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Erythropoietin a promising agent in the prevention of renal ischemia reperfusion injury

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Abstract

Erythropoietin (EPO) is an essential growth factor, which stimulates red blood cell formation by targeting hematopoietic colony forming unit (CFU-E) in the bone marrow. Erythropoietin stimulating agents (ESAs) such as recombinant human EPO (rHuEPO) are created by recombinant DNA technology in cell culture which is widely used for the treatment of anemia in chronic kidney disease (CKD). The potential protective effects of EPO against ischemia reperfusion (IR) injury have become manifest in kidney IR injury model and kidney replacement therapy. In our review, we discuss the evidence upholding EPO as essential agent in kidney IR injury and discuss the potential pathways by which it may confer this specific protection.

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Introduction

Renal ischemia reperfusion (IR) injury, an inflammatory pathophysiological process, leads to acute renal failure (ARF), delayed graft function, and early mortality in patients subjected to kidney replacement therapy (1). Acute kidney injury (AKI) is the common term that refers to a sudden decrease in renal function, resulting in loss the ability to eliminate excess salts, fluids and waste products from the blood (2). The occurrence of AKI in hospitalized patients is 2%-7% and in intensive care units (ICUs) it is more than 10%. The mortality and morbidity rate are high; in the ICUs over half of the patients subjected to renal transplantation die and patients that survive have a great risk of evolving chronic kidney disease (CKD) (3). The most common reasons of AKI is IR injury. Ischemic injury results from impairment of oxygen (O2) and nutrient supply to the kidneys, as well as from accumulation of metabolic waste products. This, in turn, leads to acute tubular and endothelial cell death by apoptosis and necrosis. The initial injury is further worsened through the subsequent reperfusion period, inflammation, damage by oxidative stress, endothelial cell injury and vascular dysfunction (4).

Material and Methods

For this review, we used a variety of sources by searching through Web of Science, PubMed, EMBASE, Scopus and Directory of Open Access Journal (DOAJ). The search was performed using combinations of the following key words and or their equivalents such as erythropoietin stimulating agents, recombinant human erythropoietin, protective, ameliorative effects and renal ischemia reperfusion injury.

Core tip

In recent years, several studies have shown that EPO diminishes tissue injury in many organs including kidneys, heart and brain by anti-apoptotic, anti-oxidative and pro-angiogenic effects. It has also anti-inflammatory actions which supports its essential protective role in renal IR injury. As a result of these activities EPO may have an important role in the future of medical sciences.

Results and Discussion

Erythropoietin (EPO) is a growth factor that was primarily known as the main regulator of erythrocyte production or erythropoiesis. However, growing evidences indicate that EPO has distinct roles independent of its actions in regulation of erythrocytes production. The favorable roles of the EPO-mediated protective effects in many tissues are not fully understood but it is demonstrated that it has anti-oxidative and

anti-apoptotic effects. It has also pro-angiogenic actions which seems to be linked to EPO-mediated cytoprotective effects. The biological actions of EPO are exerted by binding and activating it is a high affinity specific receptor (EPO receptor - EPOR). The existence of EPOR in renal tubular and mesangial cells has indicated a crucial role for EPO in the renal function. Furthermore, in recent in vivo animal studies subjected to kidney injury by exposure to cisplatin or by IR injury, EPO reinforced functional and morphologic tissue recovery; fundamentally via its anti-apoptotic effects (5). EPO has been shown to protect different organs including brain, heart, and kidney against IR injury (6). It mediates preservative roles by regulating several signaling pathways, which comprise mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt (7). Recently, additional non-erythropoietic protective roles for EPO have been reported. In the present review article, we discuss the evidence supporting EPO as an essential agent in renal IR injury and discuss the potential pathways by which it may confer this specific protection.

EPO molecule structure

EPO is a glycoprotein signaling molecule composing of 165 amino acids. EPO molecule contains four oligosaccharide side bonds and comprises up to 14 sialic acid residues. Carbohydrate part of EPO molecule contributes to 40% of its molecular weight (8). The N-linked polysaccharide portions of EPO molecule appears to have crucial role in the biosynthesis and secretion of EPO, enhances its steadiness in the blood, and limits liver metabolic excretion, thence providing the transport of EPO molecule in the blood from kidney to targeting cells of the bone marrow (9).

The position of the sialic acid portion in the EPO molecule forms different EPO isoforms with variations in actions. When the amount of sialic acid molecules on the carbohydrate part of EPO molecule raises, its serum half-life increases, whereas receptor-interacting capacity reduces (10,11). However, clearance from the body seems to have a potent impact on in vivo activity than receptor-interacting affinity. There are two receptors (EPOR) binding sites for each EPO molecule structure. EPOR has also two affinities in solution for EPO molecule: first one of high and second of low affinity (needs 1000 times the concentration of EPO to be activated) (12).

Production of EPO

EPO is particularly produced by cortical interstitial fibroblast cells in the kidneys in close association with peritubular capillary and proximal convoluted tubule and plays important role in a classical negative feedback mechanism (13). Low blood oxygen level is the main physiological stimulus that produces an expeditious rise in renal releasing of EPO by an exponential increase in the number of cortical interstitial fibroblast cells (14). Furthermore, EPO is also produced in lower amounts by the brain, liver, uterus, testis and mammary glands.

EPO receptor structure

The EPOR is a protein that in humans is expressed by EPOR gene. It has carbohydrate portion typically composing of 484 amino acids and 2 peptide residues; it is largely related to growth factor and cytokine receptor family (11). Binding study has revealed that EPOR has high affinities for EPO molecule and that EPOR with higher affinity for EPO may be in charge for the erythropoietic action of EPO, whereas EPO receptor with a lower affinity may have responsibility for non-erythropoietic effects, such as tissue preservation (15).

The EPO receptor's cytoplasmic portions consists of phosphotyrosine protein domains that are autophosphorylated by stimulation of a constituent of the Janus-protein tyrosine kinase family (JAK2), which is associated to the beta subunit of the EPOR (16). In addition to stimulating the MAPK, phosphatidylinositol 3-kinase (PI3K), and serine/threonine-specific protein kinase (protein kinase B (Akt)) signaling pathways, phosphotyrosine protein domains also act as anchoring scenes for signal transducer and substances of transcription (STATs), like STAT5. Phosphatase stimulation results in dephosphorylation of JAK and then incorporation and dissolution of the EPO and EPOR complex, which indicates the termination of EPO activity. This avoids over-activation, which may result in extravagant production of erythrocytes (17).

EPO was first brought to light for its controlling role on the production of erythrocytes. It encourages proliferation and inhibits degeneration of erythroid progenitor cells through binding to EPO receptor complex composing of second EPOR (18). Nevertheless, recently, EPO manifested to have further distinguished cytoprotective effects. It has a crucial role in diminishing local inflammation and tissue damage in many organs. These ameliorative effects are not related to interaction of EPO molecule with the EPOR2 complex, but by activating a distinctive tissue protective receptor complex (19). Immunologic studies showed that the EPOR has ability to compose a heteromeric receptor complex (EPOR2-βCR2) with the β common receptor (βCR). Nevertheless, interacting of EPO molecule with this receptor complex is indicated to encourage the tissue cytoprotective effect of EPO (19).

The cytoprotective signalling pathways of EPO have been reported in different animal models. Interacting of EPO molecule to the EPOR2-βCR2 complex results in phosphorylation of JAK2 (20). This, leads to stimulation of two main signalling pathways: signal transducer and substance of transcription-5 and phosphatidylinositol 3-kinase/AKT (PI3K/AKT). These pathways enhance regeneration; inhibit apoptosis and inflammation (6). Activation of PI3K/AKT pathway produces increment in regional blood flow, which mediated by activating endothelial nitric oxide synthase (eNOS) (21).

The ameliorative effects of EPO have been tested in renal IR injury in animal models. EPO is capable to raise phosphorylation of ameliorative signaling pathways such as JAK2, PI3K/AKT and eNOS coming after kidney IR

injury (20,22). Several studies have shown that EPO pretreatment to ischemia as well as post-reperfusion is capable to ameliorate kidney IR injury (15,22). EPO treatment improves renal function; and also inhibits inflammatory response and apoptosis in IR injury models as well as reducing inflammatory indices such as interleukin 6 (IL-6) and tumor necrosis factor (TNF-alpha) (23,24). Treatment with EPO inhibits apoptosis and necrosis following renal IR injury, which leads to improvement in renal morphology (22,23). In addition, EPO is also reduced the activity of TGF-β, suggestive diminished developing of fibrosis (25).

EPO is a cytokine which may be implicated in the amendment of cytosolic calcium metabolism by raising calcium entrance to the cells (26). Nuclear factor-kappa B (NF-kB), a cytokine and factor of inflammation, is imposed in EPO signaling cascades. The protective roles of EPO mainly rely on AKt and the following NF-kB stimulation (Figure 1). NF-kB plays a crucial role in the secretion of EPO via HIF-1 activation. AKt is able to raise NF-KB and HIF-1 stimulation escorted by elevating in release and expression of EPO (27).

The cytoplasmic domain of the EPOR comprises phosphotyrosine protein domains, which are phosphorylated by stimulation of Janus-type protein tyrosine kinase (JAK2) adhered to the EPO receptor. These phosphotyrosine protein domains act as anchoring positions for signal transducer and activator of transcription 5 (STAT5) and stimulate the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and AKt phosphorylated endothelial nitric oxide synthase (eNOS). IκB kinase (IKK) is phosphorylated by Akt activation, which phosphorylates the inhibitory IkB protein causing its detachment from NF-kB, which results in its activation (28).

Nitric oxide production by NOS activity is a physiologic controller of kidney function and crucial factor of glomerular haemodynamics (29). The direct protective role of EPO on kidney function (30,31) is clarified by enhanced expression of eNOS. Subsequent to kidney IR injury, eNOS expression is diminished at 6 hours post-reperfusion and returns back to normal expression after 24 hours (32). The cytoprotective effect of EPO on kidney is perhaps produced by raised eNOS gene expression. This indicates that enhancing eNOS gene expression by EPO treatment is most effective in the first 6 hours post-reperfusion. There is evidence that EPO stimulates the phosphorylation of serine residues on eNOS, resulting in its activation (33). EPO mediates preservative roles on the kidney by regulating a variety of signaling pathways, which comprise MAPKs and phosphatidylinositol 3-kinase (PI3K)/Akt (7). Yao and colleagues documented that PI3K/Akt signaling pathway activation has ameliorative effects on renal IR injury, as stimulated Akt mediated increases in the expression of eNOS gene and the production of nitric oxide (NO) in the endothelial cells (34). Recently, we demonstrated that up-regulation of eNOS and iNOS mediate the protective effect of EPO

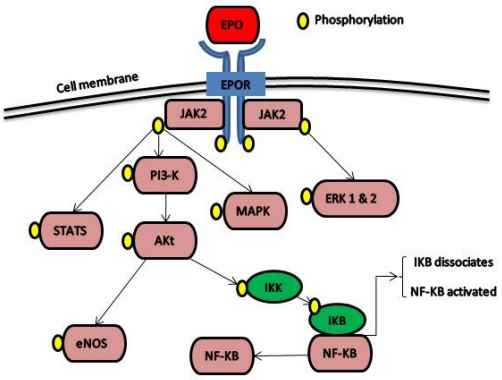

Figure 1. The signaling cascades of EPO actions.

alone or in combination with ischemic preconditioning in renal IR injury (35).

EPO and AKI

Several studies in animal models have demonstrated that erythropoietin stimulating agents (ESAs) treatment preserves renal tissues from injury and ameliorates kidney function in models of AKI induced by IR injury (22, 36) in which EPO treatment improved kidney function and tissue damage by reducing apoptosis. Furthermore, EPO was reported to decrease the levels of pro-inflammatory markers, such as IL-2 and TNF-alpha during renal IRI and invert the influence of endotoxin on the kidney superoxide dismutase (SOD) activity. These anti-inflammatory effects of EPO also indicate the participation of the NF-kB signaling pathway in its kidney preservation. In previous study, we reported the ameliorative role of EPO and IPC individually on kidney IRI in rats. In that study both treatments have been able to improve kidney function and reduce oxidative stress in the renal tissues (37). EPO pretreatment has attenuated the renal IR injury by suppressing inflammation, which was related to stimulating PI3K/Akt signalling pathway via EPOR stimulation (38). EPO protects the kidney tissues against IR injury by reducing kidney microvasculature damage and tubular epithelial cells injury. Encouraging Wnt/β-catenin signaling pathway stimulation and regulating its miRNA gene are involved in this protection (39). Ates et al demonstrated that treatment with EPO (1000 IU/kg) might reduce oxidative stress in kidney tissue and this influence is related to tyrosine kinase receptor stimulation (40). EPO treatment markedly decreased the MDA content in kidney tissues manifesting that it has a crucial role in the reduction of lipid peroxidation by several mechanisms such as enhancing the activity of antioxidant enzymes (41).

EPO treatment activates eNOS which influences directly on the endothelial cells and may be decisive for cytoprotective roles of EPO on the renal tissue. This molecule is an exceedingly effective activator for progenitor cells of endothelium, whose role is partially relying on NO bioavailability. Progenitor cells of endothelium seem to be needed in endothelial improvement after

the microvasculature damage. EPO administration diminishes ARI in part by activating vascular reform and by mobilizing progenitor cells of endothelium and enhancing tubular epithelial cells proliferation. These results propose that EPO may exert a preservative role through the interaction with the microvasculature (42).

The EPO's renoprotective roles and angiogenesis may be regulated by vascular endothelial growth factor (VEGF). In the last few years, study by Nakano et al documented that the vascular EPO/EPOR complex in blood vessels increased post-ischemic angiogenesis by up-regulating the VEGF/VEGF receptor complex, both immediately by enhancing development of new blood vessels and indirectly by mobilizing progenitor cells of endothelium and the pro-angiogenic cells in the bone marrow. It seems that angiogenesis is damaged endothelial cells are less reacting to VEGF in the lack of EPO receptor (43).

Evidence indicates that treatment with EPO particularly prevents the cell damage by activating the signaling pathways via a non-erythropoietic receptor. EPO receptor is expressed in many tissues such as kidneys, liver, brain and heart (6). Janus kinase 2 phosphorylation after the EPO receptor activation, results in activating of multiple signaling cascades such as PI3K, MAPK and NF–κB (44). Furthermore, PI3K/Akt signaling pathway activation inhibits apoptosis as well as enabling the tissues to manage cell survival and proliferation.

Conclusion

This review manifests that EPO might be a promising agent in the prevention of kidney IR injury. The protective roles of EPO and understanding of EPOR signaling cascade stimulation in the kidneys are crucial to future growing of novel clinical application of EPO.

Authors' contribution

ME searched and gathered the related articles as well as writing. BS prepared the draft. MK edited the final manuscript several times. All authors read and signed the final paper.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical consideration

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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