Recombinant human erythropoietin pretreatment alleviates renal glomerular injury induced by cardiopulmonary bypass by reducing transient receptor potential channel 6–nuclear factor of activated T-cells pathway activation

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Objective: Acute renal injury after cardiopulmonary bypass is common and associated with high mortality. We aimed to demonstrate the glomerular protective effects of recombinant human erythropoietin using an in vivo rat cardiopulmonary bypass model and to explore the possible mechanism.

Methods: Dose-related renal protective effects of recombinant human erythropoietin were studied in phase I. Male Sprague Dawley rats were randomly divided into 5 groups: sham group, cardiopulmonary bypass group, and 3 recombinant human erythropoietin–treated cardiopulmonary bypass groups (bolus doses of 500, 3000, and 5000 U/kg 24 hours before surgery). Blood and urine samples were collected just before surgery and at 2, 4, 24, 48, and 72 hours after surgery. In phase II, rats were divided into 3 groups: sham group, cardiopulmonary bypass group, and 5000 U/kg recombinant human erythropoietin group. Kidneys were harvested at 4, 24, 48, and 72 hours after surgery. Ultra-organization of glomeruli was observed. Glomerular transient receptor potential channel 6 (TRPC6) expression was studied by immunofluorescence and Western blot. Nuclei nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1) activity was analyzed by enzyme-linked immunosorbent assays and electrophoretic mobility shift assay.

Results: Pretreatment of 5000 U/kg recombinant human erythropoietin decreased the urine protein (72 hours: 7.82 ± 1.13 g/L vs 11.28 ± 1.73 g/L), serum creatinine (72 hours: 35.0 ± 3.5 μmol/L vs 60.7 ± 7.6 μmol/L), and cystatin-C (2 hours: 336.5 ± 28.2 μg/L vs 452.6 ± 63.8 μg/L) compared with the control group ($P < .01$). Cardiopulmonary bypass induced morphologic abnormalities of podocyte foot processes and slit diaphragms, which was improved by recombinant human erythropoietin. Furthermore, recombinant human erythropoietin significantly relieved glomerular TRPC6 increase and NFATc1 activation induced by cardiopulmonary bypass.

Conclusions: Pretreatment of 5000 U/kg recombinant human erythropoietin elicited potent glomerular protection against cardiopulmonary bypass. This protection may be partly due to downregulation of glomerular TRPC6-NFATc1 pathway. (J Thorac Cardiovasc Surg 2013;146:681-7)

Acute renal injury (ARI) occurs in up to 30% of patients after open surgery with cardiopulmonary bypass (CPB).[1] The development of postoperative renal dysfunction always is associated with a high mortality and accompanied by a higher incidence of gastrointestinal bleeding, respiratory infections, and sepsis.[2] Hypovolemia, ischemia–reperfusion, inflammation, and nephrotoxic factors are involved in the pathogenesis of ARI and overlap each other in leading to kidney injury.[3] Proteinuria or decline in the glomerular filter rate often is observed after CPB, especially in patients with hypertension, diabetes, or preexisting renal disease.

Erythropoietin is a hypoxia-induced hormone that is essential for normal erythropoiesis. Recent studies have shown that erythropoietin has broader actions in addition to the stimulation of erythroid precursors.[4-7] Recombinant human erythropoietin (RhEPO) was reported to have a protective effect on subsequent renal ischemia–reperfusion injury,[5] to enhance tubular epithelial regeneration, and to promote renal functional recovery in hypoxic or ischemic ARI.[6] Because rhEPO can ameliorate ischemia–reperfusion injury and the inflammatory response,[4-7] which are 2 main pathophysiologic process of ARI after CPB, it may provide renal protection against CPB. However, research concerning this is relatively limited.

Canonical transient receptor potential channel 6 (TRPC6) channel is a Ca^{2+}-selective channel that has been identified as an essential interacting protein at the renal podocyte slit diaphragm.[8] It can directly interact with the key structure and signaling proteins of podocyte,

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Abbreviations and Acronyms

ARI = acute renal injury
CPB = cardiopulmonary bypass
EMSA = electrophoretic mobility shift assay
IP = intraperitoneally
NFAT = nuclear factor of activated T cell
NFATc1 = nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
PBS = phosphate-buffered saline
RhEPO = recombinant human erythropoietin
TRPC6 = canonical transient receptor potential channel 6

podocin, and nephrin.[8] Many studies have implicated that TRPC6 has crucial roles in hereditary glomerular dysfunction and acquired glomerular diseases.[9-12] Chu and colleagues[13] recently demonstrated modulation of calcium influx through a certain subtype of TRPC channels by erythropoietin. Nuclear factor of activated T cell (NFAT) has been described as one of the most important signals downstream of TRPC6 activation. The link between TRPC6 and the calcineurin-NFAT pathway has been established in some types of cells, including cardiac myocytes and renal podocyte.[14,15] NFAT activation was considered a key intermediate step in the pathogenesis of mutant TRPC6-mediated focal segmental glomerulosclerosis, and suppression of NFAT activity may contribute to the antiproteinuric effects of calcineurin inhibitors.[16] The present study (1) explores the dose-related renal protective effects of rhEPO against glomerular injury after CPB in a rat in vivo model, (2) determines the expression of glomerular TRPC6 after CPB and regulation of this channel by rhEPO, and (3) evaluates NFATc1 activity after CPB and the effects of rhEPO on this transcription factor.

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MATERIALS AND METHODS

Animals and Drugs

Adult male Sprague Dawley rats (body weight, 400 ± 50 g) were purchased from the Animal Center of the Chinese Academy of Science, Shanghai, and randomly divided into experimental groups. All procedures were performed in accordance with *The Guide for the Care and Use of Laboratory Animals* from the National Institutes of Health (Publication No. 85-23, revised 1996). The rats were acclimated in a quarantine room and maintained on a standard pellet diet at the Animal Center of Shanghai Jiaotong University for 10 days before surgery. The following experimental protocol was approved by the Shanghai Jiaotong University Animal Care and Use Committee. The rhEPO was donated from Shenyang Sunshine Pharmaceutical Co, Ltd (Shenyang, China).

Experimental Protocol

The study consisted of 2 phases. In phase 1, a dose-effect relationship was studied and the most effective dosage was determined. Rats were randomly assigned to 5 experimental groups: (1) sham group (S group, n = 6), (2) CPB group (C group, n = 6), (3) rhEPO (500 U/kg, intraperitoneally [IP]) group (E1 group, n = 6), (4) rhEPO (3000 U/kg, IP) group (E2 group, n = 6), and (5) rhEPO (5000 U/kg, IP) group (E3 group, n = 6). In phase II, another 3 groups were enrolled: (1) sham group (S group, n = 30), (2) CPB group (C group, n = 30), and (3) rhEPO group (the most effective dosage according to phase I, n = 30). In the sham and CPB groups, a 0.9% saline solution (4 mL/kg, IP) was given 24 hours before the operation protocol. In all rhEPO groups, different doses of rhEPO were diluted in 4 mL/kg saline solution and injected IP 24 hours before the surgery protocol.

Surgical Procedure

The rat model of CPB was built according to Zhu and colleagues.[17] Rats were anesthetized with IP administration of pentobarbital (50 mg/kg) at the beginning. Additional pentobarbital was added to ensure an adequate depth of anesthesia during surgery. The right femoral artery was cannulated with a 24-gauge polytetrafluoroethylene catheter to monitor arterial pressure and collect blood samples. After administration of heparin (250 U/kg), a 16-gauge catheter, modified to a multiside-orifices cannula in the forepart, was inserted into the right jugular vein and advanced to the right atrium. A 22-gauge catheter was cannulated to the tail artery to serve as the arterial infusion line. The mini-CPB circuit comprised a venous reservoir, a specially designed membrane oxygenator, a roller pump, and sterile polyvinyl chloride tubing with an internal diameter of 3 mm for the venous and arterial lines (30 cm long). The roller pump was equipped with a silicone tube 15 cm in length with an internal diameter of 5 mm. The membrane oxygenator was specially designed with a surface area for gas exchange of 0.05 m^2 (Micro-1; Kewei Medical Instrument Inc, Dongguan, China), with its total assembly dynamic priming volume approximating 2 mL. Body central temperature was monitored with a rectal probe and kept at 36.5°C to 38.0°C by a heat lamp placed around the animal and the CPB equipment. We primed the CPB circuit with 12 mL of a solution of heparin (250 U/kg) and hetastarch. Before the initiation of extracorporeal circulation, the CPB set was examined carefully to avoid liquid and air leaks. The blood was drained from the right atrium through the jugular vein catheter to a 5-mL sterile open reservoir by gravity and siphon. A roller pump (BT00-300M; Lange Co, Baoding, China) was used to drive the blood through silicone arterial inflow tubing and then return it to the tail artery. At the initiation of perfusion, the flow rate was gradually increased to 100 mL/(kg · min) and maintained for 60 minutes; it was then turned down step by step to maintain hemodynamic stability. When the rat was weaned from CPB, the tail artery catheter was removed, and the right jugular vein catheter was drawn back to the superior vena cava. The remaining priming solution was infused gradually when the main arterial pressure was less than 60 mm Hg. After 1 hour of intensive postoperative care, the right jugular vein catheter and the femoral artery catheter were decannulated. Then, the neck, tail, and groin incisions were sutured. Throughout the experiment, the mean arterial pressure was maintained at approximately 60 to 80 mm Hg. The rats were given water and food 6 hours after the operation, and they were monitored for 24 postoperative hours.

Phase I: Specimen Collection

Mean arterial pressures were recorded during the experiment. Blood samples (1 mL) were obtained from the femoral artery immediately after heparinization and at 2, 4, 24, 48, and 72 hours after operation for serum creatinine and cystatin-C measurements. The urine also was collected for protein content determination at these time points.

Biochemical Index Measurement

Serum creatinine and cystatin C were quantified by enzyme-linked immunosorbent assays (WinhongBio, Shanghai, China) according to the manufacturer's instruction. Serum creatinine and cystatin C values were expressed as micromoles/liter and micrograms/liter separately. Urine