

Moexipril Improves Renal Ischemia/Reperfusion Injury in Adult Male Rats

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Abstract

Objective: The goal of this study is to see if moexipril can protect rats against renal ischemia/reperfusion injury.**Methods:** Overall twenty-eight males of rats were divided randomly into four groups (7 rat each group). Sham group: Except for ischemia induction, these rats underwent IP anesthesia and surgery. Induced group: This group rats were anesthetized and given a midline laparotomy to induce bilateral renal ischemia for 30 min and 2 hours of reperfusion. DMSO group: Rats received DMSO IP injection 30 min before ischemia and subjected to 30 min bilateral ischemia and reperfusion for 2 hours, DMSO is a vehicle of moexipril and considered as control. Moexipril group (pretreated group): moexipril was given in a dose 0.3 mg/kg I.P. injection 30 min before ischemia.**Results:** Renal IRI as indicated by a significant increase ($P < 0.05$) in urea, creatinine, NF-KB P65, IL-1 β , and caspase-3 level, while GSH, SOD, and Bcl-2 levels significantly ($P < 0.05$) reduced in Renal tissues of rats in the induced group compared to sham group. Moexipril pretreatment significantly ($P < 0.05$) ameliorate RIRI as suggested from significant lowering in urea, creatinine, and inflammatory markers (NF-KB P65 and IL-1 β). The renal level of oxidative marker (SOD and GSH) and anti-apoptotic marker Bcl-2 were significant decreased ($P < 0.05$) and also significantly increase ($P < 0.05$) in caspase-3 level with moexipril group in comparison to induced group.**Conclusion:** By inhibiting oxidative stress, inflammation, and the apoptotic pathway, moexipril significantly protect from renal ischemia reperfusion in rats.**Keywords:** Moexipril, renal ischemia/reperfusion injury (RIRI), inflammatory mediators, oxidative stress, apoptotic factors

Introduction

The most common cause of AKI is renal ischemia/reperfusion injury (RIRI). It was shown that 41.2% of patients worldwide did not recover following the development of AKI prior to hospitalization, and only 20% of them maintained their normal renal function.¹ A pathological condition known as RIRI is brought on by a reduction in the amount of blood flow to the kidney, which is then followed by a recovery of the kidney's perfusion and reoxygenation after the blood supply is restored.² RIRI is a common complication that can arise as a consequence of renal transplantation, hydronephrosis, nephrectomy, a decrease in blood volume, and shock that occurs during the removal of renal malignancies.³ Ischemia induces anaerobic metabolism due to a lack of oxygen, which decreases ATP production, results in intracellular acidosis from lactate synthesis, and disrupts ion-exchange channels, all of which contribute to IRI. When ion exchange channels are dysfunctional, cells swell and cytoplasmic enzyme activity decreases. The three main systems that contribute to oxidative stress in the reperfusion state due to mitochondrial damage, electrolyte imbalance, and ROS expression eventually results in cell death via the three pathways of autophagy, apoptosis, and necrosis are the NADPH oxidase system, nitric oxide synthase system, and xanthine oxidase system.⁴ The pathophysiology of IRI is heavily influenced by reactive oxygen species. Cells in hypoxic tissue are present during ischemia. Reduced oxygen levels inside the cell lead to problems with oxidative phosphorylation in the mitochondria, ATP production, calcium entrance into the mitochondria, and electron transport chain damage.^{5,6} In AKI Both innate and adaptive inflammatory responses play a role.⁷ Tissue destruction may

be aided in part by inflammatory response cells like neutrophils and macrophages at a certain point in the damage process. After renal ischemia, the first leukocytes to arrive are neutrophils. Neutrophils release proinflammatory cytokines and chemokines. Activated leukocytes have the potential to produce further endothelial cell injury as well as endothelial barrier permeability dysregulation.⁸ Ischemia causes apoptotic cell death can be triggered by either the intrinsic or extrinsic pathways.⁹ Moexipril is angiotensin-converting enzyme inhibitor (ACEI) used to treat hypertension and congestive heart failure.¹⁰ Moexipril has antioxidant properties in addition to efficiently regulating blood pressure by lowering mitochondrial reactive oxygen species production and scavenging free radicals.¹¹ By inhibiting ACE, moexipril inhibits the synthesis of the vasoconstrictor peptide (angiotensin II) from angiotensin I and limiting the breakdown of the vasodilator peptide (bradykinin).¹² Moexipril, like other ACEI, lowers intravascular hypertension, prevents systemic and coronary vasoconstriction by direct smooth muscle contraction, and decrease in adrenal aldosterone synthesis. Moreover, moexipril has many cardioprotective and vasoprotective properties, because the drug restores the balance between cardiac oxygen supply and demand, reduces ventricular pre- and afterload and reverses left ventricular hypertrophy.¹³ Moexipril has radical scavenging activities and are essential for reducing oxidative damage.¹⁴ Moexipril protects against free radical-induced neuronal damage and reduces ischemic brain injury in rodents and rats. In addition to regulating blood pressure, the neuroprotective properties of moexipril may be an important additional benefit for the treatment of hypertensive patients at high risk for stroke due to their ability to eliminate reactive oxygen species.¹⁵

Methods

Animals Preparation

Total of 35 males Sprague Dawley rats were acquired from the University of Kufa/Faculty of Science weighing 200–300g and aged 14–20 weeks. The rats were confined at the University of Kufa/Faculty of Pharmacy animal house. The animals were kept in a separate chamber, in a group caging system, with temperature and humidity controlled at $24 \pm 2^\circ\text{C}$. The rats were fed a conventional diet of food and water. After two weeks of acclimation in the isolation chamber, the experiments began.

Study Design

A total of 28 males of rats were divided randomly into four groups ($N = 7$): Sham group: Except for ischemia induction, these rats underwent IP anesthesia and surgery. (IR) Induced group: This group rats were anesthetized and given a midline laparotomy to induce bilateral renal ischemia for 30 min and 2 hours of reperfusion. DMSO group: Rats received DMSO IP injection 30 min before ischemia and subjected to 30 min bilateral ischemia and reperfusion for 2 hours, DMSO is a vehicle of moexipril and considered as control. Moexipril (pretreated) group: Rats pretreated with 0.3 mg/kg of by IP injection 30 min before ischemia and exposed to 30 min of bilateral renal ischemia and 2 hours reperfusion.¹⁵ All group rats subject to midline laparotomy incision to extract renal and blood samples.

Preparation of the Drug

Moexipril powder purchased from MedChemExpress in USA. moexipril was dissolved in DMSO 50 mg/1 ml as stock solution in accordance with the manufacturer's guidelines.

Experimental Model of Renal Ischemia/Reperfusion Injury

Sterile instruments performed all procedures. Rats were sedated for 5–10 minutes and placed on their backs with stickers to stabilize them during surgery. Shaving and povidone iodine disinfected the animals' operating area. The rats' hind feet and tails were squeezed to test their anesthesia.¹⁶ The median laparotomy incision exposed bilateral renal pedicles and rat kidneys. To access the renal arteries and veins, the liver was gently pulled up and held in place with moistened gauze while the ureter was elevated using angled forceps. blunt dissection gradually broke the connective tissues anterior and posterior to the left and right renal artery and vein, creating a conduit underneath the arteries.¹⁷ Non-invasive vascular clamps were used to occlude both renal arteries for 30 minutes, and ischemia was produced visually by seeing a gradual, uniform darkening of the kidney.¹⁸ When The clamps withdrawn, the color change from dark purple to red confirmed kidney reperfusion. The abdominal cavity incision was then double-sutured. After two hours, the animals were ethically slaughtered under deep anesthesia and blood and left kidney tissues were taken for study.¹⁹

Collection of Samples

Blood sample collection

Each rat was weighed and anesthetized using ketamine at a dose of (100 mg/kg) and, xylazine 2% at a dose of (10 mg/kg)

by IP injection. Rats were sedated for 5–10 minutes, then placed on their backs with their limbs and tails secured with stickers. A midline laparotomy incision was made, and blood was drawn immediately from the hearts left-ventricle through a heart puncture. The blood sample was placed in an anticoagulant-free tube at 37°C , centrifuged at 3000 rpm for 10 minutes, and the serum was recovered for the measurement of Urea, creatinine, NF-KB P65, and IL-1 β . Using available kits of ELISA in accordance with the manufacturer's instructions of Bioassay Technology Laboratory/China for creatinine, NF-KB P65, and IL-1 β , and ELISA kit from Sunlong Biotech co., Ltd, China for urea.

Tissue sample preparation

The left kidney of each animal was removed and cut in half. After freezing half of the sample at 80°C , it was homogenized with a high intensity-ultrasonic liquid processor in phosphate buffered saline (1:10 w/v) containing a protease inhibitor cocktail and Triton X-100 (1%).

After centrifuging the homogenate at 3000 rpm for 20 minutes at 4°C , the supernatant was collected for measurement of SOD, GSH, Caspase-3, and Bcl-2 using the ELISA technique in accordance with the manufacturer's instructions of Bioassay Technology Laboratory/China.

Tissue sampling for histopathology

For histopathological exams, the second half was immersed in 10% neutral-buffered formalin, dehydrated in alcohol, cleaned in xylene, and finally embedded in paraffin block for preservation. Hematoxylin and eosin were used to stain horizontally sliced tissue slide sections that were about 5 m thick. Light microscopy was used to investigate the sections.²⁰

Measurement of Study Parameter

Measurement of urea

The level of urea was determined by utilizing urea Elisa kit obtained from Sunlong Biotech co., Ltd, China, according to the manufacture instructions.

Measurement of creatinine

The level of creatinine was determined by utilizing creatinine Elisa kit obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of IL-1 β

The level of IL-1 β was determined by utilizing IL-1 β Elisa kit obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of NF-KB p65

The level of NF-KB P65 was measured by using NF-KB p65 Elisa kit obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of caspase-3

The level of Caspase-3 was measured by using Caspase-3 Elisa kit obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of Bcl-2

The levels of Bcl2 was measured by utilizing Bcl-2-Elisa kit obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of SOD

The level of SOD was determined by utilizing SOD Elisa kits obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Measurement of GSH

The level of GSH was determined by utilizing GSH Elisa kits obtained from Bioassay Technology Laboratory, China, according to the manufacture instructions.

Statistical Analysis

All statistical analyses were performed in GraphPad Prism 8.0.1 (GraphPad Software; La Jolla, California, USA). Unless otherwise specified, all results were presented as a mean \pm SEM. The data was analyzed using a One-Way ANOVA and then the Bonferroni multiple comparison test. In all tests, $P < 0.05$ was considered to be statistically significant. Histopathological alterations were compared between research groups using Kruskal-Wallis one-way ANOVA as well.

Results

The induction of RIRI as indicated by a significant increase in urea, creatinine, IL-1 β , NF-KB P65 and caspase-3 level, while SOD, GSH and Bcl-2 levels significantly reduced in renal tissues of rats in the induced group when compared to sham group. Moexipril significantly ameliorated renal ischemia/reperfusion as suggested from significant lowering of urea, creatinine, and inflammatory mediators NF-KB P65 and IL-1 β (Figures 1–4). Renal tissue level of anti-oxidant enzymes SOD and GSH were significant increased (Figure 5 and 6) with moexipril group in comparison to induced group. Additionally, moexipril significantly attenuated renal ischemia reperfusion-induced apoptosis reflected by lower renal caspase-3 level (Figure 7) and also significant elevation in Bcl-2 when compared to induced group (Figure 8). Furthermore, RIRI causing a significant tissue damage when compared with sham group. Pretreatment with moexipril cause a significant amelioration of tissue damage caused by IRI (Figures 9 and 10).

Discussion

Serum creatinine and urea levels are common indicators of renal impairment utilized in the assessment of IRI animal models. Serum levels of urea and creatinine were found to be significantly higher in the induced group than in the sham group. This conclusion corroborated previous work by Long and colleagues who found elevated levels of urea and creatinine in the RIRI rat model group compared to the sham group.²¹ This rise in blood urea and serum creatinine levels could be attributed to prerenal azotemia and vasoconstriction with decreased perfusion.²² Pretreatment with moexipril significantly reduced urea and creatinine levels compared to the induced and DMSO group (Figures 1 and 2), as demonstrated by this study. This is indicative of a safeguarding effect

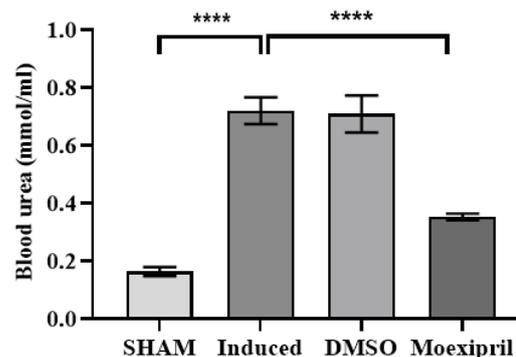


Fig. 1 Blood urea level of experimental groups. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. Serum urea concentrations were evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, **** $P < 0.0001$.

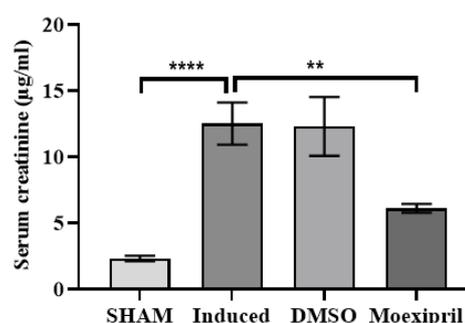


Fig. 2 Creatinine level of experimental groups. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. Serum creatinine concentrations were evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, ** $P < 0.01$, **** $P < 0.0001$.

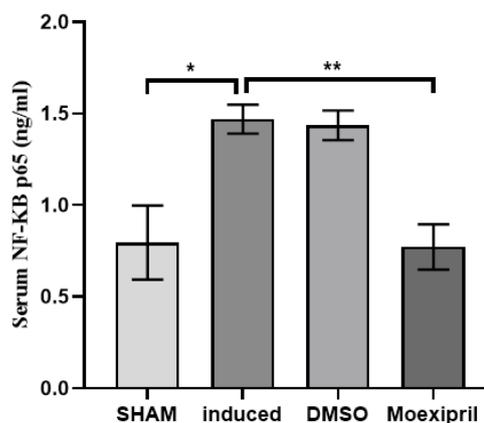


Fig. 3 NF-KB P65 level of experimental groups. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. NF-kB P65 concentrations were evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, * $P < 0.05$, ** $P < 0.01$.

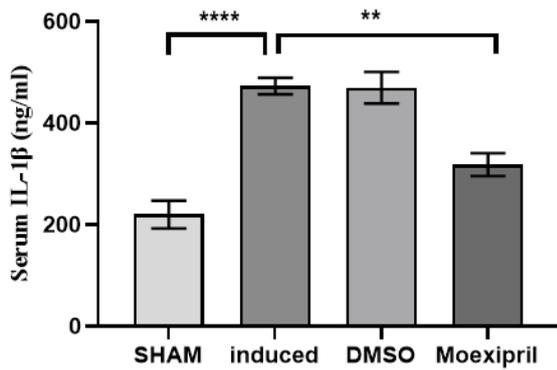


Fig. 4 IL-1 β level of experimental groups. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. IL-1 β levels were evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, ** $P < 0.01$, **** $P < 0.0001$.

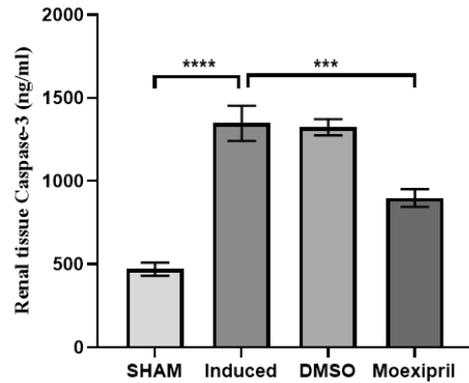


Fig. 7 Tissue caspase-3 level of experimental groups. The rats in the induced group were subjected to 30 minutes of ischemia followed by 2 hours of reperfusion .30 minutes before ischemia, rats were given either moexipril (0.3 mg/kg), DMSO, or were left untreated (sham, and induced). The caspase-3 level was determined using an ELISA kit. Following a one-way ANOVA, the data was evaluated with a Bonferroni multiple comparison test. Data are presented as mean \pm SEM, $n = 7$ rats/group, *** $P < 0.001$, **** $P < 0.0001$.

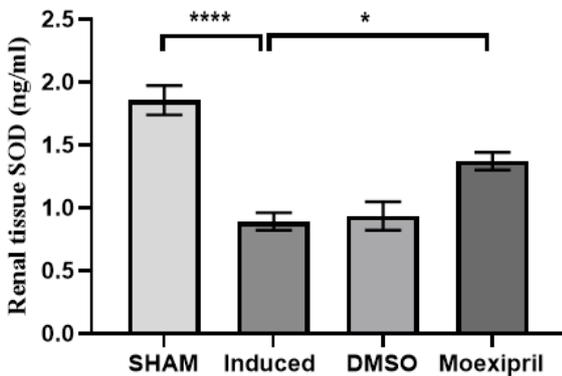


Fig. 5 Tissue SOD level of experimental groups. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. SOD level was evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, * $P < 0.05$, **** $P < 0.0001$.

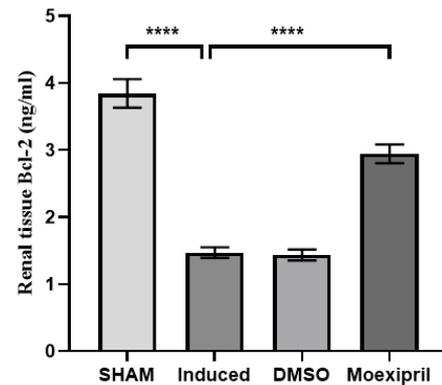


Fig. 8 Tissue Bcl-2 level of experimental groups. The rats in the induced group were subjected to 30 minutes of ischemia followed by 2 hours of reperfusion .30 minutes before ischemia, rats were given either moexipril (0.3 mg/kg), DMSO, or were left untreated (sham, and induced). The Bcl-2 level was determined using an ELISA kit. Following a one-way ANOVA, the data was evaluated with a Bonferroni multiple comparison test. Data are presented as mean \pm SEM, $n = 7$ rats/group, **** $P < 0.0001$.

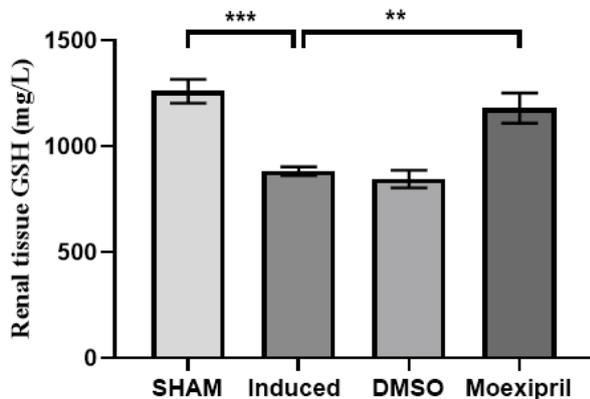


Fig. 6 Tissue GSH level of experimental groups. Rats pretreated with either moexipril (0.3 mg/kg), DMSO, or left untreated (sham and induced) 30 min before ischemia. The induced groups rats experienced 30 minutes of ischemia, then 2 hours of reperfusion. GSH level was evaluated using ELISA kit. The data was analyzed using a Bonferroni multiple comparison test after a one-way ANOVA was performed. Data are presented as mean \pm SEM, $n = 7$ rats/group, ** $P < 0.01$, *** $P < 0.001$.

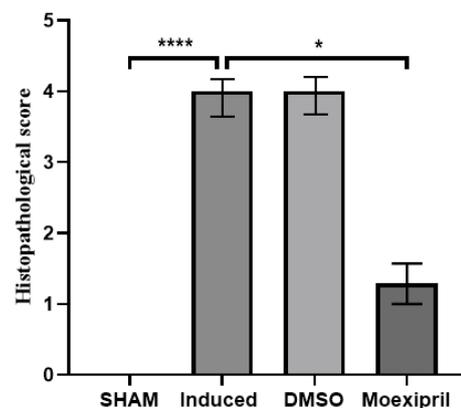


Fig. 9 Histopathological score in the experimental groups. Rats of induced group subjected to 30 min ischemia and followed reperfusion for 2 hr. Rats pretreated with either DMSO, moexipril (0.3 mg/kg), or left untreated (sham and induced) 30 min before ischemia. Changes in histopathology were measured by Kruskal-Wallis one-way ANOVA. Data are presented as mean \pm SEM, $n = 7$ rats/group, * $P < 0.05$, **** $P < 0.0001$.

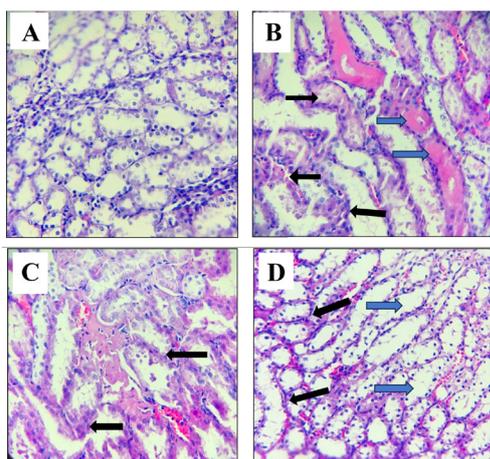


Fig. 10 (A) Sham group, normal histology of renal tubules H&E. (B) Induced group, renal tubules with score 4 damage. Cellular swelling, increased cytoplasmic eosinophilia (black arrows), eosinophilic cast (blue arrows) H&E. (C) DMSO group, renal tubules with score 4 damage. Cellular swelling, increased cytoplasmic eosinophilia (black arrows) H&E. (D) Moexipril (0.3 mg/kg) group, renal tubules with score 2 damage. Cellular swelling and increased cytoplasmic eosinophilia involving 45% of the examined tubules (black arrows), normal tubules (blue arrows) H&E.

on renal function. There has been no prior research on the impact of moexipril on blood creatinine and urea in RIRI, as far as we are aware.

The results of this study indicate that the renal tissue levels of IL-1 β and NF-KB P65 were significantly higher in the induced than in the sham group following IRI. These results agree with a study showed that when rats were subjected to bilateral renal ischemia for 30 minutes, followed by 2 h reperfusion, the inflammatory mediators IL-1 β and NF-KB P65 were significantly elevated in the control and vehicle group compared to the sham group.²³ Pretreatment with moexipril 30 min before ischemia significantly reduced IL-1 β and NF-KB P65 (Figures 3 and 4) levels relative to the induced and vehicle groups. To our knowledge, moexipril has not been studied on tissue IL-1 β and NF-KB P65 in animals.

When it comes to protecting cells from the damage caused by oxygen radicals, SOD is at the forefront. where it dismutates superoxide to H₂O₂ and O₂ to scavenge ROS. RIRI damages kidney cell mitochondria, decreasing SOD and GSH activity and preventing the body from removing oxygen free radicals, causing a redox system disturbance and worsening body damage. We found that the antioxidant levels of GSH and SOD were significantly lower in the induced group compared to the sham group. This finding is in agreement with that obtained with Hasanein and coworkers, who demonstrated that renal SOD and GSH activity were significantly lower in the I/R group than in the sham group after 20 minutes of bilateral ischemia followed by 24 hours of reperfusion in rats.²⁴ According to the results of this investigation, tissue GSH and SOD levels were much higher, and antioxidant capacity

was restored, when moexipril was administered 30 minutes before the induction of ischemia (Figures 5 and 6). Moexipril may affect lipid peroxidation and antioxidant enzyme activity in hypertensive postmenopausal women. Moexipril reduced MDA and increased SOD, catalase, and GSH activity. These data imply that moexipril may scavenge free radicals in addition to treating hypertension.²⁵

Caspase-3 is the principal apoptosis-associated protein and plays a critical role in triggering DNA fragmentation and morphological changes in dying cells. After IRI, the levels of the apoptosis mediator caspase-3 in renal tissue were found to be significantly higher in the induced group and the DMSO group compared with the sham group. This finding was also reported in other research who found that rats exposed to renal ischemia reperfusion by occluding the renal pedicles for 30 min and restoring blood for 2h had higher caspase 3 and lower Bcl2 levels than the sham group.²⁶ Moexipril pretreatment group had a substantial reduction in caspase-3 kidney level and increased Bcl-2 compared to induced group (Figures 7 and 8). Thus, moexipril has antiapoptotic effects, although there is no previous study on Bcl-2 or caspase-3 in RIRI models. Furthermore, Ravati,1999 demonstrate that Pretreatment with moexipril (0.3 mg/kg) before ischemia dramatically reduced brain damage after focal ischemia as compared to control group in rats. This demonstrated that moexipril had anti-apoptotic capabilities.¹⁵

The induced and DMSO groups had substantially higher left kidney severity scores than the sham groups. Histopathological abnormalities include cellular edema, loss of brush boundaries, cytoplasmic eosinophilia, dilated tubules, Bowman's capsule dilatation, cast formation, inflammatory response, and necrosis. These changes are consistent with earlier study findings that IR renal sections showed substantial disruption of normal kidney architecture, including glomerular injury, bleeding, loss of brush boundary, vacuolar degeneration, epithelial atrophy, and tubular casts.²⁷ Moexipril pretreatment reduced tissue damage compared to induced and DMSO groups (Figures 9 and 10). Unfortunately, moexipril has not been studied in renal parenchyma. However, moexipril upregulates Bcl-2 to decrease apoptosis, promote tissue survival, oxygen and blood supply, and kidney tissue repair nearly to normal by lowering tubular injury and inflammation.

Conclusion

Based on the result of the present study, Moexipril significantly decrease the oxidative stress, inflammatory response and apoptosis. In addition to minimizing tissue damage so we can conclude that moexipril effectively protect from RIRI in rats.

Conflict of interest

None. ■

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