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ORIGINAL ARTICLE

The administration of erythropoietin attenuates kidney injury induced by ischemia/reperfusion with increased activation of Wnt/β-catenin signaling

Xiao Chen, Cen-Cen Wang, Shu-Min Song, Shi-Yao Wei, Jian-Si Li, Shi-Lei Zhao, Bing Li*

Department of Nephrology, Second Affiliated Hospital, Harbin Medical University, Harbin, China

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Background/purpose: Understanding the mechanisms of protecting the kidneys from injury is of great importance because there are no effective therapies that promote repair and the kidneys frequently do not repair adequately. Evidence has shown that erythropoietin (EPO) has a vital renoprotective role, independent of its erythropoietic effect. However, whether EPO can contribute to kidney repair after injury and the potential mechanisms are not fully understood.

Methods: To investigate the renoprotective mechanism of EPO, a kidney ischemia/reperfusion injury (IRI) model was induced in adult male Sprague–Dawley rats. The rats were subsequently randomly treated with EPO or a vehicle 6 hours after the kidney IRI. The rats were sacrificed on Day 3, Day 5, and Day 7 post kidney IRI. Renal function and histological alterations were examined. Renal interstitial macrophage infiltration, cell proliferation, apoptosis, and angiogenesis were evaluated by immunostaining. Furthermore, the effects of EPO on the Wnt/β-catenin pathway and IRI-related micro-RNAs were investigated.

Results: The administration of EPO significantly improved renal function and reduced tubular injury. Furthermore, EPO treatment significantly prevented tubular cell apoptosis and promoted cell proliferation after IRI. Erythropoietin significantly suppressed macrophage infiltration, compared to the vehicle. In addition, treatment with EPO markedly prevented the loss of microvasculature. We have also demonstrated that, compared to the vehicle, EPO administration enhanced the expression of Wnt7b and β-catenin, and downregulated miR-21, -214, -210, and -199a.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Department of Nephrology, Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang District, Harbin, 150086, China.
E-mail address: icecreamlee@hotmail.com (B. Li).

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Conclusion: Erythropoietin protects the kidneys against IRI by attenuating injury of the renal microvasculature and tubule epithelial cells, by promoting Wnt/β-catenin pathway activation, and by regulating miRNA expression.

Introduction

Renal ischemia/reperfusion injury (IRI) is a common cause of acute kidney injury (AKI).[1,2] It results from a sudden transient drop in total or regional blood flow to the kidneys.[3] Despite advances in preventative strategies and support measures, this disease is associated with significant morbidity and mortality.[2] Many treatment strategies have been proposed to improve IRI, but few are effective.[3]

Erythropoietin (EPO) is a hematopoietic hormone produced primarily by the adult kidneys.[4] It is a multifunctional cytokine involved in a variety of processes in the kidneys, liver, and brain that are independent of erythropoiesis. Evidence has demonstrated that EPO has cytoprotective effects, which mediate antioxidative stress, antiapoptosis/necrosis, and anti-inflammatory responses,[5,6] and immunomodulation of autoimmune diseases.[7] Erythropoietin may be an efficient renoprotective agent against renal dysfunction and injury. However, the precise mechanisms of EPO involved in kidney IRI have not yet been fully elucidated.

The term "acute tubular necrosis" is used to designate AKI resulting from characteristic pathologic damage to the tubules. The improvement of renal function after kidney IRI primarily depends on the repair and regeneration of injured tubular epithelial cells.[8,9] In particular, IRI causes sterile inflammation, which increases the production of inflammatory cytokines and infiltration of neutrophils and macrophages.[10] Peritubular capillary (PTC) loss is also positively associated with tubular damage in the kidneys in ischemic AKI and in the clinical biopsy.[11,12] Our previous studies also suggested that PTC loss because of ischemia is directly correlated with impaired renal function and the development of renal fibrosis.[13,14] Promoting the repair and regeneration of injured kidney vasculature successfully reverses these pathological changes.[14,15]

Wnt signaling was originally identified as involved in the developmental fate decision and neoplasia. The canonical Wnt pathway can drive cell proliferation and maintain the dedifferentiated state of cells. It also has a critical role in kidney repair and regeneration after IRI.[16] Reparative macrophages may secrete Wnt ligands, which interact with Wnt-expressing receptors on the epithelial cell surface to repair the injured kidney.[13] Micro-RNAs (miRNAs) are endogenous noncoding RNAs that posttranscriptionally regulate gene expression. Research studies have shown that miRNAs are involved in various different biological processes such as hypoxia, differentiation, inflammation, cell proliferation, cell death, and fibrosis in kidney disease,[17,18] and in kidney IRI.[19] Whether EPO can regulate miRNAs in kidney IRI is unknown.

In this study, we aimed to reveal the multifaceted renoprotective roles of EPO in kidney IRI from various respects such as tubular cell apoptosis; macrophage infiltration; the loss of microvasculature; and the expression of Wnts, β-catenins, and IRI-related miRNAs such as miR-21, -199a, -210, and -214. Such efforts may provide better understanding of the renal-friendly benefits of EPO.

Materials and methods

Animals

Male Sprague—Dawley (SD) rats weighing 200—250 g were purchased from the Second Affiliated Hospital of Harbin Medical University Laboratories (Harbin, China). All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All rats were maintained in filter top cages and received sterilized food and acidified water. Experimental protocols were approved by the animal committee of Harbin Medical University (Harbin, China).

Animal model

Male SD rats (200—250 g) were subjected to kidney IRI by methods described previously.[13,14] The warm ischemic time was 45 minutes (i.e., the unilateral model) or 40 minutes (i.e., the bilateral model). All male SD rats were randomly divided into three groups: the sham group, the EPO group, and the vehicle group. Six hours after inducing unilateral IRI, the unilateral model rats intraperitoneally received 2000 IU/kg EPO (EPO group; $n = 24$; rhEPO; Roche, Mannheim, Germany) or received the vehicle (vehicle group; $n = 24$). The sham-operated rats, which underwent the same surgical procedure but without the placement of the vascular clamp, served as controls (i.e., sham group). The rats were sacrificed on Day 3, Day 5, and Day 7 after the operation ($n = 8$ rats per time point).

Six hours after the IRI, the rats with bilateral IRI also received rhEPO (Roche; i.e., EPO group) or the vehicle (i.e., vehicle group). Plasma samples were drawn from the tail vein on Day 1, Day 3, and Day 7 after injury to analyze the creatinine level using previously described methods.[13,14] The bilateral model rats were sacrificed on Day 7 ($n = 8$ rats per group).

Tissue preparation, immunostaining, imaging, and quantification of injury and repair

Rats were perfused with ice cold normal saline, and then preserved in opti-mumcutting temperature compound (OCT) (−80°C) and paraffin by using previously described methods.[13,14] The paraffin sections (3 μm) were processed