Antioxidative and anti-inflammatory impact of valsartan against renal ischemia-reperfusion injury; role of nitric oxide signaling pathway

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Abstract

Introduction: Renal ischemia reperfusion injury is one of the main causes of acute renal failure, which is associated with high mortality. Tissue damage caused by ischemia-reperfusion occurs due to the release of oxygen free radicals. Type I angiotensin receptor antagonists such as valsartan can be useful in the treatment of chronic kidney disease and hypertension.
Objectives: We aimed to evaluate the protective effect of valsartan against renal ischemia reperfusion via antioxidant property and nitric oxide (NO) signaling pathway.
Materials and Methods: Fifty male Wistar rats (220±10 g) were randomly divided into five groups as follows: Group 1; healthy rats without ischemia-reperfusion (control group). Group 2; rats with ischemia reperfusion (IR) (IR control group). Group 3; rats with IR which received 30 mg/kg valsartan orally. Group 4; rats with IR which received 30 mg/kg valsartan together with 40 mg/kg L-NAME. Group 5; rats with IR which received 30 mg/kg valsartan together with 40 mg/kg L-arginine. To induce ischemia-reperfusion, rats were anesthetized with thiopental and underwent surgery. Then, we induced ischemia with blocking blood vessels for 45 minutes by clamping. Biochemical parameters including urea and creatinine were measured using commercial kits. Oxidative stress and inflammatory parameters were measured by ELISA method. Renal tissues were stained with hematoxylin and eosin. Finally, the Kolmogorov-Smirnov test was used to determine the normal distribution of data.
Results: The findings of this study indicated that treatment with valsartan and valsartan plus L-arginine leads to significant decrease in the serum levels of creatinine, urea, and albumin/creatinine, malondialdehyde (MDA), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in contrast to IR control group which has increased level of these parameters. On the other hand, treatment with valsartan and valsartan plus L-arginine lead to increase in the serum levels of glutathione peroxidase (GPX), in contrast to ischemia reperfusion control group.
Conclusion: Our data revealed that valsartan as a type I angiotensin receptor antagonist could decrease oxidative stress and inflammation due to renal ischemia reperfusion injury. Hence, valsartan could propose as a therapeutic agent for kidney diseases such as renal ischemia-reperfusion injury regarded to these renoprotective effects.

Introduction

The kidneys play a key role in the filtration, metabolism and elimination of xenobiotics or their metabolic products (1). Renal ischemia reperfusion injury is one of the main causes of acute renal failure, which is associated with high mortality. It is also one of the reasons for delayed kidney transplantation function and the increase of acute or chronic renal transplant rejection in kidney transplantation (2). Renal ischemia reperfusion injury not only is an inevitable result of renal transplantation, but also is observable in other conditions such

Key point

In an experimental study we found that valsartan as a type I angiotensin receptor antagonist drugs could decrease oxidative stress and inflammation due to renal ischemia-reperfusion injury. Hence, valsartan could be proposed as a therapeutic agent for kidney diseases such as renal ischemia-reperfusion injury regarded to these renoprotective effects.

as myocardial infarction, shock and systemic blood pressure reduction (3). During ischemia-reperfusion, the morphological and functional damage induced during the

ischemic period is exacerbated during reperfusion. Tissue damage caused by ischemia-reperfusion occurs due to the release of oxygen free radicals, imbalance in intracellular and mitochondrial calcium regulation, microscopic vascular dysfunction, and complete failure to return blood flow to microscopic vessels, which intensify inflammatory response with immune cell infiltration (4,5). Reactive oxygen species are produced at high concentrations after the re-establishment of blood flow in ischemic tissues (6). In normal conditions, the antioxidant enzymes neutralize the effects of free oxygen radicals in the cells. However, during reperfusion, the protective ability of these enzymes is reduced due to the rapid production of free oxygen species, resulting in apoptosis and cell death. Therefore, the reduction of oxidative stress with pharmacological approaches is a desirable goal for treatment to slow down the damage of ischemia-reperfusion.

Type I angiotensin receptor antagonists such as losartan and valsartan are used to treat high blood pressure and control congestive heart failure (7).

Objectives
Various studies have shown that angiotensin receptor antagonists have protective effects in some tissues following various injuries such as renal injuries (8). As it has been shown, angiotensin receptor antagonists can be useful in preventing lesions after cardiac and brain strokes (9). Additionally, the drugs present in this group can show anti-platelet, anti-diabetic, vascular antiplatelet, antihypertensive effects, and also atrial antifibrillatory (10). Also, in patients with chronic kidney disease and hypertension, after the administration of these drugs, the severity of renal impairment has been reduced (11). Hence, we decided to evaluate the protective effect of valsartan against renal ischemia reperfusion via antioxidant and anti-inflammatory property and NO signaling pathway.

Materials and Methods
Animals and study design
Fifty male Wistar rats (220±10 g) in the faculty of pharmacy, Lorestan Medical Science University were examined in this study. All animals were housed in normal laboratory condition (temperature: 21-25°C and light cycle: 12 h dark- 12 h light).
Then, all rats randomly assigned into five groups;
Group 1; normal rats (control group).
Group 2; ischemic/ reperfusion (IR) Rats
Group 3; IR rats + valsartan (30 mg/kg, gavage)
Group 4; IR rats + valsartan (30 mg/kg, gavage) + L-NAME (40 mg/kg, intraperitoneal; IP).
Group 5; IR rats + valsartan (30 mg/kg, gavage) + L-arginine (40 mg/kg, IP).

Surgical procedure
To induce of ischemia-reperfusion, a single injection of thiopental (60 mg/kg, IP) was administered. After

anesthesia, the kidney was exposed. Next, we carefully clamping around blood vessels for 45 minutes. After 45 minutes, for reperfusion removed the clamps around blood vessels. Animals killed 3 days after the operation.

Biochemical and biomarker parameters measurement
In the last days, blood samples were collected and it centrifuged at 2500 rpm for 20 minutes to separation of serum. The serum was used for assessing the levels of urea, creatinine, malondialdehyde (MDA), glutathione peroxidase (GPx), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) by an auto-analyzer (12).

Renal histologic analysis
In the last day, the right kidney was exposed and separated. The specimen was stained with hematoxylin and eosin and was scored the tubular necrosis and eosinophilic casts (13).

Ethical issues
The research and the protocol of this study was in accordance with the guidelines of animal studies and was approved by Ethics Committee of Lorestan University of Medical (IR.LUMS.REC.1397.094). Accordingly, we tried to conduct the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978).

Statistical analysis
Data analysis was done using GraphPad Prism version 5.0 software. One-way ANOVA analysis followed by post hoc Dunnett test was used. The results presented by mean ± SD and the significance level will be below 0.05 ($P<0.05$).

Results
Effect of valsartan on serum level of creatinine
An increased level of creatinine was observed in the IR control group in comparison to the healthy control group. However, valsartan and valsartan plus L-arginine treatment dropped the level of creatinine compared to the other groups. Figure 1 shows the creatinine level in different groups ($P<0.05$).

Effect of valsartan on level of albumin/creatinine
As shown in Figure 2, an significant elevation in the level of albumin/creatinine in the IR control group compared to the control group was observed. On the other hand, the levels of albumin/creatinine significantly reduced in treated groups especially in valsartan and valsartan plus L-arginine compared to untreated groups and IR control group ($P<0.05$).

Effect of valsartan on serum level of urea
According to Figure 3, the level of urea increased in the IR control group more than the control group. Moreover, it depressed significantly in valsartan and valsartan plus L-arginine treated groups ($P<0.05$).
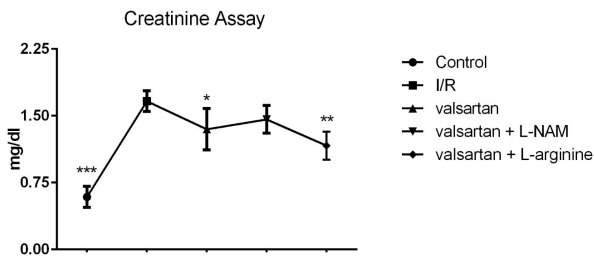
Creatinine Assay

Figure 1. The creatinine level in different groups.

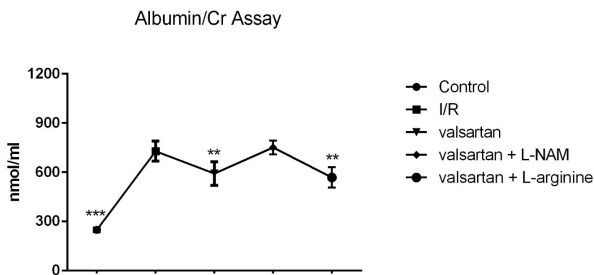## Albumin/Cr Assay

Figure 2. The level of albumin/creatinine in different groups.
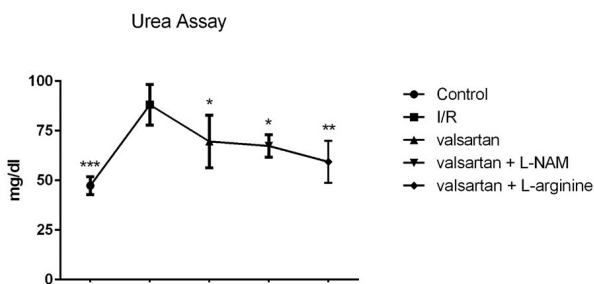
Urea Assay

Figure 3. The level of urea in different groups.

Effect of valsartan on serum level of MDA

According to Figure 4, the serum level of MDA elevated significantly in the IR control group compared to the control group. However, it depressed significantly in valsartan and valsartan plus L-arginine treated groups compared to other groups.

Effect of valsartan on serum level of GPX

The level of GPX reduced in the IR group in comparison with the control group. Moreover, valsartan and valsartan plus L-arginine caused elevation in the level of GPX, as shown in Figure 5.

Effect of valsartan on serum level of TNF-alpha

The level of TNF-alpha increased in the IR group in comparison with the control group. Moreover, valsartan and valsartan plus L-arginine caused reduction in the level of TNF-alpha, as shown in Figure 6.

Effect of valsartan on serum level of IL-6

The level of IL-6 increased in the IR group in comparison

to the control group. Moreover, valsartan and valsartan plus L-arginine caused reduction in the level of IL-6, as shown in Figure 7.

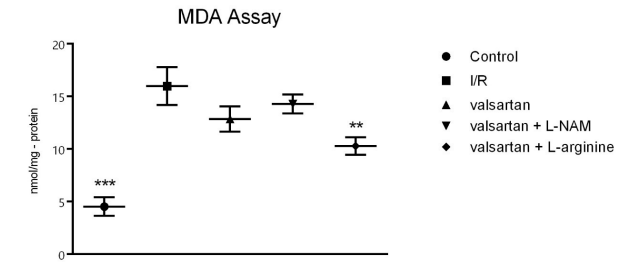## Discussion

Renal ischemia reperfusion injury is a pathologic

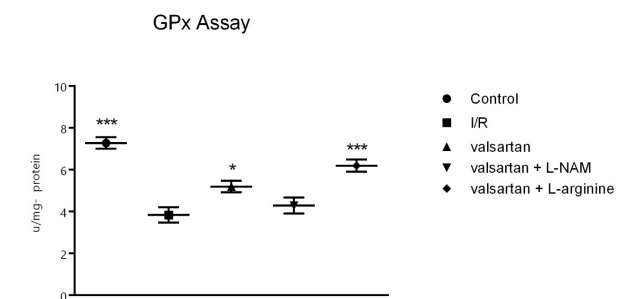## MDA Assay

Figure 4. The level of MDA in different groups.

GPx Assay

Figure 5. The level of GPx in different groups.
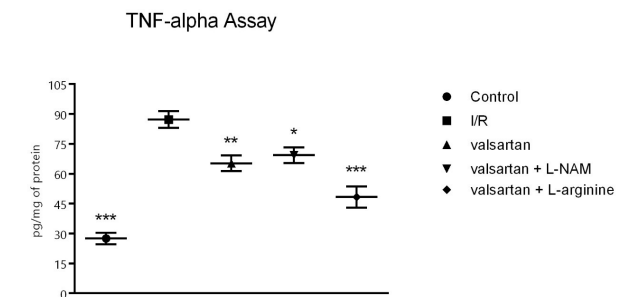
TNF-alpha Assay

Figure 6. The level of TNF-alpha in different groups.
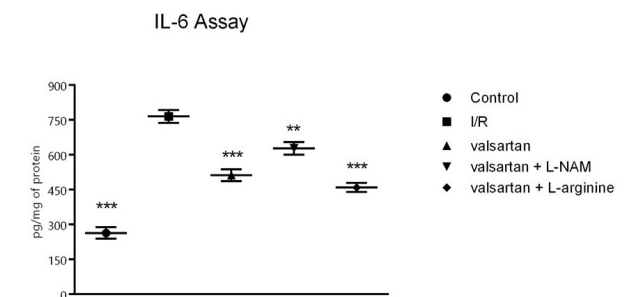
IL-6 Assay

Figure 7. The level of IL-6 in different groups.

condition which causes disruption of blood supply in kidneys. Stopping the blood supply to the kidney is called ischemic condition (5). This condition and subsequent reestablishment of blood flow leads to production of reactive oxygen species and activation of inflammatory mediators which causes damage to kidneys. Therefore, the administration of several drug compounds can involve in reduction of the oxidative stress and inflammation caused by renal ischemia reperfusion injury (14). In this study, we evaluated the effects of valsartan as an antihypertension drug and renin inhibitor in the treatment of renal ischemia reperfusion injury. The results of our study showed that the serum level of creatinine, urea, MDA, albumin/creatinine, TNF-alpha and IL-6 decreased significantly after valsartan and valsartan plus L-arginine treatment compared to IR control group. Vice versa, the serum level of GPX increased significantly after valsartan and valsartan plus L-arginine treatment compared to IR control group. We also evaluated the oxidative stress parameters including the serum levels of MDA and GPX in different groups. Our results indicated that the serum levels of MDA decreased significantly after treatment with valsartan. On the other hand, treatment with valsartan caused a significant increase in the serum levels of GPX.

The serum levels of creatinine, urea and albumin/creatinine decreased significantly after valsartan and valsartan plus L-arginine treatment compared to IR control group. Effects of valsartan have been evaluated in patients with chronic kidney disease. The results of their study showed that the serum level of creatinine in patients decreased significantly after treatment with valsartan (15). A recent study evaluated the effect of valsartan on chronic kidney disease in patients. The authors of this study concluded that pretreatment with valsartan decreased serum levels of blood urea nitrogen and creatinine 90 days after treatment, however, it was not significant (16). The effect of valsartan was also evaluated on serum levels of urea and creatinine in doxorubicin-induced renal toxicity in rats. Their results indicated that serum levels of urea and creatinine elevated significantly following doxorubicin treatment. Vice versa, treatment of rats with valsartan (10, 20 mg/kg) especially 20 mg/kg leads to significant depression in the serum levels of urea and creatinine (17).

The anti-inflammatory effects of valsartan were also assessed using measuring the serum levels of TNF-alpha and IL-6 in different groups. Our results showed that an increase in inflammatory mediators such as TNF-alpha and IL-6 was induced after the development of renal ischemia reperfusion injury. However, valsartan treatment could affect inflammation caused by renal ischemia-reperfusion injury through reducing the serum levels of TNF-alpha and IL-6. Wang et al, in a study on the ameliorative impact of valsartan on podocyte damage in rats with diabetic kidney disease, showed anti-inflammatory effects of valsartan in diabetic nephropathy. The results of their study indicated that the concentrations of inflammatory mediators such as IL-β, TNF-α and IL-6 increased significantly in the diabetic nephropathy group. However, treatment by valsartan could decrease significantly the levels of IL-β, TNF-α and IL-6 (18).

Conclusion

In conclusion, different natural compounds and chemical drugs are used for protection of kidneys against various types of renal injuries such as renal ischemia-reperfusion injury. Our data revealed that valsartan as a type I angiotensin receptor antagonist drugs could decrease oxidative stress and inflammation due to renal ischemia-reperfusion injury. Hence, valsartan could propose as a therapeutic agent for kidney diseases such as renal ischemia-reperfusion injury regarded to these renoprotective effects.

Authors' contribution
LM, SSA, SK, SSA, SP and BH conducted the research. ARD, AH and BF designed and supervised the study, prepared the final draft of the article. AH and BH analyzed the data and pathology.

Conflicts of interest
The authors declare that they have no competing interest.

Ethical considerations
Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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