Protective Role of Recombinant Human Erythropoietin in Kidney and Lung Injury Following Renal Bilateral Ischemia-Reperfusion in Rat Model

Maryam Moeini[1,2], Mehdi Nematbakhsh[1,3,4], Mohammad Fazilati[2], Ardeshir Talebi[5], Ali Asghar Pilehvarian[2], Fariba Azarkish[1,2], Fatemeh Eshraghi-Jazi[1], Zahra Pezeshki[1]

[1]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Biochemistry, Isfahan University of Payame-Noor, Isfahan, Iran, [3]Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Department of Clinical Pathology, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Prof. Mehdi Nematbakhsh,
Department of Physiology, Water and Electrolytes Research Center/Kidney Diseases Research Center/
Isfahan University of Medical Sciences,
Isfahan, Iran.
E-mail: nematbakhsh@med.mui.ac.ir

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Original Article

ABSTRACT

Background: Acute kidney injury (AKI) has been recognized as one of the most complex clinical complications in modern medicine, and ischemia/reperfusion (I/R) injury is well-known as a main reason of AKI. In addition, AKI leads to important systemic consequences such as acute lung injury. This study was designed to investigate the role of erythropoietin (EPO) on kidney function makers and tissue damage; and lung endothelial permeability and lung water content (LWC) in bilateral renal I/R injury model in rats.

Methods: Male Wistar rats were randomly divided into three groups of sham, I/R, and I/R treated with EPO (I/R + EPO) groups. The I/R and I/R + EPO groups were subjected to bilateral renal I/R injury; however, only the I/R + EPO group received EPO (500 IU/kg, i.p.) 2 h before ischemia surgery, and the same dose was continued once a day for 3 days after ischemia. The sham group underwent a surgical procedure without ischemia process.

Results: The blood urea nitrogen (BUN) and serum creatinine (Cr) levels, kidney tissue damage score (KTDS), and kidney weight (KW) per 100 g body weight significantly increased in I/R group ($P < 0.05$). EPO administration decreased levels of BUN and Cr significantly ($P < 0.05$), and KTDS and KW insignificantly ($P = 0.1$). No significant differences in kidney and serum levels of malondialdehyde, and lung vascular permeability and LWC were observed between the groups. The serum and kidney levels of nitrite were not significantly different between I/R and sham groups; however, administration of EPO increased the renal level of nitrite ($P < 0.05$).

Conclusions: EPO protected the kidney against I/R injury; however, it may not protect the lung tissue from the damage induced by renal I/R injury in rats.

Keywords: Erythropoietin, lung endothelial permeability, lung water content, rat

INTRODUCTION

Acute kidney injury (AKI) is a common clinical syndrome that is induced by kidney ischemia.[1-5] Renal ischemia and

reperfusion (I/R) injury also is one of the common complications in clinical surgeries such as renal transplantation.[6] The I/R injury triggers an immune response and leads to both local and systemic inflammations. It disturbs renal function and immune system homeostasis.[7,8] The crosstalk between the renal injury and distant organs such as lung is one of the complicated processes having very complex mechanism.[9] The most important cause of the high rate mortality induced by AKI is related to the functional role of the pulmonary system.[10] The acute lung injury after AKI or I/R injury is featured by pulmonary vascular congestion, interstitial edema, focal alveolar hemorrhage, and inflammatory cell infiltration.[11] It is also reported that pulmonary vascular permeability increases after AKI or I/R injury.[12] Erythropoietin (EPO) is a 30.4 kD glycoprotein of class I cytokine consisting of 165 amino acids.[13,14] Potentially, it exhibits a powerful tissue-protective effect against I/R.[15] EPO has been the subject of many *in vivo* and *in vitro* researches as a nephroprotectant agent after kidney injury.[6,14,16-32] Although most data available support positive effects for EPO administration, some available evidence has been shown unfavorable effects of EPO in kidney injury.[18,21,31] However, less information have supported the pulmono-protective effects of EPO after I/R injury. Some published data demonstrated that EPO pretreatment in IRI rat model may attenuate renal and lung injuries,[17,33] but it seems that more pathological information is still needed. In this study, we attempted to examine the effect of EPO administration on kidney and lung tissues simultaneously by gathering biochemical and pathological data as well as lung vascular permeability (LP) in bilateral IRI rat model.

METHODS

Animals

A total of 19 adult male (weighting 189 ± 3.36 g) Wistar rats (Animal Center, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this study. Animals were housed under standard conditions with 12 h light/12 h dark cycle and had free access to water and food. Prior to experiment, the protocols were confirmed to be in accordance with the Guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences.

Drugs

EPO (recombinant human erythropoietin alpha) and Evans Blue were purchased from Pooyesh Darou Pharmaceutical Co. (Tehran, Iran) and Sigma (St. Louis, Missouri, USA), respectively.

Experimental protocol

The animals were randomly divided into three experiment groups; sham, I/R, and I/R treated with EPO (I/R + EPO). The I/R + EPO group received EPO (500 IU/kg, i.p.) 2 h before the ischemia surgery, and administration of the drug continued for 3 days after ischemia. The I/R group followed the same regimen. Only it received saline instead of EPO. The sham group underwent the surgical procedure without ischemia process. To induce the IRI model, the animals in groups I/R and I/R + EPO were anesthetized by ketamine (75 mg/kg, i.p.) and xylaxine (10 mg/kg, i.p.). Two small incisions were made on the skin of the back of the animal, and the fascia was genteelly removed to appear the kidneys. The both kidney arteries and veins were clamped for 45 min. After removing the clamp, kidney reperfusion was performed. The animals were recovered after surgery for the following steps of an experiment. The animals in groups I/R and I/R + EPO respectively received daily saline and EPO 24, 48, and 72 h after renal IRI. The animal body weight was recorded on a daily basis. On day 3 (72 h post-IRI) and 2 h after the last injection, the animals were operated for the next step. After anaesthetization of the animals again, the trachea was cannulated by ventilation tube and the catheters were implanted into the carotid artery to obtain a blood sample and into the jugular vein for injection of Evans Blue (EB) solution (10 mg/kg). The right kidney was removed. To maintain stable anesthesia condition, animals received oxygen ventilation if needed. Finally, the rats were sacrificed 1 h after EB injection by potassium chloride solution. Tissue samples of lung and left kidney were obtained and fixed in 10% formalin solution for pathological assessment. Tissue samples of the lung were also collected to be investigated for edema and endothelial permeability. The removed right kidney was homogenated and centrifuged at 15,000*g* for 2 min, and the supernatant was used for biochemical measurements.

Measurements

Serum levels of creatinine (Cr) and blood urea nitrogen (BUN) were measured using quantitative kits (Pars Azmoon, Iran). Serum and kidney levels of nitrite (stable metabolite of nitric oxide [NO]) were measured using an ELISA assay kit (Promega Corporation, USA). Assessment of malondialdehyde (MDA) level in the serum and kidney was performed by the manual method. Briefly, a mixture of 500 µl of the sample and 1000 µl of 10% trichloroacetic acid (TCA) was centrifuged at 2000*g* for 10 min; then 500 µl of the supernatant was pulsed with 500 µl of 0.67% thiobarbituric acid (TBA). After 10 min of incubation in boiling water and then cooling, the absorbance was measured at 532 nm. Concentrations of MDA for serum and kidney samples were reported in µmol/l and nmol/g tissue, respectively.

Measurement of pulmonary water content

The lung tissue was kept in oven under 100°C until constant weight was obtained. The percentage of lung water content (LWC) was calculated as (wet lung weight – dry lung weight)*100/wet lung weight.

Measurement of LP

LP was measured by EB method that is described elsewhere.[34,35] Lung tissue was put into 4 cc formamide and kept in oven under 80°C for 24 h. The tissue EB was extracted by formamide, and then its absorbance was read at 623 nm to determine the tissue endothelial permeability (µg/g tissue) using standard curves.

Histopathological procedures

The removed kidney and lung were fixed in 10% formalin solution, and then embedded in paraffin for histopathological staining. The hematoxylin and eosin stain was applied to examine the tissue injury. To consider the kidney damage, presence of tubular atrophy, hyaline cast, ischemic necrosis, vacuolization, and debris was evaluated. Cases with ischemic necrosis higher than 5% were excluded from the study. Based on the damage intensity, we scored the samples as 1-4 while score zero 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 in the range of 1-4 while score zero was assigned to normal tissues.

Statistical analysis

The data are presented as mean ± standard error of the mean. To compare the weight change, serum levels of BUN, Cr, MDA, and NO; kidney levels of MDA and NO, kidney weight (KW), and pulmonary permeability and edema were compared between the groups by the one-way analysis of variance, followed by least significant difference. Since, the scoring is qualitative; the Mann-Whitney or Kruskal-Wallis tests were applied to compare the pathology damage scores between the groups. $P < 0.05$ were considered statistically significant.

RESULTS

Effect of I/R injury on serum Cr and BUN levels

The BUN and Cr levels increased in the I/R group in comparison with the sham group ($P < 0.05$). In the presence of EPO, the serum concentrations of Cr and BUN were lower than I/R group ($P < 0.05$) [Figure 1].

IRI effects on kidney tissue damage score and total KW

The KTDS and KW in gram per 100 g body weight in the I/R group significantly increased when compared to the sham group ($P < 0.05$). However, EPO administration reduced KTDS and KW in comparison with the I/R group ($P = 0.1$) [Figure 1]. The image of kidney tissue damage is demonstrated in Figure 2.

Effect of I/R injury on serum and kidney tissue levels of MDA and nitrite, and bodyweight

The data for the serum and kidney tissue levels of MDA and nitrite, and bodyweight change is tabulated in Table 1. No significant differences were observed in the MDA and nitrite levels between the I/R and sham groups. However, EPO did not change the MDA level, but the kidney nitrite level in the I/R + EPO group was greater than that in the I/R group ($P < 0.05$). No bodyweight change was detected between the groups.

Effect of I/R injury on LP, LWC, and lung tissue damage score

No significant difference in the LP and LWC were observed between the groups. However, EPO administration significantly increased the

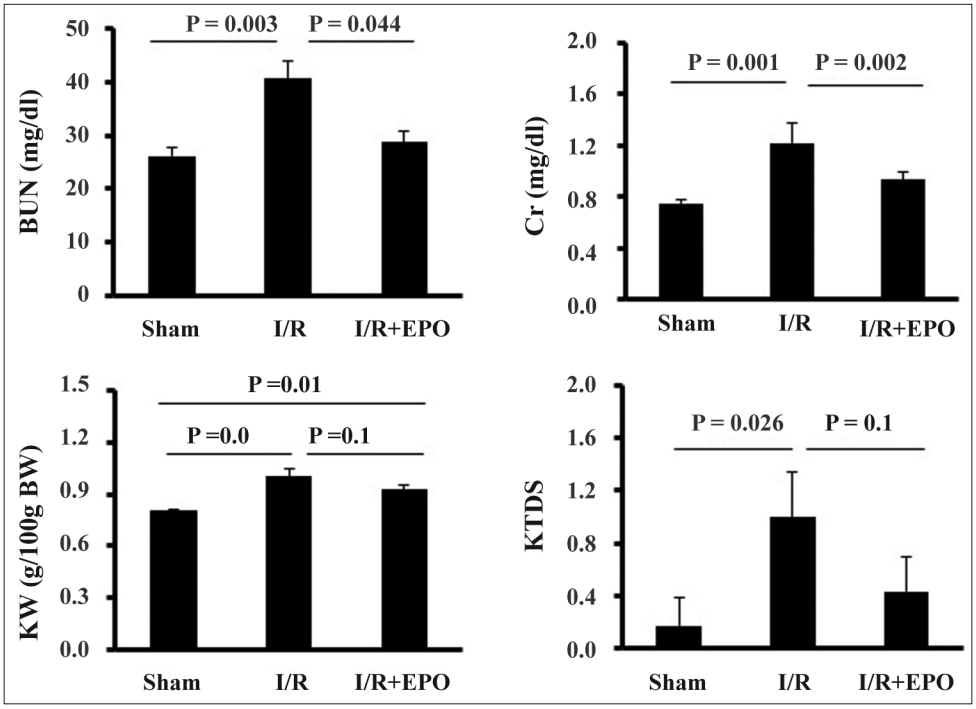

Figure 1: Serum creatinine and blood urea nitrogen levels, total kidney weight per 100 g body weight, and kidney tissue damage score in the sham, ischemia/reperfusion (I/R), and I/R treated with erythropoietin groups

Table 1: Serum and kidney tissue levels of MDA and nitrite, and body weight change in the sham, I/R, and I/R+EPO groups

Group	Serum MDA (µmol/l)	Kidney MDA (nmol/g tissue)	Serum nitrite (µmol/l)	Kidney nitrite (nmol/g tissue)	Body weight change (g)
Sham	2.69±0.49	1.72±0.31	12.71±2.60	0.17±0.02	7.5±2.4
I/R	3.41±0.67	2.92±1.06	7.65±0.94	0.14±0.01	5.6±2.7
I/R+EPO	3.32±0.81	2.34±1.03	9.12±2.34	0.19±0.03*	6.5±2.4

*Significant difference from the I/R group, $P<0.05$. MDA=Malondialdehyde, I/R=Ischemia/reperfusion, EPO=Erythropoietin

lung tissue damage when compared to the sham group ($P < 0.05$) [Figure 3]. The sample lung tissue damage is demonstrated in Figure 2.

DISCUSSION

Findings of this study indicated that EPO has protective effects on renal injury induced by bilateral renal IR, without positive effects on lung injury. Our results are confirmed by the previous studies.[27,36-38] The increased KW in the IR group may be related to renal edema or cell proliferation in kidney tissue, which is in agreement with the reports of Forbes *et al*.[39] The protective effect of EPO against renal IRI may be associated with its antioxidant, anti-apoptotic, anti-inflammatory, and angiogenic properties.[13,15,36,37,40-42] The body weight loss induced by IR occurs due to inability of the kidney for salt and water retention[43] or cachexia and polyuria.[12] It was also reported that EPO (500 U/kg) administration after renal IRI had no effect on bodyweight.[44]

MDA is well-known as a final product of lipid peroxidation.[14] In our study, the serum and tissue levels of MDA increased non-significantly after renal IR. Previous studies have reported increased level of MDA after IRI.[14,45] However, in the study performed by Rasulian, no change in the serum level of MDA was reported; probably due to increased activity of super oxidase dismutase.[46]

The vasodilatory action of NO on vascular smooth muscle cells is well-known.[47] Decreased NO serum and tissue levels in the IR group may be related to reduced endothelial nitric oxide synthase (eNOS) in ischemic AKI.[48] On the other hand, EPO activates eNOS[13,47,49] that is agreement with our results for the EPO treated group. In the

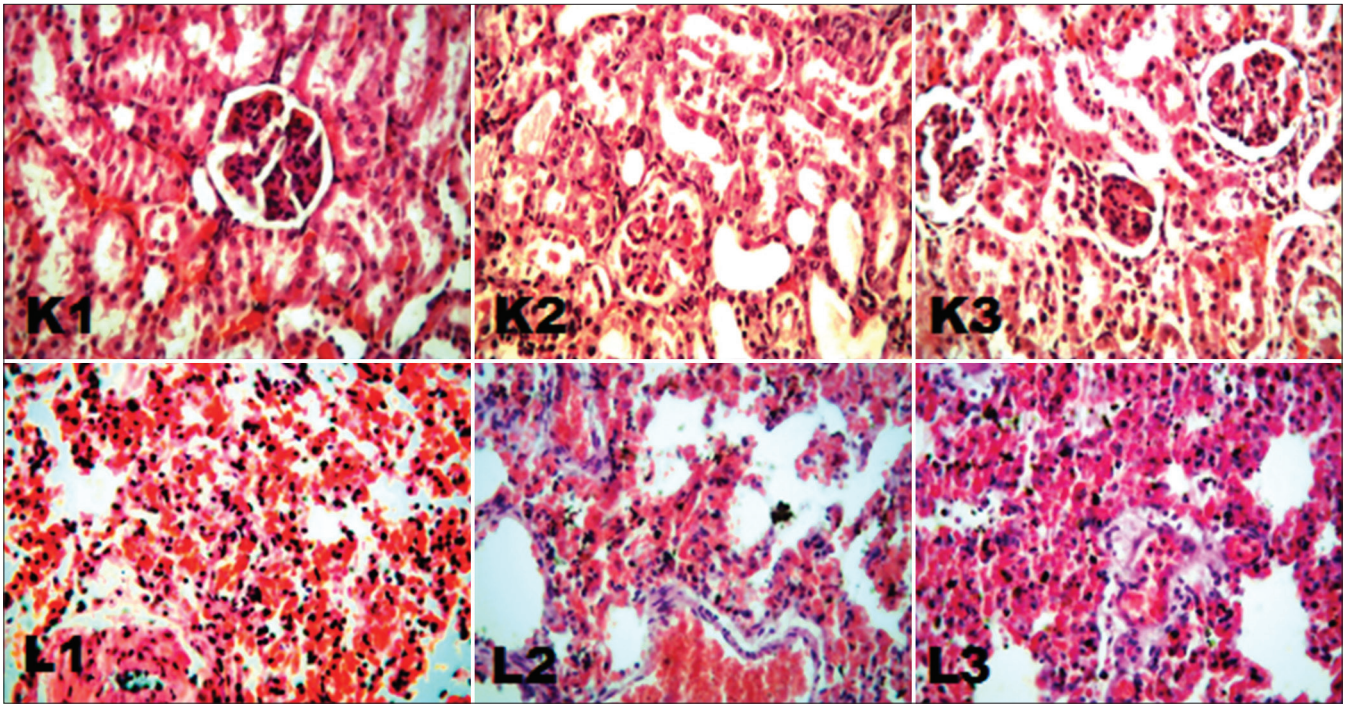

Figure 2: Kidney (k) and lung (l) tissue images (magnification ×100). K1-K3 and L1-L3 demonstrate the kidney and lung tissues image of groups 1-3. More tissue damages were observed in group 2 (K2 and L2). Erythropoietin indicates less kidney tissue damage (K3). However, it may promote the lung tissue damage (L3)

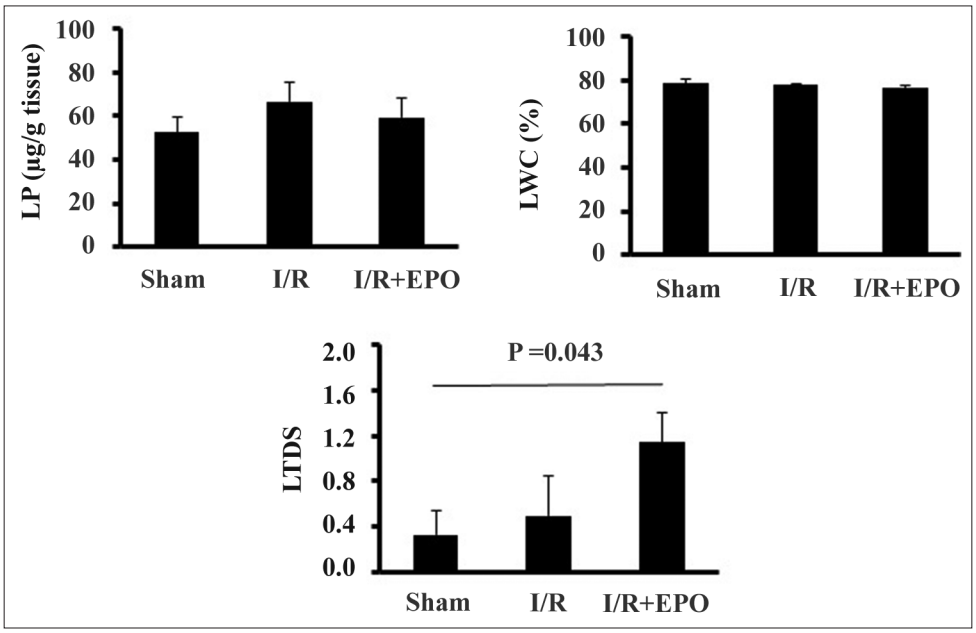

Figure 3: Lung endothelial permeability, lung water content, and lung tissue damage score in the sham, ischemia/reperfusion (I/R), and I/R treated with erythropoietin groups

current study, no change in LWC and LP were observed within 72 h after ischemia. Kramer *et al*. demonstrated that lung interstitial edema, vascular permeability occur 24 h and 48 h after renal IRI, but not 96 h after renal IRI. This is correlated with the level of Cr itself,[50] as we observed in the current study. In other study, pulmonary edema was observed after renal ischemia.[51] A limitation of

the current study is not measuring the parameters at day 2 after ischemia.

Lung tissue damage score (LTDS) was characterized by the presence of congestion, inflammatory cells, and hemorrhage; and EPO did not decrease its level. Wu *et al*. reported that low dose of EPO (300 IU/kg) increased lung injury after endotoxin shock via enhancing production of pro-inflammatory cytokines.[38,52] Furthermore, other study reported that low dose of EPO increase production of tumor necrosis factor-α and IL-6 in partial hepatectomy model.[53] These findings showed that EPO (500 IU/kg) probably induces lung tissue damage by activating pro-inflammatory pathways.

CONCLUSIONS

The present study indicated that EPO have protective effects against renal injury induced by renal ischemia-reperfusion, but it did not improve the lung tissue damage induced by I/R injury.

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