Protective Effect of Erythropoietin in Renal Ischemia-Reperfusion Injury

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Abstract: Ischemia and/or reperfusion injury (IR) is one of the most common causes of acute renal failure. Erythropoietin (EPO), is main hematopoietic hormone that has recently been shown to exert important cytoprotective and anti-apoptotic effects in experimental I/R and toxicity models.

Recent studies suggest that administration of exogenous EPO prevents ischemia-induced kidney damage. In this review we focused on anti-apoptotic, anti-inflammatory and anti-oxidant action of EPO in renal ischemia-reperfusion models and its application in nephropathies. EPO seems to be a very promising agent for protecting cellular survival during both acute and chronic ischemic and toxic nephropathies.

Keywords: Erythropoietin, kidney, ischemia reperfusion, apoptosis, oxidative stress, inflammation.

INTRODUCTION

Erythropoietin (EPO) was first characterized as a hematopoietic factor and has been used clinically for the treatment of anemia. EPO promotes the proliferation and differentiation of erythroid precursors and functions as an antiapoptotic factor for the hematopoietic cells. Erythropoietin is a well known hypoxia-regulated gene and this regulation occurs mainly at the mRNA level by hypoxia-inducible factor-1 (HIF-1). A deficiency in tissue oxygen results in increased EPO production mainly in the kidney and liver [1, 2]. At lower levels similar increases occur in other organs and cell types. Hypoxia induces cells to respond through multiple gene products such as EPO and VEGF which will supply oxygen delivery to the tissues, or enzymes of different metabolic pathways such as induction of glycolytic enzymes that will adapt the cellular metabolism to decreased oxygen availability [3].

EPO receptor expression is shown in non-erythroid tissues and cells such as the brain [4], retina [5], heart [6], kidney [7], smooth muscle cells [8], myoblasts [9] and vascular endothelium [10]. EPO is a member of the cytokine type I superfamily [11]. Typical of the cytokines, EPO has multiple functions outside of the bone marrow. The discovery of novel biological functions of endogenous EPO signaling in non-hematopoietic tissues has been started to associate with the possibility of the ability of exogenous EPO to modulate organ function and cellular responses to different types of injury. In recent years, however, it has been recognized that many of these functions are parallel to its action in hematopoiesis, where EPO induces survival and maturation of the cells. Over the last 10 years, a prominent role for EPO has been defined in the nervous system and there is a growing interest in the potential therapeutic use of EPO for neuroprotection [12]. In this review, we will outline the mechanism by which EPO acts in renal ischemia reperfusion injuries with a focus on inhibition of apoptosis, inflammation and oxidative stress.

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RENAL ISCHEMIA-REPERFUSION INJURY

Ischemia and/or reperfusion injury (IR) is one of the most common causes of acute kidney injury (AKI). Acute renal failure (ARF) is associated with a high degree of morbidity and mortality. ARF is a common condition which develops in 5% of hospitalized patients [13]. Ischemia and/or reperfusion initiate changes and disturbances in the functions of vascular endothelial cells, tubular epithelial cells and immune system homeostasis in the kidney. The most common causes of ARF are ischemic and toxic injury to tubule epithelium. Cell injury results in disruption of the epithelium and disturbances in its normal reabsorptive functions, Apoptosis of the cells may be seen along the tubule epithelium and cell necrosis that may become prominent at the late stages. IR injury has more destructive effects rather than ischemia alone. Microvascular dysfunctions, imbalance of vasoactive substances, increased reactive oxygen species (ROS) and nitrogen (RNS) species, increased endothelial injury, local activation of inflammation, disturbed mitochondrial respiration are the main mechanisms that are underlying IR induced damages. Different processes are started to disturb structural and functional integrity of the kidney after a toxic or ischemic insult. Fig. (**1**) shows the events that occur after ischemia and reperfusion [14, 15].

USAGE OF EPO IN NEPHROPATHIES: (EPO IN RENAL ISCHEMIA-REPERFUSION INJURY)

Recent studies have focused attention on the cytoprotective effect of EPO in various models of toxic and ischemic injuries of cardiac [15], renal [5], and nervous system [16, 17]. It was recently shown that EPO has also antiapoptotic effects on cardiomyocytes *in vitro* and *in vivo* in a rat model of myocardial infarction, where it normalizes hemodynamic functions. It was found that activation of

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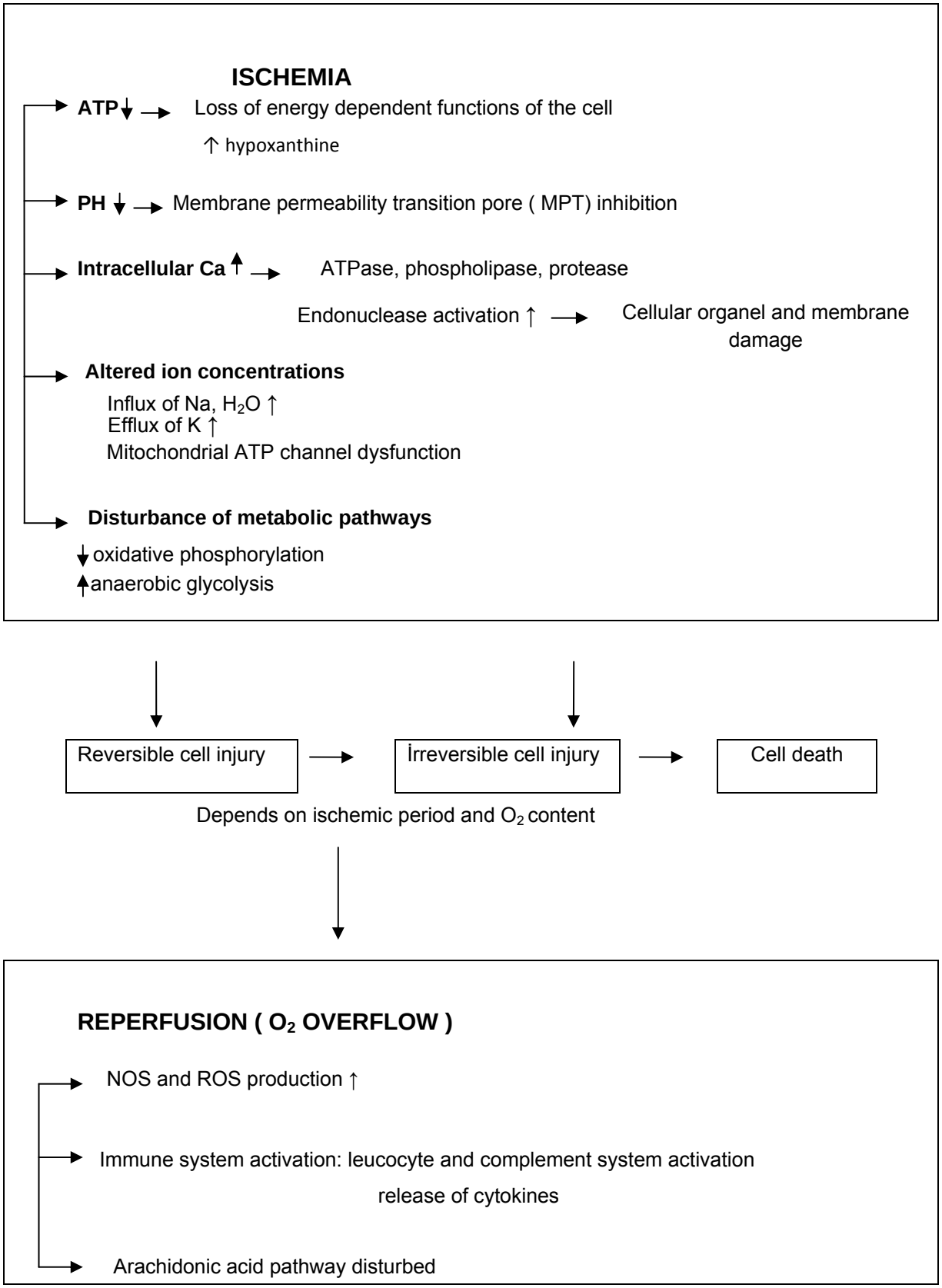

Fig. (1). Mechanisms involved in ischemia reperfusion injuries.

potassium channels and protein kinases by erythropoietin was an important mechanism for increasing cardioprotection [18, 19]. EPO mimics ischemic preconditioning like mechanisms, this mechanism was found not only in self defence mechanism of the same organ but also acts as disseminated endocrine function against ischemia of any other organ system [20]. Moreover, several independent research groups have reported that EPO protects cultured neurons against glutamate toxicity [17] and reduces ischemic neuronal damage and neurobiological dysfunction in rodent models of stroke [21-23], suggesting a preventive effect of EPO against ischemic or toxic injuries. In addition to its hematopoietic effect, EPO plays an important role during acute renal failure repair process by rapidly correcting anemia [24] and enhancing renal tubular regeneration in toxic models [25]. EPO also stimulates renal carcinoma cell proliferation [26].

ANTIAPOPTOTIC EFFECT

The mechanisms and intracellular signaling pathways involved in mediating the protective effects of Epo in kidney pathologies have been investigated in animal studies and in cultures of cell lines. In an early study, which has shown EPO given intraperitoneally protected the kidney from ischemic injury, investigators found that there was a functional protection in EPO- treated animals with significant attenuation in the rise of creatinine induced by ischemia at the 24 h post-reperfusion. EPO reduced proximal tubular epithelial cell apoptosis and death by leading increased expression of $Bcl\text{-}_2$, an anti-apoptotic protein that prevents mitochondrial depolarization, and the molecular chaperone heat shock protein-70 (HSP-70) in both sham operated and animals subjected to I/R. The increase in HSP-70 expression was time- and dose-dependent, *via* activation of the JAK2/STAT transcription factor pathway [27].

In vivo and *in vitro* studies support the results showing that the cytoprotective effect of Epo was associated with upregulation of the anti-apoptotic proteins BCL-XL and XIAP, and down regulation of Bad, Bax in the tubular epithelium of kidney [28-30]. In cultured kidney epithelial cells, protein kinase inhibitors targeting the mitogen-activated protein (MAP) kinase and phosphoinositide-3 kinase (PI3K)-AKT pathways [30], blocked the ability of EPO to reduce cellular apoptosis induced by hypoxia, similar results have been found in neuronal cell injury models [31]. Sharples *et al.* found that preincubation with EPO (1 to 50 U/ml) for 1 h significantly protected against hydrogen peroxide-mediated cell death in a dose-dependent manner in HK-2 cells, an immortalized human proximal tubule epithelial cell line [32]. They showed that co-incubation with LY294002 or wortmannin which are specific inhibitors of PI3K, significantly reduced the protection of EPO pretreatment in a dose-dependent manner. They also have similar results with Tyrphostin (AG490), an inhibitor of JAK2 phosphorylation. Their findings with others [29, 33] supported the conclusion that EPO directly protects proximal tubule epithelial cells by activating EPO receptor/JAK-2 kinase, activating PI3K leading to activation of Akt, upregulation of $Bcl\text{-}X_L$ and XIAP, and preventing the activation of caspase-3, 8, 9 and DNA fragmentation, and the final result is the death of cell by apoptosis [32]. Induction of HIF-1 α and related gene expression in renal tissue is another accepted renoprotective mechanism of EPO [34].

INFLAMMATION

Several studies have demonstrated that complement system and inflammatory pathway are activated by I/R injury in the kidney. Pro-inflammatory cytokine and chemokine production start to increase in damaged tissue. These chemokines attract neutrophils and macrophages to the injured kidney [35]. Patel *et al.* showed that pre and post treatments with 1000 iu/kg EPO significantly decrease polymorphonuclear leukocyte (PMN) infiltration and tissue myeloperoxidase (MPO) activity [36]. Further studies showed that EPO treatment inhibits renal inflammation during I/R damage by decreasing proinflammatory cytokines, TNF-α, IL-6 and NF-κB activation [37, 38]. Our study supported these findings; the upregulation of TNF-α, IL-6 production reduced by 500 IU/kg EPO pretreatment. EPO has a preconditioning like effect and this effect is mediated by ATP dependent K (K-ATP) channel activation [39]. It was shown that K-ATP channels are important for cardioprotective effect of EPO in myocardial ischemia and infarction [19]. We found that channel activation *via* EPO takes part in cytoprotection of EPO against H2O2 induced damage in hepatocytes [40]. K channels are found both in cell membrane and mitochondria and general inhibition of these channels is important for vascular tonus and inflammatory cell functions. The mechanisms proposed to explain the reperfusion-associated injury include vasodilatation, increased blood flow and release of reactive oxygen species, such as superoxide radical and hydrogen peroxide, recruitment and activation of leukocytes, and subsequent release of mediators of the inflammatory process. Besides, Pompayer *et al.* showed that treatment with glibenclamide is associated with inhibition of neutrophil recruitment and amelioration of renal dysfunction following renal I/R [41].

The involvement and upregulation of death receptors Fas (CD95), TNF-R1, and toll like receptors (TLR) have been implicated in the pathogenesis of renal I/R injury, and TNF-α and IL-6 production is induced after reperfusion by the upregulation of NF-κB *via* p38 [35]. Based on the information in the literature, however, it is not fully clear whether EPO-mediated anti-apoptotic effect is associated with NF-κB activation in renal I/R injury. Recently, data provided by *in vitro* studies suggests that EPO-mediated protection against ischemia-induced injury may involve cross-talk between JAK2 and NF-κB resulting in an early (within 24 h of reperfusion) activation of NF-κB signaling pathways [42]. But according to the experimental results of Spandou *et al.* [43], EPO administration did not lead to NF-κB activation after reperfusion even though it is proved in neuronal tissue. Lipton and Digicaylioglu suggested that neuronal EPO Rs activates a neuroprotective pathway by activation of JAK2 and it leads to phosphorylation of the inhibitor of NF-kb (Ikb) and subsequent nuclear translocation of the transcription factor NF-kb and NF-kb dependent transcription of neuroprotective genes [42]. Besides these findings, EPO has direct immunomodulatory action on human monocytes and macrophages in their response to LPS by regulating cytokine production [44].

OXIDATIVE DAMAGE

Renal I/R damage results in a rapid and extensive oxidative stress. Decreasing the level of oxidative stress will minimize the secondary destruction after renal injury [45]. Catalase and GSH are among the most important intracellular anti-oxidant substances against reactive oxygen species. GSH is involved in the scavenging of both inorganic and organic peroxides [46]. EPO may exert its antioxidative effects directly by inducing intracellular antioxidative mechanisms such as heme oxygenase-1 and glutathione peroxidase. EPO is also capable of functioning as an antioxidant, both directly and indirectly [47]. In neuroprotection afforded by EPO, the modulation of cellular glutathione peroxidase activity was proposed to be a potential underlying mechanism. Similar results in renal models showed that EPO has the capability of decreasing oxidative stress after I/R injuries. Ates *et al.* found that EPO 1000 iu/kg treatment might improve oxidative damage in renal tissue and this effect is mediated by tyrosine kinase receptor activation [37]. rHuEpo markedly reduced the MDA in recent studies showing that Epo has the potential to inhibit lipid peroxidation by several possible mechanisms like increasing the activity of antioxidant enzymes.

Characterization of the nonerythropoietic biological effects of EPO and understanding the mechanisms of EPO-EPOR signaling activation in nonhematopoietic organs and cell types are critical to the future development of novel applications for EPO and its derivatives. The optimization of the use of recombinant EPO beside its current clinical indications including anemia in acute and chronic kidney diseases it appears to promise a novel future treatment for patients with toxic and ischemic nephropathies.

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