

The role of ATP-sensitive potassium channel blockers in ischemia-reperfusion-induced renal injury versus their effects on cardiac ischemia reperfusion in rats

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Abstract. – In renal ischemia reperfusion (I/R), opening of adenosine-triphosphate (ATP)-sensitive potassium (K_{ATP}) channels results in massive influx of neutrophils in both renal and lung tissues. Our study was focused on the role of ATP-dependent potassium channel modulators, glimepiride and glibenclamide on I/R induced renal injury in rats. Additionally we evaluated their effects on normal heart and on ischemic reperfused heart subjected to ischemic preconditioning protection afforded by diazoxide. To test this hypothesis, we used renal I/R and cardiac I/R experiment. Renal ischemia reperfusion induced marked renal dysfunction associated with significant increase in arterial pressure, TNF- α levels, superoxide anion production, and myeloperoxidase activity. Treatment with glibenclamide or glimepiride, demonstrated a significant improvement in the reperfusion-induced injury in both kidney and lung. Glimepiride has no effect on superoxide anion production. However glibenclamide induced a significant improvement in these measurements as compared to glimepiride group.

Before coronary artery ligation, neither diazoxide nor glimepiride pretreatment influenced significantly the electrocardiographic parameters in comparison with control group. Conversely, glibenclamide supplementation induced a significant elevation in these parameters. After left coronary artery ligation, reperfusion of the ischemic hearts caused a significant elevation in the measured electrocardiographic parameters. These elevations were significantly ameliorated by the pretreatment with diazoxide. In conclusion, the administration of glibenclamide significantly abolished the protective effects of diazoxide, while the pretreatment with glimepiride didn't abolish it. So, glimepiride offers some promise for therapy of renal I/R with minimizing the undesirable cardiac side effects.

Key Words:

Renal ischemia reperfusion injury, Cardiac ischemia reperfusion injury, Neutrophil infiltration, Diazoxide, Glibenclamide, Glimepiride.

Introduction

Renal ischemia reperfusion (I/R) is an important cause of renal dysfunction in renal transplantation and is associated with an increased rate of acute and chronic rejection^{1,2}. In addition, it is the most common cause of acute renal failure in shock, sepsis, and renal artery stenosis³.

The impairment of organ function derived from ischemia/reperfusion injury is still an important problem in solid organ transplantation. Although the restoration of blood flow is the treatment of choice following acute ischemia of vascular territory, restoration of oxygen delivery or reperfusion after ischemia can be accompanied by significant injury to local and remote organs, such as the lung, and systemic inflammatory events that may limit the beneficial effects of blood flow restoration⁴. The reperfusion-associated injury could be attributed to oxygen-derived free radicals and intracellular calcium (Ca^{+2}) overload, which are associated with further injury, as well as, recruitment and activation of leukocytes and subsequent release of mediators of the inflammatory process^{3,5}.

In the kidney, fall in intracellular ATP concentration induces opening of adenosine-triphosphate (ATP)-sensitive potassium (K_{ATP}) channels,

that plays an important role in the massive influx of neutrophils early after reperfusion, exerting a crucial role in the patho-physiology of post-ischemic renal failure, by the release of various inflammatory mediators as cytotoxic proteases and oxygen-derived free radicals^{3,4,6}.

Renal I/R is a complex neutrophil-mediated syndrome, in which neutrophil and TNF- α are among the cell types and inflammatory mediators, respectively, thought to be of major relevance in the patho-physiology of I/R injury¹. Many of the pathological changes that follow I/R injury in various organs are believed to be induced by infiltrating activated neutrophils². Myeloperoxidase (MPO), an heme enzyme secreted by activated neutrophils, is an accepted indicator of neutrophil accumulation and oxidative activity⁷. Superoxide anion (O_2^-) generated by neutrophils, monocytes and macrophages is of a central importance in host defense mechanism and it is involved in augmenting neutrophils influx into the tissues⁸.

ATP-sensitive K^+ (K_{ATP}) channels belong to a family of inward rectifying potassium channels⁹, whose structure is believed to comprise a tetramer of four inwardly rectifying K^+ channel subunits, that are called the Kir subunits (forming the ionic pore), coupled to four regulatory sulphonylurea receptors (SUR) subunits^{10,11}. This structure allowed to several subclasses of those ionic channels¹²⁻¹⁴. The four regulatory SUR subunits contain the binding site for the anti-diabetic sulphonylureas, and the pharmacological specificity of each sulphonylurea depends on the type of SUR protein present in each tissue¹¹. Another protein called the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is expressed abundantly in the kidney and associates with both ATP and sulphonylurea sensitivity^{10,15,16}. The K_{ATP} is also abundant in the cardiac myocytes. Therefore, sulphonylurea may also bind the K_{ATP} channels of the cardiac myocytes with subsequent development of cardiac complications¹³.

Glimepiride is supposed to have several benefits over conventional drugs such as glibenclamide. The benefits include rapid onset and longer duration of action¹⁷, and more specificity than glibenclamide for the SUR₁ receptor in the pancreatic beta cells¹⁸.

As treatment of renal I/R injury is still only supportive, the development of therapeutic interventions to prevent or reduce a renal tissue injury after I/R remains the focus of research. In the present study we have used the renal I/R model

to assess the role of ATP-dependent potassium channel modulation, comparing the protective effects of glimepiride and of glibenclamide on renal I/R inflammatory injury and neutrophil aggregation. Moreover, we have observed whether the inhibition of the inflammatory injury is accompanied by an improvement of renal dysfunction in rats. For comparison, we have also evaluated the effects of the potassium channel opener diazoxide. Because the protective effect of K_{ATP} channel blockade in renal I/R stands in sharp contrast to the harmful effects on the cardiac tissues, our study was extended to evaluate the possible harmful effects of both sulphonylureas drugs on normal heart and on ischemic reperfused heart subjected to ischemic preconditioning (IP) protection afforded by diazoxide which is one of the preconditioning mimetic agents.

Materials and Methods

Animals: one hundred and fourteen (n=114) adult albino rats weighing 250 ± 10 g were used in this study: seventy two of them were used for the in renal I/R experiment and forty two rats for the in cardiac I/R experiment. They were purchased from the Egyptian Organization for Biological Products and Vaccines (Egypt), and allowed free access to food and water *ad libitum*. They were kept under constant conditions with 12/12 h light/dark cycles and left for acclimatization for one week before the start of the study. The care and handling of the animals used in the present study was in accordance with the guidelines of the National Institutes of Health (NIH).

Drugs: glibenclamide, glimepiride (Aventis Pharmaceutical Company, Egypt) and diazoxide (Sigma Chemical Company, Egypt) were used. Glibenclamide, glimepiride and diazoxide were given intraperitoneally at doses of 20 mg/kg, 5 mg/kg and 30 mg/kg respectively^{4,19}. All drugs were dissolved in dimethyl sulfoxide (DMSO)²⁰.

(I) Renal I/R Experiment

Animal model of renal I/R: animals were given water but no food for 24 hours before the experiment. Rats were anaesthetized with intraperitoneal injection of urethane in a dose of 1.25 mg/kg²¹. The renal I/R protocol was performed according to Pompermayer et al⁴. Left nephrecto-

my was performed through a left flank incision. Renal ischemia was performed by right flank incision and dissecting the right renal pedicle so as to expose the renal vessels. A thick cotton thread was passed over the renal vascular pedicle to induce ischemia. Both ends of the thread were exteriorized through the back of the rat and tied tightly above a length of latex tubing on the external side of the skin. Ischemia was confirmed visually by blanching of the kidney. The renal pedicle was released 45 minutes after occlusion by cutting the thread and pulling it out, and the wound was closed with 3-0 silk suture followed by 4 or 24 hours of reperfusion. Animals were allowed to recover from anesthesia without further interventions.

Experimental Protocol

All rats were subjected to left nephrectomy, then they were divided into six experimental groups (12 rats each). Each group was divided into two sub-groups according to the period of reperfusion at 4 hours (sub-group A, n=6) or 24 hours (sub-group B, n=6). Diazoxide was given as a single intraperitoneal (i.p.) injection at 40 minutes before reperfusion for both the 4 and 24 hours reperfusion.

Glibenclamide and glimepiride were given as single dose 40 minutes before reperfusion for 4 hours reperfusion but for 24 hours reperfusion they were given in two doses, one 40 minutes before reperfusion and the other 8 hours after reperfusion⁴.

Group 1 – *Sham operated control group (Sham)*: rats were submitted to left nephrectomy and were used as control for the reperfusion-induced injury.

Group 2 – *Renal ischemia reperfusion group (I/R)*: rats were submitted to 45 minutes of ischemia, and then the renal pedicles were released and followed by reperfusion period. This group was kept without treatment and served as a positive control.

Group 3 – *Renal I/R + solvent control group (I/R + solvent)*: rats were subjected to I/R and received DMSO i.p. and served as a control group for the tested drugs.

Group 4 – *Renal I/R + diazoxide group (I/R + Diazoxide)*: rats were subjected to I/R and were pretreated with diazoxide.

Group 5 – *Renal I/R + glibenclamide group (I/R + Glibenclamide)*: rats were subjected to I/R and were pretreated with glibenclamide.

Group 6 – *Renal I/R + glimepiride group (I/R + Glimepiride)*: rats were subjected to I/R and were pretreated with glimepiride.

Urine samples were collected using the metabolic cages. Rats from each group were anaesthetized with (i.p.) urethane 1.25 mg/kg²¹. Arterial pressure changes were measured through cannulation of the carotid artery. Blood samples were collected via the tail veins, then rats were sacrificed, kidney and lung tissues were also obtained and divided into two portions. The first one was immediately frozen at -80°C for the different biochemical determinations, while the other part was embedded in 10% neutral buffered formalin and processed to perform histopathological assay 4-6 µm thick paraffin sections were subjected to the hematoxyline and eosin stain.

Rats in sub-groups A were sacrificed 4 hours after the time of reperfusion, while rats in sub-groups B were sacrificed 24 hours after the time of reperfusion.

Measurement of Renal Function

Renal dysfunction was evaluated by measuring serum levels of blood urea nitrogen and creatinine by standard urease assays and picric acid reactions by colorimetric methods²², using Bioclin kit (Santa Coloma, Spain).

Determination of Blood Glucose Level

Serum glucose level was determined enzymatically according to the principle of²³, using Spinreact Diagnostics Kits (Santa Coloma, Spain).

Measuring Systemic Arterial Blood Pressure

Recording was done by using the PT400 blood pressure transducer that was connected to the FC137 strain gauge coupler which was fixed to a channel of the oscillograph. A mid line longitudinal skin incision started just below the neck and extended to the sternum was done to expose the trachea and the common carotid artery. A tight ligature of the artery at its distal end (cephalic end), and a loose ligature around its proximal end (thoracic end) were applied. A bulldog clamp was applied and a small snip across the artery was opened by a small sharp scissors. A polyethylene arterial cannula filled with the heparinized

saline solution was inserted gently towards the heart, the ligature was tied around the cannula and the bulldog clamp was removed. About 10 cm polyethylene tube with a clamp was connected to one side limb of the PT400 transducer, and the other limb of the transducer was connected through polyethylene tube with a clamp to the arterial cannula. Calibration was done by using the oscillograph at speed 0.25 mm/second²⁴.

Assay of Renal Myeloperoxidase (MPO) Activity

Assaying of MPO activity was described by^{4,25,26}. Briefly, kidney or lung tissue (0.5 gm) were homogenized in 10 ml of homogenization buffer pH (4.7) [0.1 mol/L NaCl, 0.02 mol/L NaPO₄ and 0.015 mol/L sodium ethylene diamine tetracetic acid (EDTA)], centrifuge at 260 × g for 10 minutes and the pellet underwent (dissolved) hypotonic lysis (0.2% NaCl) solution followed 1 minutes later by addition an equal volume of solution containing (1.6% NaCl and 5% glucose). After further centrifugation, the pellet was then suspended in resuspension buffer pH 5.4 (0.05 mol/L NaPO₄ containing 0.5% hexadecyltrimethylammonium bromide) and rehomogenized. One milliliter aliquots of the suspension were frozen and thawed three cycles in liquid nitrogen, then centrifuged for 15 minutes at 3000 g. the pellet was discarded. MPO activity was assayed by measuring the change in optical density at 450 nm using tetramethylbenzidine, as substrate (1.5 mmol/L) and H₂O₂ (0.5 mmol/L). Results were expressed as MPO relative units/100 mg tissue. One unit of MPO activity was defined as the quantity of enzyme degrading one mmol peroxide at 25°C. The activity of purified known human neutrophil MPO was used as the standard (Sigma Chemical Co, Egypt).

Evaluation of TNF-α in Lung and Kidney

Kidneys and lungs were homogenized using 0.1 M phosphate buffer (pH 7.4) containing 0.05% (wt/vol) sodium azide at 4°C. Homogenates were sonicated for 20 seconds and centrifuged (2000 g for 10 minutes at 4°C). The resulting supernatants were used for assaying TNF-α levels using ELISA (BioSource Europe S.A., Belgium)²⁵.

Assay of Superoxide Anion Production in Kidney and Lung Tissues

Tissue O₂⁻ was determined according to the modified method of Hassoun and Sthos²⁷ following the principle of Babior et al.²⁸. Briefly, tissue

was homogenized in phosphate buffer saline and centrifuged at 16,000 × g for 15 minutes in cooling centrifuge. The supernatant was added to 0.5 mM cytochrome C (Sigma Chemical Co, Egypt). This mixture was centrifuged again at 3000 × g for 10 minutes, the supernatant fractions were collected for subsequent spectrophotometrically measurement at 550 nm.

(II) Cardiac I/R experiment

Cardiac ischemia reperfusion model by left coronary artery ligation was used for evaluating the side effects of both sulfonylureas (glibenclamide and glimepiride) on both normal and ischemic preconditioning rat's hearts.

Animal model of cardiac ischemia reperfusion

Rats were anaesthetized with intra-peritoneal injection of urethane in a dose of 1.25 mg/kg. The chest was opened in the fourth intercostal space, and the heart was exposed and a loose loop of silk was placed around the left main coronary artery, approximately 2 mm from its origin. Both ends of the ligature were led out of the thoracic cavity through flexible tubing. The heart was set back in its place and artificial respiration was started. The standard electrocardiogram (lead II, ECG) was recorded using subcutaneous needle electrodes. The animals were allowed to stabilize for 10 minutes, then the loose loop of the coronary artery ligature was tightened and fixed by clamping on the silk and thus regional myocardial ischemia was produced for 6 minutes and then followed by reperfusion for 5 minutes¹⁹.

Experimental Protocol

Rats were divided into seven experimental groups (6 rats for each). Diazoxide, glibenclamide and glimepiride were given as a single i.p. dose 30 minutes before coronary artery ligation¹⁹.

Group 1 – Control group (cardiac I/R): rats were submitted to six minutes of ischemia, and then the coronary ligation were released and followed by reperfusion period. This group was kept without treatment and served as control group.

Group 2 – Solvent treated group (cardiac I/R +

solvent): rats were subjected to cardiac I/R and received DMSO i.p. 30 minutes before ligation and served as a control group for the tested drugs.

Group 3 – *Diazoxide treated group (cardiac I/R + Diazoxide)*: rats were subjected to cardiac I/R and were pretreated 30 minutes before ligation with diazoxide.

Group 4 – *Glibenclamide treated group (cardiac I/R + Glibenclamide)*: rats were subjected to cardiac I/R and were pretreated 30 minutes before ligation with glibenclamide.

Group 5 – *Glimepiride treated group (cardiac I/R + Glimepiride)*: rats were subjected to cardiac I/R and were pretreated 30 minutes before ligation with glimepiride.

Group 6 – *Diazoxide + Glibenclamide treated group (cardiac I/R + Diazoxide + Glibenclamide)*: rats were subjected to cardiac I/R and were pretreated 30 minutes before ligation with both diazoxide and glibenclamide.

Group 7 – *Diazoxide + Glimepiride treated group (cardiac I/R + Diazoxide + Glimepiride)*: rats were subjected to cardiac I/R and were pretreated 30 minutes before ligation with both diazoxide and glimepiride.

The anaesthetized rats of each group were subjected to ECG monitoring using the two-channel oscillograph at speed of 50 mm/second before and after coronary ligation. The electrocardiograph was adjusted so that each 20 mm height represented 1 mv. The heart rate, T-wave voltage and ST segment displacement were measured.

Statistical Analysis

SPSS program version 15 was used. The statistical significance was evaluated by using Student's *t* test for comparison between the different groups. *P* value <0.05 was considered significant.

Results

(I) Renal I/R Experiment

Reperfusion of ischemic kidney induced significant elevation in serum levels of urea and creatinine (in both 4 & 24 hours reperfusion periods)

with a significant reduction in urine volume ($P < 0.001$) compared with sham-operated group, suggesting a significant degree of glomerular dysfunction caused by renal I/R. These functional changes were prevented by treatment with sulphonylureas (glibenclamide & glimepiride) supplementation in comparison with I/R group. However glibenclamide induced a significant improvement in renal function as compared to glimepiride group. Additionally renal I/R induced a significant elevation in blood glucose level in comparison with the sham-operated group. While treatment with glibenclamide & glimepiride significantly reduced this elevated glucose level as compared to I/R group (Table I A, B).

Table II A, B, Figure 1 showed that the renal ischemic reperfusion model had a significant elevation in arterial blood pressure compared with sham-operated group ($P < 0.001$). These elevated measurements were improved by treatment with sulphonylureas (glibenclamide & glimepiride) in comparison with I/R group (0.001). However glibenclamide induced a significant improvement in these measurements as compared to glimepiride group ($P < 0.05$).

Table III A, B showed that reperfusion for (4 & 24 hours) I/R, leads to a significant elevation ($P < 0.001$) in both renal and lung MPO activity and O_2^- production in comparison with the Sham-operated group. Treatment with glibenclamide significantly reduced both MPO activity and O_2^- production in renal and lung tissues compared to the I/R. However, treatment with glimepiride significantly reduced MPO activity but, failed to affect the reperfusion-induced increased in the O_2^- production ($P < 0.001$).

Renal I/R induced a marked elevation in the concentration of TNF- α in both the renal and lung tissues. Treatment with glibenclamide or glimepiride suppressed the reperfusion-induced TNF- α ($P < 0.001$) in the kidney and abolished its production in the lungs in both (4 & 24 hours reperfusion with more potency of glibenclamide than glimepiride (Figure 2).

Diazoxide administration caused a significant increase in all studied measurements with significantly reduced urine volume in comparison with both glibenclamide & glimepiride ($P < 0.001$) (Tables I, III & Figure 2). As expected by its vasodilatory effects, administration of diazoxide induced significant ($P < 0.001$) reduction in the elevated blood pressure as compared with I/R group (Table II A, B, Figure 1).

(II) Cardiac I/R experiment

Table I. Effect of diazoxide (D) glibenclamide (Glib) and glimepiride (Glim) on serum glucose (mg/dl), creatinine (mg/dl), BUN (mg/dl) and urine volume (ml $\times 10^{-1}$ /min) in rats exposed to renal I/R for 4 hours (a), for 24 hours (b).

Groups A	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
Glucose	84.8 \pm 10.8	160.0 \pm 17.5 [†]	162.5 \pm 18.5 [†]	165.6 \pm 14.4 [†]	115 \pm 9.8 [#]	108 \pm 9.7 [#]
Creatinine	0.83 \pm 0.27	2.18 \pm 0.44 [†]	2.06 \pm 0.46 [†]	2.26 \pm 0.42 [†]	1.06 \pm 0.18 [#]	1.5 \pm 0.31 ^{#§}
BUN	27.3 \pm 4.08	65.0 \pm 7.8 [†]	65.1 \pm 7.1 [†]	71.6 \pm 5.8 [†]	28.1 \pm 4.9 [#]	42.6 \pm 5.9 ^{#§}
Urine volume	1.26 \pm 0.25	0.11 \pm 0.034 [†]	.11 \pm 0.028 [†]	0.10 \pm 0.044 [†]	1.2 \pm 0.22 [#]	0.93 \pm 0.18 ^{#§}
Groups B	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
Glucose	94.5 \pm 4.0	178 \pm 13.3 [†]	167.8 \pm 13.5 [†]	180.0 \pm 10.7 [†]	113 \pm 10.0 [#]	111 \pm 10.8 [#]
Creatinine	0.91 \pm 0.15	2.2 \pm 0.28 [†]	2.3 \pm 0.2 [†]	2.41 \pm 0.47 [†]	1.16 \pm 0.18 [#]	1.58 \pm 0.14 ^{#§}
BUN	28.6 \pm 8.5	72.3 \pm 5.3 [†]	70.6 \pm 7.5 [†]	75.3 \pm 7.8 [†]	32.6 \pm 5.8 [#]	45.0 \pm 6.4 ^{#§}
Urine volume	1.5 \pm 0.36	0.09 \pm 0.016 [†]	0.098 \pm 0.017 [†]	0.086 \pm 0.013 [†]	1.4 \pm 0.31 [#]	1.05 \pm 0.13 ^{#§}

n = 6

[†]Significantly different from sham group at $p < 0.001$; [#]Significantly different from I/R group at $p < 0.001$. [§]Significantly different from glibenclamide group at $p < 0.05$.

Before coronary artery ligation, neither diazoxide nor glimepiride pretreatment influenced significantly the electrocardiographic parameters (heart rates, T-waves voltages and ST segment elevation) in comparison with the control group. However, glibenclamide supplementation induced a significant elevation ($P < 0.001$) in all these parameters (Table IV & Figure 5).

After left coronary artery ligation, reperfusion of the ischemic hearts caused a significant elevation in the measured electrocardiographic parameters. These elevations were significantly ($P < 0.001$) ameliorated by pretreatment with diazoxide Table IV & Figure 5. Administration of glibenclamide significantly ($P < 0.001$) abolished

the protective effects of diazoxide, while pretreatment with glimepiride didn't abolish it.

Histological Findings

As shown in Figure 3, histological examination of renal sections stained with H& E showed no histopathological changes in the kidney of Sham-operated group in cortex and medulla (a) while kidney sections of I/R group showed immunocompetent cell aggregation, tubular cell necrosis as well as shedding of the lining epithelium and cytoplasmic eosinophilia (b). I/R pretreated with diazoxide was similar to I/R group in addition the renal tubules showed hyaline casts (c). Treatment with glibenclamide was nearly similar to Sham group except that there

Table II. Effect of diazoxide (D) glibenclamide (Glib) and glimepiride (Glim) on systolic and diastolic blood pressure (BP) (mm Hg) in rats exposed to renal I/R for 4 hours (A), for 24 hours (B).

Groups A	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
Systolic B.P.	98.3 \pm 8.2	170.8 \pm 5.8 [†]	169.1 \pm 5.8 [†]	112.5 \pm 2.7 [#]	122.2 \pm 4.7 [#]	131.6 \pm 2.5 ^{#§}
Diastolic B.P.	66.6 \pm 6.0	136.6 \pm 4.0 [†]	133.3 \pm 19.4 [†]	81.6 \pm 6.1 [#]	87.0 \pm 7.6 [#]	101.6 \pm 13.6 ^{#§}
Groups B	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
Systolic B.P.	97.5 \pm 5.2	172.5 \pm 5.2 [†]	171.6 \pm 6.1 [†]	113.3 \pm 9.8 [#]	119.2 \pm 5.8 [#]	130.8 \pm 3.8 ^{#§}
Diastolic B.P.	70.8 \pm 7.3	132.5 \pm 5.2 [†]	133.3 \pm 4.08 [†]	83.3 \pm 6.8 [#]	86.6 \pm 7.5 [#]	98.3 \pm 5.1 ^{#§}

n = 6

[†]Significantly different from sham group at $p < 0.001$; [#]Significantly different from I/R group at $p < 0.001$. [§]Significantly different from glibenclamide group at $p < 0.05$.

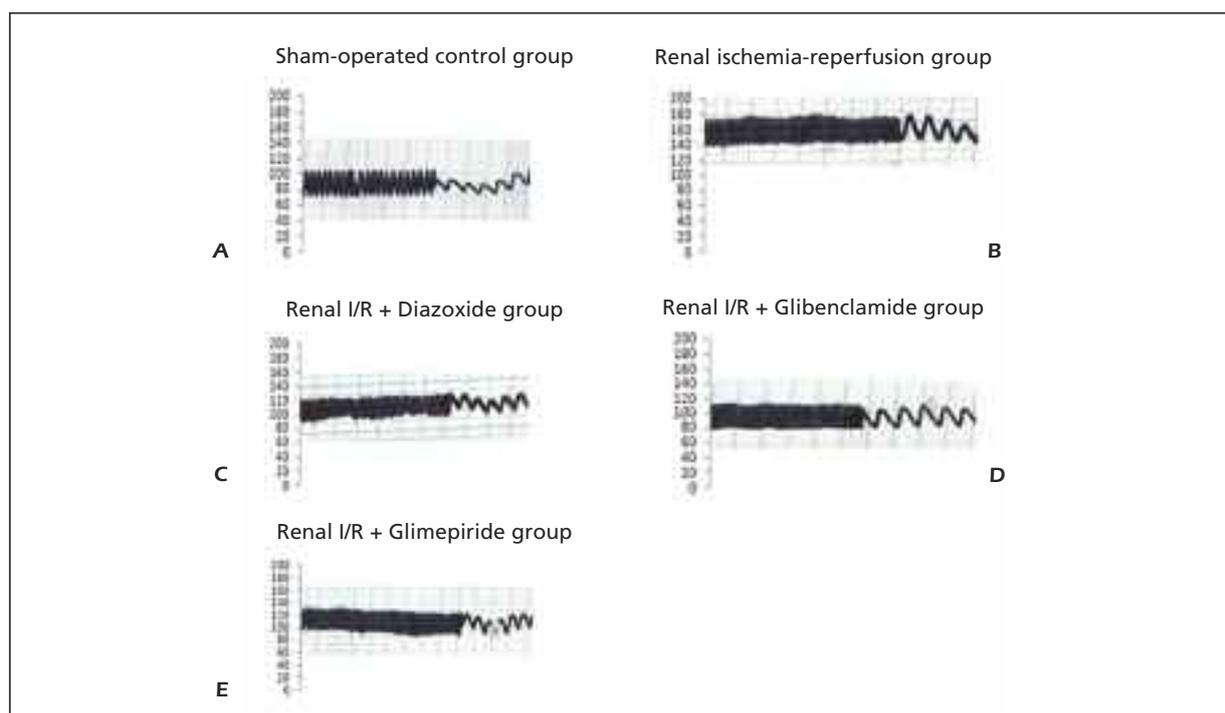


Figure 1. Effect of diazoxide, glibenclamide and glimepiride on systolic and diastolic blood pressure in rats exposed to renal ischemia and reperfusion (I/R).

Table III. Effect of diazoxide (D) glibenclamide (Glib) and glimepiride (Glim) on myeloperoxidase (MPO) activity (U/100 mg tissue) and O_2^- (nmol cytochrome C reduced /min/g tissue $\times 10^{-4}$) in the kidney and lung tissues of rats exposed to renal I/R for 4 hours (A), for 24 hours (B).

Groups A	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
MPO						
Kidney	0.03 \pm 0.01	0.45 \pm 0.05 [†]	0.43 \pm 0.08 [†]	0.47 \pm 0.06 [†]	0.05 \pm 0.01 [#]	0.103 \pm 0.016 ^{#§}
Lung	1.3 \pm 36	12.1 \pm 4.6 [†]	11.46 \pm 1.55 [†]	12.8 \pm 2.7 [†]	2.5 \pm 0.54 [#]	5.9 \pm 0.93 ^{#§}
O_2^-						
Kidney	1.05 \pm 0.18	3.1 \pm 0.5 [†]	3.18 \pm 0.37 [†]	3.0 \pm 0.53 [†]	1.26 \pm 0.24 [#]	2.9 \pm 0.19 [§]
Lung	2.9 \pm 0.66	6.3 \pm 1.0 [†]	6.4 \pm 0.63 [†]	6.4 \pm 0.86 [†]	3.63 \pm 0.5 [#]	5.9 \pm 1.3 [§]
Groups B	Sham	I/R	I/R + solvent	I/R + D	I/R + Glib	I/R + Glim
MPO						
Kidney	0.048 \pm 0.007	0.55 \pm 0.12 [†]	0.58 \pm 0.15 [†]	0.59 \pm 0.08 [†]	0.06 \pm 0.017 [#]	0.155 \pm 0.03 ^{#§}
Lung	2.0 \pm 0.49	11.2 \pm 1.83 [†]	9.56 \pm 1.55 [†]	10.3 \pm 1.7 [†]	2.65 \pm 0.7 [#]	4.8 \pm 0.7 ^{#§}
O_2^-						
Kidney	1.08 \pm 0.19	3.2 \pm 0.3 [†]	3.3 \pm 0.42 [†]	3.16 \pm 0.48 [†]	1.25 \pm 0.27 [#]	3.0 \pm 0.67 [§]
Lung	2.2 \pm 0.44	6.4 \pm 0.9 [†]	5.9 \pm 0.66 [†]	6.4 \pm 0.86 [†]	2.4 \pm 0.47 [#]	5.8 \pm 0.66 [§]

n = 6

[†]Significantly different from sham group at $p < 0.001$; [#]Significantly different from I/R group at $p < 0.001$; [§]Significantly different from glibenclamide group at $p < 0.05$.

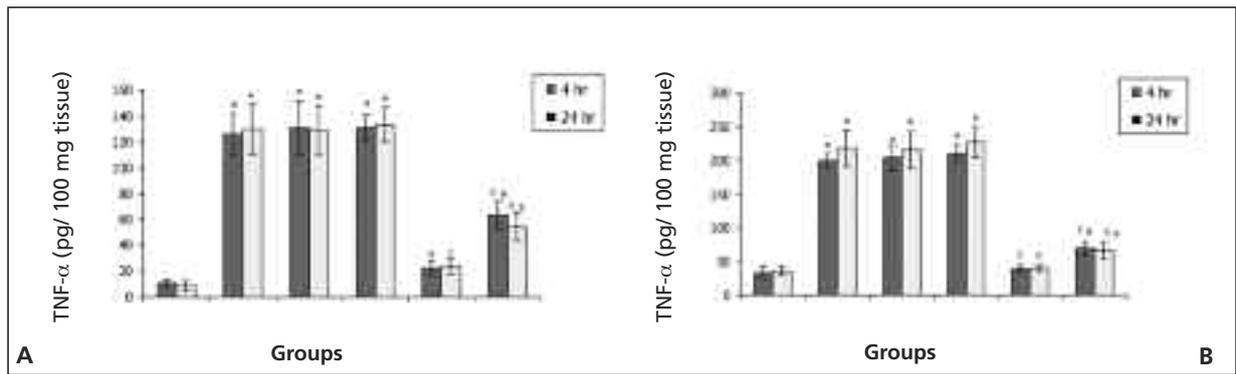


Figure 2. Effect of diazoxide (D), glibenclamide (Glib), and glimepiride (Glim) on TNF- α (pg/ 100 mg tissue) in the kidney (**A**) and lung tissues (**B**) of rats exposed to renal I/R for 4 hr and 24 hr. n = 6. *Significantly different from sham group at 4 and 24 h ($p < 0.001$); #Significantly different from I/R group at 4 and 24 h ($p < 0.001$); ^aSignificantly different from glibenclamide group at 4 and 24 h ($p < 0.05$).

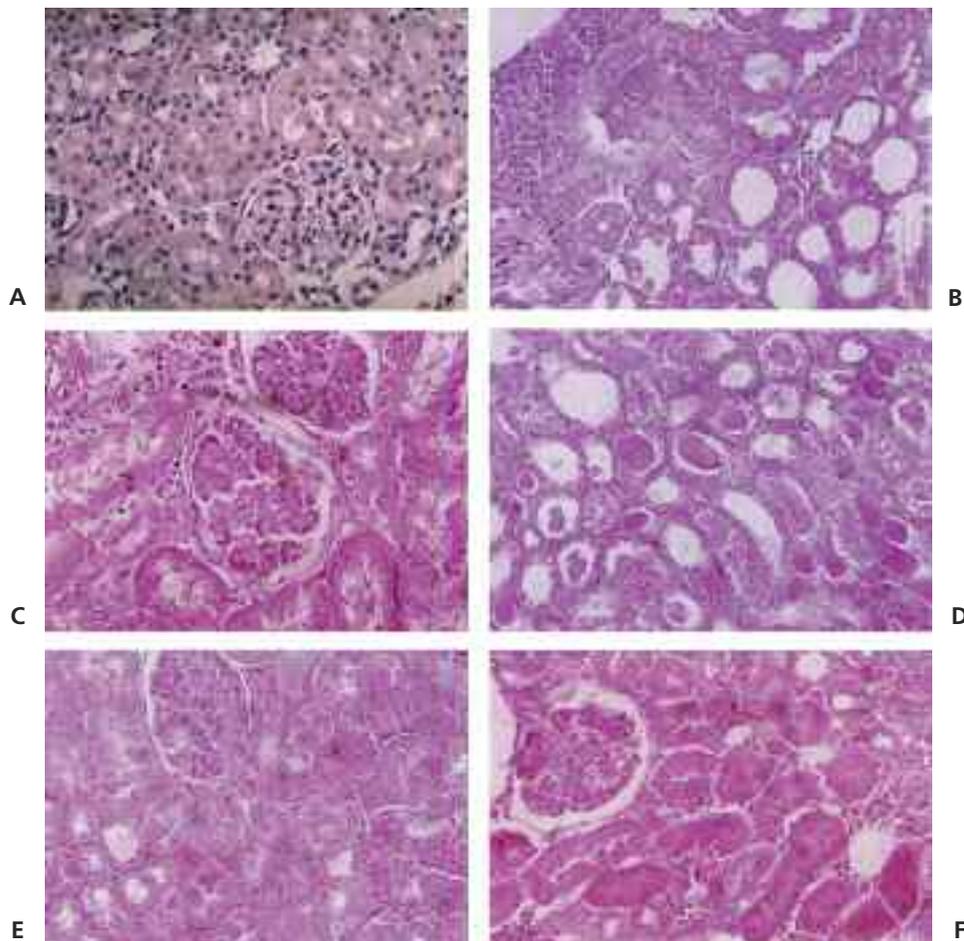


Figure 3. **A**, A photomicrograph renal sections stained with H & E showed no histopathological changes in kidney of sham operated group in cortex and medulla. **B, C**, While kidney sections of I/R group showed immunocompetent cell aggregation, tubular cell necrosis as well as shedding of the lining epithelium and cytoplasmic eosinophilia. **D**, I/R pretreated with diazoxide was similar to I/R group in addition to renal tubules showed hyaline casts. Treatment with glibenclamide was nearly similar to sham group except that there was a light edema of tubular cells (**E**), however glimepiride had less improvement than glibenclamide (**F**). (H & E 400 \times).

Table IV. The effect of diazoxide (D) glibenclamide (Glib) and glimepiride (Glim) on heart rate (beats/min), T waves (mv) and ST segment elevation (mm) in rats before and after coronary artery ligation.

Groups	Before ligation			After ligation		
	Heart rate	T-wave voltage	ST segment elevation	Heart rate	T-wave voltage	ST segment elevation
Cardiac I/R	278.3 ± 20.4	0.21 ± 0.04	1.7 ± 0.5	466.6 ± 37.2	0.65 ± 0.058	6.6 ± 0.58
Cardiac I/R + solvent	268.3 ± 19.4	0.18 ± 0.04	2.0 ± 0.66	460 ± 33.5	0.68 ± 0.04	6.3 ± 0.41
Cardiac I/R + D	270 ± 21.9	0.2 ± 0.037	1.9 ± 0.37	375 ± 23.2 [#]	0.32 ± 0.051 [#]	2.9 ± 0.58 [#]
Cardiac I/R + Glib	388.3 ± 22 [†]	0.45 ± 0.058 [†]	4.9 ± 0.73 [†]	463.3 ± 51.25	0.68 ± 0.7	6.4 ± 0.58
Cardiac I/R + Glim	275 ± 23.5	0.24 ± 0.037	1.8 ± 0.25	453.3 ± 37.3	0.63 ± 0.068	6.2 ± 0.52
Cardiac I/R + D + Glib	395 ± 21.5 [†]	0.46 ± 0.06 [†]	5 ± 0.490 [†]	463.3 ± 34.5	0.58 ± 0.06	5.9 ± 0.66
Cardiac I/R + D + Glim	266.6 ± 18.6	0.2 ± 0.052	1.8 ± 0.51	377.5 ± 22.1 [#]	0.35 ± 0.04 [#]	3.1 ± 0.52 [#]

n = 6

[†]Significantly different from cardiac I/R group before ligation at $p < 0.001$; [#]Significantly different from cardiac I/R group after ligation at $p < 0.001$.

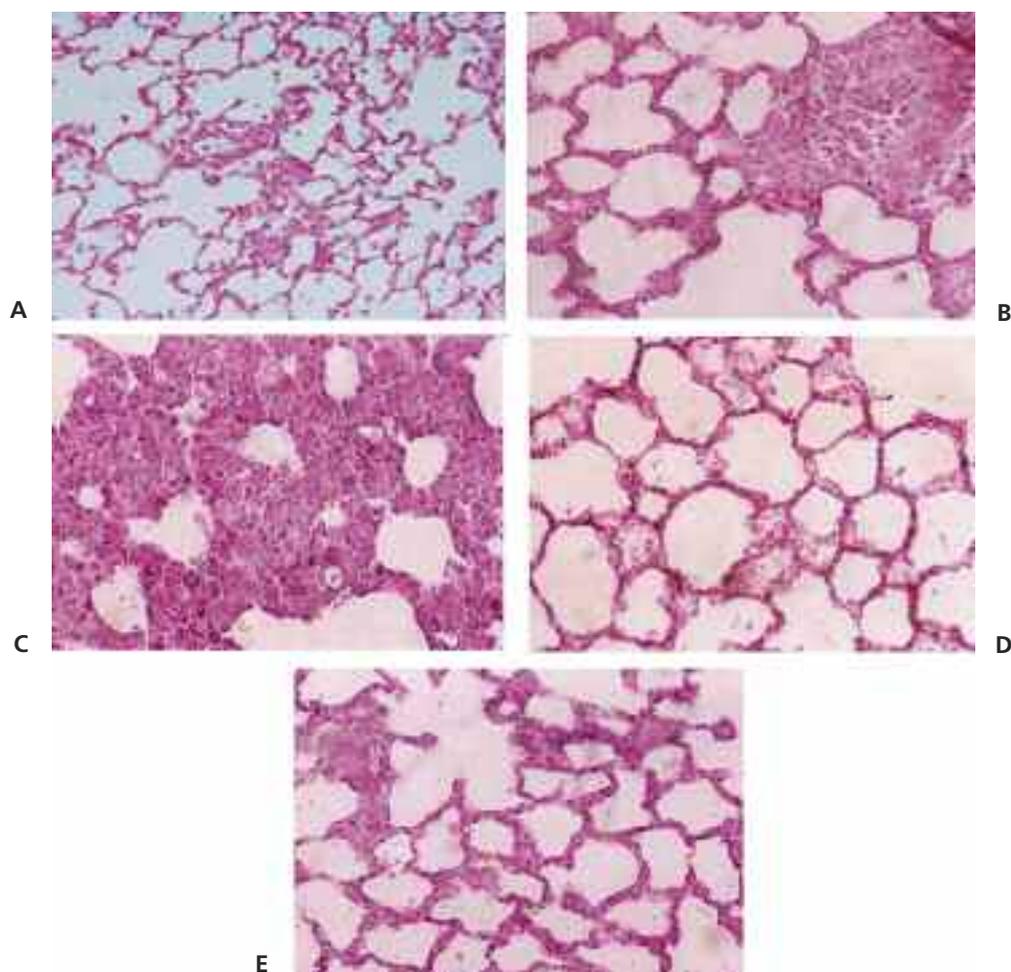


Figure 4. *A*, A photomicrograph showed sections of the sham lung stained with H & E showed normal architecture with no histopathological changes. *B*, *C*, Sections of I/R and I/R pretreated with diazoxide showed marked increase in thickness of the inter-alveolar septa due to mononuclear cellular infiltration. This thickening of the inter-alveolar septa caused narrowing of the alveolar cavities. *D*, *E*, Treatment with glibenclamide and glimepiride preserved the normal histology of the lung. There was a significant decrease in the thickening of inter-alveolar septa. (H & E 400 ×, except a: 100 ×).

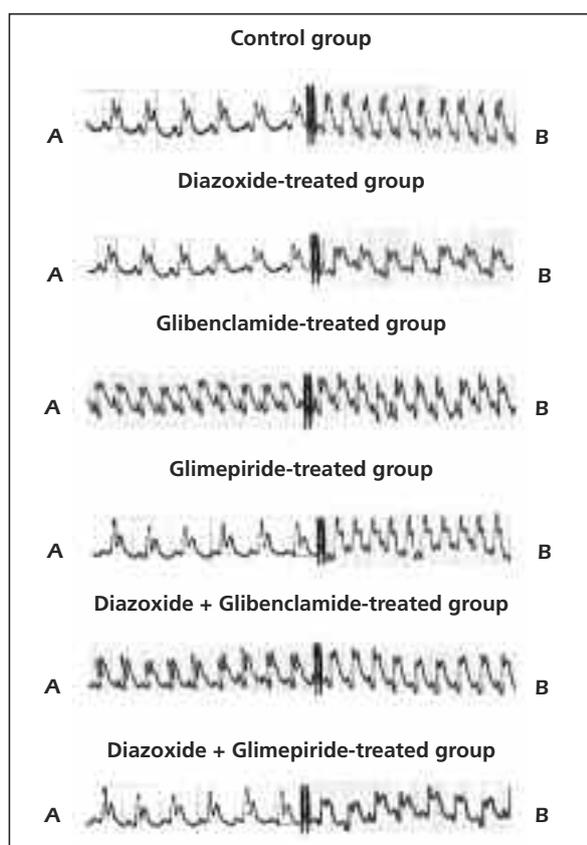


Figure 5. The effect of diazoxide, glibenclamide and glimepiride on ECG tracing in rats before (A) and after (B) coronary artery ligation.

was a light edema of tubular cells (d). Glimepiride induced less improvement than glibenclamide (e).

Figure 4 shows sections of the Sham lung stained with H & E showing normal architecture with no histopathological changes (a). In contrast, sections of I/R and I/R pretreated with diazoxide showed marked increase in the thickness of the inter-alveolar septa due to mononuclear cellular infiltration. This thickening of the inter-alveolar septa caused narrowing of the alveolar cavities (b), (c). Treatment with glibenclamide and glimepiride preserved the normal histology of the lung. There was a significant decrease in the thickening of inter-alveolar septa (d & e).

Discussion

Reperfusion of an ischemic vascular bed is accompanied by local inflammatory injury that limits the potential benefits of blood flow restora-

tion. So, renal ischemia followed by reperfusion results in severe injury that may contribute to renal damage^{29,30} and accompanies a lung inflammation, which complicates the reperfusion syndrome and contributes to the overall lethality^{29,31}. So, strategies that limit reperfusion-associated renal damage may be useful in these conditions.

Our data demonstrated that, after 4 hours of renal reperfusion, there was a marked elevation of the TNF- α level in kidney tissues, and these effects were extended and still present at 24 hours. This increase was due, in ischemic reperfused injury, to complement activation and neutrophil stimulation with accompanying oxygen radical-mediated injury²⁹. Oxidants were released during the reperfusion of ischemic tissue; moreover, neutrophil activation stimulated transcription factors involved in TNF- α expression^{4,6,29}. This notion was confirmed in our study by the findings that renal tissue levels of O_2^- significantly were increased in association with the accumulated neutrophils and elevated TNF- α level in 4 hours of reperfusion and extended also to 24 hours. These results agreed with those of Rahgozar et al.³ who emphasized that reperfusion of ischemic tissues imposes an oxidant burden in which the reduced form of molecular oxygen, hydrogen peroxide, contributes to injury. Furthermore, the activation of oxidant-sensitive enzymes involved in TNF- α production represents an additional mechanism by which the oxidant stress induces a cellular damage.

Our study was extended by measuring the myeloperoxidase activity which is an accepted indicator of neutrophil accumulation and oxidative activity. An associated renal dysfunction accompanies the increase in the activity of MPO. Toybin et al.³⁰ discovered that activated neutrophils produce superoxide, which can be dismutated into hydrogen peroxide. Neutrophil MPO enzyme converts hydrogen peroxide to hypochlorous acid that reacting with superoxide produces hydroxyl radicals. The elevated renal dysfunction also demonstrated in our study by an increase of both BUN and creatinine, raised blood pressure and decreased urinary volume can be due to the increase of TNF- α level, O_2^- production and MPO activity.

As sulfonylureas are widely used as hypoglycemic drugs, we measured the blood glucose levels at 4 and 24 hours of renal reperfusion and we remarked that glycemia was high in rats submitted to renal I/R. These high levels of glucose may be due to the surgical stress carried out.

However, treatment with sulfonylureas ameliorated this elevated levels.

In the current study, renal I/R-induced histopathological changes included tubular necrosis, vascular congestion and neutrophil accumulation in the outer medulla. These findings could be considered the main site of inflammation where neutrophils are activated is the postcapillary venules feeding the proximal tubules in the kidney. In addition, the activated neutrophil-induced endothelial cell injury might lead to ischemia of the proximal tubules in the outer medulla.

During renal I/R toxic products accumulate intracellular; after re-establishment or re-connection of the vasculature to the circulation, oxygen is re-applied and repair mechanisms are set into place. During that time, accumulated toxic metabolites are flushed into the system, which may affect other organs and may negatively influence the process of regeneration in the ischemic organ. These events occur secondary to the opening of ATP-sensitive potassium channels after fall in intracellular ATP concentration, causing massive influx of neutrophils in association with the release of various inflammatory mediators and oxygen-derived free radicals. These effects called the "uremic lung"^{29,31}. Our data revealed the marked neutrophil and cytokines accumulation in the lung tissues that begin as early as 4 hours and extended at 24 hours.

In this study we found that the treatment with both the glibenclamide and the glimepiride significantly ameliorated the reperfusion-induced neutrophil accumulation and elevation of TNF- α level in both the kidneys and lungs. We also observed that the reperfusion-induced myeloperoxidase activation in both the kidneys and lungs was inhibited by using both sulfonylurea drugs. This effect was secondary to their ability to improve the production of TNF- α and neutrophil aggregation following renal I/R. Furthermore, we found that sulfonylurea, because their anti-inflammatory effects, improved all the functional renal parameters as BUN, creatinine and the urinary volume. In addition, sulfonylurea ameliorated the level of blood pressure and the histo-pathological changes associated with the renal I/R. In conclusion, the inhibition of the K_{ATP} channel due to the sulfonylurea is the mechanism by which these drugs prevent the renal I/R injury in both the kidneys and lungs. The activation of ATP-sensitive potassium channels during hypoxia plays an important role in the neutrophil accumulation and the development of inflammatory response that accompany the renal I/R injury⁴.

In studying the protective effects of both glibenclamide and glimepiride on renal I/R, we noticed that the glibenclamide is more effective than the glimepiride in inhibiting the local and remote injury following I/R of the renal vascular pedicle in rats. This inhibitory effect may be due to the anti-oxidant effects of glibenclamide, that was emphasized in this study by the inhibitory effects of glibenclamide on the O_2^- production, while glimepiride had no such effect. Another explanation was afforded by Lee and Chou¹⁸ who emphasized that the glimepiride was more specific than the glibenclamide for the SUR_1 receptor in the pancreatic beta cells. However, protein making up the K_{ATP} channel in the pancreatic beta cells and in the principal cells of the kidney were different³². So, most probably the $Kir_{1.1a}$ /CFTR receptor that is expressed in the kidney was more efficiently inhibited by the glibenclamide more than by the glimepiride. Further researches are required before any definitive conclusions can be made.

As sulfonylureas have attracted a great deal of interest in experimental cardiology and the protective effect of K_{ATP} channel blockade in renal I/R stands in sharp contrast to the harmful effects on the cardiac tissues, our study was extended to evaluate the possible harmful effects of both sulfonylureas drugs on normal and ischemic reperfused heart. It is well established that K_{ATP} channels are present in cardiac cells and also in vascular smooth muscle cells, and are implicated in the regulation of myocardial and vascular function. So, drugs which open or inhibit the opening of these channels, might profoundly modify the cardiovascular functions both under physiological and pathological conditions³³. Questions are now raised about the potential adverse effects of these drugs on the cardiovascular functions.

The mechanism of protection against arrhythmia associated with ischemia reperfusion is that the myocardial sarcolemmal ATP-dependent potassium channels, which are normally closed by high ATP concentration, open during ischemia when the ATP generation decreases favoring potassium efflux. This reduces the action potential duration (APD) of the cardiomyocyte thus decreasing the time of calcium influx and overload inducing arrhythmia³⁴. Moreover, ischemic preconditioning is considered to be one of the most potent mechanisms of protection against myocardial I/R injury¹⁰. The mitochondrial K_{ATP} channel opening plays a central role in the acquisition of this protection²⁰.

Our data showed that, neither diazoxide nor glimepiride pretreatment influenced significantly the cardiac parameters before coronary artery ligation. However, glibenclamide significantly increased the heart rate associated with elevated T-wave voltage and ST segment. These elevated parameters increased more after transient coronary artery ligation followed by reperfusion. Moreover, the harmful effects on the rat's hearts that occur secondary to transient coronary artery ligation, were improved by a pretreatment with diazoxide. Opening of the mitochondrial K_{ATP} channels and increasing the total outward potassium current shortens the action potential duration, thereby decreases the calcium influx. Thus, causes an energy preservation and a mediation of ischemic preconditioning^{10,20}.

We observed that pretreatment with glibenclamide abolishes the cardio-protective effect of diazoxide pretreatment presumably by inhibiting the mitochondrial K_{ATP} channel opening in the myocyte²⁰. These results agreed with Negroni et al.³⁴ who referred that glibenclamide interferes with the beneficial action of K_{ATP} opening during ischemia reperfusion events thus prolonging the APD and calcium entry for a longer period of time, which is potentially harmful to the heart. Conversely, the improvements that were associated with preconditioning afforded by diazoxide were not significantly changed when the preconditioning protocol took place in the presence of glimepiride. A possible explanation is that glimepiride, unlike glibenclamide, doesn't block the mitochondrial K_{ATP} channels known to play a crucial role in preconditioning protection¹³. Glimepiride appears to be significantly less harmful than glibenclamide to the normal and ischemic reperfused hearts subjected to IP protection afforded by diazoxide which is one of the preconditioning mimetic agents.

In conclusion, glimepiride at equivalent protective dose in renal I/R has less cardiovascular side effects than conventional sulfonylureas. Therefore, it offers some promise for therapy of renal I/R minimizing the undesirable cardiac side effects.

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