephrol., 18:482-9.

Radhakrishnan J, Kiryluk K, 1997. Acute renal failure outcomes in children and adults. Kidney Int., 69:17-9.

Sachse A, Wolf G, 2007. Angiotensin II-induced reactive oxygen species and the kidney. J Am Soc Nephrol., 18:2439-46.

Singh D, Chander V, Chopra K, 2004. The effect of quercetin, a bioflavonoid on ischemia/reperfusion induced renal injury in rats. Arch Med Res., 35:484-94.

Slater TF, Sawyer BC, 1971. The stimulatory effects of carbon tetrachloride and other halogeno alkanes or peroxidative reactions in rat liver fractions in vitro. Biochem J, 123:805– 14.

Thadhani R, Pascual M, Bonventre JV, 1996. Acute renal failure. N Engl J Med., 334:1448-60.

Thomas MC, Mathew TH, Russ GR, Rao MM, Moran J., 2001. Early peri-operative glycaemic control and allograft rejection in patients with diabetes mellitus. a pilot study. Transplantation, 72: 1321–1324

Van den Berghe G, Wouters P, Weekers F et al, 2001. Intensive insulin therapy in the surgical intensive care unit. N Engl J Med, 345: 1359–1367

Wald H, Markowitz H, Zevin S, Popovtzer MM, 1990. Opposite effects of diabetes on nephrotoxic and ischemic acute tubular necrosis. Proc Soc Exp Biol Med, 195: 51–56

Ziyadeh FN, 1996. Significance of tubulointerstitial changes in diabetic renal disease. Kidney Int, 49: 10–13.

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