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Original Article

Effects of alliin on renal ischemia reperfusion induced lung injury

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Abstract

Aim: Renal ischemia reperfusion (I/R) injury is a problematic process which leads to renal dysfunction and may be mortal for the patients in intensive care units. Increased oxidant molecules and decreased antioxidant molecules were detected in renal I/R-induced lung injury. In the present study, it was aimed to investigate the effect of Alliin on oxidative stress in renal I/R-induced lung injury.
Material and Methods: A total of 32 Wistar Albino male rats were randomly divided into 4 groups (n = 8): Sham, I/R, I/R+Alliin 100 mg/kg and I/R+Alliin 200 mg/kg group. The effects of Alliin on oxidant and antioxidant molecules were evaluated via biochemical methods.
Results: Alliin treatment increased antioxidant parameters and decreased oxidant parameters.
Conclusion: Alliin has been shown to reduce oxidative stress and renal I/R-induced lung injury.

Keywords: Alliin; kidney ischemia reperfusion; lung; oxidative stress

INTRODUCTION

Acute kidney injury (AKI) is a common complication in hospitalized patients. It often leads to chronic kidney disease together with multiple organ failure and increases the rate of deaths associated with AKI (1,2). AKI occurs in many clinical conditions such as renal ischemia, renal transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, cardiopulmonary by-pass and elective urological operations (3). Providing reperfusion after prolonged ischemia activates vascular endothelial cells and increases the formation of reactive oxygen species (ROS) (4,5). AKI-associated acute lung injury (ALI) is clinically characterized by increased pulmonary edema similar to acute lung injury (6). ALI has traditionally been characterized by the accumulation of inflammatory cells both in alveolar and capillary endothelium and the loss of respiratory barrier functions (7). AKI often leads to ALI in critically ill individuals. Mortality rate of combined AKI and ALI may increase up to 80% (8). Medical treatments that reduce kidney and lung ischemia reperfusion (I/R) injury may be useful in reducing morbidity and mortality rates (9). It was shown that oxidant molecules increased and antioxidant molecules decreased in renal I/R induced lung injuries (10,11).

Recent studies confirmed the beneficial role of plant-derived natural products since they have anti-inflammatory effects and have potential to remove free radicals (12,13). Alliin (S-allyl cysteine sulfoxide, C 6 H 11 NO 3 S) is an organosulfide compound derived from garlic (14). Previous studies have shown that Alliin has significant antioxidant effects and plays role in inflammation suppression (15; 16). Alliin can also function as an important inhibitor by controlling ROS formation and inhibiting mitogen-activated protein kinase (MAP kinase) in some oxidant stress injuries (17). In this study, we evaluated whether Alliin has a protective effect in lung injury induced by renal I/R model.

MATERIAL and METHODS

Ethics and Experimental Design

Thirty-two Wistar Albino male rats weighing 220-250 gr were used in the study. Legal permission was obtained from the Animal Experiments Ethics Committee of our University for the experimental protocols (Meeting Date: 28.03.2019 Meeting Number:4 Decision No:74). All the procedures in the study were performed in line with the ethics committee protocol. Wistar albino rats obtained from our University Experimental Animals Research and Application Center. During the course of the experiment,

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rats were conserved in 12 h light / 12 h dark cycle at 20-22 °C, and the ad libitum feeding of standard chow and normal tap water was allowed in rats. 4 groups were formed. Each group consisted of 8 animals:

Group 1 (Sham)
Animals were surgically opened in the dorsal region and closed without any intervention.

Group 2 (I/R)
The dorsal areas of the animals were shaved, disinfected and animals were anaesthetized with a mixture containing ketamine (75mg/kg) and xylazine (8mg/kg). Both kidneys were palpated and surgical intervention was performed. Renal pedicles of kidneys were clamped via microvascular clamp to induce ischemia. After one hour, the clamps were removed and reperfusion was achieved. Animals were decapitated after 24 hours.

Group 3 (I/R+100 mg/kg Alliin)
Group 2 procedures were performed and 100 mg/kg Alliin was administered intraperitoneally 5 minutes before reperfusion.

Group 4 (I/R+200 mg/kg Alliin)
Group 2 procedures were performed and 200 mg/kg Alliin was administered intraperitoneally 5 minutes before reperfusion.

At the end of the study, animals were decapitated under anesthesia and lung tissues were collected. Organs were washed with isotonic saline solution, labeled and stored at -80 °C for further analysis.

Biochemical analyses
100 mg sample was taken from each group and mechanically homogenized by adding 0.9 ml phosphate buffer (pH: 7.40). The homogenized tissues were centrifuged at +4^{0}C for 5 minutes at 3000 rpm. Supernatant was analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), myeloperoxidase (MPO), total antioxidant status (TAS) and total oxidant status (TOS).

MDA levels, which are lipid peroxidation index, were measured according to the method of Ohkawa et al. (18). Absorbance was measured at 532 nm and the results were expressed as nmol/mg protein. MPO activity was measured according to Bradley technique (19). Kinetic readings were performed at 460 nm for 5 minutes in ELISA microplate reader. The results are given as unit/g protein. SOD measurement was performed by the method of Sun et al. (20) and measured at 550 nm. SOD activity was expressed as unit/mg protein.

TAS and TOS were measured using commercial kits (Rel Assay Diagnostics) with microplate reader, and TOS/TAS ratio was accepted as oxidative stress index (OSI).The OSI level was measured as: OSI = [(TOS, µmol/L) / (TAS, mmol/L)×10] (21).

Statistical analysis
SPSS 20 (SPSS Corporation, Chicago, IL, USA) statistical program was used for data analysis, results were expressed as mean±standard deviation (SD) and $p<0.05$ was considered statistically significant. One-way ANOVA was used for statistical analysis and Tukey post hoc test was used to determine the difference between the groups.

RESULTS

TAS level decreased in I/R group compared to sham group and increased in I/R+100 mg/kg- 200 mg/kg Alliin groups ($p<0.001$) compared to I/R group. TOS level increased in I/R treated group compared to sham group and decreased in I/R+100 mg/kg- 200 mg/kg Alliin groups compared to I/R group ($p<0.001$) (Table 1).

MDA and MPO activities increased in I/R group sham group and decreased in I/R+100 mg/kg-200mg/kg Alliin groups compared to I/R ($p<0.001$) (Figures 1 and 2, respectively). SOD levels decreased in I/R group compared to control group and increased in I/R+100 mg/kg-200mg/kg Alliin groups compared to I/R group ($p<0.001$) (Figure 3).

Table 1. TAS, TOS and OSI levels of the groups

Groups / Parameters	TAS (µmol/L)	TOS (µmol/L)	OSI
Sham	1.72 ± 0.17	8.59 ± 0.72	0.50 ± 0.07
I/R	0.77 ± 0.11*	15.77 ± 1.48*	2.09 ± 0.40*
I/R+100 mg/kg Alliin	1.35 ± 0.11#	9.29 ± 1.60#	0.73 ± 0.08#
I/R+200 mg/kg Alliin	1.69 ± 0.10#	9.03 ± 1.31#	0.53 ± 0.09#

* p<0.001 compared to Sham group, # p<0.001 compared to I/R group. Data were presented as mean±SD (n = 8)

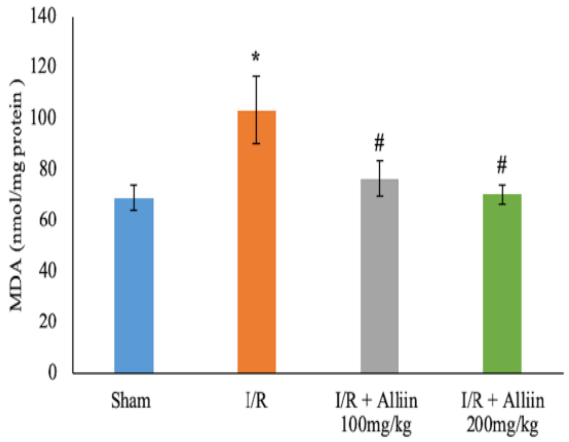

Figure 1. The effect of Alliin on MDA levels in renal I/R-induced lung injury model. *p<0.001 compared to sham group, #p<0.001 compared to I/R group. Data were presented as mean±SD (n = 8)

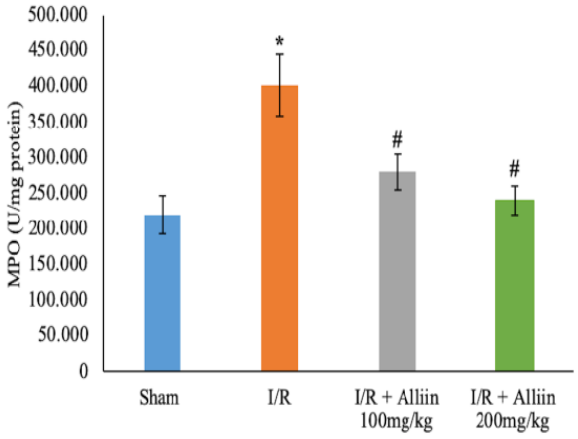

Figure 2. The effect of Alliin on MPO levels in renal I/R-induced lung injury model. *p<0.001 compared to sham group, #p<0.001 compared to I/R group. Data were presented as mean±SD (n = 8)

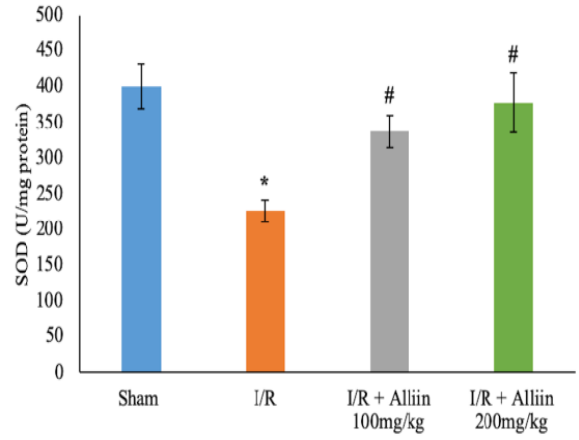

Figure 3. The effect of Alliin on SOD levels in renal I/R-induced lung injury model. *p<0.001 compared to sham group, #p<0.001 compared to I/R group. Data were presented as mean±SD (n = 8)

DISCUSSION

Epidemiological studies have demonstrated a relationship between AKI and dysfunction of extrarenal organs such as the lung (22,23). AKI related ALI is characterized by increased pulmonary edema (6). ALI has traditionally been characterized by loss of respiratory membrane barrier functions both in alveolar and capillary endothelium (7). Reperfusion injury may lead to mechanical ventilation need and therefore lung injury affects post-operative morbidity and mortality rate of patients (9). During pulmonary I/R, cellular apoptosis is induced by various factors such as ROS and it plays a key role in pulmonary insufficiency (24). Increased ROS contributes to the activation of inflammatory pathways and deterioration of organ function. Therefore, ROS can be considered as signaling molecules that trigger several important mechanisms of reperfusion injury. In addition, ROS produced by neutrophils increases tissue damage through proteases (25,26). Renal I/R also cause tubular damage in kidneys and alveolar damage in lungs as remote organ damage (27).

ROS-mediated cellular damage occurs when the oxygen exceeds the cellular detoxification capacity. Endogenous antioxidant enzymes such as SOD protect cells from the harmful effects of ROS. The activity of these enzymes indicates the magnitude of the oxidative stress that occurs during I/R damage. ROS and ROS-mediated lipid peroxidation are highly effective in the pathogenesis and complications of I/R (28). As in present study, I/R related low SOD levels increased with Alliin treatment and this data is consistent with the literature. Oral treatment of Alliin increased the activities of various antioxidant molecules, including SOD, in isoproterenol-induced myocardial injury in rats (29). Similar findings were reported in another vitro experimental model (30).

In the present study MDA, the end product of lipid peroxidation, increased in I/R group compared to sham group and damaged the membrane lipids. This observation is consistent with previous studies in which lipid peroxide levels were increased (10,31). It has been shown that serum MDA levels were decreased in volunteers who use Alliin in tablet form (32). It has been reported that garlic organosulfide compounds can reduce both inflammation and MDA levels that occur during dengue viral infection of human cells (33).

MPO content reflects neutrophil accumulation and activity (34), because MPO is almost found in neutrophils (35). Alliin decreased MPO levels as dose-dependent in lipopolysaccharide-induced lung injury (36). Alliin reduced MPO and MDA activity in mice with dextran sulfate sodium-induced colitis and lipopolysaccharide-induced RAW264.7 cell model (37). In current study, increased MPO levels due to I/R reduced with Alliin treatment.

TAS and TOS reflect the redox balance between oxidation and antioxidation. TAS measurement is an indicator for the activity of all antioxidants and TOS is a ROS indicator (38,39). Oxidative stress is the imbalance between oxidant and antioxidant mechanisms which goes in oxidant's favor. OSI is the ratio of TOS to TAS and is an indicator of the degree of oxidative stress (38,39). Alliin performed protective antioxidant activities against free radical damage in several experimental studies (29,40-42). In present study, I/R increased OSI, TOS values and decreased TAS level but Alliin reversed all. These results were consistent with the literature data.

CONCLUSION

In this study, Alliin performed protective effects against renal I/R-induced lung injury with antioxidant features. Treatment with different doses of Alliin reduced lung injury in experimental animals with renal I/R. However, some histopathological studies should be done and signaling pathways should be investigated in order to have a deeper understanding of effects of Alliin.

Competing interests: The authors declare that they have no competing interest.

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