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

Jing-Guang Liao1*, Min-Yan Li1, Xiao-Hua Wang2, Qiang Xie3

1Pharmacy Department, Panyu Maternal and Child Care Service Centre of Guangzhou, 511400, Guangzhou, China
2Urology Department, Panyu Maternal and Child Care Service Centre of Guangzhou, 511400, Guangzhou, China
3Department of Cardiology, Panyu Maternal and Child Care Service Centre of Guangzhou, 511400, Guangzhou, China

ARTICLE INFO
Article history:
Received 15 Apr 2016
Received in revised form 25 Apr 2016
Accepted 20 May 2016
Available online 11 Aug 2016

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-kB) 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-kB 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-kB 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-kB 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-kB pathway mediated inflammatory responses.

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 process4–6. 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 responses7–9.

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 brain10, heart11, liver12,13 and kidney14. 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 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 each group were executed to collect blood specimens and kidney tissue was obtained and protein extraction was added into it and tissue was frozen transitorily by liquid nitrogen and then kept in the fridge at −80 °C. Gel electrophoresis imaging system was developed. The gray value of the protein band and the protein content was calculated.

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 and Scr 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 [(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) μg/L], [(48.52 ± 8.25) μg/L], [(31.23 ± 5.61) μg/L], [(126.64 ± 19.15) μg/L], [(105.38 ± 16.37) μg/L] and the contents of Scr [(79.51 ± 9.32) μmol/L], [(9.42 ± 1.16) mmol/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) μg/L], [(19.34 ± 3.06) μg/L], [(21.49 ± 3.18) μg/L], [(48.52 ± 8.25) μg/L], [(38.36 ± 6.53) μg/L] and the contents of Scr [(89.32 ± 10.23) μmol/L], [(128.48 ± 18.52) μmol/L] were all significantly higher than those in the IRI group (Table 1).

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 contents of TNF-α [(25.27 ± 5.26) μg/L], [(9.33 ± 1.05) μg/L], [(56.71 ± 9.26) μg/L], [(10.41 ± 1.76) μg/L], [(113.62 ± 16.76) μg/L], [(10.28 ± 1.55) μg/L], [(33.57 ± 5.61) μg/L], [(15.78 ± 2.27) μg/L], [(78.32 ± 19.16) μg/L], [(17.18 ± 2.89) μg/L], [(146.76 ± 17.85) μg/L], [(214.42 ± 30.38) μg/L] were all significantly higher than those in the IRI group (Table 2).
Table 1
Comparison of the serum biochemical indexes of the three groups.

<table>
<thead>
<tr>
<th>Biochemical indexes</th>
<th>Group</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mmol/L)</td>
<td>Sham</td>
<td>6.82 ± 0.82</td>
<td>7.75 ± 0.92</td>
<td>6.10 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>9.42 ± 1.16a</td>
<td>32.58 ± 5.29a</td>
<td>52.31 ± 8.52a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>7.64 ± 1.03b</td>
<td>13.29 ± 2.82b</td>
<td>21.84 ± 4.18b</td>
</tr>
<tr>
<td>Scr (µmol/L)</td>
<td>Sham</td>
<td>35.22 ± 5.82</td>
<td>38.31 ± 4.39</td>
<td>30.15 ± 5.23</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>79.51 ± 9.32a</td>
<td>283.42 ± 42.38a</td>
<td>402.95 ± 62.41a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>52.16 ± 7.29b</td>
<td>89.32 ± 10.23b</td>
<td>128.48 ± 18.92b</td>
</tr>
</tbody>
</table>

Compared with the sham group, a: P < 0.05; Compared with the IRI group, b: P < 0.05.

Table 2
Comparison of the contents of serum inflammatory factors in rats of the three groups.

<table>
<thead>
<tr>
<th>Biochemical indexes</th>
<th>Group</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ng/L)</td>
<td>Sham</td>
<td>10.33 ± 1.68</td>
<td>13.18 ± 1.71</td>
<td>11.38 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>19.34 ± 3.06a</td>
<td>48.52 ± 8.25a</td>
<td>126.64 ± 19.15a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>13.65 ± 2.26b</td>
<td>21.49 ± 3.18b</td>
<td>38.56 ± 6.53b</td>
</tr>
<tr>
<td>IL-1 (µg/L)</td>
<td>Sham</td>
<td>23.51 ± 4.68</td>
<td>30.42 ± 6.51</td>
<td>26.36 ± 3.72</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>40.27 ± 6.72a</td>
<td>105.38 ± 16.37a</td>
<td>193.52 ± 25.28a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>31.23 ± 5.37b</td>
<td>50.33 ± 7.85b</td>
<td>84.14 ± 11.38b</td>
</tr>
</tbody>
</table>

Compared with the sham group, a: P < 0.05; Compared with the IRI group, b: P < 0.05.

IL-6 [(277.65 ± 34.15) vs. (142.97 ± 22.78) ng/L], [(514.64 ± 76.85) vs. (150.12 ± 19.57) ng/L], [(883.25 ± 104.57) vs. (147.75 ± 20.36) ng/L] were all apparently higher than those of the sham group. In the kidney tissue in rats of the EPO group, the contents of TLR4 [(1.83 ± 0.27) vs. (1.12 ± 0.15)] were all significantly higher than those of the IRI group, [(1.00 ± 0.14)] and NF-κB [(1.28 ± 0.17) vs. (1.17 ± 0.18)].

Table 3
Comparison of the contents of inflammatory factors in the kidney tissue of the three groups.

<table>
<thead>
<tr>
<th>Biochemical indexes</th>
<th>Group</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB</td>
<td>Sham</td>
<td>1.39 ± 0.22</td>
<td>3.36 ± 0.49a</td>
<td>4.85 ± 0.71a</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>1.95 ± 0.24a</td>
<td>3.36 ± 0.49a</td>
<td>4.85 ± 0.71a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>1.28 ± 0.17b</td>
<td>1.66 ± 0.20b</td>
<td>2.37 ± 0.40b</td>
</tr>
</tbody>
</table>

Compared with the sham group, a: P < 0.05; Compared with the IRI group, b: P < 0.05.

3.4. The contents of TLR4/NF-κB in the kidney tissue

After 4, 12 and 24 h reperfusion, the contents of TLR4 and NF-κB in the kidney tissue in rats of the three groups were different (P < 0.05). In the kidney tissue in rats of the IRI group, the contents of TLR4 [(1.83 ± 0.27) vs. (1.00 ± 0.16), (3.18 ± 0.52) vs. (1.16 ± 0.14), (4.46 ± 0.74)] and NF-κB [(1.95 ± 0.24) vs. (1.00 ± 0.14), (3.36 ± 0.49) vs. (1.12 ± 0.15), (4.85 ± 0.71)] were all significantly higher than those of the sham group. In the kidney tissue in rats of the EPO group, the contents of TLR4 [(1.39 ± 0.22) vs. (1.83 ± 0.27)], [(1.78 ± 0.24) vs. (3.18 ± 0.52)], [(2.52 ± 0.34) vs. (4.46 ± 0.74)].

Table 4
Comparison of the contents of inflammatory factors in the kidney tissue of the three groups.

<table>
<thead>
<tr>
<th>Biochemical indexes</th>
<th>Group</th>
<th>4 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR4</td>
<td>Sham</td>
<td>1.00 ± 0.16</td>
<td>1.16 ± 0.14</td>
<td>1.08 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>1.83 ± 0.27a</td>
<td>3.18 ± 0.52a</td>
<td>4.46 ± 0.74a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>1.39 ± 0.22b</td>
<td>1.78 ± 0.24b</td>
<td>2.52 ± 0.34b</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Sham</td>
<td>1.00 ± 0.14</td>
<td>1.12 ± 0.15</td>
<td>1.17 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>IRI</td>
<td>1.95 ± 0.24a</td>
<td>3.36 ± 0.49a</td>
<td>4.85 ± 0.71a</td>
</tr>
<tr>
<td></td>
<td>EPO</td>
<td>1.28 ± 0.17b</td>
<td>1.66 ± 0.20b</td>
<td>2.37 ± 0.40b</td>
</tr>
</tbody>
</table>

Compared with the sham group, a: P < 0.05; Compared with the IRI group, b: P < 0.05.

4. Discussion

In this study, acute ischemic renal injury was simulated by building a kidney IRI model. Creatinine and urea nitrogen are
products of the protein catabolism in the body, which are metabolized mainly through the kidney. When the renal function is injured beyond its compensative capacity, the levels of BUN and Scr increase obviously. The contents of BUN and Scr are used to evaluate renal function and reflect the renal injury degree in clinic. In this study, a kidney IRI model was established by clipping first and then opening the renal artery. It was found by analyzing the contents of BUN and Scr within 24 h after reperfusion that the contents of BUN and Scr in rats of the IRI after 4, 12 and 24 h reperfusion rose significantly, which indicated that the kidney tissue was injured after experiencing ischemia reperfusion and thus greatly weakened the discharge capacity for creatinine and urea nitrogen. EPO plays a positive role in promoting the hematopoesis of the myeloid tissue, which was first used to treat anemia. In recent years, studies on the biological effect of EPO have manifested that EPO can protect IRI of multiple tissues such as brain [10], heart [11], liver [12] and kidney [14]. In this study, EPO pretreatment was given before building a kidney IRI model. It was found by analyzing the contents of BUN and Scr that the content of BUN and Scr after 4, 12 and 24 h reperfusion in rats of the EPO group were all lower than those of the IRI group, which verified that EPO contains a protective effect on the kidney IRI.

At present, there was no unified understanding of the pathogenesis of IRI. Cell apoptosis, oxidative stress, inflammatory response and vascular endothelial injury are all physiopathologic changes in the procedure of IRI. Therefore, inflammatory response caused by ischemia reperfusion is an important pathological link in the procedure. A large number of the gathered inflammatory cells and the inflammatory factors can induce cell apoptosis, enhance oxidative stress and aggravate vascular endothelial injury [15]. TNF-α, IL-1 and IL-6 are important inflammatory mediators in the body [16-18]. After analyzing the contents of the above inflammatory mediators in serum and renal tissue in rats of the three group, it was confirmed that the contents of TNF-α, IL-1 and IL-6 in serum and renal tissue increased after IRI, while EPO pretreatment could obviously reduce the contents of TNF-α, IL-1 and IL-6 in serum and renal tissue. Therefore, it could be concluded that the substantially produced inflammatory mediators is the key step causing kidney IRI, and EPO could relieve the kidney injury caused by IRI by inhibiting the production of inflammatory mediators. To date, the regulatory mechanism of inflammatory mediators in the process of kidney IRI is not clear. TLRs is a kind of pattern recognition receptor which can activate tumor necrosis factor receptor associated factor-6 by connecting molecules TIR domain-containing adaptor protein and myeloid differentiation marker 88 and then activate NF-κB and make it transfer into the nucleus. After entering the nucleus, NF-κB can promote the transcriptions of TNF-α, IL-1 and IL-6 [19-21]. The analysis of the expression quantities of TLR4 and NF-κB in the kidney tissue confirmed that the expression quantities of TLR4 and NF-κB in the kidney tissue after ischemia reperfusion were elevated, while the expression of TLR4 and NF-κB can be inhibited after EPO pretreatment, which can be included that TLR4/NF-κB is the important mechanism of inflammatory responses mediating the process of kidney IRI. EPO can reduce the synthesis of inflammatory factors by inhibiting the TLR4/NF-κB pathway so as to alleviate kidney IRI.

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

Conflict of interest statement

The authors report no conflict of interest.

References


