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EDITORIAL COMMENTARY

EPO: renoprotection beyond anemia correction

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Abstract Until recently the major physiological function of erythropoietin (EPO) was thought to be the induction of erythropoiesis. However, a growing body of evidence indicates that EPO has tissue-protective properties and prevents ischemia induced tissue damage in several organs including the kidney. A pivotal intracellular pathway mediating the beneficial effects of EPO is the activation of Akt, i.e. serine/threonine protein kinase B. As a result, Akt phosphorylates the proapoptotic factor Bad, which in turn causes inhibition of programmed cell death (apoptosis). In the present article we review data on the non-hematological effects of recombinant human EPO (rHuEPO) in different experimental settings of acute and chronic kidney injury, and discuss clinical renoprotective strategies with rHuEPO or analogues substances that are not related to anemia correction.

Keywords Akt activation · Apoptosis · Erythropoietin (EPO) · Renoprotection

Introduction

The hematopoetic cytokine erythropoietin (EPO) was originally described as the long sought glycoprotein required for the proliferation, differentiation and survival of committed erythroid progenitor cells. Consequently, treatment with recombinant human EPO (rHuEPO) was a major breakthrough in the therapy of anemic states. Currently, the main indication for rHuEPO is treatment of anemia due to EPO deficiency in patients with preterminal and terminal renal failure. In addition, rHuEPO is also widely used in anemic patients with malignancies, and for autologous blood donation in perioperative surgical patients. However, a steadily growing body of evidence indicates that the therapeutic benefits of rHuEPO could be far beyond correction of anemia, and several review articles have been published recently on the non-hematological effects of rHuEPO in the cardiovascular and nervous system [1–3]. In this article we focus on non-hematological effects of rHuEPO in various experimental settings of acute and chronic kidney injury. Since these tissue protective effects of rHuEPO are not the result of correction of anemia related tissue hypoxia, we will also discuss potential molecular pathways involved. Finally, we propose clinical renoprotective strategies with rHuEPO or analogues substances.

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Molecular pathways of EPOs tissue protective action

Binding of a single EPO molecule to two adjacent EPO receptors on the membrane of target cells leads to homodimerization of the receptors and the triggering of different intracellular signaling cascades. By using EPO receptor fusion proteins it has been demonstrated that distinct conformations of the receptor exist which may activate different intracellular pathways. Thus, it may depend on the extracellular binding site of rHuEPO which receptor conformation is achieved and which signalling pathway is subsequently activated [4]. It is also possible that a single EPO receptor chain interacts with other membrane proteins forming novel heteromeric receptor complexes, as has been reported for the common beta receptor chain subunit (CD131) [5]. Such a heterodimeric receptor complex, which also

possibly contains other molecules, could exert a different signalling behaviour than the "classical" EPO receptor. After tyrosine phosphorylation the activated EPO receptor exhibits more than 40 binding sites, and a pivotal molecule which binds to the receptor and induces intracellular signalling is JAK2 tyrosine kinase. Activation of this kinase leads to tyrosine phosphorylation and dimerization of STATs. The JAK2-STAT5 signalling pathway is responsible for the effects of EPO on red blood cell differentiation and proliferation, but can also mediate protection against programmed cell death (apoptosis) [6]. It is therefore probable that the JAK-STAT5-pathway also plays an important role in the tissue-protective properties of rHuEPO not related to anemia correction. Another important signalling pathway triggered by EPO is the phosphatidylinositol 3-kinase (PI3K), which activates Akt (i.e. serine/threonine protein kinase B), a signaling pathway pivotal for cell survival. Akt subsequently phosphorylates the proapoptotic factor Bad, which in turn dissociates from a cell survival factor, Bcl-XL, resulting in inhibition of apoptosis [7]. The induction of Akt activity seems to be imperative for tissue protection by rHuEPO, since prevention of Akt phosphorylation abolished the beneficial action of rHuEPO in settings of experimental cardiovascular and neuronal injury [1–3]. Moreover, additional intracellular targets of EPO have been identified such as protein kinase C and heat shock proteins. The role of these pathways not related to erythropoesis unquestionably warrants further research. While the above pathways seem to be activated via the "classical" EPO receptor, it is presently unknown whether different ligands of the EPO receptor may induce different signalling pathways or whether the single EPO receptor chain associates and forms dimers with other membrane proteins. Theoretically, these "novel" receptors may be the target of newly designed rHuEPO compounds such as the carbamylated form of the hormone [8].

Treatment with rHuEPO in acute renal failure

EPO receptors have been documented in vascular as well as non-vascular kidney tissue, specifically on renal tubular cells [9]. Moreover, it has been shown that rHuEPO activates different "surviving" intracellular pathways in kidney tissue such as the PI3K/Akt pathway, or the heat shock protein 70. It is therefore not surprising that administration of rHuEPO was shown to be protective in various experimental settings of acute renal failure (ARF) such as ischemia/reperfusion injury [10–17] (Table 1). These in-vivo investigations unequivocally documented that treatment with even a single (high) dose of rHuEPO amelioriates renal dysfunction by reducing apoptotic cell death thus preserving kidney tissue. The encouraging experimental results have certainly prepared the ground for studies exploring the therapeutic potential of rHuEPO in humans with ARF, but before that some safety issues must be resolved. Particularly the dose-

Table 1 In-vivo studies on acute protective effects of recombinant human erythropoietin (rHuEPO) on the kidney

Species [reference]	Experimental model of acute renal failure	Dose, route and time of application	Mechanism(s) of tissue protection
Rat [10]	Hemorrhagic shock	300 U/kg rHuEPO i.v. before resuscitation	Reduced caspase activity (reduced apoptosis)
Rat [11]	Cisplatin nephrotoxicity	100 U/kg rHuEPO i.p. daily for 9 days	Increased regeneration of renal tubular cells
Rat [12]	Ischemia/reperfusion	3,000 U/kg rHuEPO i.v. 24 hours before ischemia	Heat shock protein 70 activation, reduced caspase activity (reduced apoptosis)
Rat [13]	Ischemia/reperfusion	300 U/kg rHuEPO i.v. 30 min before ischemia, 5 min before reperfusion or 30 min after ischemia	Reduced caspase activity (reduced apoptosis)
Rat [14]	Ischemia/reperfusion	200 U/kg rHuEPO i.p. before ischemia, 6 an 24 hours after reperfusion, thereafter daily	Reduced down-regulation of renal aquaporins (water channels) and sodium transporters
Rat [15]	Ischemia/reperfusion	5,000 U/kg rHuEPO i.p. 30 min before ischemia	Reduced apoptosis, increased regeneration of renal tubular cells
Mouse [16]	Ischemia/reperfusion	1,000 U/kg rHuEPO s.c. for 3 days before ischemia or 5 min before reperfusion	Reduced oxidative stress and lipid peroxidation
Rat [17]	Ischemia/reperfusion	5,000 U/kg rHuEPO or 25 μg/kg darbepoetin alpha i.p. immediately after ischemia or 6 hours after ischemia	Reduced apoptosis, increased regeneration of renal tubular cells

i.v. - intravenously, i.p. - intraperitoneally, s.c. - subcutaneously

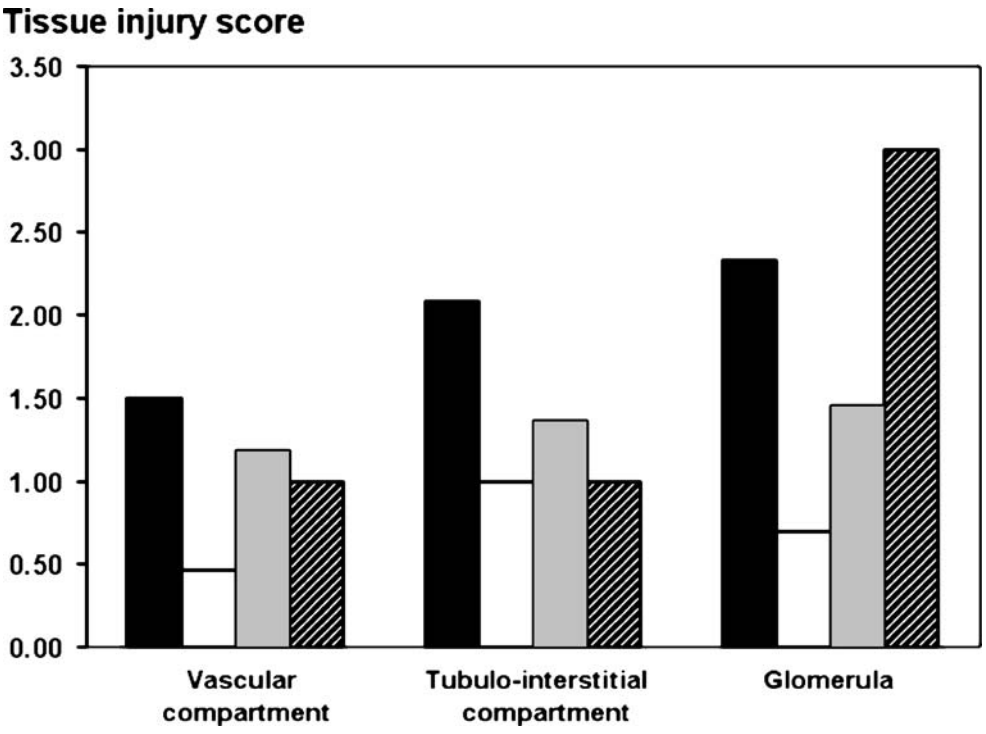

Fig. 1 Median tissue injury scores in different compartments of remnant kidney animals treated with placebo or escalating doses of darbepoetin alpha. Renal issue was obtained 6 weeks after 5/6 nephrectomy. *Black bars*=saline (placebo), *white bars*=0.1 μg/kg body weight darbepoetin (low dose), *grey bars*=0.3 μg/kg body weight darbepoetin (intermediate dose), *hatched bars*=with 1.0 μg/kg body weight darbepoetin (high dose). Low dose darbepoetin prevented tissue injury in all three renal tissue compartments (all $p<0.05$ vs. placebo), whereas the protective effect on renal vessels and particularly the glomerula vanished with increasing darbepoetin doses. High dose darbepoetin treatment caused a marked increase in hematocrit ($p<0.05$ vs. placebo and low dose darbepoetin)

dependent effects on the number and activation state of thrombocytes, and the stimulation of platelet adherence to (injured) endothelium could mitigate the beneficial effects of rHuEPO on damaged renal (vascular) tissue [18]. A recent randomized controlled study in premature infants who received rHuEPO chronically in order to stimulate erythropoiesis revealed increased thrombin receptor-activating peptide (TRAP-6)-induced expression of P-selectin on platelets in newborns treated with rHuEPO accompanied by a significant increase in the number of activated platelets [19]. However, it is conceivable that administration of a single "high dose" (or few repetitive doses) of rHuEPO in order to prevent renal tissue apoptosis in the course of ischemic injury may be a promising approach to limit loss of kidney tissue also in patients with ARF.

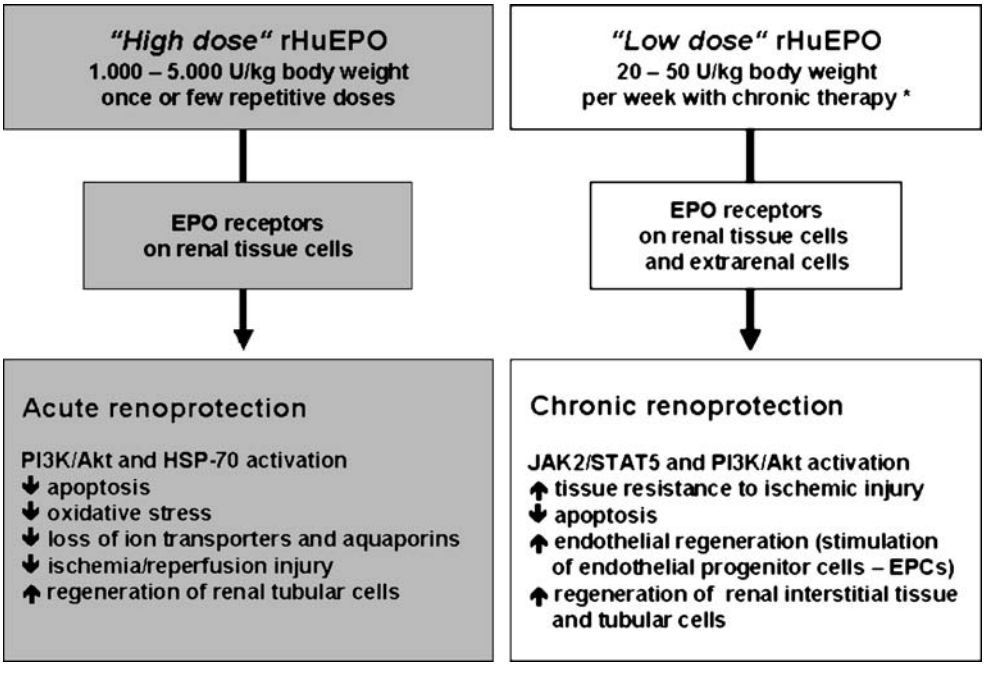

Fig. 2 Established and hypothetical renoprotective mechanisms, and indications for high and low dose therapy with recombinant human erythropoietin (rHuEPO) in clinical settings of acute and chronic kidney injury (*=alternatively: 0.1–0.3 μg/kg body weight darbepoetin alpha per week)

Renoprotection by rHuEPO in chronic renal failure

We could demonstrate that chronic treatment with the long-acting rHuEPO analogue darbepoetin alpha conferred renal vascular and tissue protection and preserved renal function in the established 5/6 nephrectomy remnant kidney model in the rat, i.e. a model that features progressive injury to the renal microvascular endothelium leading to glomerular sclerosis and ischemia-induced tubulointerstitial damage [20]. Treatment with rHuEPO not only reduced renal dysfunction, but also significantly improved survival of uremic rats. In this experimental setting we found persistent activation the Akt pathway in endothelial and epithelial glomerular cells, and reduced apoptotic cell death in renal tissue. Importantly, we used a hematologically non-effective dose of darbepoetin which did not affect hematocrit levels in treated animals. This could be of considerable clinical relevance, since "low dose" rHuEPO treatment may be a safe strategy to avoid potential adverse effects of high rHuEPO doses, i.e. doses that cause an increase in hematocrit (with accompanying changes in viscosity) and activate thrombocytes. Indeed, we could show that in 5/6 nephrectomized rats escalating doses of darbepoetin alpha mitigate the protective effects on the remnant kidney tissue and even worsen microvascular renal injury, i.e. glomerulosclerosis (Fig. 1). Thus, administration of rather low doses of rHuEPO may be a feasible way to limit renal tissue damage in patients with chronic kidney diseases (CKD). Indeed, results of a recently published clinical trial have revealed that early initiation of rHuEPO therapy in patients with CKD and mild to moderate anemia significantly slowed the progression of renal disease and delayed the initiation of renal replacement therapy [21]. Importantly, anemia correction in this study was achieved with relatively low rHuEPO doses over a time period of 6 months. However, with respect to the observed beneficial effect on progression one cannot discriminate between the effect of rHuEPO treatment and the effect of anemia (i.e. hypoxia) correction. Presumably, establishing a minimal effective dose in future studies will allow better cost/benefit estimates of rHuEPO treatment in the setting of CKD. Moreover, rHuEPO analogues that maintain tissue protective effects but are devoid of the action on erythropoesis (and thrombopoesis?) may represent a valuable alternative. Such molecules like carbamylated rHuEPO (CEPO) have already been tested in experimental studies revealing tissue protective properties comparable to that of "classic" rHuEPO, but without any effect on hematocrit and procoagulative activity [8, 22]. The potential of such rHuEPO analogues to prevent loss of renal tissue in patients with CKD awaits further investigation.

Conclusions

There is accumulating evidence that the renal therapeutic benefits of rHuEPO could be far more wide-reaching than anticipated by ameliorating of anemia and accompanying tissue hypoxia. As more studies are undertaken to evaluate the non-hematological actions of rHuEPO, it should become clearer which additional effects contribute to organ protection. In addition to the well documented anti-apoptotic effect of rHuEPO in renal tissue, stimulation of regenerative progenitor cells by rHuEPO may play a role in organ protection/regeneration as well [1, 2, 23]. It is therefore conceivable that therapy with rHuEPO—whether acute high dose or chronic low dose (Fig. 2)—may join the arsenal of approaches directed against the sequels of ARF and/or progressive CKD.

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