Erythropoietin ameliorates renal dysfunction during endotoxaemia

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Abstract

Background. Sepsis has a high mortality (50–80%) when associated with acute renal failure (ARF). Oxidant injury and proinflammatory cytokines and chemokines have been shown to increase with endotoxaemia-related ARF. Since erythropoietin (EPO) has been demonstrated to possess anti-oxidant and anti-inflammatory properties, EPO may have therapeutic efficacy for treating ARF associated with endotoxaemia.

Methods. Wild-type mice were given 2.5 mg/kg of intraperitoneal (i.p.) endotoxin, lipopolysaccharide (LPS), and studied 16 h later. Thirty minutes prior to LPS, the mice were given either EPO or vehicle.

Results. During endotoxaemia, EPO was found to significantly attenuate the renal dysfunction, as assessed by glomerular filtration rate (48.1 ± 12.4 μl/min vs 136.7 ± 30.2, \( P < 0.05 \)). Renal blood flow and mean arterial pressure were not significantly different between the two groups. The renal dysfunction during endotoxaemia was associated with a decrease in renal superoxide dismutase (SOD). The EPO-related renal protection was associated with reversal of the effects of endotoxin on renal SOD.

Conclusion. This is the first demonstration of a renal protective effect of EPO on endotoxin-related renal dysfunction.

Keywords: acute renal failure; lipopolysaccharide; sepsis; super oxide dismutase

Introduction

Sepsis is the major cause of acute renal failure (ARF) in intensive care units [1] and sepsis-related ARF has a 50–80% mortality [2,3]. There is, therefore, a need for pre-clinical, experimental studies that provide insight into potential interventions to decrease the occurrence of sepsis-related ARF in patients.

In a recent study, the administration of human recombinant erythropoietin (EPO) abolished the renal dysfunction secondary to ischaemia-reperfusion (I/R) caused by either haemorrhagic shock or acute myocardial infarction in rats [4]. This protective effect of EPO has also been demonstrated with I/R injury secondary to bilateral renal artery clamping [5,6]. These are very exciting findings, particularly since EPO receptors have been identified in renal tubular cells [7] and EPO is readily available for use in humans. Of interest, however, the same dose of EPO which afforded renal protection against I/R was found not to provide renal protection against endotoxaemia [lipopolysaccharide (LPS)] [4]. This is an important observation, since endotoxin release from gram-negative bacteria accounts for much of the hemodynamic, proinflammatory and anti-oxidant effect of sepsis [8].

A difference in I/R and endotoxaemia is to be expected, however, since endotoxaemia is a more severe inflammatory syndrome. In this regard, tumour necrosis factor \( \alpha \) (TNF\( \alpha \)) is a well-established pathogenetic factor in endotoxaemia in mice [9,10], and yet recent studies in mice did not demonstrate an increase in plasma TNF\( \alpha \) with either I/R [11] or nephrotoxicity with cisplatin [12] unless LPS was also administered.

Because of the more severe inflammatory response with endotoxaemia than I/R, as assessed by plasma TNF\( \alpha \), larger doses of EPO may be needed to afford renal protection during endotoxaemia than I/R. Since human recombinant EPO has been found to be safe in humans and is FDA approved, the potential role of EPO in endotoxaemia is in need of further study which is the purpose of the present investigation.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Ethics Review Committee at the University of Colorado Health Sciences Center. C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Male mice aged 8–10 weeks were used throughout the study. Mice were
mained on a standard rodent chow and had free access to water.

**Materials**

Chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified.

**Endotoxemic ARF model in mice**

Mice were injected with 2.5 mg/kg LPS (LIST Biological Laboratories, Campbell, CA, USA) intraperitoneally (i.p.). Human recombinant EPO was given at 4000U/kg i.p. 30 min before the LPS administration. The dose of EPO was determined on the basis of the publication by Patel et al. [6]. Sixteen hours after LPS injection, renal function and blood pressure were measured.

**Measurement of glomerular filtration rate (GFR), renal blood flow (RBF), and mean arterial pressure (MAP)**

GFR was measured using FITC-inulin clearance, RBF was measured by a blood flow meter and MAP was measured through the carotid artery [13].

**Determination of total superoxide dismutase (SOD) activity**

Total SOD activity in renal tissue was determined using colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA). The lipid fraction was extracted from homogenized tissue, partially purified by solid-phase extraction and then derivatized to the pentafluorobenzyl ester trimethylsilyl ethers.

**Measurement of serum EPO and C-reactive protein levels**

Mouse serum EPO levels before and after LPS were determined using Erythropoietin Quantikine ELISA Kit (R & D system, Minneapolis, MN, USA). C-reactive protein (CRP) levels were measured using mouse CRP ELISA kit (Newberg, OR, USA).

**Measurement of serum TNF-α**

Electrochemiluminescence (ECL) assay was used to measure serum TNF-α as described previously in detail [14].

**Measurement of serum NO**

Mouse serum NO levels were measured using Nitrate/nitrite colorimetric assay kit (Cayman, Ann Arbor, MI, USA).

**Detection of Apoptosis**

Both morphologic and TUNEL staining were used to detect apoptosis in the kidneys. Morphological criteria were used to count apoptotic cells on PAS-stained tissue by a pathologist experienced in the evaluation of renal apoptosis. Morphologic characteristics included cellular rounding and shrinkage, nuclear chromatin compaction and formation of apoptotic bodies [15]. Apoptotic tubular cells were quantitatively assessed per HPF in the cortex and outer stripe of the outer medulla by the renal pathologist without knowledge of protocol. At least 10 fields were counted for each slide. TUNEL staining was done using TACS.XL® DAB In Situ Apoptosis Detection Kit (Trevigen®, Gaithersburg, MD, USA).

**Detection of Neutrophil infiltration**

Neutrophil infiltration was quantitatively assessed on PAS-stained tissue by the renal pathologist by counting the number of neutrophils per high powered field (HPF) (400×). At least 10 fields were counted in the cortex and the outer stripe of the outer medulla for each slide.

**Statistical analysis**

Values are expressed as means ± SEM. Multiple comparisons were assessed by unpaired t-test. Nonparametric one-way ANOVA based on the ranks was used to analyse neutrophil infiltration data. $P$-value <0.05 was considered significant.

**Results**

**Effect of EPO on renal function during endotoxaemia**

Renal function decreased dramatically at 16 h after LPS injection ($48.1±12.4\mu l/min$ vs $212.4±25.0\mu l/min$, $n=6$, $P<0.01$). The administration of EPO (4000IU/kg) 30 min before LPS significantly improved the renal function ($136.7±30.2\mu l/min$ vs $48.1±12.4\mu l/min$, $n=6$, $P<0.05$) (Figure 1). However, there was no change in either mean arterial pressure MAP ($78.5±5.3\mu l/min$ vs $78.3±4.2\mu l/min$, $P=NS$) or RBF ($1.43±0.31\mu l/min$ vs $1.45±0.13\mu l/min$, $P=NS$) with EPO treatment during endotoxaemia.

**Fig. 1.** Effect of EPO on renal function during endotoxaemia. EPO (4000IU/kg, i.p.) was administered 30 min before LPS (2.5mg/kg, i.p.). Renal function was measured at 16 h after LPS treatment. GFR (i) was measured by inulin clearance. Data are presented as mean ± SEM.
Effect of EPO on renal SOD activity

Renal SOD activity decreased significantly at 16 h after LPS (2.5 mg/kg, i.p.) (1.30 ± 0.19 IU/mg vs 2.32 ± 0.20 IU/mg, P < 0.01) (Figure 2). This reduction was reversed by the administration of EPO (4000 U/kg, i.p.) 30 min before LPS (2.15 ± 0.23 IU/mg vs 1.30 ± 0.19 IU/mg, P < 0.05, Figure 2).

Effect of EPO serum C reactive protein (CRP) during endotoxaemia

CRP was increased during endotoxaemia (68.4 ± 3.7 vs 43.6 ± 2.1 ng/ml, P < 0.05) and EPO administration further enhanced the rise in this acute-phase reactant (98.6 ± 8.0 vs 68.4 ± 3.7 ng/ml, P < 0.01).

Effect of LPS on serum EPO concentrations

There was a modest increase in serum EPO at 16 h after LPS from 223.8 ± 27.7 to 342.9 ± 447 pg/ml, P < 0.05.

Effect of EPO on serum NO levels during endotoxaemia

Serum levels increased significantly with LPS treatment. (286.7 ± 18.0 μM, n = 7 vs 4.6 ± 0.6 μM, n = 10, P < 0.001). But there is no change in serum NO levels with EPO treatment (242.7 ± 20.2 μM, n = 8 vs 286.7 ± 18.0 μM, n = 7, P = NS).

Effect of EPO on serum TNF-α levels during endotoxaemia

Serum TNF-α levels increased significantly with LPS treatment (26.3 ± 6.1 pg/ml, n = 6 vs 3.4 ± 1.7 pg/ml, P < 0.01). Serum TNF-α levels decreased with EPO treatment but did not reach statistical significance (15.9 ± 5.3 pg/ml, n = 8 vs 26.3 ± 6 pg/ml, n = 6, P = NS).

Discussion

EPO is stimulated during hypoxia and upregulates erythroid progenitor cell proliferation and differentiation by inhibiting apoptosis [17]. In this regard, with hypoxia activation of the initiator caspases 8 and 9 as well as the executioner caspase 3 of apoptosis have been shown to be inhibited in vivo with EPO [6]. EPO administration also has been shown to decrease ischaemia-reperfusion injury in several organs [18–20] including the kidney [5,6].

However, in a previous study of endotoxaemia-related renal injury, EPO administration failed to afford renal protection [4]. The same dose of EPO nevertheless protected against renal dysfunction secondary to ischaemia-reperfusion [4]. The murine model used in the present investigation (2.5 mg/kg i.p.) has been shown to be associated with ischaemia as evidenced by a rise in plasma norepinephrine, epinephrine, and renin activity [13] and proinflammatory cytokine response [9]. A modest increase in serum EPO was observed with endotoxaemia. While previous studies in the rat have shown that LPS (0.1 or 1.0 mg/kg) administration before hypoxia (8% O2) inhibited plasma and renal mRNA of EPO [21], this effect was not observed in our mouse model with a higher dose by body weight of LPS.

Most important, renal protection, as assessed by inulin clearance, was observed during endotoxaemia with exogenous EPO administration. This is, therefore, the first demonstration that EPO can ameliorate the renal dysfunction during experimental endotoxaemia. The renal protection with EPO occurred in the absence of a change in mean arterial pressure and RBF as measured by the flow probe technique. This beneficial effect on GFR could relate to a protective effect of EPO on glomerular permeability, or a counter-balancing decrease in glomerular afferent and increase in glomerular efferent arteriole resistance as RBF was unaltered.

Histological studies did not support an anti-apoptotic role of EPO in the present studies.
Specifically, there is no evidence of apoptosis within 16 h after endotoxin administration by either morphological or TUNEL staining. These results are similar to those recently reported in septic patients with ARF [22]. While apoptosis was demonstrated in lymphocytes and intestinal epithelium, only one of the 20 patients demonstrated the presence of apoptosis in the kidney.

Oxidant injury is known to occur during endotoxaemia [23] and anti-oxidant treatment by chemically dissimilar anti-oxidants has been shown to provide protection against endotoxaemia-related renal dysfunction [24]. Moreover, EPO has been shown in haemodialysis patients to exert an anti-oxidant effect [25]. Renal anti-oxidant concentration was also affected by EPO. Specifically, during endotoxaemia renal SOD was observed to be decreased significantly, an effect which was prevented by EPO therapy. This effect of EPO on the renal anti-oxidant SOD may have been a direct effect, but an indirect effect of EPO secondary to the renal protection cannot be excluded.

There is substantial evidence for the occurrence of inflammation in the present mouse model of endotoxaemia-related ARF. TNF-α has been consistently shown to be increased in this model [9,26]. Moreover, TNF inhibitors with soluble TNF receptor and decreased production of TNF with pentoxifylline [9,26] have been shown to afford protection against endotoxaemia-related ARF. Thus, the known anti-inflammatory effect of EPO is another factor [27] along with the agent’s anti-oxidant property, which may afford renal protection in endotoxaemia-related ARF.

CRP was increased during endotoxaemia and EPO administration further enhanced the rise in this acute-phase reactant. This result is intriguing since transgenic mice expressing CRP [28] and mice receiving CRP [29] have been found to be resistant to endotoxaemia. The mechanism for this protective effect has been attributed to an increase in cytokine binding by CRP during endotoxaemia.

In conclusion, the present results are the first to demonstrate a protective effect of exogenous EPO against the renal dysfunction associated with experimental endotoxaemia. The mechanism involved in this protective effect appears to involve anti-oxidant and anti-inflammatory properties.

Conflict of interest statement. None declared.

References

EPO ameliorates renal dysfunction.


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