



Protective effect of aliskiren against renal ischemia reperfusion via antioxidant property and nitric oxide signaling pathway

Leila Mohmoodnia¹, Sahar Koushki², Michele Moruzzi³, Sajad Papi⁴, Reza Mohammadrezaei Khorramabadi², Behrooz Farzan⁵, Babak Hadian⁶, Amin Hasanvand^{7*}

¹Cancer Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

²Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran

³Medical Research Center, Universitätsklinikum of Leipzig, Leipzig (GE), Germany

⁴Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran

⁵Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

⁶Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

⁷Hepatitis Research Center, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran

*Correspondence to

Amin Hasanvand Ph.D. E-mail:
Dr.hasanvan@yahoo.com, Amin.
Hasanvand@lums.ac.ir

Received 13 December 2018

Accepted 18 March 2019

Published online 7 April 2019

Keywords: Aliskiren, Renal ischemia reperfusion, Antioxidant property, Kidney, Acute renal failure, Reactive oxygen species,

Abstract

Introduction: Renal ischemia reperfusion (RIR) is created following different mechanisms such as oxidative stress and inflammation.

Objectives: The roles of chemical drugs, including aliskiren, have been evaluated in various kidney diseases. Hence, we assessed the effect of aliskiren on renal ischemia reperfusion.

Materials and Methods: Fifty male Wistar rats (220±10 g) were grouped randomly in five groups; 1. Healthy control group, 2. Ischemia of reperfusion (IR) control group, 3. Rats with IR which received 30 mg/kg aliskiren orally, 4. Rats with IR which received 30 mg/kg aliskiren together with 40 mg/kg L-NAME, 5. Rats with IR which received 30 mg/kg aliskiren together with 40 mg/kg L-arginine. To induce ischemia-reperfusion, rats were anesthetized treated with thiopental and went under surgery. Then, we revealed the left and right kidneys, and we induced ischemia with blocking blood vessels for 45 minutes by clamping. Biochemical parameters including urea and creatinine were measured using commercial kits with auto analyzer. Oxidative stress and inflammatory parameters were evaluated using ELISA method. Renal tissues were stained with hematoxylin and eosin. Finally, Kolmogorov-Smirnov test was applied to determine the normal distribution of data.

Results: Our results showed that treatment with aliskiren and aliskiren plus L-arginine causes a significant decrease in the serum levels of creatinine, urea, albumin/creatinine and malondialdehyde (MDA), in contrast with IR control group which has increased level of these parameters. On the other hand, treatment with aliskiren and aliskiren plus L-arginine leads to increase in the serum levels of glutathione peroxidase (GPX) and superoxide dismutase (SOD) in contrast with IR control group.

Conclusion: The protective effect of aliskiren has been proven in different kidney diseases such as RIR and diabetic nephropathy. Our results demonstrated that aliskiren could be proposed as a therapeutic agent against renal ischemia complications.



Citation: Mohmoodnia L, Koushki S, Moruzzi M, Papi S, Mohammadrezaei Khorramabadi R, Farzan B. Protective effect of aliskiren against renal ischemia reperfusion via antioxidant property and nitric oxide signaling pathway. Immunopathol Persa. 2019;5(1):e10. DOI:10.15171/ipp.2019.10.

Introduction

Ischemia of reperfusion (IR), is the reintroduction of blood flow into an ischemic or oxygen-deficient tissue. The absence of oxygen and nutrients in the blood during the ischemic period and the return of blood circulation, induce oxidative stress that leads to inflammation and damage to tissues such as the brain, heart, kidney, liver, lungs and skeletal tissue of the body (1, 2). Renal ischemia reperfusion (RIR) is one of the factors leading to acute renal failure. RIR is very common episode that happens during

the kidneys rejection and transplantation. The IR causes tissue damage, and when the ischemia occurs subsequently deficiency of oxygen and nutrients develops. Then, restoring the blood flow to the tissue develop inflammation, oxidative stress and apoptosis (3). As mentioned before, oxidative stress plays a crucial role in the development of RIR. In fact, the production of reactive oxygen species occurs in early moments of reperfusion. Several studies showed that reactive oxygen species is an important mediator for damages caused by RIR (4). It is

Key point

Ischemia of reperfusion (IR) is the reintroduction of blood flow into an ischemic or oxygen-deficient tissue. The absence of oxygen and nutrients in the blood during the ischemic period and the return of blood circulation induce oxidative stress that leads to inflammation and damage to tissues such as the brain, heart, kidney, liver, lungs and skeletal tissue of the body. In this study we decided to evaluate protective effect of Aliskiren against renal ischemia reperfusion via antioxidant property and nitric oxide signaling pathway.

also demonstrated that RIR could lead to decreased levels of antioxidant enzymes such as superoxide dismutase (SOD) (5). Studies have shown that the condition of IR generates inducible nitric oxide synthase (iNOS-synthase), which leads to increased synthesis of nitric oxide synthesis (NO-oxide nitric). Finally, it reacts with superoxide anions and damages the kidney tissue by forming proximity nitrite (6).

Various types of natural compounds and chemical drugs are used for the treatment of RIR (7). Aliskiren is recognized as an orally active, non-peptidic inhibitor of renin. Aliskiren is recognized as an orally active, non-peptidic inhibitor of renin. It has therapeutic application for the treatment of hypertension because it is only a direct renin inhibitor. Aliskiren acts as a renin inhibitor which blocks the renin system by inhibiting plasma renin activity (8,9).

Objectives

In this study we aimed to evaluate the protective effect of aliskiren against RIR via antioxidant property and NO signaling pathway.

Materials and Methods**Chemicals**

Aliskiren, ELISA kits, hematoxylin and eosin (H&E) stain.

Animals and study design

Fifty male Wistar rats (220±10 g) were used in this study. The rats were kept in normal laboratory condition and were adapted to the laboratory situations such as limitless access to food and water, under control temperature (21-25°C) and appropriate light cycle (12 h dark- 12 h light).

Then, they were classified randomly as follow;

Group 1: Healthy rats without ischemia-reperfusion (control group).

Group 2: Rats with IR (IR control group).

Group 3: Rats with IR which received 30 mg/kg aliskiren orally.

Group 4: Rats with IR which received 30 mg/kg aliskiren together with 40 mg/kg L-NAME.

Group 5: Rats with IR which received 30 mg/kg aliskiren together with 40 mg/kg L-arginine.

Surgical procedure

Prescribing drugs in the groups was done from the three

days before the induction of ischemia-reperfusion to three days later. To induce ischemia-reperfusion, rats were anesthetized and treated with thiopental before surgery. Then, we revealed the left and right kidneys, and we induced ischemia with blocking blood vessels for 45 minutes by clamping. After 45 minutes, we removed the clamps and performed reperfusion. 72 hours after reperfusion, we killed animals and collected the samples.

Biochemical parameters measurement

Biochemical parameters including urea and creatinine were measured using commercial kits with auto-analyzer.

Oxidative stress parameters measurement

Blood samples were collected from the heart of the animal and after centrifugation; the supernatant was isolated and used to evaluate the parameters. Oxidative stress was evaluated by the concentration of malondialdehyde (MDA), glutathione peroxidase (GPX), and SOD. The MDA will be evaluated using the thiobarbituric acid reaction assay by measuring absorbance at 532 nm. The activity of GPX, and SOD were measured by using commercial kits (10,11).

Renal histologic analysis

For renal histologic analysis, 24 hours after reperfusion, the right kidney was removed and was cut with microtome and stained with H&E, for see the tubular necrosis and eosinophilic casts (12).

Ethical issues

The study was also approved by Ethics Committee of Lorestan University of Medical Sciences (#IR.LUMS.REC.1397.094). Prior to the trial, the protocols like animal care, nutrition and prescribing method, anesthesia and euthanasia procedures were approved to be in agreement with the rules of the ethical committee of this university.

Statistical analysis

For analyzed the data, One-way analysis of variance (ANOVA) analysis followed by post hoc Dunnett test was used by GraphPad Prism Version 5.0. The results are shown as Mean ± SD (significance level: $P < 0.05$).

Results**Effect of aliskiren on the serum level of creatinine**

The results showed that creatinine level significantly increased in the IR control group in comparison with the healthy control group. However, it depressed significantly in aliskiren and aliskiren plus L-arginine treated groups compared to the other groups. Figure1 shows the creatinine level in different groups ($P < 0.05$).

Effect of aliskiren on level of albumin/creatinine

The level of albumin/creatinine has been indicated in Figure 2. This figure demonstrated that level of albumin/

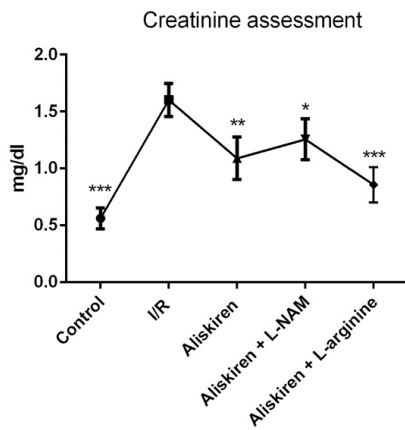


Figure 1. Creatinine level in different groups.

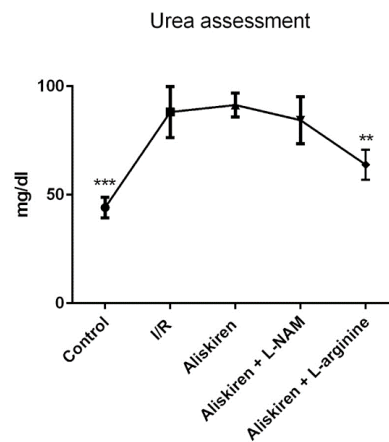


Figure 3. Level of urea in different groups.

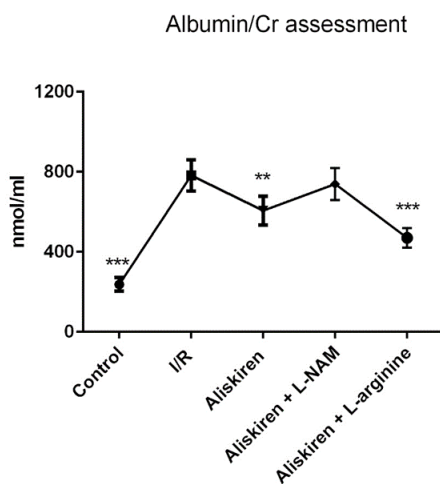


Figure 2. Level of albumin/creatinine in different groups.

creatinine is higher in the IR control group compared to the control group. Moreover, the levels of albumin/creatinine significantly reduced in treated groups especially in aliskiren and aliskiren plus L-arginine compared to untreated groups and IR control group ($P < 0.05$).

Effect of aliskiren on the serum level of urea

The IR control group has a urea level more than the control group. On the other hand, it depressed significantly in aliskiren and aliskiren plus L-arginine treated groups as shown in Figure 3 ($P < 0.05$).

Effect of aliskiren on the serum level of MDA

As shown in Figure 4, the serum level of MDA increased significantly in IR control group compared to the control group. However, it decreased significantly in aliskiren and aliskiren plus L-arginine treated groups compared to other groups.

Effect of aliskiren on the serum level of GPX

The serum level of GPX decreased in the IR group in comparison with the healthy control group. On the other

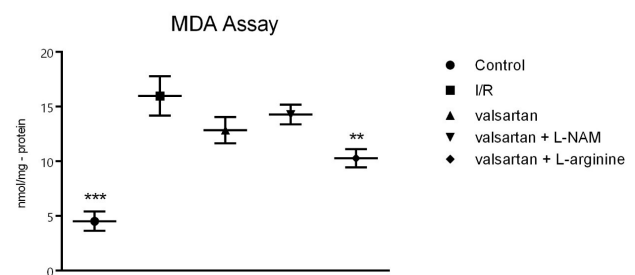


Figure 4. Level of MDA in different groups.

hand, aliskiren and aliskiren plus L-arginine caused increase in the level of GPX, as shown in Figure 5.

Effect of aliskiren on the serum level of SOD

The level of SOD in serum decreased in the IR control group in comparison with the healthy control group. Otherwise, aliskiren and aliskiren plus L-arginine treatment caused increase in the level of SOD, as shown in Figure 6.

Discussion

Ischemia is one of the common causes of acute renal failure, which results from a severe reduction or loss in blood flow to the kidneys. RIR lesions create and develop due to oxidative stress and inflammation (13). Therefore, the use of some drug compounds can play an important role in reducing the oxidative stress and inflammation caused by RIR (14). We evaluate the effects of aliskiren as an antihypertension drug and renin inhibitor in the treatment of RIR. Researchers have indicated that angiotensin II (Ang II) has a vasoconstrictive effect and intensifies the ischemia of the kidney which ultimately leads to oxidative stress and inflammation (15). Several studies have proven that angiotensin converting enzyme inhibitors (ACE inhibitors) such as aliskiren could attenuate IR injury (16, 17). Our study showed that the serum level of creatinine, urea, MDA, albumin/creatinine decreased significantly after aliskiren and aliskiren plus L-arginine treatment

GPx assessment

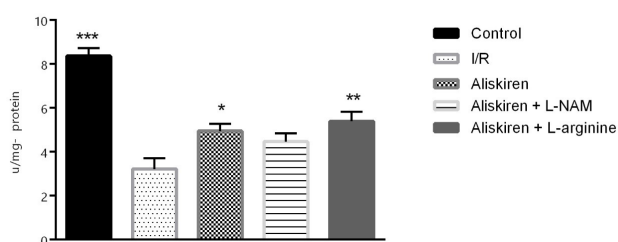


Figure 5. Level of GPx in different groups.

SOD assessment

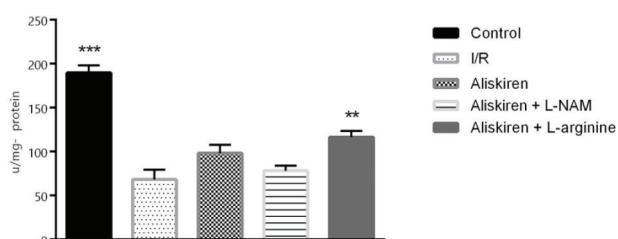


Figure 6. Level of SOD in different groups.

compared to IR control group. Otherwise, the serum level of GPx and SOD increased significantly after aliskiren and aliskiren plus L-arginine treatment compared to IR control group.

A part of our research demonstrated that level of albumin/creatinine elevated significantly in the IR control group compared to the control group. Moreover, the levels of albumin/creatinine significantly reduced in treated groups especially in aliskiren and aliskiren plus L-arginine compared to untreated groups and IR control group. The effect of aliskiren administration had been evaluated in type 2 diabetes and nephropathy. The results indicated that daily administration of 300 mg of aliskiren decreased the mean urinary albumin/creatinine ratio by 20% in patients with type 2 diabetes and nephropathy who treated with aliskiren compared to patients which received placebo. Moreover, albuminuria and proteinuria reduced after treatment with aliskiren for 50% (18). Another similar study which assessed renoprotective effect of aliskiren in patients with type 2 diabetes showed that urinary albumin/creatinine decreased after treatment with aliskiren in comparison with placebo (14%) (19).

Our results concluded that administration of aliskiren leads to protection of kidneys via significantly decreased in renal function test such as urea and creatinine level. Another related study showed that aliskiren pretreatment (3 mg/kg) could significantly reduce the serum levels of urea and creatinine as compared with the IR control group (16). It is also demonstrated that aliskiren has anti-diabetic and renoprotective effects against diabetic nephropathy in rats. These results showed that the serum levels of

kidney function tests significantly decreased for urea and creatinine; however, albumin significantly increased after aliskiren treatment such that the serum levels of urea and creatinine had 74.2% and 38.3% decrease after treatment, respectively. On the other hand, albumin had an increase after aliskiren treatment (20). The role of aliskiren has been established in lowering of albuminuria. The results of a related study indicated that aliskiren at a dose of 10 or 30 mg/kg/d for 10 weeks plays a role in the reduction of albuminuria in diabetic rats (21).

After that, oxidative stress was examined in the kidneys underwent IR. Our data demonstrated that the rats of IR control group had higher level of MDA and lower level of GPx and SOD compared to the healthy control group. On the other hand, aliskiren treatment significantly reversed the levels of these parameters. Wang et al, in a similar study to our study assessed the protective effects of aliskiren on ischemia–reperfusion-induced renal injury in rats. Their results demonstrated that aliskiren treatment decreased the level of MDA and increased SOD levels which is consistent with the results of our study (16). The findings of another related work which evaluated the effect of aliskiren against diabetic nephropathy in rats showed a significant decreased level of MDA and increased SOD levels for after aliskiren treatment (20). Our results highlighted that the kidney of the IR rats has lower levels of SOD and have a higher level of MDA, as compared with the sham control rats. Pretreatment with aliskiren reversed these changes to some degree. Oxidative stress has been proven to play a key role in IR injury in many studies.

Conclusion

In conclusion, different compounds such as natural plant compounds and also synthetic drugs could use for protection against renal ischemia reperfusion. aliskiren is one of these drugs which its renoprotective effect has been proven against renal ischemia reperfusion in different studies. It could act renoprotective effects through antioxidant and anti-inflammatory properties.

Acknowledgments

The authors would like to thanks to the Lorestan University of Medical Sciences, Khorramabad, Iran, because of their financial support. The authors also thank the head and staff of the Razi Herbal Medicines Research Center of Lorestan University of Medical Sciences.

Authors' contribution

LM, SK, SP and RMK conducted the research. AH, MM, BF and BH designed and supervised the study, prepared the final draft of the article. BH analyzed the data and pathology. AH and LM, finalized the manuscript. All authors read and signed the final paper.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double

publication) have been completely observed by the authors.

Funding/Support

This study was supported by Lorestan University of Medical Sciences with (Grant #A-10-1758-7).

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