Beyond hematopoietic property; administration of erythropoietin for nephroprotection

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Acute kidney injury (AKI) is defined as an abrupt or rapid decline in renal filtration function. Erythropoietin (EPO) as a hematopoietic and multifunctional hormone is produced primarily by kidney. Many investigations have shown that EPO as an antioxidant agent has shown several effects such as anti-apoptotic, antioxidant, and anti-inflammatory and also angiogenic activities. The biological activities of EPO are mediated by binding to its receptor (EPOR). The potential role of EPO in kidney is related to the presence of functional EPOR in renal mesangial cells, tubular epithelial cells and the glomerulus. Antioxidants and reactive oxygen species (ROS) scavengers such as EPO, can protect the kidneys against conditions that induce nephrotoxicity. Most studies in the field of renoprotective effects of EPO have focused on AKI models. In this paper we sought to review the ameliorative effects of EPO against various agents or conditions that induce nephrotoxicity including ischemia/reperfusion injury (IRI), cisplatin, gentamicin, rhabdomyolysis, amikacin and vancomycin.

Introduction

Erythropoietin (EPO) is a glycoprotein and cytoprotective multifunctional hormone. More than 90% of systemic EPO is produced primarily by peritubular interstitial fibroblasts in renal cortex and outer medulla of the kidney in adults and is secreted in order to increase the rate of production of red blood cells in the bone marrow by stimulating the proliferation of erythroid progenitors and precursors, in response to hypoxia in the renal tissues (1,2). Numerous studies have shown several effects of EPO including anti-apoptotic, antioxidant, and anti-inflammatory and also angiogenic activities (1). Acute kidney injury (AKI) is characterized by a rapid decline in renal function, electrolyte and fluid imbalance and generally classified as pre-renal, intrinsic, and post-renal dysfunction. Furthermore, it develops rapidly over a few hours or a few days (3). Numerous experimental investigations have attempted to explain the ameliorative
effects of EPO as a nephron-protective drug in many AKI models. The biological activities of EPO are mediated by binding to its EPO receptor (EPOR) and the potential role of EPO in the kidney is related to the presence of functional EPOR in renal mesangial and tubular epithelial cells. Ischemia and reperfusion causes serious damages and disturbances to tissues and organs. Renal ischemia is the most common cause of AKI and serious problem during partial nephrectomy, cardiopulmonary bypass, kidney transplantation and hydronephrosis, and leads to renal injury. Antioxidants and reactive oxygen species (ROS) scavengers such as EPO, can protect the kidneys against ischemia/reperfusion injury (IRI) (4). Rhabdomyolysis as one of the causes of AKI, is a serious syndrome due to a direct or indirect muscle injury and EPO is effective for amelioration of rhabdomyolysis-induced AKI (5,6). Cisplatin as an anticancer drug (7), gentamicin and amikacin as aminoglycoside antibiotics (8,9) and vancomycin as a bacterial antibiotic (10) can induce nephrotoxicity or AKI. Many studies have shown the ameliorative effects of EPO against this agents-induced nephrotoxicity. This paper will focus on renoprotective impact of EPO in models of AKI including renal IRI, nephrotoxicity induced by cisplatin, gentamicin, rhabdomyolysis, amikacin and vancomycin, as well as reviewing the recent researches about structure of EPO molecule and EPOR and antioxidant properties of EPO. The aim of this paper is to provide a base for investigations and therapeutic impact of EPO in humans with AKI.

Materials and Methods
For this review we used a variety of sources by searching through PubMed, Embase, Scopus and directory of open access journals (DOAJ). The search was performed by using combinations of the following key words and or their equivalents; erythropoietin, acute kidney injury, ischemia/ reperfusion injury, antioxidant, nephrotoxicity. Manuscripts published in English as full-text articles and or as abstracts were included to the study.

The structure of EPO molecule and EPO receptor
EPO is a glycoprotein hormone with molecular weight of 34 kDa and class I cytokine, which is composed of 165 amino acids and 4 acidic oligosaccharide side chains (11). They are connected to core polypeptide by 1 O-linked glycosylation on 1 serine residue and 3 N-linked glycosylation on 3 asparagine residues. The N-linked polysaccharide side chains cause the simplification in systemic transit of EPO from kidney to bone marrow via enhancing the biosynthesis and secretion of EPO in blood and limiting hepatic clearance. EPO needs a receptor for recognition and decoding into intracellular signaling cascades, to exert its biological effects (11,12). Additionally, each EPO molecule has two binding sites for EPOR and it should act in cells with EPOR and all its physiological actions seems to be mediated by EPOR as a homodimer on the cell membrane. EPOR is a membrane glycoprotein with molecular weight of 66 kD, which is composed of two peptide chains and 484 amino acids. Moreover, EPOR belongs to a cytokine and growth factor receptor family and EPO binds to two EPORs (12). EPOR isoforms have two affinities for EPO including high and low affinities. Higher affinity for EPO binding may have erythropoietic effects, whereas lower affinity for EPO may be responsible for non-erythropoietic effects such as protecting the tissue (11,12).

Antioxidant properties of EPO
Oxidative stress (OS) is described as an imbalance between the production of free radicals like hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^\cdot$), superoxide anion (O$_2$$\cdot^-$) and peroxyl radicals (LOO$^\cdot$) and capability of the body to neutralize or detoxify their harmful effects via neutralization by antioxidants. Antioxidants are molecules in the cells, which inhibit these reactions through donating an electron to the free radicals. EPO can protect cells via reducing OS, as one of the most important causes of cellular damage. Many investigations have detected the direct and indirect anti-oxidative impacts of EPO (11). In direct anti-oxidative pathway, EPO can increase antioxidative enzymes such as glutathione peroxidase, catalase and superoxide dismutase and enhance cellular antioxidant capacity. Besides, upregulation of heme oxygenase-1 (HO-1) is one of the most significant mechanisms involved in direct anti-oxidative pathway. Many investigations reported the direct antioxidant effects of EPO via direct action inducing HO-1 expression (11,12). One study on cultured renal endothelial cells showed that EPO was able to diminish intracellular-OS, to decrease the OS-induced cell death and to provide cytoprotection against H$_2$O$_2$, through increased HO-1 expression. Moreover, in an in vivo study, administration of EPO to Dahl salt-sensitive rats was effective for reducing proteinuria and renal injury. Also this renoprotective effect of EPO was attributed to up-regulation of HO-1 in kidney, which provided cytoprotection against OS (12). Treatment with EPO increased the expression of monocyte HO-1 mRNA and induced HO activity in astrocytes and cultured human bone marrow erythroid progenitor cells. In indirect anti-oxidative pathway, EPO can inhibit iron-dependent oxidative damage through indirectly depleting body iron. Furthermore, EPO indirectly reduces cellular OS by increasing young red blood cells, which can help to ameliorate iron-dependent oxidative damage (11).

EPO in renal IRI
The effect of EPO on renal IRI as one of the most common causes of AKI, was extensively investigated among many AKI models. Many studies suggested that administration of exogenous EPO was effective for reducing ischemia-
induced kidney damage, through antioxidant, anti-inflammatory and anti-apoptotic mechanisms. Ischemia followed by reperfusion initiates changes leading to damages in the functions of renal tubular epithelial cells, vascular endothelial cells and immune system homeostasis in kidney (13). Various factors play serious roles in the development of renal IRI such as generation of OH⁻, O₂⁻ as species of reactive oxygen and also peroxynitrite (OONO⁻) and nitric oxide (NO) as species of reactive nitrogen species and decrease of antioxidant defense. Besides, several investigations revealed that lipid peroxidation is a free radical generating system that is closely related to renal IRI, and malondialdehyde is an index of the degree of lipid peroxidation (3,4). To find the impact of EPO on IRI, Ahmadiasl et al, conducted an experimental study on rats. They detected, administration of EPO (5000 U/kg, intraperitoneal injection; i.p.) as an antioxidant agent, 20 minutes before ischemia increased superoxide dismutase and glutathione peroxidase. Likewise, they detected a decrease in urea level and malondialdehyde too (4). Moreover, administration of EPO (5000 U/kg, i.p.) 10 minutes before ischemia showed the same results. They found, EPO is able to decrease serum creatinine following an increase in total antioxidant capacity level (3).

EPO is an anti-inflammation agent for renal IRI (14). Transcription nuclear factor κB (NF-κB) probably plays an essential role in the pathophysiology of renal IRI and might have an important role in EPO-mediated protective effects (15). According to an experimental study in rats this effect of EPO was mediated by inhibiting the nuclear translocation of NF-κB signaling pathway following renal IRI and decreasing the gene expression of pro-inflammatory cytokines including IL-1b, IL-6, IL-10 and TNF-a, and also chemokine. In this study treatment of the rats with 2000 U/kg, 30 minutes before ischemia was effective in decreasing the myeloperoxidase positive cells, serum creatinine and urea nitrogen, which increased during the renal IRI (14). Other study by Spandou et al showed the ameliorative effect of EPO (300 U/kg, i.p.) 20 minutes before ischemia in renal IRI rat model. In this study treatment with EPO as a single dose before the onset of ischemia, revealed a significant decrease in serum urea and creatinine. Also down-regulation of Bax as an important indicator of apoptosis, in renal tubular epithelial cells and decreased expression of NF-κB in the EPO-treated rats was observed (15).

Administration of EPO (2000 IU/kg) 6 hours after the renal IRI, improved renal function, attenuated pathological alteration, macrophage infiltration and peritubular capillary loss, reduced tubular cell apoptosis and increased cell proliferation after renal IRI in rats. The pathological changes, which attenuated in EPO treatment group, were included accumulation of necrotic materials within the lumen, degeneration and necrosis of tubular epithelial cell, swelling and deformation of renal tubule and infiltration of inflammatory cell. MicroRNAs have a significant role in regulating cell survival, hypoxia, apoptosis and inflammation related to renal IRI. EPO administration down-regulated miR-21, miR-214, miR-210 and miR-199a and increased the expression of b-catenin and Wnt7b mRNA (16). Sharples et al detected the protective effects of EPO (300 U/kg) against kidney injury and dysfunction caused by IR in rats. They demonstrated, a single systemic injection of recombinant human EPO (rhEPO), either pre-ischemia or before the onset of reperfusion reduced tubular injury, glomerular dysfunction, DNA fragmentation and apoptotic cell death through Janus kinase 2 (JAK2) signaling and the phosphorylation of protein kinase B (Akt) by phosphatidylinositol 3-kinase (PI3K). Accordingly, rhEPO prevented the activation of caspase 3, 8, and 9 (17).

EPO and nephrotoxicity induced by cisplatin

Cisplatin is a cytotoxic drug administered for cancer chemotherapy. However, it has several side effects such as nephrotoxicity or AKI. Furthermore, the clinical administration of cisplatin is limited due to the increase of dose-dependent nephrotoxicity in about thirty percent of patients (18). In an experimental study by Mohamed et al, 60 rats were divided into three groups including control group (normal saline), cisplatin group (9.0 mg/kg) and EPO/cisplatin group (100 IU/kg/d). In EPO treatment group, rats received rhEPO for 2 weeks. In this study, EPO showed protective effects against damages, which were induced by cisplatin including a significant increase in the elevated oxidative and nitrosative stress markers and tubular cell damage such as tubular cell atrophy and glomerulosclerosis and also cystic dilatation of the most renal tubules. They also found increment of serum creatinine level, serum blood urea nitrogen, malondialdehyde, expression of HO-1, vascular endothelial growth factor and inducible NO synthase and down regulation of anti-apoptotic protein Bcl2 in most tubular cells. All these abnormalities were reversed by EPO administration (7).

Moreover, administration of rhEPO (5000 U/kg) 15 minutes and 2 days before and 2 days after cisplatin administration was effective for inhibiting the cisplatin-induced nephrotoxicity by a possible mechanism involving activation of the PI3K/Akt pathway, which is involved in endoplasmic reticulum stress-mediated apoptosis (18). Rjiba-Touati et al demonstrated that pre-administration, co-administration or even post-administration of rhEPO especially in cases of pretreatment with EPO protected the rats against cisplatin-induced renal OS and nephrotoxicity via reduction of malondialdehyde and protein carbonyl levels and prevention of glutathione depletion (19). Similarly, the renoprotective effect of EPO against cisplatin-induced kidney damage was shown in a study by Pezeshki et al too (20). However, in another study,
Eshraghi-Jazi et al determined the gender differences in the protective impact of rhEPO against cisplatin-induced nephrotoxicity. In this study, 33 Wistar rats were divided into six groups in which groups 1 and 2 were treated first by 100 IU/kg/d rhEPO for 3 days and then were administered 7 mg/kg cisplatin; in groups 3 and 4 rats were administered the same single dose of cisplatin and then were treated with rhEPO for 7 days and the groups 5 and 6 were administered a similar regimen of group 3 and 4 except for saline instead of rhEPO. Treatment with EPO showed a significant decrease in malondialdehyde, creatinine and blood urea nitrogen in male rats, but not in females (21).

EPO and nephrotoxicity induced by gentamicin
Gentamicin is a broad spectrum aminoglycoside antibiotic which is effective against aerobic gram-negative rods. Nephrotoxicity is one of the serious side effects of gentamicin and other aminoglycoside antibiotics (8). Nephrotoxicity of gentamicin is characterized by a decrease in glomerular filtration rate and increase of blood urea nitrogen and serum creatinine. Moreover, it causes tubular necrosis, desquamation of proximal tubule epithelial cells, epithelial edema, tubular fibrosis and glomerular hypertrophy (22). Many studies have suggested that gentamicin induces nephrotoxicity via many mechanisms such as induction of apoptosis, ROS, necrosis, elevation of endothelin I, up-regulation of transforming growth factor beta (TGF-β) and increase of monocyte/macrophages infiltration (8,22). Previously, we showed that rhEPO (100 U/kg) was able to inhibit or reduce tubular cell damage induced by gentamicin. In this investigation 40 male Wistar rats were divided into four groups including group 1 as a sham group; in group 2 the rats received 100 mg/kg of gentamicin for 10 days; in group 3 the rats at first were injected gentamicin for 10 days and then were administered 100 U/kg recombinant human EPO for the next 10 days and in group 4 the rats received a combination of gentamicin (80 mg/kg) and rhEPO (100 U/kg) for 10 days. This study disclosed that recombinant human EPO was useful for ameliorating serum creatinine, blood urea nitrogen and tubal necrosis (8).

EPO and rhabdomyolysis-induced AKI
Rhabdomyolysis is a breakdown of muscle tissue that releases a damaging protein into the blood and extracellular space which can lead to complications such as renal failure (6). Yang and colleagues demonstrated that treatment with rhEPO decreased biochemical substances such as creatinine, blood urea nitrogen, glutamic oxaloacetic transaminase, creatine phosphokinase and glutamic pyruvic transaminase levels. Additionally, rhEPO-treatment group revealed fewer NF-kB-positive cells and fewer inducible NO synthase-positive cells in the renal tubular cells of rats. In this study, 24 rats were divided into three groups including glycerol group, normal saline/EPO group and glycerol/EPO group. Ten minutes following induction of rhabdomyolysis by glycerol, the rats received an intravenous injection of rhEPO (300 U/kg) (5).

EPO in vancomycin and amikacin induced nephrotoxicity
Vancomycin is a bacterial antibiotic that is used against resistant strains of *Streptococcus* and *Staphylococcus*. Also it is associated with a number of adverse side effects such as nephrotoxicity principally manifested by increased serum creatinine or blood urea nitrogen concentrations. Vancomycin causes toxic oxidative effects on the proximal renal tubules and can change mitochondrial function (10,23). Cetin et al reported the antioxidant property of EPO on vancomycin-induced nephrotoxicity in rats. In this study, 24 rats were divided into three groups, including control, vancomycin (200 mg/kg) for 7 days and vancomycin plus EPO (150 IU/kg) groups, in which the EPO treatment was started 24 hours before administration of vancomycin and continued for 7 days. At the end of this study, with EPO treatment, histopathological changes such as tubular dilatation, vacuolization, tubular epithelial cell desquamation and interstitial edema were improved. Furthermore, superoxide dismutase activity increased and malondialdehyde levels decreased via EPO treatment (23). Amikacin is an aminoglycoside antibiotic, which is used to treat various types of bacterial infections. Numerous studies have demonstrated that amikacin is able to induce nephrotoxicity via oxidative reactions. The first experimental investigation regarding the effect of EPO on renal tubular cell injury was published in 2012. In this study, Wistar rats which pretreated with EPO (2000 IU/kg) showed an improvement in serum urea and tubular necrosis (9).

Conclusion
In summary, this review showed that EPO is suitable as a nephroprotective agent. The nephroprotective efficacy of EPO is associated with its direct and indirect antioxidant properties. The activities of EPO are mediated by binding to EPOR and the positive role of EPO in the kidney is related to presence of functional EPOR in renal mesangial cells and renal tubular epithelial cells. These findings revealed an association between administration of EPO and reduction of renal injuries induced by cisplatin, gentamicin, rhabdomyolysis, vancomycin and amikacin in rats and renal IRI as one of the most common causes of AKI. Previous studies and this review showed the encouraging results on potential therapeutic impact of EPO in AKI.

Authors’ contribution
AHA, AB, EZ and MB searched the data. AHA and AB prepared the manuscript. All authors read and signed the final paper.
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Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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