Journal of Acute Disease 2016; ■(■): 1–5

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Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Original article http://dx.doi.org/10.1016/j.joad.2016.08.008

The protective effect of erythropoietin pretreatment on ischemic acute renal failure in rats

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ARTICLE INFO

Article history:
Received 15 Apr 2016
Received in revised form 25 Apr 2016
Accepted 20 May 2016
Available online xxx

Keywords:
Erythropoietin
Acute kidney injury
Ischemia-reperfusion injury
Inflammatory response

ABSTRACT

Objective: To investigate the protective effect of erythropoietin (EPO) pretreatment on ischemic acute renal failure in rats and its molecular mechanism.

Methods: Male Sprague–Dawley rats were selected as experimental animals and they were randomly divided into the sham operation group (sham group), ischemia-reperfusion injury group (IRI group) and EPO pretreatment group (EPO group). Each group had 15 rats. Serum specimens and renal specimens were collected after a IRI model was built for 4, 12 and 24 h. The contents of creatinine, urea nitrogen tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6 and IL-8 in serum and the contents of TNF-α, IL-1, IL-6, IL-8, toll like receptor 4 (TLR4) and nuclear factor-kappa B (NF-κB) in the kidney tissue were determined.

Results: After 4, 12 and 24 h reperfusion, there were differences between the contents of creatinine, urea nitrogen TNF-α, IL-1, IL-6 and IL-8 in serum and the contents of TNF-α, IL-1, IL-6, IL-8, TLR4 and NF-κB in rats of the three groups ($P < 0.05$). The contents of creatinine, urea nitrogen TNF-α, IL-1, IL-6 and IL-8 in serum and the contents of TNF-α, IL-1, IL-6, IL-8, TLR4 and NF-κB in the kidney tissue in rats of the IRI group were significantly higher than those of the sham group; and the contents of creatinine, urea nitrogen TNF-α, IL-1, IL-6 and IL-8 in serum and the contents of TNF-α, IL-1, IL-6, IL-8, TLR4 and NF-κB in the kidney tissue in rats of the EPO group were distinctly lower than those of the IRI group.

Conclusions: EPO pretreatment can protect the renal function of rats with ischemic acute renal failure by inhibiting the TLR4/NF-κB pathway mediated inflammatory responses.

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1. Introduction

Acute kidney injury is a clinically troublesome disease mainly resulted from ischemia causes such as cardiopulmonary bypass, hemorrhagic shock, vascular occlusion in renal transplantation and so on. It mainly causes acute ischemic injury through ischemia-reperfusion injury (IRI)[1–3]. The pathophysiological procedure of kidney IRI is quite complicated. Cell apoptosis, oxidative stress, inflammatory response and vascular endothelial injury all participate in the IRI process[4–6]. The excessively activated inflammatory responses and the over-compounded inflammatory factors play the key role in IRI process and the pathological and physiological changes of many other related diseases, which can relieve kidney injury and improve renal function by inhibiting the activation of inflammatory responses[7–9].

Erythropoietin (EPO) is a soluble glycoprotein existing in the body's circulation. EPO is compounded and secreted by interstitial cells and it can promote the production of erythrocytes. It was first used in the treatment of anemia. Recent researches have proved that other than the hematopoietic function, EPO also possesses multiple biological functions which can protect the IRI of brain[10], heart[11], liver[12,13] and kidney[14]. However, the concrete molecular mechanism of the protective effect still remains unclear. Toll like receptors (TLRs) are considered as the key upstream molecules in the regulation process of inflammatory responses. There are researches confirming that

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Peer review under responsibility of Hainan Medical College. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Please cite this article in press as: Liao J-G, et al., The protective effect of erythropoietin pretreatment on ischemic acute renal failure in rats, Journal of Acute Disease (2016), http://dx.doi.org/10.1016/j.joad.2016.08.008

the expression of TLR4 in tubular epithelial cells increases significantly and thereby activates NF-κB and facilitates the expressions of various inflammatory factors by the myeloid differentiation marker 88-dependent method in the occurrence process of kidney IRI. In the following researches, we mainly analyzed whether EPO protects ischemic acute kidney injury by adjusting the TLR4/NF-κB pathway in the kidney tissue.

2. Materials and methods

2.1. Experimental materials

A total of 45 male Sprague–Dawley rats with specific pathogen free level (weighting 220–250 g) were bought from the Animal Center of Southern Medical University. The animal experiment was approved by the hospital's Ethics Committee. Tumor necrosis factor-alpha (TNF-α), IL-1, IL-6 and IL-8 ELISA kits were purchased from Shanghai Westang Bio-Tech Co. TLR4 and NF-κB monoclonal antibodies were from Santa-Cruz and the tissue protein extraction reagent was from Wuhan Boster Co.

2.2. Animal experimental methods

Those 45 rats were randomly divided into the sham operation group (sham group), IRI group and EPO group with 15 rats in each group. Twenty-four hours before building models, rats in the EPO group were intraperitoneally injected with 3000 IU/kg EPO, while rats in the sham and IRI groups were intraperitoneally injected with 3000 IU/kg normal saline. Acute kidney injury models of the IRI and EPO groups were built by the following steps. After intraperitoneal anesthesia with 10% chloral hydrate, a median abdominal incision was made and the kidney and renal pedicles were dissociated after entering the enterocoelia. A bulldog clamp was used to clip the bilateral renal artery. Sixty minutes later, the bulldog clamp was removed and the renal tissue received reperfusion. Rats in the sham group were only treated with the opening enterocoelia and dissociating the renal artery and no bulldog clamp was needed to clip the blood vessel. After 4, 12 and 24 h reperfusion, 5 rats of each group were executed to collect blood specimens and kidney specimens. Serum obtained from centrifugalization of the blood samples was stored in a refrigerator at −80 °C and the renal tissue was frozen transitorily by liquid nitrogen and then kept in the fridge at −80 °C.

2.3. Index detection methods

Serum samples were collected. The automatic biochemical analyzer was used to test the contents of serum creatinine (Scr) and blood urea nitrogen (BUN), while ELISA kits was applied to detect the contents of TNF-α, IL-1, IL-6 and IL-8. The renal tissue was obtained and protein extraction was added into it and then they were grinded and homogenized. After that, the homogenization was transited into the Eppendorf tube and centrifuged and the supernatant was collected. ELISA kits was again applied to detect the contents of TNF-α, IL-1, IL-6 and IL-8, and Western blot method was employed to test the expression quantities of TLR4 and NF-κB. The experiment conditions of the Western blot method included 130 V vertical electrophoresis for 80 min, 100 V transfer tank for electrophoresis for 90 min, 2% skim milk in isolation for 2 h, 1:1000 TLR4, incubation by NF-κB monoclonal antibody overnight, incubation by 1:1000 horseradish peroxidase-marked secondary antibody for 1–1.5 h. Gel electrophoresis imaging system was developed. The gray value of the protein band and the protein content was calculated.

2.4. Statistical methods

Data were input and analyzed by using SPSS17.0 software. Measurement data of the three groups were analyzed by One-way ANOVA. Differences showed statistical significance ($P < 0.05$).

3. Results

3.1. Serum biochemical indexes

After 4, 12 and 24 h reperfusion, the contents of BUN and Scr of the three groups were different ($P < 0.05$). The contents of BUN in the IRI group [(9.42 ± 1.16) *vs.* (6.82 ± 0.82) mmol/L], [(32.58 ± 5.29) *vs.* (7.75 ± 0.92) mmol/L], [(52.31 ± 8.52) *vs.* (6.10 ± 0.77) mmol/L] and the contents of Scr [(79.51 ± 9.32) *vs.* (35.22 ± 5.82) μmol/L], [(283.41 ± 42.38) *vs.* (38.31 ± 4.39) μmol/L], [(402.95 ± 62.41) *vs.* (30.15 ± 5.23) μmol/L] were all significantly higher than those in the sham group. In the EPO group, the contents of BUN [(13.29 ± 2.82) *vs.* (32.58 ± 5.29) mmol/L], [(21.84 ± 4.18) *vs.* (52.31 ± 8.52) mmol/L], [(7.64 ± 1.03) *vs.* (9.42 ± 1.16) mmol/L] and the contents of Scr [(52.16 ± 7.29) *vs.* (79.51 ± 9.32) μmol/L], [(89.32 ± 10.23) *vs.* (283.41 ± 42.38) μmol/L], [(128.48 ± 18.52) *vs.* (402.95 ± 62.41) μmol/L] were all obviously lower than those in the IRI group (Table 1).

3.2. The contents of serum inflammatory factors

After 4, 12 and 24 h reperfusion, the contents of serum TNF-α, IL-1, IL-6 and IL-8 in the three groups were different ($P < 0.05$). In the IRI group, serum levels of TNF-α [(19.34 ± 3.06) *vs.* (10.33 ± 1.68) μg/L], [(48.52 ± 8.25) *vs.* (13.18 ± 1.71) μg/L], [(126.64 ± 19.15) *vs.* (11.38 ± 1.41) μg/L], IL-1 [(40.27 ± 6.72) *vs.* (23.51 ± 4.68) μg/L], [(105.38 ± 16.37) *vs.* (30.42 ± 6.51) μg/L], [(193.52 ± 25.28) *vs.* (26.36 ± 3.72) μg/L], IL-6 [(214.42 ± 30.38) *vs.* (115.22 ± 17.43) ng/L], [(336.41 ± 50.27) *vs.* (130.64 ± 22.36) ng/L], [(477.54 ± 62.51) *vs.* (109.62 ± 18.45) ng/L] were all significantly higher than those in the sham group. In the EPO group, the serum levels of TNF-α [(13.65 ± 2.26) *vs.* (19.34 ± 3.06) μg/L], [(21.49 ± 3.18) *vs.* (48.52 ± 8.25) μg/L], [(38.56 ± 6.53) *vs.* (126.64 ± 19.15) μg/L], IL-1 [(31.23 ± 5.37) *vs.* (40.27 ± 6.72) μg/L], [(50.33 ± 7.85) *vs.* (105.38 ± 16.37) μg/L], [(84.14 ± 11.38) *vs.* (193.52 ± 25.28) μg/L], IL-6 [(165.63 ± 20.24) *vs.* (214.42 ± 30.38) ng/L], [(336.41 ± 50.27) *vs.* (189.61 ± 23.42) ng/L], [(477.54 ± 62.51) *vs.* (223.54 ± 34.18) ng/L] were all obviously lower than those in the IRI group (Table 2).

3.3. The contents of inflammatory factors in the kidney tissue

After 4, 12 and 24 h reperfusion, the contents of TNF-α, IL-1 and IL-6 in the kidney tissue of the three groups were different ($P < 0.05$). In the kidney tissue in rats of the IRI group, the

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