

N-acetylcysteine Prevents Kidney and Lung Disturbances in Renal Ischemia/Reperfusion Injury in Rat

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ABSTRACT

Background: One of the most common causes of acute kidney injury (AKI) is kidney ischemia/reperfusion injury (IRI). The distant organ injury such as acute lung injury is one of the side effects of AKI or kidney IRI. In this study, we performed bilateral renal IRI in rats and the protective role of N-acetylcysteine (NAC) in kidney and lung was investigated.

Methods: Rats ($n = 30$) were randomly assigned to four experiment groups. The group 1 was assigned as sham-operated group. Before kidney IRI performance, the others groups were treated with saline (group 2), 150 mg/kg (group 3) or 500 mg/kg (group 4) of NAC, and the treatment were continued daily after IRI for next 3 days. At day 3, the all groups' animals were subjected for the measurements.

Results: The serum level of blood urea nitrogen (BUN) and creatinine (Cr) in the control group increased significantly ($P < 0.05$), and administration of NAC (150 mg/kg) decreased the serum levels of Cr and BUN. However, only the serum level of Cr decreased significantly ($P < 0.05$). NAC did not improve kidney weight and damage; however, its low dose (150 mg/kg) attenuated the lung injury score ($P < 0.05$) when compared with the control group. No significant differences were observed in lung water content and endothelial permeability, serum levels of malondialdehyde and nitrite between the groups.

Conclusions: Low dose of NAC as a protectant agent may protect the kidney function and lung tissue damage after kidney IRI.

Keywords: Kidney, lung endothelial permeability, lung water content, NAC, rat, renal IRI

INTRODUCTION

Epidemiologic studies have shown that the mortality rate of combined acute kidney injury (AKI) and acute lung injury (ALI) is about 80%.^[1-5] It is also reported that 5-6% of intensive care unit patients suffer from AKI, which may require dialysis.^[4-6] One of most common causes of AKI is kidney ischemia/reperfusion

injury (IRI). Kidney IRI occurs in various clinical settings, including shock, sepsis, kidney transplantation, partial nephrectomy, vascular surgery, and elective urologic operations.^[7,8] The kidney IRI may disturb tubular structure and kidney functions including eliciting epithelial cell necrosis, interstitial inflammation, and interstitial microvasculopathy.^[9,10] Due to these malfunctions and disturbances in the kidney, the patients' prognosis become worst after an AKI.^[9] Special attention is also needed to monitor distant organs during renal failure.^[1,3,5-7] The distant organ injury is one of the side effects of AKI or kidney IRI. The lung has a complex and large microcapillary network, which is affected by ischemic AKI.^[3] Accordingly, renal ischemia may induce ALI via loss of the normal balance of immune system, inflammatory mechanism, soluble mediator metabolism, apoptosis, water clearance, and oxidative stress.^[6,7,10,11] Experimental studies have demonstrated increasing of pulmonary vascular permeability, lung edema, focal alveolar hemorrhage, inflammatory cell infiltration, and abnormalities of salt and water transporters following ischemic AKI.^[2-6,11,12] These factors likely affect patients' survival.^[6] Therefore, in kidney IRI, monitoring of distant organs is extremely important to prevent complicated side effects of ischemia. Due to development of oxidant agents during kidney IRI, administration of an antioxidant after kidney IRI may protect not only the kidney but also the distant organ from the ischemia-induced damage.

N-acetylcysteine (NAC) is a drug with few side effects, which is commonly used in clinic.^[13,14] It is an antioxidant with low-molecular weight and has the thiol group.^[14-17] It is also a scavenger for free radicals such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}).^[13,16,18,19] In addition, NAC acts as a precursor for L-cysteine and reduced glutathione (GSH).^[14,17-20] The sulfhydryl-containing compounds, especially reduced GSH, are important in protection of cells against hydroperoxide damage. NAC causes vasodilatation particularly by enhancing the effects of nitric oxide (NO), inhibits platelet aggregation,^[17-19] and promotes cell growth and survival.^[16] NAC has been used in different clinical problems such as chronic bronchitis, liver toxicity after acetaminophen overdose, cancer, and cardiovascular diseases.^[15,17,18,20] Its protective role

was reported in lung injury induced by skeletal muscle and liver IR and lung IRI after lung transplantation.^[18,20] Due to its well-documented antioxidant effect, in this study, we attempt to investigate the protective role of NAC in lung and kidney after bilateral renal IRI. To achieve this purpose, two doses of NAC were administrated in rat IRI model and biochemistry markers, lung water content, and lung endothelial permeability were compared with the control and sham groups.

METHODS

Animals

Male Wistar rats weighting 180-220 g were housed at the room temperature of 23-25°C with a 12-h light/dark cycle and were allowed 1 week to acclimatize to the conditions. The rats were fed with rat chow and water *ad libitum*. The protocol of experiment was approved in advance by the Isfahan University of Medical Sciences Ethics Committee.

Drugs

The NAC; Flumil Antidote 20% from Pharmazam S. A. (Barcelona, Spain) and Evans Blue (EB) from Sigma (St. Louis Missouri, USA) were purchased.

Experimental protocol

Rats ($n = 30$) were randomly assigned to four groups of experiments; namely sham-operated (group 1, $n = 10$), control (group 2, $n = 7$), low-dose of NAC (group 3, $n = 8$), and high dose of NAC (group 4, $n = 5$). On the day of the experiment, the animals in groups 3 and 4 received a single dose of NAC [150 and 500 mg/kg, intraperitoneal (i.p)], and 2 h later they were anesthetized with the mixture of xylaxine (10 mg/kg, i.p) and ketamine (75 mg/kg, i.p). Incisions were made on skin and tissue of lumbar area and then the kidneys were carefully excised. Special care was paid to avoid damage to the organ. In order to achieve kidney IRI in animals, renal artery and vein were simultaneously occluded in both kidneys by placing a clamp on the vessels for 45 min. Then, the clamp was removed with care to make sure that blood flows into the kidneys. The animals without desirable restoration of blood flow or with vessel damage in this stage were excluded from the experiment. The same surgical procedure was

done on the animals in group 2, but they received saline instead of NAC. All surgical procedures except clamping the vessels were applied to the sham group. Furthermore, neither NAC nor saline was administered to the animals in this group.

After surgical procedures, the animals were kept in the animal room and observed for next 3 days. Each day after renal IRI, the animals in groups 2, 3, and 4 received their treatment (NAC or saline). The animal body weight (BW) was recorded on a daily basis. On day 3 and 1 h after last NAC or saline injection, the rats were anesthetized again. The tracheae were cannulated to facilitate ventilation, and catheters were implanted into the jugular vein and carotid artery. Blood sample was taken from carotid artery, and right kidney was removed, homogenized, and centrifuged at 6000 g for 10 min. The supernatant was removed and centrifuged again at 15000 g for 2 min for measuring selected biochemical parameters. Then, EB solution (10 mg/kg) was injected via the jugular vein, and the animals were sacrificed 1h later by lethal injection of intravenous potassium chloride (10% KCL). Lung and left kidney tissue samples were fixed in 10% formalin for pathological examinations. Two other samples from the lung were also immediately weighed for determination of water content and pulmonary endothelial permeability.

Measurements

Serum creatinine (Cr) and blood urea nitrogen (BUN) levels were determined using quantitative kits (Pars Azmoon, Iran). Levels of nitrite (stable NO metabolite) in the serum and right kidney were measured using a colorimetric assay kit (Promega Corporation, USA). Malondialdehyde (MDA) level of the serum and homogenized kidney supernatant were quantified according to the manual method. Briefly, 500 μ L of the samples were mixed with 1000 μ L of 10% trichloroacetic acid. The mixture was centrifuged at 2000 g for 10 min; 500 μ L of the supernatant was added with 500 μ L of 0.67% thiobarbituric acid. Then, this solution was incubated in warm water bath with temperature of 100°C for 10 min. After cooling, the absorbance was measured at 532 nm. The serum and kidney concentrations of MDA were reported according to μ mole/l and nanomole/g tissue, respectively.

Lung water content determination

Pulmonary edema was determined by determination of water content percentage (%). The lung samples were dried in the oven (100°C) for at least 48 h until constant weights were obtained. The percentage of water content was calculated by the following formula:

$$\text{Water content\%} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100$$

Determination of pulmonary endothelial permeability

Endothelial permeability of pulmonary system was measured by EB method that is described elsewhere.^[21,22] Briefly, samples of lung tissue were immediately weighed and put into 4 cc formamide, placed in an oven (80°C) for 48 h, and then the absorbance was measured at 623 nm. The standard curve of EB concentration was plotted and the EB concentration (μ g) to lung weight (gr) was determined as pulmonary endothelial permeability.

Histopathological procedures

The removed kidneys and lungs were fixed in 10% formalin solution, embedded in paraffin for histopathological staining. The hematoxylin and eosin staining was applied to examine the tissue injury. To consider the kidney damage, we evaluated presence of tubular atrophy, hyaline casts, ischemic necrosis, vacuolization, and debris. Also, to have the cases with complete reperfusion, the cases with ischemic necrosis more than 5% were excluded from the study. Based on the damage intensity in the kidney and damage percentage, the samples were scored 1-4, where 0 was assigned to normal tissue. To consider the lung tissue damage, presence of congestion, inflammation, and fibrosis were evaluated. Based on the damage intensity, the samples were scored from 1 to 4, where 0 was assigned to normal tissue.

Statistical analysis

Statistical data are presented as mean \pm standard error of the mean. The groups were compared with each other by one-way analysis of variance, followed by least significant difference with regard to the serum levels of BUN, Cr, NO, and MDA; and kidney levels of MDA and NO, kidney weight, BW changes, lung water content, and endothelial

permeability. Due to the qualitative nature of scoring, Mann-Whitney or Kruskal-Wallis tests were used to compare the pathological damage score of the groups. *P* values more than 0.05 were considered statistically significant.

RESULTS

Effect of IRI on serum BUN and Cr levels

The serum levels of BUN and Cr in the control group increased significantly when compared with the sham group ($P < 0.05$). Administration of NAC (150 mg/kg) decreased the serum levels of Cr and BUN in comparison with the control group. However, only the serum level of Cr decreased significantly ($P < 0.05$) [Figure 1].

Effect of IRI on kidney weight and damage

The kidney weight (KW) (tissue weight per 100 g BW) and kidney tissue damage increased significantly in the control and NAC groups, and the groups were significantly different from the sham group in this respect ($P < 0.05$). These data also indicated that NAC did not improve KW and damage [Figure 1]. The sample of Kidney tissue images is shown in Figure 2.

Effect of IRI on serum and tissue MDA levels

The serum and kidney tissue levels of MDA increased non-significantly in the control group when compared with the sham-operated group. NAC did not have a significant effect on decreasing the MDA level in serum and kidney tissue [Figure 1].

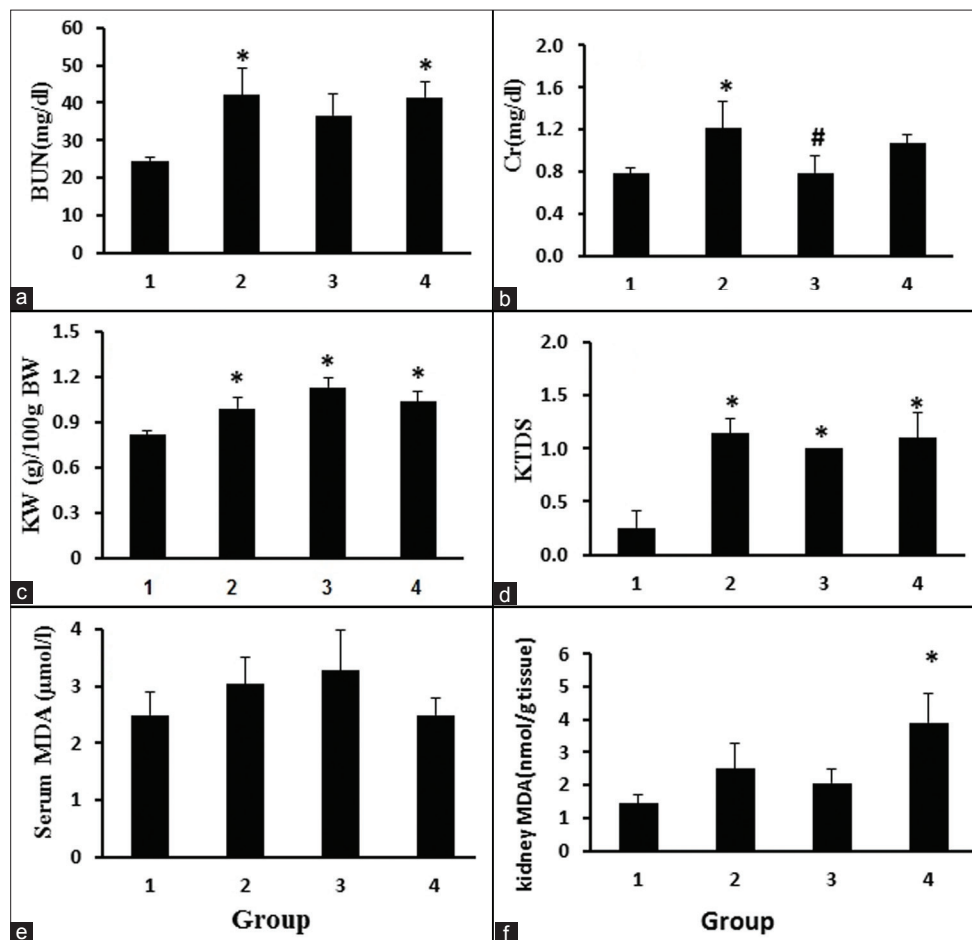


Figure 1: (a-b) Blood urea nitrogen (BUN) and serum level of creatinine (Cr). (c) Total kidney weight (KW)/100 g body weight, (d) Kidney tissue damage score (KTDS). (e-f) Serum and kidney malondialdehyde levels. *and # indicate significantly different from the sham and the control groups ($P < 0.05$), respectively

Effect of IRI on BW changes

Ischemia process induced weight loss in the control group compared with the sham group. Moreover, administration of NAC (150 mg/kg)

ameliorated weight loss in comparison with the control group. In contrast, administration of 500 dose of NAC significantly decreased BW when compared with the sham and control groups ($P < 0.05$) [Figure 3].

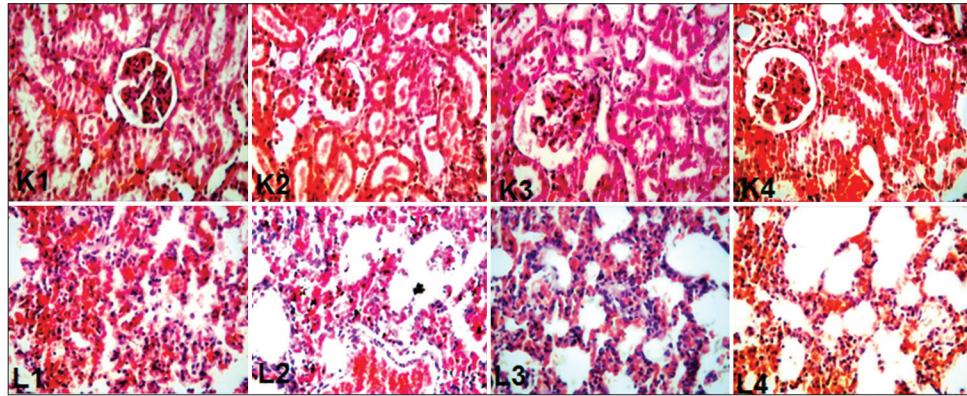


Figure 2: Kidney (K) and lung (L) tissue images (magnification $\times 100$). K1 to K4 and L1 to L4 demonstrate the kidney and lung tissues image of groups 1 to 4. More tissue damages were observed in group 2 (K2 and L2). The low dose of N-acetylcysteine (150 mg/kg, K3 and L3) indicate less tissue damage

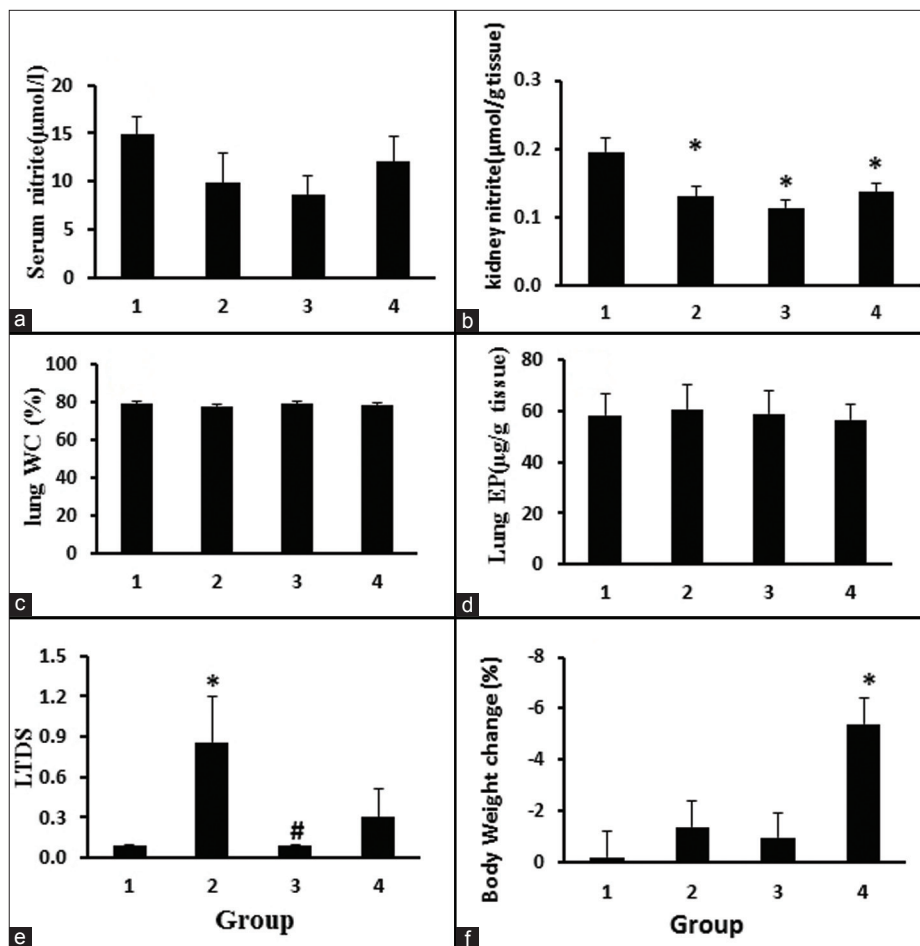


Figure 3: (a-b) Serum and kidney nitrite. (c) Percentage (%) of lung water content. (d) Lung endothelial permeability. (e) Lung tissue damage score (LTDS). (f) Percentage (%) of body weight change. *, #, and †: Significantly different from the sham group, control group, and group 3, respectively

Effect of IRI on serum and kidney tissue nitrite levels

The nitrite level in the control and NAC groups significantly decreased in kidney tissue ($P < 0.05$), while the decrease in the serum level was not statistically significant when compared with the sham-operated group. The data also indicated that NAC did not affect the nitrite [Figure 3].

Effect of IRI on lung tissue

No significant differences were observed in lung water content and endothelial permeability among the groups. However, the pathological data indicated significant differences among the groups. Lung tissue damage score (LTDS) was higher in the control group than in the sham group ($P < 0.05$). Administration of NAC reduced the lung injury score; however, only low dose of NAC (150 mg/kg) influenced the injury to reduce LTDS significantly ($P < 0.05$) [Figure 3]. The sample of lung tissue images is shown in Figure 2.

DISCUSSION

In this study, we evaluated the protective role of NAC on kidney injury and likely distant lung injury following bilateral renal IRI. The main finding of the present study was that it was shown that the low-dose of NAC was more effective than the high dose in protecting against the kidney IRI. Various reports have explained that renal IRI affects NO and MDA levels, weight and histology of kidney, and also serum biochemical factors such as Cr and BUN.^[14,23-27] Our result showed that low dose of NAC reduces serum Cr and BUN levels in rats with renal IRI. It was also demonstrated that plasma Cr level is reduced by administration of NAC.^[14,26,28,29] It seems that reduction of Cr level in the current study as well as other studies is not probably associated with alteration of glomerular filtration rate,^[29,30] but possibly associated with activating creatine kinase^[31] and increasing the tubular secretion of Cr.^[30]

KW and damage was significantly increased in the control and NAC groups when compared with the sham group. Accordingly, neither high nor low doses of NAC improved KW and damage. However, findings of earlier studies^[14,28,32] and the Cr level obtained in the current study indicate improvement of kidney function and lack of renal damage recovery. The discrepancy between kidney

function marker; Cr and renal tissue damage may be related to alteration of Cr, which is associated with tubular secretion as mentioned before.^[30]

BW loss was observed after renal IRI. Rodent BW loss after kidney IRI is most likely due to inability of the kidney to retain salt and water. This effect causes polyuria and cachexia.^[12,27,33] Greater BW loss was observed in the NAC (500 mg/kg) group. This may be related to the dose administered, which has a diuretic effect^[34] and inhibits epithelial cell sodium reabsorption.^[35] Probably, high dose NAC solution inhibited sodium absorption across renal epithelial cells and caused dehydration and subsequently weight loss in this group.

MDA is a marker of lipid peroxidation. MDA increase following IR was reported in several studies before.^[36,37] However, Rasoulia *et al.*,^[38] could not detect the change of serum MDA after kidney IRI. This effect is probably due to increased activity of superoxide dismutase or other antioxidant enzymes.^[38,39] For the kidney tissue, MDA level decreased by low-dose NAC non-significantly, while it increased significantly by high-dose NAC. It seems that low concentrations of NAC is a powerful scavenger of free radicals, while high doses of NAC might themselves exert deleterious effects under certain circumstances.^[40]

Our study indicated that both kidney tissue and serum nitrite levels in the control group decreased, which are the evidence of reduction in NO production after kidney IRI. According to previous studies, NO production by endothelial NO synthase (eNOS) and inducible NOS (iNOS) increased early after renal IRI and then decreased.^[24,41-43] Considering the cited articles, declined levels of nitrite in our study is possibly due to reduction of activity of NO synthase forms; particularly the eNOS. Lung damages, including inflammatory cell infiltrations, hemorrhage, and vascular congestion significantly increased after ischemia. This is consistent with the results of Kramer *et al.*^[44] They reported no changes in lung water content and endothelial permeability 96 h after renal IRI. However, previous studies revealed an enhancement of lung water content and endothelial permeability at hours 24, 36, and 48 after renal ischemia.^[2,27,44,45] Kramer *et al.*,^[44] showed lung edema and permeability caused by renal ischemia coincident with Cr changes. On the contrary, maximum Cr concentration is reported

24 h after ischemia.^[24,44,46] So, one of the limitations of the current study was not having samples for 24-h post ischemia NAC improved lung damage score. This is in agreement with other studies that reported pretreatment with NAC reduce lung tissue damage^[47] and also NAC ameliorate inflammatory response in different models of lung injury.^[48,49] In addition, Vassilev *et al.*,^[50] showed that low dose of NAC protects lung against endotoxin and oxidants, while high dose of NAC increased the mortality.

CONCLUSIONS

Low dose of NAC improved just functional markers of kidney after IRI, without improvement of renal structure. However, lung tissue injuries improved by administration of NAC.

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