

Cellular protection by erythropoietin: new therapeutic implications?

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List of non-standard abbreviations:

asialoEPO: desialylated erythropoietin

β cR: beta-common receptor

CEPO: carbamylated erythropoietin

EPOR: erythropoietin receptor

HIF-1: hypoxia-inducible factor 1

Jak2: janus kinase 2

K_{ATP}: ATP-sensitive potassium channel

K_{Ca}: calcium-activated potassium channel

NF- κ B: nuclear factor-kappa B

NO: nitric oxide

PI3 kinase: phosphatidylinositol-3 kinase

rhEPO: recombinant human erythropoietin

TGF- β : transforming growth factor-beta

TNF- α : tumor necrosis factor-alpha

Abstract

Erythropoietin (EPO), the principal hematopoietic hormone produced by the kidney and the liver in fetuses, regulates mammalian erythropoiesis and exhibits diverse cellular effects in non-hematopoietic tissues. The introduction of recombinant human EPO (rhEPO) has marked a significant advance in the management of anemia associated with chronic renal failure. At the same time, experimental studies have unveiled its potential neuroprotective and cardioprotective properties, occurring independently of its hematopoietic action. As with other cytoprotective agents, administration of exogenous rhEPO can confer cerebral and myocardial protection against ischemia-reperfusion injury, in terms of reduction in cellular apoptosis and necrosis as well as improvement in functional recovery. Very recent studies even suggest that this drug could have beneficial applications in oncology, protecting against chemotherapy cardiotoxicity. The purpose of this letter is to review current information regarding the various conditions in which rhEPO and its derivatives could confer cellular protection. We also address clinical perspectives and novel therapeutic strategies which could be developed based on these studies. Thus, EPO appears to be a very promising agent for protecting cellular survival during both acute and chronic diseases and its future should be considered with enthusiasm.

Introduction

The hormone erythropoietin (EPO), produced by the kidney and the liver in fetuses, is well known to regulate mammalian erythropoiesis. Exogenous EPO, the recombinant human EPO (rhEPO), introduced since around two decades, is presently used for the treatment of anemia resulting from a variety of conditions such as chronic renal failure and chemotherapy. However since the last decade, the existence of EPO and its receptor (EPOR) localized outside of the liver and the kidney, such as the brain and heart has been shown. At the same time, several experimental studies using rhEPO have unveiled the potential neuroprotective and cardioprotective role of EPO against ischemia, occurring independently of its hematopoietic action (Bogoyevitch, 2004; Joyeux-Faure et al., 2005).

The cell possesses a remarkable ability to adapt to stress by changing its phenotype in a manner that renders it more resistant to subsequent injury. This powerful adaptative phenomenon, called preconditioning, is illustrated by the fact that a sublethal stress (such as ischemia or pharmacological agent administration) applied to an organ enhances its tolerance to a subsequent lethal stress. When preventively administered, rhEPO is able to mimic ischemic preconditioning, protecting neuronal and cardiac cell against various stresses, such as lethal ischemia or cytotoxic drugs (Baker, 2005). On the other hand, rhEPO administered after the stress (i.e. after a cardiac or cerebral ischemia) is also able to protect the cells against the development of deleterious consequences, indicating that it can be used not only in prevention but also in the treatment of ischemic episode. However, the molecular mechanisms underlying the cellular protective effects of rhEPO remain largely unknown since only few potential actors have been identified (Bogoyevitch, 2004).

In this article, we review current information regarding the various conditions in which rhEPO and its derivatives could confer cellular protection. We also report recent data concerning the mechanisms underlying the cytoprotective effects of rhEPO such as the role of

EPOR and the activation of the following cellular signaling pathways. Finally, we address clinical perspectives and novel therapeutic strategies which could be developed based on the experimental studies.

Structure and expression of EPO, EPOR and rhEPO

The circulatory EPO is a 30.4-kDa glycoprotein containing 165 amino acids. This protein has 4 glycosylated chains including 3 *N*-linked and 1 *O*-linked acidic oligosaccharide side chains. These glycosylated chains are necessary for the production and secretion of the mature EPO as well as for its biological activity protecting it against oxygen radicals. The biological activity of EPO also requires 2 disulfide bonds formed between cysteine amino acids (Chong et al., 2002).

Besides the kidney and liver, additional organs have been found to secrete EPO, including peripheral endothelial cells, vascular smooth muscles cells, neurons, astrocytes, microglia and cardiomyocytes (for a review see Maiese et al., 2005), and the number of new discovered secretory sites for EPO continues to grow up. EPOR is also expressed in previously cited cells secreting EPO.

Production and secretion of EPO and EPOR expression are regulated by the tissue oxygen supply. Indeed, a deficiency in tissue oxygen results in hypoxia-dependent gene transcription of EPO and EPOR in the kidney and the liver, via the activation of the hypoxia-inducible factor 1 (HIF-1) pathway. EPO may also be produced in the brain, possibly crossing the blood-brain barrier to reach the systemic circulation. Many other stimuli may lead to activation of HIF pathway and increased expression of EPO and EPOR, such as hypoglycemia and reactive oxygen species. Finally, several cytokines including tumor necrosis factor α (TNF- α), interleukin 1 β and interleukin 6 are also able to increase EPO and EPOR expression (Maiese et al., 2005).

There are five rhEPO currently available: epoetin-alpha, epoetin-beta, epoetin-omega, epoetin-delta, and darbepoetin-alpha. These agents all have the same amino-acid sequence, but glycosylation varies as a result of type- and host cell specific differences in the production process. Darbepoetin-alpha is an erythropoietin analogue, carrying two additional glycosylation sites, which produces a longer half-life and potency.

Neuronal protection induced by rhEPO

In neuronal injury models, rhEPO administration has been shown to protect against ischemia and free radical injury (Maiese et al., 2004). Of the many examples of this successful approach, local cerebral administration of rhEPO prevents ischemia-induced learning disability and neuronal death (Sadamoto et al., 1998; Bernaudin et al., 1999). Several *in vivo* studies have confirmed the beneficial neuroprotective effect of rhEPO. For example, rhEPO (5000 IU kg⁻¹, intraperitoneal injection) administered either at the time of the middle cerebral artery occlusion or after it, decreases the cerebral infarction by as much as 75% (Brines et al., 2000; Siren et al., 2001). Moreover, intravenous administration of rhEPO (350 – 1000 IU kg⁻¹) at reperfusion prevented motor neuron apoptosis and neurological disability induced by a spinal cord ischemic injury (Celik et al., 2002). Application of systemic rhEPO following experimental subarachnoid hemorrhage restores the autoregulation of cerebral blood flow, reverses basilar artery vasoconstriction and enhances neuronal survival and functional recovery (Olsen, 2003). More importantly, a clinical trial on the effect of EPO in the treatment of stroke shows a strong trend for reduced cerebral infarction and improved follow-up and outcome scales in rhEPO-treated patients receiving 33,000 IU per day for 3 days following stroke (Ehrenreich et al., 2002).

Neuroprotection conferred by EPO could come from different mechanisms, independent of changes in erythrocyte numbers. Indeed, EPO can reverse vasospasm, reducing the basilar

artery vasoconstriction (Grasso et al., 2002), potentially through a direct effect on vascular endothelium (Chong et al., 2002). EPO also modulates inflammation (Brines et al., 2000) and recruits stem cells (Shingo et al., 2001). Moreover, EPO can act directly on neurons, attenuating the production of damaging molecules such as reactive oxygen species or glutamate-stimulated excitotoxicity (Digicaylioglu and Lipton, 2001). This likely contributes to lower levels of apoptosis and necrosis.

Myocardial protection induced by rhEPO

Similarly, rhEPO administration has been shown to protect cardiomyocytes against cellular damage induced by ischemia or cytotoxic agents, in isolated cells as well as in animal models.

RhEPO-induced cardioprotection against ischemic injury

Protection against ischemic injury has first been investigated. Indeed, an antiapoptotic effect of rhEPO has been described in rat cardiac myocytes (Calvillo et al., 2003) and myoblasts (Parsa et al., 2003) subjected to hypoxia.

The cardioprotective effect of rhEPO in whole organ was investigated in numerous studies using different times of administration with regard to the ischemic insult (Joyeux-Faure et al., 2005). When administered either before (Cai et al., 2003; Joyeux-Faure et al., 2006) or at the onset of ischemia (Parsa et al., 2003), rhEPO is able to efficiently prevent deleterious consequences induced by this stress. Others studies show the beneficial effect of rhEPO when administered immediately at the beginning of reperfusion (Lipsic et al., 2004). This molecule can thus be considered as a pharmacological preconditioning agent used to prevent ischemic damage as well as a protective agent used in the treatment of cardiac ischemic insult.

Cardioprotective effect conferred by rhEPO has many aspects, reflected by different indexes measured through numerous studies (Joyeux-Faure et al., 2005). Indeed, it has been shown

that rhEPO administration preserves the ventricular function (Cai et al., 2003; Joyeux-Faure et al., 2006), reduces inflammation (Liu et al., 2006), lethal arrhythmias (Hirata et al., 2005), apoptosis (Cai et al., 2003; Calvillo et al., 2003) and necrosis (Lipsic et al., 2004; Bullard et al., 2005) induced by ischemia-reperfusion sequence.

RhEPO-induced protection in chronic heart failure

In models of chronic ischemia, development of the cardiac hypertrophy, fibrosis and necrosis is abrogated by a single administration of rhEPO which is able to attenuate the progression of the ischemic tissue damage. These cardioprotective effects could be due to different properties of rhEPO such as its antiinflammatory, antiapoptotic or proangiogenic properties (Moon et al., 2003; Krause et al., 2006).

Moreover, when rhEPO is chronically administered in a ischemic heart failure model, this treatment reduces inflammatory cytokine expression, oxidative damage, infarct size and improves cardiac function, potentially through the better neovascularization induced (van der Meer et al., 2005; Li et al., 2006).

More recently, it has been observed that repeated administrations of rhEPO are able to prevent the cardiomyopathy induced by doxorubicin, a chemotherapeutic agent known to be very cardiotoxic (Hamed et al., 2006; Li et al., 2006). Finally, rhEPO attenuates the cardiomyopathy associated with an experimental autoimmune myocarditis induced in the rat by myosin immunization (Mitsuma et al., 2006).

EPO derivates and cellular production

Because of the adverse effects associated with long-term rhEPO administration such as hypertension or thrombosis, several engineered EPO derivates have been developed that have cytoprotective effects, but do not stimulate significant erythropoiesis. Hematopoietic effects

of EPO on the bone marrow are mediated by the homodimeric EPOR. Desialylated EPO (asialoEPO), which has the same EPOR affinity but with a very short plasma half-life reducing the hematopoietic response, remains neuroprotective (Erbayraktar et al., 2003). In addition, carbamylated EPO (CEPO), another EPO analog (with all lysines transformed to homocitrulline by carbamylation) that do not bind to the homodimeric EPOR and lack erythropoietic activity, confers neuroprotection as well as cardioprotection against various cellular injury similarly to rhEPO (Fiordaliso et al., 2005; Moon et al., 2006). With these compounds, it is now possible to trigger EPO-mediated cytoprotective pathways without cross-talk with hematopoietic system.

Components of the mechanism of rhEPO induced cytoprotection

As illustrated previously, many studies using EPO derivatives without hematopoietic effect have shown that rhEPO cytoprotective effect is dissociated from its ability to alter erythrocyte number. The molecular signals by which rhEPO provides its benefit against various cellular stresses is currently unclear but some actors have been identified and common pathways have been proposed (Baker, 2005; Joyeux-Faure et al., 2005; Maiese et al., 2005).

EPO binding to the EPOR causes receptor homodimerization, with subsequent activation of the receptor associated Janus kinase 2 (Jak2), leading to tyrosine phosphorylation of EPOR (Smith et al., 2003). Signaling through the EPOR is promoted by tyrosine phosphorylation of the cytosolic domain and the recruitment of secondary signaling molecules such as the phosphatidylinositol-3 kinase (PI3 kinase). PI3 kinase then activates the antiapoptotic Akt pathway, which maintains the mitochondrial membrane potential, prevents the cellular release of cytochrome C and modulates caspase activity. Jak2 kinase is also able to phosphorylate and to activate the downstream antiapoptotic targets STAT3 and STAT5 or various kinases (p38

or p42/44 mitogen-activated protein kinases and protein kinase C) with antiapoptotic or antinecrotic properties.

Various mediators activated by these different pathways have been proposed. Among them, nitric oxide (NO) synthesis, ATP-sensitive potassium (K_{ATP}) channel or calcium-activated potassium (K_{Ca}) channel opening seem to mediate the EPO-induced cytoprotection (Shi et al., 2004; Joyeux-Faure et al., 2006).

In addition to preventing cellular death by apoptosis or necrosis, EPO also has been found to play a role in progenitor cell development through activation of nuclear factor- κ B (NF- κ B) which is a key mediator of inflammatory response (Digicaylioglu and Lipton, 2001).

Other data support the concept that tissue protection conferred by EPO is mediated through a heteroreceptor complex comprising EPOR and the common β receptor (β cR) (Brines et al., 2004). Indeed, CEPO that it signals only through β cR and not the homodimeric EPOR, yet is neuro and cardioprotective (Fiordaliso et al., 2005; Moon et al., 2006). Thus, rhEPO could induce cytoprotection through EPOR and/or β cR, which is a common receptor for various cytokines. Finally, it has been suggested that the effect of rhEPO on cell survival might involve an interplay of growth factors and cytokines, e.g., transforming growth factor (TGF), tumor necrosis factor (TNF), and IL-6 (Moon et al., 2003).

Further investigations are today required to confirm the identity of the EPO receptor subtype as well as the different actors involved in the EPO-induced cytoprotection.

Potential therapeutic benefits of rhEPO administration

Clinical use in patients with anemia and chronic kidney diseases as well as a safety clinical study on normal patients (Ehrenreich et al., 2002) has shown rhEPO to be safe and well tolerated, suggesting that this drug can fulfill the role as an ideal cytoprotective agent. Robust

randomize clinical trials are now needed to explore the therapeutic potential of rhEPO in the following areas.

As neuroprotective agent, rhEPO has already proven its efficacy in the treatment of stroke in a clinical trial on few patients (Ehrenreich et al., 2002), as previously mentioned. Further studies on a larger population could provide additional information on optimal rhEPO administration timing and dosage, completing these exiting results.

The future of rhEPO as cardioprotective agent seems very promising. Prospective studies have already shown significant functional improvement in chronic heart failure patients treated with antianemic rhEPO doses (Silverberg et al., 2001). In these studies, however, the presence of a direct effect of rhEPO on the myocardium independent from its effect on bone marrow was not directly assessed. On the other hand, since rhEPO prevents the cardiomyopathy induced by doxorubicin experimentally (Hamed et al., 2006; Li et al., 2006), this cytoprotective agent could be used in oncology against the cardiotoxic effects of anthracycline chemotherapy. Erythropoietin may also be a suitable exogenous agent to protect the heart against ischemia or to treat an ischemic episode. Thus, foreseeable ischemic events such as cardiac surgery, angioplasty or preservation of donor hearts for transplantation represent an opportunity to assess the cardioprotective effects of rhEPO. Moreover, rhEPO administration may represent a novel therapy to reduce cardiac cell dysfunction and death in patients presenting with acute myocardial ischemia. Many clinical trials in the field are currently in progress and will explore these hypotheses.

RhEPO may also have additional therapeutic effects *in vivo*, such as recruitment of vascular progenitor cells (van der Meer et al., 2004), that may promote tissue repair following various stresses.

Finally, the development of EPO derivatives, such as CEPO, opens a new field of cellular protectants with more specific properties and could result in clinical trials to test their cytoprotective effects in a near future.

In conclusion, accumulating evidence suggests that the therapeutic benefits of rhEPO administration could be much broader than initially anticipated. The future of rhEPO therapy in ischemic diseases as well as against cytotoxic agent side effects seems promising. A better understanding of its protective mechanisms based on experimental investigation could lead to carefully conducted clinical trials comparing the relative effectiveness of this protection with more conventional therapeutic strategies. We can hope that acute rhEPO administration will represent pharmacological approach to cytoprotection in upcoming years, leading to reduced cellular damage associated with ischemic event or toxic drugs.

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