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REVIEW ARTICLE

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Beneficial and ominous aspects of the pleiotropic action of erythropoietin

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Abstract The primary function of the glycoprotein hormone erythropoietin (Epo) is to promote red cell production by inhibiting apoptosis of erythrocytic progenitors in hemopoietic tissues. However, functional Epo receptors (Epo-R) have recently been demonstrated in various nonhemopoietic tissues indicating that Epo is a more pleiotropic viability and growth factor. Herein, in vitro and in vivo effects of Epo in the brain and the cardiovascular system are reviewed. In addition, the therapeutic impact of Epo in oncology is considered, including the question of whether Epo might promote tumor growth. Convincing evidence is available that Epo acts as a neurotrophic and neuroprotective factor in the brain. Epo prevents neuronal cells from hypoxia-induced and glutamate-induced cell death. Epo-R is expressed by neurons and glia cells in specific regions of the brain. Epo supports the survival of neurons in the ischemic brain. The neuroprotective potential of Epo has already been confirmed in a clinical trial on patients with acute stroke. With respect to the vasculature, Epo acts on both endothelial and smooth muscle cells. Epo promotes angiogenesis and stimulates the production of endothelin and other vasoactive mediators. In addition, Epo-R is expressed by cardiomyocytes. The role of Epo as a myocardial protectant is at the focus of present research. Epo therapy in tumor patients is practiced primarily to maintain the hemoglobin concentration above the transfusion trigger and to reduce fatigue. In addition, increased tumor oxygenation may improve the efficacy of chemotherapy and radiotherapy. However, tumor cells often express Epo-R. Therefore, careful studies are required to fully exclude that recombinant human Epo (rHuEpo) promotes tumor growth.

Keywords Erythropoietin · Erythropoietin receptor · Hemopoiesis · Anemia · Neuroprotection · Cardioprotection · Malignancy · Tumor cell growth

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Introduction

Erythropoietin (Epo) has been well characterized as the renal glycoprotein hormone which promotes the survival, proliferation, and differentiation of erythrocytic progenitors in hemopoietic tissues. Recombinant human Epo (rHuEpo) and related compounds have proved most useful for treatment of the anemia associated with chronic renal failure and, more restrictedly, certain types of nonrenal anemias (for references see [97, 134]). Epo maintains erythropoiesis by binding to specific transmembrane receptors (Epo-R) which are expressed primarily by erythrocytic progenitors such as the burst-forming units–erythroid (BFU-E) and the colony-forming units–erythroid (CFU-E). The functional human Epo-R is a member of the cytokine class I receptor superfamily and presents as a homodimer of two identical glycoprotein chains of 484 amino acids [169]. Each of the chains is composed of an extracellular domain with conserved cysteines and a WSXWS motif, a hydrophobic transmembrane sequence, and a cytoplasmic domain to which the protein tyrosine kinase JAK2 (Janus kinase 2) is affiliated. The Epo molecule binds to both Epo-R subunits [157], whereby the dissociation constants for the two binding sites differ greatly (1 μM vs 1 nM). With respect to novel recombinant Epo analogues, it is important to note that the affinity for the receptor decreases with Epo glycosylation [61]. The carbohydrate portion of Epo is thought to prevent Epo-R binding through electrostatic forces [53]. Epo binding induces a conformational change and a tighter connection of the two Epo-R subunits [44, 160, 183].

Consequently, the two JAK2 molecules of the cytoplasmic regions of the Epo-R subunits undergo autophosphorylation [160, 207] and catalyze the phosphorylation of tyrosine residues of Epo-R, thereby providing docking sites for signaling proteins containing SRC homology 2 (SH2) domains [13, 185]. The complex network of Epo-R signaling involves (1) the expression of the antiapoptotic protein bcl-x_L [81], (2) the activation of mitogen-activated protein kinase (MAPK) and phosphatidyl-inositol 3-kinase (PI-3K/Akt), and (3) homodimerization of the signal transducer and activator of transcription 5 (STAT5). The concerted action of these mediators increases the rates of survival, proliferation, and differentiation of the erythrocytic progenitors [109, 151, 219]. In vitro studies have shown that the Epo-induced signaling pathways return to nearly basal levels after 30–60 min of stimulation [196]. The effect of Epo is terminated by the action of the hemopoietic cell phosphatase (HCP) which catalyzes JAK2 dephosphorylation [110, 216]. The Epo/Epo-R complex is then internalized and degraded [181, 196].

Whereas most of the erythrocytic progenitors die without Epo [54, 113], an increasing number of BFU-E and CFU-E escape from apoptosis and proliferate in the presence of Epo, thereby producing large progeny of proerythroblasts and normoblasts. The time from the CFU-E to the reticulocyte is about 7 days and involves several cell divisions. Therefore, there is a lag of a few days before reticulocytosis occurs following a rise in the plasma Epo concentration due to an increase in endogenous Epo gene expression in association with hypoxic stress or on the administration of rHuEpo.

Originally, stimulation of erythropoiesis was thought to be the sole physiological function of Epo [93]. However, recent advances in analytical techniques have led to the demonstration of Epo-R mRNA, Epo-R protein, Epo binding to Epo-R, and intracellular signaling in a variety of nonhemopoietic cells and organs, including the brain, cardiovascular tissues (endothelium, vascular smooth muscle, cardiomyocytes), the liver, gastrointestinal tissues, pancreatic islands, the kidney, the testis, and the female reproductive organs (for references see [101, 127, 138, 168]). Thus, Epo is a more pleiotropic survival and growth factor than initially thought (Fig. 1). It is assumed that Epo has neurotrophic and neuroprotective [43, 49, 98, 99, 126, 128], vascular [128, 177], and cardioprotective [155, 177] functions. Herein, relevant experimental studies and preliminary clinical observations are summarized with respect to the value of rHuEpo therapy in cerebral and cardiac disorders. On the other hand, given that Epo is a pleiotropic growth factor, the worrying question has to be raised as to whether tumor cells express Epo-R and whether Epo can induce or promote tumor growth. This aspect is highly relevant to the rationale of rHuEpo treatment of renal and nonrenal anemias, particularly the anemia associated with cancer.

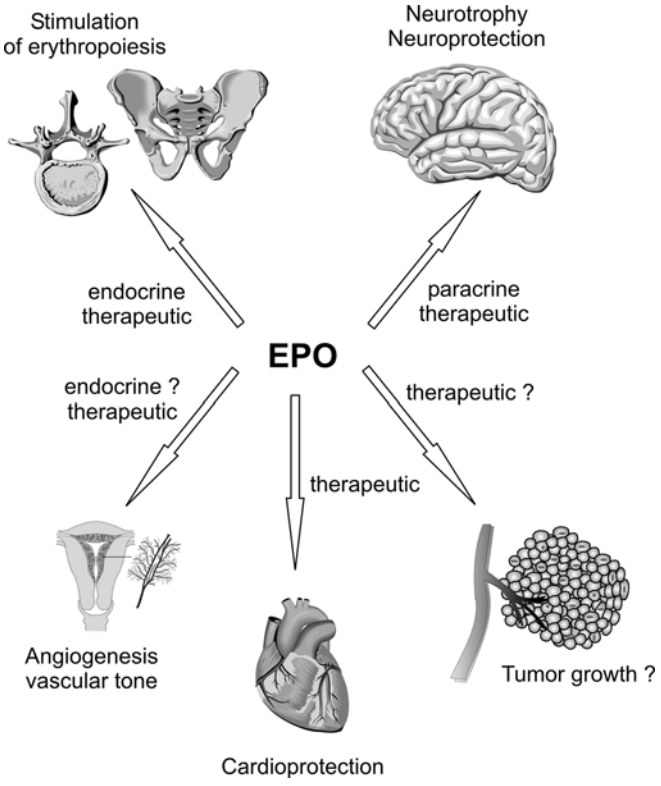

Fig. 1 Target tissues of erythropoietin (EPO). Circulating EPO acts primarily on erythropoietic progenitors. In addition, the hormone stimulates angiogenesis. Brain-derived EPO exerts its action in a paracrine way. Neuroprotective and cardioprotective effects of the pharmacological administration of rHu-EPO have been reported. Whether EPO stimulates tumor growth is a matter of debate

Effects of Epo on neuronal cells

Expression of Epo-R by neurons and neurotrophic effects

Masuda et al. [127] first detected low affinity binding sites for Epo in rat PC12 pheochromocytoma and mouse basal forebrain SN6 cell cultures. PC12 cells respond to exogenously added Epo with a rapid increase in the cytosolic Ca^{2+} concentration and the release of monoamines. More recent studies with PC12 cells have confirmed that Epo modulates dopamine release and NO production [107, 112] and affects the rate of the expression of several genes as assessed by cDNA array screening [161]. Another seminal finding was reported by Konishi et al. [111] who showed that Epo increases choline acetyltransferase activity in primary cultures of mouse neurons and the survival of septal cholinergic neurons in rats with fimbria-fornix transections. Several groups of investigators have subsequently demonstrated Epo-R mRNA and protein in distinct areas of rodent and mammalian brain [57, 119, 124]. Epo binding sites are mainly located in the hippocampus, capsula interna, cortex, and midbrain of mice [57]. Hypoxia exposure leads to an increase in Epo mRNA levels in human and mouse brain [18, 45, 119, 165, 176]. In human embryos, both Epo-R and Epo become detectable in the brain 5 weeks post-conception

[52, 103, 105, 118]. Both neurons and astrocytes express Epo-R [18, 19, 105, 176, 180]. In addition, brain capillary endothelial cells express Epo-R, as shown in rat [213] and human [26] biopsies. Interestingly, rat capillary endothelial cells express two different forms of Epo-R mRNA [213]. Epo-R and Epo have also been detected by immunohistochemistry in peripheral rat nerves [35]. The histological assignment has been confirmed in several cell culture studies. Epo-R expression has been demonstrated in primary cultures or cell lines of neuronal origin [18, 19, 45, 127, 137, 143, 210], astrocytes [18, 124, 143, 180], microglia [143], and brain-derived capillary endothelial cells [18, 213].

Trophic effects of Epo have been demonstrated in cultures of cortical and cholinergic neurons [111, 137]. Epo also stimulates the proliferation and differentiation of neuronal stem and progenitor cells [173, 179]. Clearly, however, while the Epo/Epo-R system is required for prenatal and postnatal erythropoiesis, this does not appear to be the case with respect to the neuronal Epo/Epo-R system. Suzuki et al. [182] have established a transgenic mouse line which expresses Epo-R exclusively in hemopoietic tissues. The transgenic mice develop normally and show no neurologic abnormalities despite the lack of Epo-R in the brain and other nonhemopoietic tissues.

Neuroprotective effects of Epo in vitro

Glutamate is considered one of the major mediators of neuronal cell death due to hypoxia [46]. Hypoxic neurons release large amounts of glutamate, which can act both on ionotropic receptors (NMDA-R, AMPA-R/kainate-R) and metabotropic receptors [130]. Preconditioning of rat hippocampal and cerebral cortical cells in primary culture with Epo has been shown to reduce glutamate cytotoxicity [137]. In fact, preconditioning with Epo protects neurons from both NMDA-induced and NO-induced apoptosis [18, 56]. When acutely added, Epo fails to prevent rat hippocampal and cerebral cortical neurons in primary culture from NO-induced death although it inhibits the NMDA-R-mediated increase in the cytosolic Ca^{2+} concentration [166]. Recent in vitro studies indicate that the PI-3K/Akt pathway is of primary importance in the neuroprotective action of Epo by maintaining mitochondrial membrane potential in anoxic primary hippocampal neuronal cell cultures [48]. Destabilization of the mitochondrial membrane potential leads to the release of cytochrome C, which activates the caspases 8, 1, and 3 that promote DNA fragmentation [50]. Other studies regarding Epo/Epo-R signaling in neuronal cells are described elsewhere [47, 49].

Neuroprotective effects of Epo in vivo

Evidence for a neuroprotective action of Epo in the brain was first provided by the group of Sasaki in 1998 [165, 166]. Infusion of rHuEpo into the lateral ventricles of Mongolian gerbils with experimental cerebral ischemia prevents ischemia-induced learning disability and rescues hippocampal CA1 neurons from death, whereas infusion of soluble Epo-R augments neuronal degeneration and impairs learning ability [166]. rHuEpo infused into the cerebral ventricles of rats with permanent middle artery occlusion reduces ischemia-induced place navigation disability, cortical infarction, and thalamic degeneration [165]. Further studies have shown that locally administered rHuEpo upregulates the expression of bcl-x_L in the hippocampal CA1 field [203], inhibits NO production [33], and maintains cognitive functions [40] in gerbils with experimental cerebral ischemia. The role of NO is not fully understood, however, since Genc et al.[70, 71] have reported that Epo exerts neuroprotective effects in vivo by increasing NO production. NO can be cytotoxic for neuronal cells, but it may also improve O_2 supply by means of vasodilation. In addition, NO induces Epo-R expression in neuronal cell cultures [148].

Preconditioning stressors such as hypoxia render tissues more tolerant to subsequent stress events. Accordingly, brief periods of sublethal cerebral ischemia protect rats from subsequent stroke caused by permanent middle cerebral artery occlusion [174]. Hypoxic preconditioning also reduces hypoxia-induced ischemic brain injury in neonatal rats [191] and kainate-induced neuronal damage in adult rats [64]. The beneficial effect of hypoxia preconditioning is partly mediated by Epo which is increasingly produced in the hypoxic brain. Indeed, a recent study has shown that the protective effect of hypoxic preconditioning is significantly reduced in mice when Epo signaling is locally blocked by infusion of soluble Epo-R into the cerebral ventricle [158].

It was earlier assumed that systemically administered Epo would not enter the brain because of the blood–brain barrier [102, 104, 125]. However, when biotinylated rHuEpo was administered intraperitoneally (i.p.) to rats, peroxidase reaction product was observed in surrounding capillaries and later localized to scattered neurons in the brain [26]. Jumbe [99] has shown the cerebrospinal fluid to serum concentration ratios to be about 1×10^{-3} following the intravenous (i.v.) administration of rHuEpo (5000 U/kg) or darbepoetin alfa (25 μg/kg) in rats. The calculated mean area under the concentration–time curve (AUC_{0-8}), by noncompartmental analysis, was 340 mU h/ml for rHuEpo and 3.6 ng h/ml for darbepoetin alfa in cerebrospinal fluid vs 370,000 mU h/ml and 4500 ng h/ml in serum, respectively. The i.p. administration of rHuEpo (25–100 U) has been reported to reduce postischemic malonyl dialdehyde levels, NO formation, brain edema, and hippocampal CA1 neuronal loss in gerbils with bilateral carotid occlusion [33]. Other investigators have reported that the systemic administration of high doses of rHuEpo to experimental animals reduces the volume of infarction 24 h after middle cerebral artery occlusion [175], reduces mortality rate [29], prevents neuronal damage [6], increases cerebral blood flow [20, 78], and reduces neurologic deficits [79, 80]. Investigations in rats, in whom crush injury was mimicked by the aneurysm

clamp technique or in whom traumatic brain injury was induced, have shown that rHuEpo prevents motor neuron apoptosis and neurologic disability [42] and improves recovery of motor function [77]. Possibly due to the prevention of death of neuronal cells rHuEpo reduces the inflammatory reaction in hypoxic brain [197]. A recent, elegant gene therapy study in rats has shown that i.v. injected naked plasmid DNA encoding Epo induces neuroprotective effects similar to rHuEpo [202]. Based on animal studies, investigators have also put forward the concept that rHuEpo may be of value in retinal diseases involving apoptosis of photoreceptors or of retinal ganglionic cells [82, 100].

Furthermore, the Epo/Epo-R system has been proposed to play a role in peripheral nerves. Epo-R immunoreactivity has been detected in the somata and axons as well as in Schwann cells of rat sciatic nerves [35]. rHuEpo protects mechanically injured dorsal root ganglionic neurons from undergoing apoptosis [170]. In an experimental model of diabetes, rHuEpo has proved to partially reverse the alterations in nociception and to restore Na^+/K^+-ATPase activity in nerve fibers [22].

Clinical experience with rHuEpo in stroke patients

Knowing that brain ischemia is a leading cause of death and disability in humans and that there is need for additional stroke therapy strategies, Ehrenreich et al. [62] performed a clinical trial with rHuEpo in patients with acute stroke. Initially, a safety study was carried out in which 13 patients received rHuEpo i.v. (3.3×10^4 U) once daily for the first 3 days after stroke. The mean concentration of Epo in the cerebral spinal fluid of the patients increased to 17 U/l (compared to the normal value of about 1 U/l [21, 125, 144]). Serum Epo levels in the patients approximated 5000 U/l 3 h after rHuEpo infusion [62] (compared to a normal serum level of about 15 U/l in nonanemic humans [96]). Thereafter, a double-blind randomized proof-of-concept study was carried out on 40 patients who received either rHuEpo or saline. Study inclusion criteria were age <80 years, ischemic stroke within the middle cerebral artery territory, symptom onset <8 h before rHuEpo or saline administration, and deficits on stroke scales. The results of the trial indicated a strong trend for reduction in infarct size in the rHuEpo-treated patients as indicated by magnetic resonance imaging. The reduction in infarct size was associated with a marked neurological recovery and clinical outcome 1 month after stroke. Thus, rHuEpo therapy may add to the beneficial effect of conventional clot-dissolving strategies in stroke patients [62].

Résumé and future directions

Epo produced in the brain exerts a local function that is distinct from that in erythropoiesis. Epo and Epo-R are expressed both by neurons and astrocytes. Similar to renal Epo, cerebral Epo production increases on hypoxic stress. The response to hypoxia is accomplished by the stabilization and activation of the hypoxia-inducible transcription factors 1 and 2 (HIF-1 and HIF-2), which induce the expression of several genes encoding proteins that protect tissues from O_2 and energy deprivation. There is convincing evidence that Epo is an antiapoptotic and mitogenic factor for neuronal cells. In experimental animals, locally or systemically administered rHuEpo is neuroprotective against a variety of insults, including cerebral ischemia, subarachnoid hemorrhage, head injury, and experimental autoimmune encephalomyelitis. With respect to the therapeutic use of rHuEpo as a neuroprotective agent in humans, the blood–brain barrier is a critical issue. The concept has been put forward that there are specific transport mechanisms to ship Epo from the systemic circulation into the central nervous system [26]. In addition, Epo may even get easier access to the brain after hypoxic lesion of the blood–brain barrier. Along these lines, hypoxia stimulates the production of vascular endothelial growth factor, which is one of the main mediators of the leakage of the blood–brain barrier in brain ischemia and trauma [66, 140]. In view of the limited penetration of intact Epo across the blood–brain barrier, Erbayraktar et al. [65] have tested the efficacy of asialo-rHuEpo, which has a plasma half-life of 1.4 min and exerts no erythropoiesis-stimulating activity. This drug could be administered at high doses without producing erythrocytosis. Reportedly, asialo-rHuEpo crosses the blood–brain barrier after i.v. administration, binds to neurons within the hippocampus and cortex in a pattern corresponding to the distribution of Epo-R, and is neuroprotective in cerebral ischemia, spinal compression, and sciatic nerve crush [65].

Effects of Epo in the cardiovascular system

Effects of Epo on endothelial cells

Epo-R is expressed on human vascular endothelial cells from coronary, pulmonary, and cerebral arteries, the umbilical vein, and dermal vessels [9, 12]. Epo stimulates in vitro the proliferation and migration of human, murine, and bovine endothelial cells [8, 141, 162]. In addition, Epo induces a proangiogenic phenotype of endothelial cells and neovascularization [39, 92]. It also promotes blood vessel formation in the uterus of ovariectomized mice [214]. Very recent studies have shown that rHuEpo administration in humans produces an increase in the number of circulating endothelial progenitor cells, which may be beneficial in augmenting the neovascularization of ischemic tissues [11, 86]. In vitro, rHuEpo causes a rapid tyrosine phosphorylation of cytosolic proteins and the translocation of STAT5 in human umbilical vein endothelial cell (HUVEC) cultures [84]. A recent differential display analysis of HUVEC extracts has revealed four groups of genes that are upregulated by rHuEpo, including those encoding proteins in the control of vascular function (e.g., thrombospondin-1), gene transcription (e.g., c-myc

purine-binding transcription factor PuF), mitochondrial function (e.g., cytochrome C oxidase subunit 1), and regulators of signal transduction [68]. However, it must be noted—and this holds true for most of the endothelial cell culture studies cited below—that the concentration of rHuEpo added to the cultures was much higher than that generally reached in the vascular bed.

Epo can stimulate the production of several endothelial-derived modulators of the vascular tone, including some with vasoconstrictive and some with vasorelaxant properties. The highly potent vasoconstrictor endothelin is released [37, 106] via a Ca^{2+}-dependent mechanism [24, 38, 198]. This effect has been implicated clinically in the arterial hypertension observed in rHuEpo-treated patients with chronic renal failure [37]. Clearly, however, rHuEpo therapy-induced increases in arterial blood pressure result primarily from the elevated blood viscosity and the abolishment of tissue hypoxia-associated vasodilation [51]. Indeed, while tenfold overexpression of Epo in transgenic mice activates the tissue endothelin system [159], the plasma endothelin concentration is not consistently elevated in rHuEpo-treated patients with chronic renal failure [28]. Studies in partially nephrectomized uremic rats have shown that immunoreactive endothelin-1 is increased in arterial walls on rHuEpo therapy, but not in circulation [115]. In the same experimental model, selective endothelin-1 $receptor_A$ (ET_A) blockade but not nonselective $ET_{A/B}$ blockade prevents the aggravation of hypertension [27]. Interestingly, the acute administration of rHuEpo produces increases in plasma endothelin-1 levels and in blood pressure in spontaneously hypertensive rats (SHR), but not in normotensive Wistar-Kyoto rats (WKR; [188]). Thus, rHuEpo may aggravate a preexisting state of hypertension.

Other mediators reported to be released in response to rHuEpo treatment of cultured endothelial cells include the vasoconstricting prostanoids prostaglandin $F_{2\alpha}$ and thromboxane B_2 [24], plasminogen activator inhibitor-1 [142], and the vasodilating vascular endothelial growth factor (VEGF) [147]. The vasodilatory action of VEGF is known to be mediated by NO [90, 154]. The exact nature of the interference of Epo with the endothelial NO system remains to be clarified, since the induction of NO synthase protein and activity [12, 190, 206, 212] as well as no change [146] or depression [149, 201] have been reported. In vivo, the Epo-induced rise of the red blood cell count increases blood viscosity and endothelial shear stress, which is the major regulating stimulus for the endothelial NO system. Thus, in Epo transgenic mice (hematocrit 0.85), the intravascular NO bioavailability is increased due to marked induction of the endothelial NO synthase [85, 199].

Effects of Epo on vascular smooth muscle

Vascular smooth muscle cells also have been shown to express Epo-R [4, 7] and been investigated extensively in relation to the rHuEpo therapy-associated arterial hypertension. In reduced complexity experiments using isolated vessels and cell cultures, Epo induced vasoconstriction [87] and vascular smooth muscle contraction [136], whereas in the isolated hemoglobin-perfused kidney setting no increase in renal vascular resistance was observed [153]. Epo signaling in vascular smooth muscle cells is Ca^{2+} dependent [5, 114, 145] and includes the activation of the phospholipase C cascade and the activation of oncogenes (myc, jun, fos) [76] promoting DNA replication and cellular growth. Thus, by endorsing vascular smooth muscle cell growth, Epo could be a factor in vascular hypertrophy and arterial hypertension. Other intracellular signals activated by Epo include the MAPK [5, 7] and PI-3K/Akt pathways [4], which are intimately involved in the inhibition of apoptosis [60, 208].

Myocardial protection by Epo

To foster new therapeutic approaches for the treatment of myocardial disease, it is critical to dissect the underlying subcellular pathways used by potential cytoprotectants. In this regard, rHuEpo has become especially attractive. The prerequisite of Epo action, the expression of Epo-R, has been proven for murine [211] as well as human embryonic cardiomyocytes [103], and for human adult cardiomyocytes [189] and cardiac tissue (own unpublished observation). Epo has been shown to act as a mitogen on neonatal rat cardiomyocytes, a process which involves tyrosine kinase and protein kinase C [200]. Of note, the murine knockout of either the Epo gene or the Epo-R gene results in a phenotype of severe cardiac malformations with embryonic lethality at embryonic day 13.5 [211]. However, more recent studies have shown that a tissue-specific knockout of Epo-R selectively in nonhemopoietic tissues does not result in a defect or abnormality in the myocardium [182]. Thus, the impaired heart development seen in earlier studies was likely caused by the decrease in red cell numbers, and thereby, reduced O_2 transport capacity of the blood.

Recent experimental studies have provided strong evidence that Epo protects the myocardium (Table 1). In hypoxia-exposed mice, treatment with rHuEpo enhances cardiac contractility, while treatment with anti-Epo-antibody has the opposite effect [178]. In addition, it has been reported that Epo administration reduces myocardial infarction volume, protects against ischemia reperfusion injury, and promotes beneficial ventricular remodeling in mice, rats, and rabbits [32, 34, 135, 155, 156]. Importantly, beneficial effects were not only seen with preemptive Epo administration 24 h prior to the coronary artery occlusion but also when Epo was given after reperfusion was started. Thus, in a real-life setting with delayed access of patients to reperfusion treatment, i.e., emergency coronary bypass grafting for acute coronary occlusion, rHuEpo administration several hours later still might have a beneficial effect on infarct size, extent of reperfusion injury, and functional recovery of the myocardium. Importantly, cardioprotective effects of Epo were

seen without an increase in hematocrit, thus eliminating O_2 delivery as an etiologic factor in myocyte survival and function.

The myocardial protection by Epo is based on the antiapoptotic effect of Epo on cardiac myocytes and possibly cardiac fibroblasts [156]. Epo-induced myocardial survival involves a PI-3/Akt-dependent process leading to a reduction of the number of apoptotic myocytes [48, 156, 189]. Since apoptotic cell death of myocytes is a key feature of myocardial damage from infarction and ischemia/reperfusion [60, 133], the reduction of the number of apoptotic myocytes might result in improved myocardial survival and function.

Impact of rHuEpo therapy in oncology

Rationale for the use of rHuEpo

Patients with malignant tumors often present with normochromic and normocytic anemia. Although red cell survival may also be shortened, the anemia is primarily of the hypoproliferative type. Iron availability is reduced despite normal iron stores. Proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interferons inhibit the proliferation of erythrocytic progenitors [129] and suppress Epo gene expression [94]. The concentration of serum Epo is relatively low for the degree of anemia in many cancer patients as first shown by Miller et al. [131]. Anemia (hemoglobin <120 g/l) is a negative prognostic factor for successful tumor therapy and disease-free survival [73, 187, 193]. A thoughtful review of the role of tumor hypoxia on tumor progression has been provided very recently [194]. rHuEpo or its analogue, darbepoetin alfa [75], can substitute for the lacking endogenous Epo and counteract the cytotoxic effects of chemotherapy and radiotherapy on erythrocytic progenitors. In vitro studies with Epo-producing human hepatic cells [209] and isolated perfused canine kidneys [67] have shown that cytotoxic drugs may inhibit Epo synthesis, apart from their suppressing erythropoiesis directly. On the other hand, however, clinical studies indicate that Epo production is maintained on both platinum and nonplatinum chemotherapy [36].

The primary goals of rHuEpo therapy are to maintain the patient's hemoglobin concentration above the transfusion trigger, to reduce asthenia or fatigue, and to increase exercise tolerance [55, 74, 150]. The alleviation of tumor and tumor therapy-associated anemia may also restore brain functions [98, 117]. Although it is generally taken for granted that rHuEpo treatment leads to anemia improvement in cancer patients, a recent critical review has emphasized the use of inappropriate quality of life instruments in almost all clinical studies reported so far and suggested more reliable research [25]. In general, 10–30% of the tumor patients still have to be transfused despite rHuEpo therapy [14]. An increase in hemoglobin concentration ≥10 g/l or in reticulocyte count $\geq 40\times10^9$/l after 4 weeks of rHuEpo treatment is considered a reliable indicator of response [41]. According to evidence-based clinical practice guidelines published by the American Society of Clinical Oncology and the American Society of Hematology [163], the use of rHuEpo is recommended as a treatment option for patients with chemotherapy-associated anemia with a hemoglobin concentration below 100 g/l. The dosage of rHuEpo (or its analogue darbepoetin alfa) should be titrated to maintain a hemoglobin concentration of 120 g/l to avoid cardiovascular disorders.

Two recent clinical trials have shown that hemoglobin concentrations in cancer patients should not be raised into the normal range. First, a multicenter trial that sought to assess the effect of rHuEpo treatment to maintain normal hemoglobin concentrations on survival in breast cancer patients under chemotherapy was terminated early because of an increase in mortality in the first 4 months of the study [116]. However, there were difficulties in the interpretation of that study which showed imbalances of risk factors between the rHuEpo and the placebo groups, and the lack of occurrence of anemia in the placebo group [116]. Second, a randomized, double-blind, placebo-con-

Table 1 Investigative models for study of the effects of EPO on myocardiocytes

Disease Model	Species, in vitro model	Finding	Reference
Myocardial infarction-reperfusion	Rat	Improved functional myocardial recovery, 50% reduction of apoptosis	Calvillo et al. [34]
Myocardial hypoxia, ischemia-reperfusion	Mouse/rat	Improved myocardial recovery of function, reduction of apoptotic myocytes by 50%	Cai et al. [32]
Hypoxia, myocardial infarction	Rat, myocyte culture	Reduction of apoptosis	Tramontano et al. [189]
Myocardial infarction	Rat	Reduction of infarct size by 75%, attenuation of functional myocardial decline, anti-apoptosis	Moon et al. [135]
Hypoxia, myocardial infarction	Rabbit, myoblast culture	Improved myocardial function following infarction, mitigation of myocyte apoptosis	Parsa et al. [155]
Ischemia-reperfusion	Rabbit, fibroblast culture	Enhanced cardiac function and recovery, reduced apoptosis	Parsa et al. [156]

trolled trial on 351 patients with head and neck cancers receiving radiotherapy has shown an advantage in progression-free survival in the placebo-treated compared to the rHuEpo-treated group [89]. However, apart from some imbalances in prognostic factors (such as smoking) and in patient numbers, a main problem of this study seems to have been the overcorrection of anemia [192]. The mean hemoglobin concentration was 154 g/l (±17 g/l) in patients treated with rHuEpo for 9 weeks [89]. Evidence suggests that the hemoglobin concentration leading to the highest tumor oxygenation ranges from 130 g/l to 140 g/l, because intratumoral blood flow is disturbed at higher hemoglobin levels [192]. The association between O_2 capacity of the blood and tumor oxygenation, on the one side, and tumor growth and efficiency of radiotherapy and chemotherapy, on the other side, is plausible [59, 69, 72, 91, 186, 195]. As discussed elsewhere [95], however, neither the experimental nor the clinical support for this association is fully convincing. Even less well understood is the mechanism underlying the recent observation that rHuEpo treatment improves tumor oxygenation independently of its effect on erythropoiesis [23]. In this study rHuEpo was administered to rats before or after mammary adenocarcinoma transplantation. At similar blood hemoglobin concentrations, O_2 measurement histograms revealed significantly less hypoxic tumor areas in animals during rHuEpo therapy [23].

Direct influence of Epo on tumor cell growth

Since rHuEpo was introduced as a drug for treatment of renal anemia almost 20 years ago, several groups of investigators have carefully studied whether Epo can induce or promote tumor growth. Clinically, no evidence has been reported so far indicating that the erythrocytic growth factor Epo directly stimulates tumor cell proliferation. In addition, an elegant study in transgenic mice transfected with a construct that linked the human Epo gene to an erythroid-specific regulatory element has shown that the continuous stimulation of erythropoiesis leads to erythrocytosis but not to erythroleukemia [122]. However, there is at least one case report of Epo-dependent leukemic transformation of myelodysplastic syndrome (MDS) to acute monoblastic leukemia (AML) [31]. A careful examination has shown Epo-R expression on leukemia cells in 60% of patients with all French–American–British types of AML and in 29% of acute lymphoblastic leukemia (ALL) cases [184]. In vitro a proliferative response to Epo was observed in 16% of patients. Patients with both Epo-R expression and in vitro response to Epo had shorter remission duration than those without Epo-R [184]. Thus, close observation for leukemic transformation is necessary in patients with MDS on rHuEpo therapy.

In light of the demonstration of Epo-R expression in various nonerythroid tissues, it is important to regard studies aimed at investigating direct effects of Epo on tumor cells. Kayser and Gabius [108] first suggested that human tumors may express Epo-R. In their study 81% of human lung carcinoma tissues possessed Epo-binding sites as detected by use of biotinylated rHuEpo. Epo-R transcripts and Epo-R protein were subsequently demonstrated in human renal carcinoma [204], tumors of the cervix and other organs of the female reproductive tract [3, 215, 216], and in various specimens of common pediatric tumors such as neuroblastomas, brain tumors, hepatoblastomas, and Wilms' tumors [15]. By immunohistochemistry, Epo-R has been shown to be expressed in breast carcinoma [2, 10, 88] and in vestibular schwannoma [58].

Of major concern are studies indicating that local inhibition of Epo-R signaling results in tumor regression. Yasuda et al. [215] first showed that the application of anti-Epo-antibody or of soluble Epo-R into transplants of uterine or ovarian tumors in nude mice produces a decrease in tumor size. Similarly, the administration of anti-Epo-antibody, soluble Epo-R, or an inhibitor of JAK2 resulted in a delay in tumor growth with 45% reduction in maximal tumor depth in a tumor-Z chamber model with rat mammary adenocarcinoma cells [10]. Yasuda et al. [215] have put forward the interesting hypothesis that Epo could be essential for the development of tumor progression through its antiapoptotic effect on the endothelium. In addition, in the absence of an intact Epo/Epo-R system, the increase in the number of cells undergoing apoptotic death may promote local immune reactions by attracting neutrophilic granulocytes and monocytes [215]. Mittelman et al. [132], in studying murine myeloma models, have shown that rHuEpo treatment induced complete tumor regression in 30–60% of mice with a syngeneic progressively growing tumor. This regression was related to a tumor-specific immune response to the myeloma cells which was mediated by T cells [132]. The effects of Epo on antitumor immune responses are an important field for future investigations.

Previous reports on the expression of Epo-R and the effects of rHuEpo on cultured tumor cells are also conflicting. Epo-R has been detected in cultures of various malignant human cell lines [1, 3, 10, 58, 152, 171, 204, 205, 217]. Some of these studies suggested a link between Epo-R expression and tumor cell proliferation [1, 2, 10, 204]. For example, rHuEpo (range: 0.5–100 U/ml) stimulated the growth of human renal carcinoma cells in culture [204]. The addition of rHuEpo (≥10 U/ml) produced a significant increase in the release of angiogenic growth factors from tumor cell cultures, namely VEGF and placenta growth factor, from pediatric tumor cell lines [15]. The authors have suggested that Epo antagonists (with transfusion support) could be potentially used in conjunction with antiangiogenic agents and chemotherapy [15]. In other studies no relationship between Epo-R expression and tumor growth was apparent [17, 58, 83, 164, 171, 205, 210]. In fact, no growth stimulation of human primary tumor specimens [16] or of permanent hemopoietic or nonhemopoietic malignant cell lines [17, 139, 164] was observed, even when tumor cells were incubated with Epo at concentrations that were by several orders of magnitude higher than those achieved physio-

logically or by the administration of rHuEpo. Westphal et al. [205] recently investigated Epo-R and granulocyte colony-stimulating factor (G-CSF) receptor expression in various human benign and malignant cell lines. Treatment with rHuEpo (up to 1000 U/ml) had no effect on the rate of proliferation and tyrosine phosphorylation when Epo-R-positive tumor cell lines were tested. In studying various tumor cell lines, Liu et al. [120] found that neither rHuEpo nor granulocyte-monocyte colony-stimulating factor (GM-CSF) influence basal viability. However, pretreatment with these growth factors resulted in a reduction of the cytotoxic effects of cisplatin in cell lines with high growth factor receptor expression [120].

Conclusions

The primary function of Epo is to inhibit apoptosis of erythrocytic progenitors and, thereby, to stimulate the growth of young red blood cells. rHuEpo has proven to be a most useful replacement therapy for the prevention of the anemia associated with chronic kidney disease. The drug raises hematocrit and blood hemoglobin concentration in a dose-dependent and predictable way and abolishes the need for red cell transfusion with its risks of incompatibility reactions, infections, and iron overload. Present pharmacological interest focuses on the use and development of recombinant erythropoiesis-stimulating drugs with prolonged survival in the circulation by producing analogues with additional carbohydrate chains [61, 63] or with attached polyethylene glycol polymers [121]. Next to the renal anemias, possible indications for the administration of rHuEpo may be the anemias associated with autoimmune diseases, acquired immunodeficiency syndrome (AIDS), malignancies, and surgical interventions. The detection of functional Epo-R in nonhemopoietic tissues has recently aroused experimental and clinical interest in the use of rHuEpo as a survival factor for nonhemopoietic cells and tissues.

Convincing evidence has accumulated that Epo acts as a neurotrophic and neuroprotective factor in the central nervous system. In vitro, Epo protects neuronal cells from hypoxia-induced and glutamate-induced cell death. In animal models, Epo promotes the survival of neurons and synapses and reduces the size of infarct areas in the ischemic brain. A first clinical trial has shown neuroprotective potential of rHuEpo in patients with acute stroke [62]. It is hoped that the drug may prove useful for treatment of other neuronal disorders such as brain trauma, inflammatory diseases, and degenerative diseases [30, 98, 123, 167].

Epo exerts mitotic effects on vascular tissues and induces the production and release of vasoactive mediators from the endothelium. Of major clinical relevance are recent observations showing that rHuEpo promotes the mobilization of endothelial progenitor cells which may be beneficial in the vascularization of ischemic tissues, including the heart [11, 86]. Knowledge concerning the ability of Epo to function as a specific myocardial protectant has recently started to emerge and the present experimental and clinical evidence mandate further, thorough investigation of the therapeutic potential of rHuEpo for myocardial protection. At present, it seems clear that cardiomyocytes express Epo-R and respond to Epo with activation of signaling pathways resembling those known from erythropoietic cells.

The role of Epo in tumor therapy needs to be further explored. Anemia-associated tissue hypoxia promotes angiogenesis, growth, and metastasis of tumors [172,194]. In addition, the efficacy of radiotherapy and chemotherapy depends on the availability of O_2. In most cancer patients rHuEpo therapy increases the blood hemoglobin concentration and tumor oxygenation, thereby increasing the sensitivity of the tumor cells to radiotherapy and chemotherapy [73, 187]. The rHuEpo doses should be titrated to maintain a hemoglobin concentration of 120 g/l in tumor patients [163] because a further increase bears the risk of cardiovascular disorders. In vitro studies have shown that tumor cells may express Epo-R. These findings merit further exploration. However, based on almost 20 years of the use of rHuEpo in the clinical routine and on the observation of anemic persons with high endogenous Epo levels, there is at present no reason to give patients a fear that Epo may induce or promote tumor growth.

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