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Review

The Role of Erythropoietin as an Inhibitor of Tissue Ischemia

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Erythropoietin is a hypoxia-induced cytokine that stimulates erythropoiesis through the promotion of erythroid precursor cell proliferation and differentiation. Recent evidence supports that erythropoietin has a broad spectrum of tissue protecting actions affecting other systems than hemopoietic. Lately, research has focused on the nonhemopoietic effects of erythropoietin against tissue ischemia due to the unexpected observations of erythropoietin receptor expression by various cells, such as endothelial cells, neuronal cells, cardiac myocytes, and vascular smooth muscle cells. It has been shown that erythropoietin exerts its cardioprotective action during cardiac ischemic injury through reducing the infract size and enhancing new vessel formation over a longer time frame. Erythropoietin plays a crucial role in neuroprotection in many types of ischemic injury in the central and the peripheral nervous system. It is also strongly believed that erythropoietin exhibits a critical role in many other disorders that are pathogenetically related to acute tissue ischemia. This article reviews the proposed implications of erythropoietin in tissue ischemia and discusses the possible mechanisms for this action along with its potential therapeutic applications.

Key words: erythropoietin, ischemia, nervous system, cardiovascular system, ischemia/reperfusion injury

1. Introduction

Erythropoietin is a 165 aminoacid glycoprotein hormone with approximately 30 kD molecular weight. It is synthesized primarily by kidneys in adults and by kidneys and liver in the fetus. The ratio between kidney and liver erythropoietin in the adult is 9:1 [1]. Its primary role involves prevention of programmed cell death (apoptosis) of erythrocyte precursors [2]. Erythropoietin induces erythropoiesis by promoting proliferation and differentiation of erythroid progenitor cells with the main target cell being the colony-forming unit erythroid (CFU-E). Although erythropoietin is the main regulator of this process, other growth factors, such as granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), interleukins IL-1, IL-3, IL-6 IL-4, IL-9, and IL-11, granulocyte-macrophage (GM)-CSF, and insulin growth factor-1 (IGF-1) are believed that contribute in different levels of maturation of the erythrocyte [3, 4].

Erythropoietin gene expression is regulated mainly by hypoxia [5, 6]. Hypoxia-inducible factors (HIF) 1, 2 and 3, as well as nuclear factor kappa B (NF-kB) are the key regulators of erythropoietin gene expression [7]. Recent data revealed that hypoxia-inducible factor 2a (HIF-2a) has prominent role in controlling erythropoietin gene expression in hepatic cells [8, 9]. According to some investigators this role of HIF-2a may be also applied in other tissues [10,11].

In addition to its well known effect on red blood cell mass in response to changes in tissue oxygenation, many investigations have shown that erythropoietin also exerts protective role against tissue ischemia. It is believed that this is achieved both directly by activating multiple biochemical mechanisms that provide antiapoptotic, antioxidative, and anti-inflammatory response to hypoxia/anoxia and indirectly via its angiogenic potential by inducing oxygen systematic supply to the ischemic tissue.

This article reviews the proposed implications of erythropoietin in tissue ischemia and discusses the possible mechanisms for this action along with its potential therapeutic applications.

2. Non Erythropoietic Role of Erythropoietin

Erythropoietin has a broad spectrum of tissue protecting actions affecting other systems than hemopoietic [12-15]. Beginning with the unexpected observations of erythropoietin receptor (Epo-R) expression by various cells, such as endothelial cells, neuronal cells, cardiac myocytes, and vascular smooth muscle cells [16-19], research lately focused on the nonhemopoietic effects of erythropoietin and its potential use against tissue ischemia.

Cardiovascular System

Erythropoietin exerts its cardioprotective action during cardiac ischemic injury through reducing the infract size and enhancing new vessel formation over a longer time frame [20]. It is well known that erythropoietin induces vasoconstriction-dependent hypertension and stimulation of angiogenesis in erythropoietin-treated animals and humans [21]. The effect of erythropoietin in blood pressure has been extensively described in the past after the initiation of treating anemia in chronic renal failure patients [22-24]. The exact mechanism remains unclear, though, different hypotheses involving cyclooxygenase-dependent endothelium derived contracting factors (EDCFs), stimulation of vascular cell growth, endogenous vasodilatory factors, and a direct angioconstrictive action of erythropoietin have been described [25, 26]. Erythropoietin effect on heart disorders has been showed that involves a mechanism with greater immediacy than that of direct oxygen supply due to haemoglobin levels increase [27].

Silverberg et al. [28] evaluated the influence of recombinant erythropoietin in patients with chronic cardiac failure and suggested that erythropoietin usage improves both cardiac and renal function, while it decreases mortality rates and hospitalization due to heart failure. Similar observational studies in the past, have reported better clinical outcome, improvement of survival and lower complication rate when erythropoietin is administrated to patients undergoing hemodialysis in order to normalize haemoglobin levels [29, 30]. On the contrary, recent evidence from randomised trials showed an increased risk of cardiovascular events and increased mortality in the same patient group [31, 32]. Bearing in mind the high cost of erythropoietin treatment and the insecure outcome in patient life expectancy, scepticism over the benefits of excessive use of erythropoietin is necessary.

Erythropoietin exerts both acute and long term cardioprotective effects through its receptor. In vitro studies have shown that Epo-R is expressed in various cell lines originated from the cardiovascular system [16, 33, 34]. The presence of Epo-R was demonstrated in rat cardiac myocytes where the administration of erythropoietin prevented apoptosis by activating phosphatidylinositol 3 kinase (PI3-K)/Akt intracellular pathway [33]. According to Depping et al. [35] the expression of Epo-R was confirmed in ventricular myocytes and endothelial cells in adult human heart.

Apoptotic mechanisms are considered to be associated with crucial cardiac cell death following myocardial infraction that potentially is complicated with cardiac failure [36, 37]. Inhibition of apoptosis has been strongly correlated with erythropoietin-mediated cardioprotection against acute ischemic injury. The antiapoptotic effects of erythropoietin have been associated with the inactivation of Bcl-2 associated death-promoting protein (BAD) through PI3-K/Akt pathway as well as the increased expression of the antiapoptotic proteins Bcl-2 and Bcl-$_{XL}$ [20, 38]. In a rat isolated-heart model, pre-treatment with erythropoietin increased the functional recovery of hearts during ischemia/reperfusion injury [39]. Further studies showed that specific pathways, such as PI3-K/Akt, mitogen-activated protein kinase (MAPK) pathways and protein kinase C (PKC) are implicated in erythropoietin cardioprotective action before, during or after ischemia [40-42].

Although apoptosis prevention is a main mechanism for erythropoietin acute cardioprotection, the role of nitric oxide (NO), a modulator of vascular tone and inhibitor of processes involved in atherothrombosis, should also be considered [43-45]. The endothelial isoform of NO is the prevailing form in the cardiovascular system. Endothelial nitric oxide synthase (eNOS) is activated by the signal transducer and activator of transcription-5 (STAT-5) and RAS/MAPK signalling molecules. In a transgenic mice model overexpressing human erythropoietin, the authors demonstrated fail to develop hypertension and myocardial infraction [46]. This was attributed to up-regulation of eNOS expression, increased NO production, and NO-mediated vascular relaxation compared to wild type animals.

Long–term protection of ischemic myocardium by erythropoietin is attributed to both new vascular formations by directly enhancing angiogenesis and increased vasculogenesis from endothelial progenitor cells (EPCs) [20]. It has been found that erythropoietin promotes EPC mobilization which in turn was associated with neovascularization of ischemic tissue [47]. Interestingly, the number of circulating EPCs in patients with coronary artery disease was significantly correlated with serum levels of erythropoietin [47]. Moreover, erythropoietin promotes NO synthesis in EPCs and reendothelialization of injured arterial vessels [48].

High-dose chronic erythropoietin treatment may result to increased hematocrit associated side effects, such as hypertension and thromboembolism. Enhancement of erythropoietin cardiac tissue protective activity without increasing the number of red blood cells is the key in the management of heart disorders.

Nervous System

It has been shown that special pathways for erythropoietin and its receptor are present not only in the central nervous system but also in the peripheral nerves, cerebrospinal fluid, and the retina [49-53].

Erythropoietin is suggested to be a cytokine with a neurotrophic and a neuroprotective role in the central and peripheral nervous system. Several studies have shown the neuroprotective action of erythropoietin [13, 14], while others have demonstrated that erythropoietin may facilitate nerve regeneration after peripheral nerve injury [54]. This role in neural system appears to resemble the actions of erythropoietin in progenitor cells of the hematopoietic tissue and is thought to be multifactorial. Hypoxia and neurological damage enhance erythropoietin and Epo-R production in the central nervous system [52, 55, 56]. HIF-1, a member of the PAS (PER/arylhydrocabon-receptor nuclear translocator/single minded) family of hypoxia-regulated transcription factors is the main factor responsible for hypoxia-mediated induction of erythropoietin expression in the retina [57].

Despite previous evidence it is now believed that erythropoietin crosses the blood-brain barrier in an injured brain [58]. This evolution confirms the importance of erythropoietin role in the initial treatment of the acutely injured nervous tissue. Furthermore, it offers an effective treatment for these conditions, since its intravenously or subcutaneous administration provides effective and targeted therapy. In addition, the results from the administration of recombinant human erythropoietin (rhEpo) in acute stroke patients confirmed exultantly both its safety and effectiveness in protecting cerebral tissue from ischemia [59].

Several hypotheses have been proposed in order to explicate the neuroprotective role of erythropoietin. Erythropoietin exhibits neurotrophic, anti-inflammatory, and antioxidant action [14, 49, 60-63]. It has been shown that it decreases susceptibility to glutamate toxicity [64, 65] and promotes apoptosis through induction of apoptotic agents [14, 51, 62-65]. Erythropoietin reduces nitric oxide-mediated injury [66-68], with its protective role on glia [69-71]. The improvement of blood flow to the injured tissue is a further potential mechanism of erythropoietin's protective role in the nervous system.

When a single molecule of erythropoietin binds its receptor enzymic phosphorylation of PI3-K and the nuclear factor NF-kB occurs. NF-kB, which is activated by erythropoietin after oxidotic stress [67], is an inflammatory reaction genes regulator [72]. Erythropoietin activates JAK2, which in turn activates NF-kB resulting in increased expression of apoptosis inhibiting genes, such as XIAP and c-IAP2. Activation of Akt by erythropoietin inhibits various metabolic paths that are connected to cell death, such as those which are related to glycogen synthetase kinase 3β (GSK3β), related to BAD, caspase-9, etc [73]. Furthermore, JAK2 induces activation by phosphorylation of Jun kinase (JNK) and signal transducers and activators of transcription-5 (STAT-5) [74, 75]. STAT-5 enters the nucleus and induce transcription of genes involved with inhibition of apoptosis, such us Bcl-x_L genes [74].

In the recent years, increasing knowledge of the nervous system physiology allowed the analysis of the pathogenetic mechanisms involved in various abnormal conditions. Disorders such as early brain injury, attention deficit hyperactivity disorder, and retinopathy of prematurity have been etiologically related to hypoxia. Erythropoietin actions seem to reverse this hypoxia-induced sequelae. A recent study proposed potential benefit by erythropoietin administration in patients with various psychiatric disorders [76]. Such an ischemic etiological profile may alternate not only the treatment approach used so far, but could also develop a new dynamic in the research of pathogenetic factors of vague disorders, such as Alzheimer's disease.

Other Roles Proposed for Erythropoietin

Based on observations on the role of erythropoietin in other tissues, it is strongly believed that erythropoietin plays a critical role in disorders that are pathogenetically related to acute tissue ischemia.

Erythropoietin has been used in the past for correcting both cancer and chemotherapy induced anemia [77-80]. Furthermore, the treatment outcome of both radiotherapy and chemotherapy in cancer patients receiving erythropoietin seems to be superior based on the beneficial effects of erythropoietin to tumour hypoxia [81]. However, the expression of erythropoietin receptor in neoplasmatic cells initially raised the question whether erythropoietin promotes tumour growth. Recently, Hamadmad and Holh [82] demonstrated that erythropoietin induces chemotaxis of cancer cells via MAPK/Erk and Rho/RhoA kinase pathways. These reports are in accordance with the finding from a trial conducted by Leyland-Jones et al. [83] where the usage of erythropoietin for the treatment of anemia in metastatic breast cancer patients resulted in decreased survival. In the same study, the primary cause of death in erythropoietin group was disease progression, significantly higher than the control group.

Although these reports are not debating the positive affects of erythropoietin, they make the need for further research in this field essential [84]. A recent meta-analysis confirmed the increased risk of venous thromboembolism and increased mortality rates after erythropoietin administration [85]. Safety concerns over the usage of erythropoiesis stimulating agents in treating anemia in patients with cancer are well established. Understanding the mechanism of the exact role of erythropoietin in neoplasmatic cells would possibly

clarify these issues and potentially indicate alternative therapeutic measures against cancer.

The protective role of erythropoietin in tissue ischemia extended the investigation of the potential role of erythropoietin in other conditions related with ischemia. In critical ill patients, erythropoietin has been proposed as an ameliorator of the clinical outcome [86]. Studies suggesting the role of erythropoietin in survivor of skin and muscular flaps in animal studies magnify the significance of this cytokine in trauma patients [87, 88].

Although erythropoietin failed to reduce red blood cell transfusions in critically ill patients, it finally declined mortality in this group of patients with a higher decrease applied in trauma patients [86]. Since there was no difference in blood transfusion rate, the decrease in the mortality rate is not considered as a consequence of blood transfusion. The contribution of a nonhematopoietic mechanism in the superior mortality rate in erythropoietin treated patients is believed by the authors to be logical explanation to this contradictory event.

The presence of erythropoietin receptor in retina establishes a major role to erythropoietin in the treatment of several pathologic conditions etiological related to ischemia in these highly oxygen dependent tissue. Despite the discouraging results reported in non randomised trials exploring the results of erythropoietin in retinopathy [89], a new dynamic has been developed as knowledge on erythropoietin and nervous system advances [90]. Further study is needed in order to clarify the role of this ischemia protective cytokine in conditions, such as diabetic retinopathy and retinopathy of prematurity.

The role of angiogenic potential on survival of various flaps after erythropoietin administration has been extensively investigated. It is known that erythropoietin results in neovascularization and increased healing through stimulation of endothelial cell mitosis [88]. According to Jaquet et al. [91] erythropoietin found to have equal angiogenic potential with vascular endothelial growth factor (VEGF) which was confirmed by histologic evaluation on flaps. Moreover, exogenous administration of erythropoietin stimulated angiogenesis by day 3 in a genetically diabetic mouse model by increasing VEGF mRNA and protein expression [92]. However, Kim et al. [93] supported that the angiogenic potential of erythropoietin is unlikely to occur in the first 24 hours and therefore is not sufficient enough to explain the protective effect of erythropoietin on ischemia/reperfusion injury in their rat transverse rectus abdominis musculocutaneous (TRAM) flap model.

Novel erythropoiesis stimulating protein (NESP) or darbepoetin alfa has been introduced for the treatment of anemia in several conditions with comparable results with those of erythropoietin. It has a different pharmacokinetic profile from erythropoietin that allows less frequent administration in order to maintain similar haemoglobin levels [94-98]. Since the mechanism of action of NESP is relevant to the one of erythropoietin, its role in other tissues remains to be explored. Further study is needed before establishing the exact role of both erythropoietic inducing factors in tissue ischemia. Based on the disadvantages of high cost and potential low safety profile of recombinant erythropoiesis-stimulating agents in some patient groups, the potential benefit of interfering in erythropoietin gene expression through HIF-2 mechanisms emerges as an alternative for the treatment of certain types of anemia.

3. Erythropoietin in Ischemia/Reperfusion Injury

Reperfusion injury is defined as the tissue injury following restoration of blood flow to an ischemic region. Ischemia/reperfusion-induced injury is characterized by a greater rate of tissue damage than the original ischemic insult itself and it is thought to be the result of the acute interruption of blood flow within the microcirculation. The injury is attributed to a complex interaction between neutrophils and endothelial cells when the formers release enzymes, free radicals, and cytokines [99]. This cascade usually leads to endothelium destruction, capillary obstruction, and finally to impairment of oxygen supply to the tissue [100-103].

Many studies have demonstrated that erythropoietin has a protective effect on tissue injury associated with ischemia/reperfusion injury in many tissues, including brain, retina, cardiac, liver, kidney, lung, and intestine [104-109]. This erythropoietin mediated protective effect against ischemia/reperfusion injury has been related to its antiapoptotic, antioxidative, and antiiflammatory properties.

Although apoptosis serves to eliminate dying cells in proliferating or differentiating cell populations [110], inappropriate induction of programmed cell death in pathologic conditions, such as ischemia/reperfusion injury, results in tissue dysfunction. Previous studies have shown that rhEpo protects against the ischemia/reperfusion injury, mainly through its antiapoptotic action [105, 109, 111]. According to Brimes and Gerami [112], the mechanism mediating the antiapoptotic effects of erythropoietin probably incudes the JAK2-STAT-Bcl-2 pathway.

Bcl-2 may also be responsible for the antioxidant activity of erythropoietin [67]. It is believed that erythropoietin exhibits its antioxidant action either directly through the scavenging action of its sugar-moiety or indirectly by activating antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) [109, 113-118]. In an intestinal ischemia/reperfusion injury model in rats, Guneli et al. [109] revealed that although enzymatic activity of SOD and GSH-Px was not significantly changed after intraperitoneal administration of erythropoietin, significant alterations were obtained in the level of CAT activity. The authors concluded that erythropoietin may be capable to increase the activity of the antioxidant system via the increase or restoration of CAT levels which decreases due to ischemia/reperfusion injury.

Oxidative stress is an important determinant in the pathogenesis of ischemia/reperfusion injury. Accumulation of activated neutrophils at the injury site triggers the inflammatory cascade through the production of various cytotoxic proteins which in turn promote the radical induced ischemia/reperfusion injury [119-121]. Erythropoietin by suppressing the inflammatory response after the ischemia/reperfusion injury seems to be a protective agent in tissues subjected to this type of damage [65, 122, 123]. Erythropoietin mediated neutropenia reversal following reperfusion is believed to be the underlying mechanism for the inhibition of neutrophil recruitment in the inflamed tissue.

The role of erythropoietin on eNOS is still controversial. It is supported that although eNOS-derived NO may play a protective role at the onset of ischemia/reperfusion injury, the superoxide anions produced during oxidative stress may react with NO and turn it into oxidant peroxynitrite which is cytotoxic [124, 125]. Many authors have shown that eNOS production and eNOS-derived NO overproduction is decreased after erythropoietin administration [66, 109, 126-128]. However, other investigators have reported increased expression of eNOS after erythropoietin therapy [129]. Therefore, before direct conclusions can be made, the role of erythropoietin in eNOS expression after ischemia/reperfusion injury should be elucidated.

4. Conclusion

Erythropoietin is believed to serve a manifold role in several tissues with the primary target being the protection from ischemia. It acts both by ensuring adequate oxygen supply through its role to erythropoiesis but also it intervenes directly to the tissue targets with multiple set of actions that reserve cell survivor through its nonhematopoietic effects. Further research would provide us with evidence that could alter the way erythropoietin is currently used in the clinical practice.

Conflict of interest

The authors have declared that no conflict of interest exists.

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