Therapeutic Potential of Erythropoietin and its Structural or Functional Variants in the Nervous System

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Summary: The growth factor erythropoietin (EPO) and erythropoietin receptors (EPOR) are expressed in the nervous system. Neuronal expression of EPO and EPOR peaks during brain development and is upregulated in the adult brain after injury. Peripherally administered EPO, and at least some of its variants, cross the blood-brain barrier, stimulate neurogenesis, neuronal differentiation, and activate brain neurotrophic, anti-apoptotic, anti-oxidant and anti-inflammatory signaling. These mechanisms underlie their tissue protective effects in nervous system disorders. As the tissue protective functions of EPO can be separated from its stimulatory action on hematopoiesis, novel EPO derivatives and mimetics, such as asialo-EPO and carbamoylated EPO have been developed. While the therapeutic potential of the novel EPO derivatives continues to be characterized in preclinical studies, the experimental findings in support for the use of recombinant human (rh)EPO in human brain disease have already been translated to clinical studies in acute ischemic stroke, chronic schizophrenia, and chronic progressive multiple sclerosis. In this review article, we assess the studies on EPO and, in particular, on its structural or functional variants in experimental models of nervous system disorders, and we provide a short overview of the completed and ongoing clinical studies testing EPO as neuroprotective/neuroregenerative treatment option in neuropsychiatric disease. **Key Words:** Ischemia, cognition, motor function, hematocrit, thrombocytes, safety.

INTRODUCTION

The growth factor erythropoietin (EPO) has been originally known for and named after its potent stimulation of erythropoiesis.[1] Apart from their hematopoietic actions, EPO and EPO variants are directly neuroprotective in cell culture models and after application in the brain.[2–6] Moreover, several EPO variants that do not bind to EPO receptors (EPOR) in myeloid cells, and that lack hematopoietic activity, display potent tissue protective activities.[7,8] Expression of EPO and its receptors in the nervous system, as well as its multifaceted protective actions in cell culture and animal models (e.g., induction of anti-apoptotic, anti-oxidant and anti-inflammatory signaling in neurons, glial and cerebrovascular endothelial cells, stimulation of angiogenesis, and neurogenesis), have been reported extensively and reviewed in detail elsewhere.[7–17] Therefore, this review will focus on 1) preclinical work published on EPO variants and 2) clinical studies in neuropsychiatric disease performed with EPO.

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EPO SIGNALING IN THE NERVOUS SYSTEM

EPO and its receptor are expressed in the developing brain.[18–20] The weak constitutive expression in the adult brain can be rapidly increased by hypoxia and acute metabolic stress as evidenced by detection of EPO in cerebrospinal fluid or postmortem brain tissue after traumatic brain injury, subarachnoid hemorrhage, and stroke.[12,13,21–24] Hypoxia-induced expression of EPO and the classical EPOR in brain cells may contribute to ischemic tolerance,[16,25] whereas neutralization of the brain endogenous EPO augments ischemic damage.[4] In fact, EPOR-deficient mice show increased apoptosis in the brain and enhanced hypoxia sensitivity.[26] Interestingly, EPOR is also up-regulated in chronic brain disease (e.g., schizophrenia and Alzheimer's disease [AD]), potentially reflecting ongoing metabolic distress.[27,28]

EPO acts by binding to its specific transmembrane receptor (EPOR). The classical EPOR in hematopoietic

cells is a cytokine type I receptor characterized by an extracellular N-terminal domain with conserved cysteines and a WSXWS-motif, a single hydrophobic transmembrane segment, and a cytosolic domain with no intrinsic kinase activity.[29] Homodimerization of the two transmembrane EPOR molecules binds one EPO molecule and leads to a conformational change, which in turn activates EPOR associated Janus family tyrosine kinase 2 (JAK2) molecules. Once activated, JAK2 phosphorylates distal parts of the receptors, which subsequently serve as docking sites for downstream signaling molecules. Multiple signal transduction pathways are activated downstream of EPOR/JAK2.[8,29] In neurons these include signal transducers and activators of transcription (Stat), phosphatidylinositol 3-kinase (PI3K)/Akt, Ras/extracellular signal regulated kinase (ERK1/2), nuclear factor-kappa-B (NF-κB), and calcium.[6,7,30,31]

Brines and Cerami[7] have proposed that the cytoprotective effects of EPO and its nonhematopoietic derivative, the carbamoylated EPO (CEPO) are mediated by a heteromeric receptor complex comprised of one EPOR subunit and a dimer of the common beta-chain shared by the members of the interleukin-3 receptor family.[32,33] The molecular assembly of this proposed heteroreceptor has been projected from that of the known stoichiometry of the granulocyte-macrophage colony-stimulating factor receptor complex.[7,32] The authors demonstrated that the common beta-chain can be co-immunoprecipitated with EPOR antibodies from the P19 embryonal carcinoma cell line.[32] Loss of some of the cytoprotective effects of EPO and CEPO in mice lacking the common beta-chain,[32,33] together with the immunohistochemical observations on similarities in the expression pattern of the common beta-chain and the classical EPOR in rat spinal cord[32,34] support their hypothesis, but a concrete proof for the existence of the proposed heteromeric receptor structure in primary brain cells or tissues is lacking. In a recent study, no expression of the common beta-chain could be detected in neuronal cell lines that respond to EPO.[35] Furthermore, studies in mice harboring a brain specific genetic ablation of the classical EPOR have shown that expression of the EPOR is indispensable for EPO-induced neurogenesis and neuroprotection.[36–38] In contrast, CEPO stimulates adult neurogenesis in the EPOR-knock-out mice suggesting that the cellular signaling by CEPO does not involve the classical EPOR.[38] Future studies are still needed to clarify whether the classical EPOR, the proposed heteromer or another yet unidentified brain specific EPOR mediate the actions of EPO and its structural variants on brain cells.

EPO STRUCTURAL AND FUNCTIONAL VARIANTS: ANALOGUES, DERIVATIVES, MIMETICS AND ENDOGENOUS STIMULATORS OF EXPRESSION

Stimulation of erythropoiesis using recombinant human EPO (rhEPO) has been a medical and economic success story. To improve/modify the properties of EPO with respect to its pharmacokinetic and pharmacodynamic profile and, last but not least, to compensate for expired EPO patent protection and gain an erythropoiesis stimulating agent (ESA) market share, a number of EPO variants (see FIG. 1) are actively under development.[1,39]

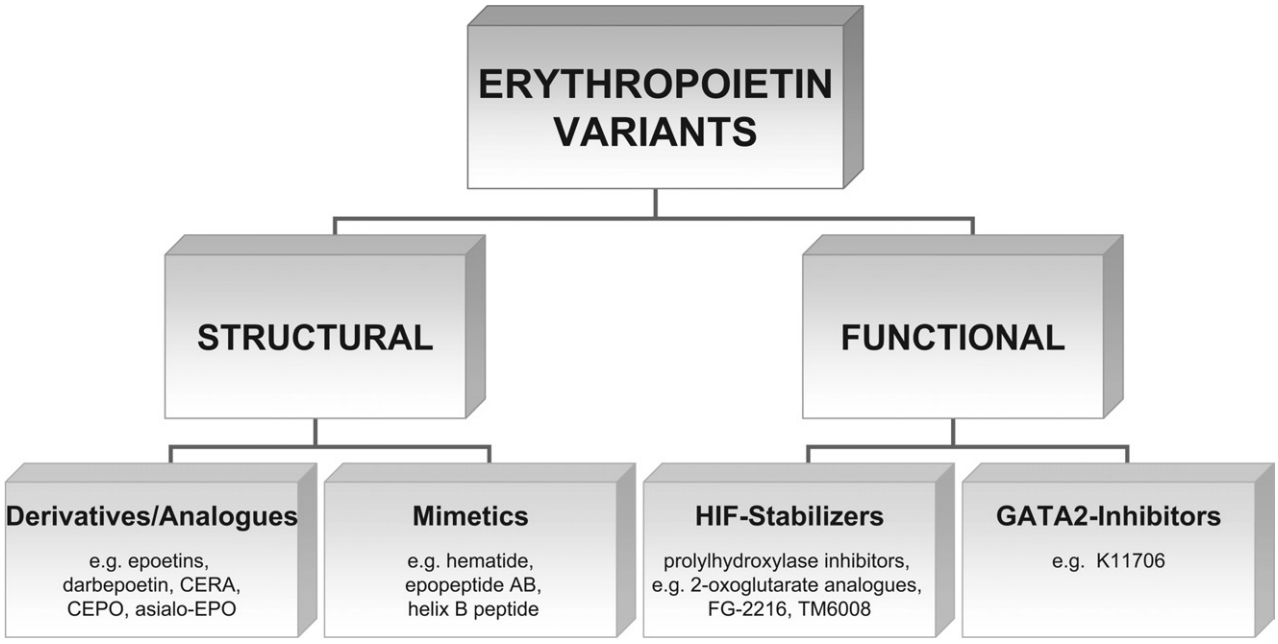

FIG. 1. Classification of erythropoietin (EPO) structural and functional variants. CEPO = carbamoylated EPO; CERA = continuous erythropoiesis receptor activator; GATA2 = GATA binding protein 2; HIF = hypoxia-inducible factor.

Table 1. *Overview of Structural and Functional Erythopoietin Variants*

Name of Compound	Group	Molecular Weight (kDa)	EP	NP	NP Dose Equivalent rhEPO	BBB Permeability
Epoetin alpha/beta	rhEPO	30–34	+	+	1	+
Epoetin delta	rhEPO	26–32	+	n.d.	n.d.	n.d.
Epoetin omega	rhEPO	34	+	n.d.	n.d.	n.d.
Darbepoetin	rhEPO	37	+	+	1	+
CERA	rhEPO	60	+	n.d.	n.d.	n.d.
Carbamoylated rhEPO (CEPO)	rhEPO	40	−	+	1	+
Carbamoylated darbepoetin (Caranesp)	rhEPO	40	−	+	1	Likely
Asialo-EPO	rhEPO	30–34	−	+	1	+
EPO-S100E	rhEPO		−	+	1	Likely
HBP (Helix B peptide), pHBSP (pyroglutamate Helix B surface peptide)	EMP	20 aa 11 aa	−	+	<1	Likely
Epopeptide AB	EMP	17 aa	−	+	>1	n.d.
Hematide	EMP	20 aa 5 kDa	+	+	>1	n.d.
K-11706/K7174	GATA-2 inhibitor		+	n.d.		n.d.
Compound A	HIF-stabilizer		n.d.	+		Likely
TM 6008/TM6089	HIF-stabilizer		+	+		Likely
FG-2216/ FG-4592	HIF-stabilizer		+	n.d.		Likely
Betahydroxybuturate	HIF-stabilizer		+	+		Likely

aa = amino acids; BBB = blood-brain barrier; CERA = continuous erythropoiesis receptor activator; EAE = experimental autoimmune encephalomyelitis; EP = erythropoiesis; EMP = EPO mimetic peptide; EPO = erythropoietin; GATA-2 = GATA binding protein 2; HIF = hypoxia-inducible factor; ip = intraperitoneal; iv = intravenous; kDa = kiloDalton; n.d. = not determined; NP = neuroprotection; NP dose equivalent rhEPO = dose equivalent to rhEPO for neuroprotection; rhEPO = recombinant human erythropoietin; sc = subcutaneous; T1/2 = half life.

Table 1. *Continued*

Remarks	References
Produced in hamster ovary cell lines T1/2: rodents: 2.5h iv; 6h ip, human: 8.5h iv, 24h sc, transport across BBB may be receptor-mediated, potent neuroprotective actions *in vitro, in vivo* models of nervous system disease and in clinical trials	1,7,27,39,46,69, 119,124
Lacks N-glycolyl-neuraminic acid (Neu5Gc), produced in human fibrosarcoma cell lines, pharmacokinetic profile similar to Epoetin alpha/beta; T1/2: humans 5.12h iv	1
Produced in hamster kidney cells	1
Hyperglycosylated rhEPO, also known as novel erythropoiesis stimulating protein (NESP); T1/2: rats: 6.9h iv; human: 25.3h iv, 48.8h sc, less frequent dosing than rhEPO; neuroprotective *in vivo* models of stroke, sciatic nerve injury, improvements in recognition memory in rats after hippocampal lesions; transport across BBB may involve extracellular pathways	1,46,55,68,85,101
Polyethylenglycol-linked epoetin beta, T1/2: 6 days sc, less frequent dosing than rhEPO, long-acting agent	1,39
Suggested to bind to EPOR- β common receptor heteromer, neuroprotective activity retained in brain targeted EPOR KO-mice, does not bind EPOR in myeloid cells; T1/2: rodents 4-6h ip, transport across BBB may be receptor-mediated; Neuroprotective *in vitro* models: hippocampal neurons, slice cultures, neuronal cell lines; *in vivo* models: post-treatment in stroke and traumatic brain injury, spinal cord injury, EAE, diabetic neuropathy, radiation toxicity, reduced risk for thrombosis as compared to rhEPO	7,32–34,38,44,45, 58,73,80,95,99, 105,115
Neuroprotective in an *in vivo* model of stroke	44
Desialated rhEPO; Short T1/2, rats 1.4min iv, 0.5h ip, 2.5h sc, transport over BBB may involve specialized glial cells ensheathing the capillaries; Neuroprotective in animal models of stroke, spinal cord compression, sciatic nerve crush model, EAE, no neuroprotective effects in a transgenic model of Mb Huntington, motoneuron survival *in vivo*	32,43,89,95,115
Neuroprotective in an *in vivo* model of stroke	33,44
Suggested to bind to EPOR- β common receptor heteromer; short T1/2, for pHBSP in rats and rabbits 2min iv; Neuroprotective in animal models of stroke, sciatic nerve compression, diabetic retinopathy, improved performance in novel object recognition test	41
First identified neurotrophic sequence of EPO; neuroprotective and neurotrophic in neuroblastoma cells, facilitates neuronal sprouting *in vivo*	42
PEGylated peptide, unrelated to EPO; T1/2: rodents 31h iv, monkey: 60h iv neuroprotective and EPO-mimetic actions on transmitter release in neuroblastoma cells lines and hippocampal slices	47–50
Induces EPO gene expression, effects on neural tissues not reported	39
Orally active peptide inhibitor of prolyl hydroxylases, induces EPO gene expression; neuroprotective in an *in vivo* and *in vivo* model of stroke	53
Small molecule inhibitors of prolyl hydroxylases, induce EPO gene expression: T1/2: rats TM6008 (50mg/kg, oral):1.5h, TM6089 (iv): 0.6h; neuroprotective *in vivo* and *in vivo* model of global forebrain ischemia in gerbils	51
Orally active small molecule inhibitors of prolyl hydroxylases; induces EPO gene expression	1,39
Small molecule inhibitor of prolyl hydroxylases, induces EPO gene expression, neuroprotective *in vivo* and *in vivo* models of stroke, similar effects by ketotic metabolic status	52,54

More challenging and certainly more important than producing *"me-too ESA compounds"* will be the development of predominantly neuroprotective/tissue protective EPO variants. Since the original discovery of the neuroprotective potential of EPO more than a decade ago,[2–4,40] attempts to separate the hematopoietic actions of EPO from its tissue protective effects have resulted in identification of novel nonhematopoietic EPO derivatives.[7,8,33,41–45] In addition to the development of structurally related EPO derivatives[1,8,39,46] or mimetics[41,42,47–50] (i.e., structural variants) that target EPOR, compounds that act by induction of EPO gene expression (i.e., functional EPO variants), such as HIF-stabilizers[51–54] and GATA-2 inhibitors[1,39] are actively pursued as alternatives to EPO for stimulation of erythropoiesis, and ultimately also for neuroprotection (FIG. 1). The advantages and disadvantages of agents that ubiquitously induce a wide spectrum of hypoxia-inducible genes are presently not known. In particular, the question whether these agents have benefits over the use of rhEPO in treatment of nervous system disorders remains to be addressed. The main properties of EPO variants are summarized in Table 1.

ERYTHROPOIETIN VARIANTS AS NEUROPROTECTIVE/NEUROREGENERATIVE TREATMENT: PRECLINICAL STUDIES

Stroke and cerebral ischemia

The beneficial effects of EPO and its variants in models of experimental stroke and cerebral hypoxia–ischemia can be attributed to a multitude of cytoprotective mechanisms, including inhibition of apoptosis, anti-inflammatory and anti-oxidant actions, restoration of blood-brain barrier integrity, stimulation of neurogenesis and angiogenesis.[6,33,44,55–64] Earlier studies used the direct intracerebroventricular route of administration of EPO to demonstrate its potent tissue protective activity in focal and global models of cerebral ischemia.[2–4] EPO, as a large, highly glycosylated negatively charged molecule, was not expected to penetrate the blood-brain barrier.[65,66] The first evidence for a neuroprotective effect of EPO by peripheral route of administration was published by Brines et al.[67] with the demonstration that an intraperitoneal injection of high dose rhEPO (Epoetin alpha, 5000 U/kg) up to 6 h after reperfusion resulted in reduction of infarct volumes in a focal stroke model in rats. Immunohistochemical detection of biotinylated rhEPO 5 h after its intraperitoneal injection at the therapeutically effective dose (5000 U/kg) showed that rhEPO crosses the blood-brain barrier in the rat.[67] The transfer of circulating EPO across the blood-brain barrier in therapeutically effective concentrations has since been confirmed in several species, including man.[21,27,68–72] It is important to note, however, that the amount of EPO reaching the brain is only in the range of 1% of the intravenously applied protein, thus explaining the high doses required for brain protection. Intriguingly, EPO and its derivatives reduce histological damage and improve functional outcome when given as intraperitoneal or even intranasal post-treatment after experimental stroke and hypoxia-ischemia.[33,44,55–64,73] A typical example of the functional improvement by EPO in sensorimotor deficits after a focal stroke is presented in Figure 2 (Sirén and Ehrenreich, unpublished own preclinical data in preparation of the "Göttingen EPO Stroke Study," see as follows).

A comprehensive dosing study using post-treatment with EPO starting at 6 h after an embolic middle cerebral artery (MCA) occlusion in rats demonstrated dose-dependent reduction of functional deficits and infarct volume up to 28 days after MCA occlusion.[58] The hematocrit was transiently increased at all dose levels of EPO with a peak at 14 days after the initial dose.[58] CEPO (50 μg/kg) induced protective effects equal to the highest dose of EPO (5000 U/kg equivalent to 46 μg/kg) without increasing hematocrit.[58] As summarized in Table 1, neuroprotection in experimental stroke can be achieved with CEPO and other nonhematopoietic derivatives of EPO (asialo-EPO, Caranesp, EPO-S100E) at equipotent doses to that of rhEPO. Whether the lack of effect on hematopoiesis in the acute clinical setting represents a clear advantage over the use of rhEPO remains to be verified in future studies. Like the native protein, the nonhematopoietic derivatives of EPO are large proteins with the potential to induce antibody formation.[74,75]

Traumatic brain injury and spinal cord injury

EPO and EPO variants improve morphological, functional and cognitive recovery in experimental models of traumatic brain injury.[67,76–86] Brain edema after experimental injury can effectively be attenuated by post-treatment with EPO.[76,78,83] Mechanisms which account for the beneficial actions after traumatic injuries include inhibition of apoptosis, anti-inflammatory and anti-oxidant actions, restoration of blood-brain barrier integrity, stimulation of neurogenesis, and angiogenesis,[7,8,67,76–84] but it is not yet clear which of the neuroprotective effects of EPO are responsible for the long-term prevention of trauma-induced brain atrophy, cognitive and neurobehavioral dysfunction.[86] Recovery of both motor function and reduction of the histopathological damage by EPO and its nonerythropoietic derivatives CEPO and asialo-EPO have been reported in various, but not all, models of spinal cord injury.[33,34,43,87–90]

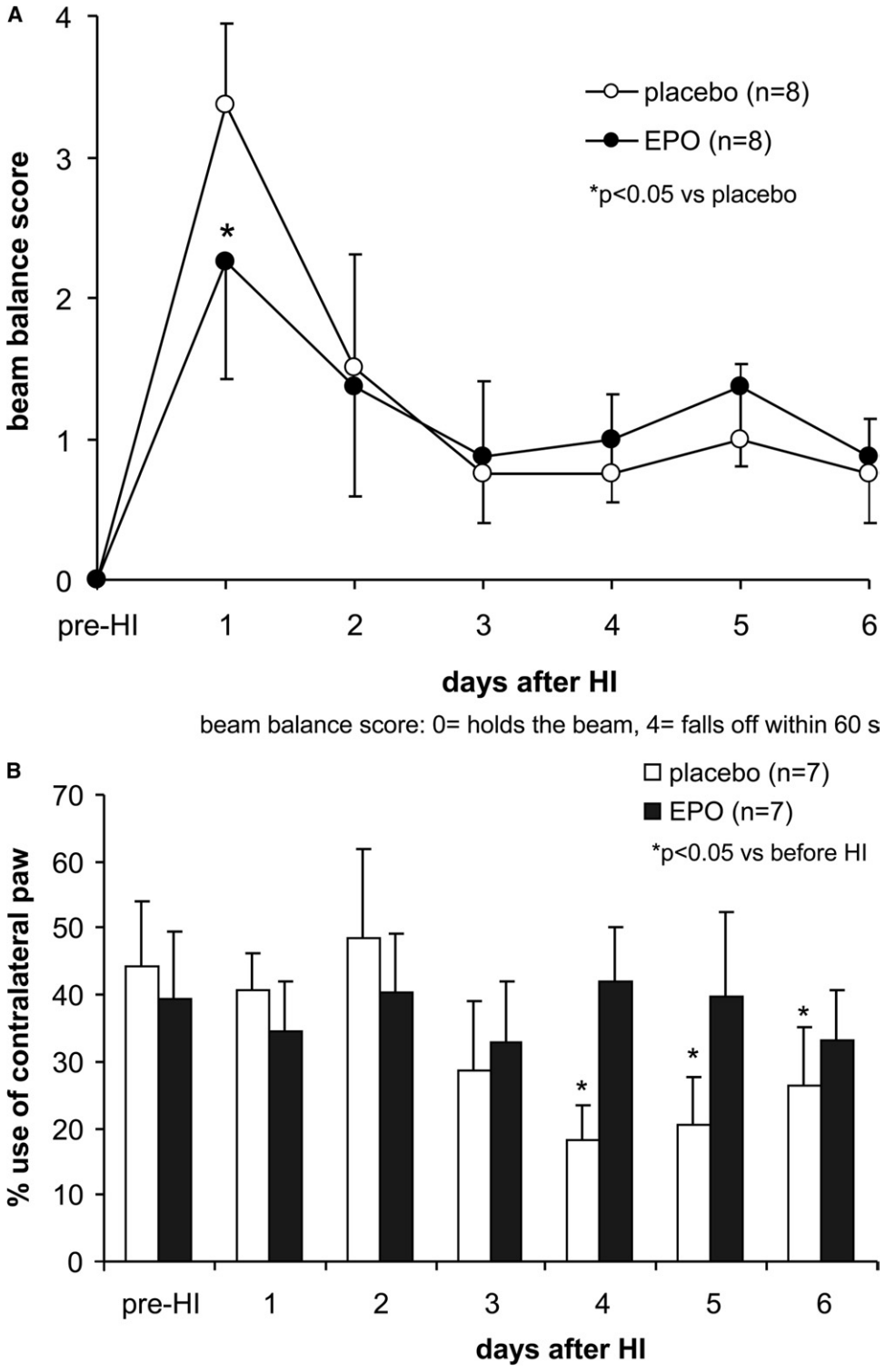

FIG. 2. Erythropoietin (EPO) reduces motor deficits and sensorimotor neglect in an experimental stroke model in rats. Unilateral hypoxia-ischemia (HI) was induced by ligation of the right carotid artery followed by exposure to hypoxia (8% oxygen) for 40 min in 28-day-old Wistar/Imamichi rats. Epoetin alpha (5000 U/kg) was given intraperitoneally immediately before and 24 and 48 h after exposure to hypoxia. After habituation to test environment and handling, the rats were tested daily for motor function (balance on a 1.6 cm diameter round beam for 60 s, panel [A]), and for sensorimotor asymmetry (the cylinder test, panel [B]) 4 days before (pre-HI) and 6 days after HI insult. In the cylinder test, each weight-bearing contact of the initial forepaw with the wall during full rear was counted for a 3-min period. Mean ± standard error of the mean. Statistical analysis: two-sided *t*-test.

Neuroinflammation, retinal disease, and peripheral nerve damage

In experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), EPO derivatives improve functional recovery, reduce tissue damage, inflammatory responses, and blood-brain barrier leakage.[33,67,91–96] Neuronal damage associated with cerebral malaria and radiosurgically-induced brain injury in mice can also be reduced by EPO and CEPO.[97–99] Translational relevance of these data is emphasized by a recent clinical study demonstrating that high plasma levels of EPO are associated with reduced risk of neurological sequelae in children with cerebral malaria.[100] Beneficial effects of both EPO and its nonhematopoietic derivatives and mimetics have been described in models of peripheral axonal nerve injury, injury-induced Wallerian degeneration, diabetic, and HIV-associated neuropathy.[41,101–105] In these conditions, the anti-cytokine, anti-apoptotic, anti-oxidative, and trophic effects on both neurons and oligodendrocyte progenitor cells are likely to reduce inflammation and preserve myelination and neuronal function.[33,67,91–98,102,103] The initial observation by Grimm et al.[106] of a potent neuroprotective effect of EPO in light-induced retinal degeneration has been confirmed in many other models of retinal disease in which EPO derivatives are in consideration for clinical use.[7,106–109] Here, a local intravitreal administration has been proposed to avoid neovascularization and angiogenesis after systemic EPO therapy.[108,110] Interestingly, timing of the therapy seems to have a decisive role for the outcome in models of hypoxia-induced retinopathy, whereas early administration of EPO protects retinal neurons against hypoxia-induced apoptosis and prevents hypoxia-induced neovascularization, late EPO treatment fails to protect the retina and enhances pathological neovascularization.[107]

Neurodegeneration

Based on their pharmacological profile, EPO derivatives would be expected to counteract degenerative processes in experimental models of chronic neurodegeneration, such as AD. Although increased expression of EPOR in the brains of AD patients[28] together with the findings demonstrating protection by EPO against beta-amyloid toxicity in cultured neurons[111] support a role for EPO derivatives in this prototype of neurodegenerative disorders, no data on effects of EPO derivatives in animal models of AD are available thus far. Slight improvements in functional outcome with variable efficacy to reduce histological damage have indeed been reported in models of Parkinson disease and amyotrophic lateral sclerosis (ALS) in which these compounds presumably act by inducing anti-oxidant enzymes, inhibiting apoptosis, and stimulating axonal regeneration.[112–115] Furthermore, EPO improved graft survival of embryonic ventral mesencephalic dopamine neurons when transplanted into the striatum of 6-hydroxy-dopamine lesioned rats.[116] However, treatment with asialo-EPO failed to reduce cell death or modify disease progression in a mouse model of Huntington's disease.[117]

Clearly, the rapidly growing evidence of neuroprotection by EPO (and EPO variants) in animal models of brain disease and peripheral nerve injury awaits its translation into clinical studies in nervous system disorders. In the case of acute ischemic stroke, the preclinical evidence meets most of the Stroke Therapy Academic Industry Roundtable (STAIR) criteria,[118] such as testing by several laboratories, using both temporary and permanent stroke models, peripheral application as post-treatment at several doses, and exploration of therapeutic window, characterization of its pharmacokinetic profile in respect to blood-brain barrier penetration after peripheral administration, measurement of histological, and functional outcome with prolonged survival. Conceptually, the nonhematopoietic variants of EPO would be expected to provide an advantage over EPO in the clinical setting for brain and nervous system indications. In diseases like MS, however, the very weak hematopoietic effects of EPO, leading additionally to disease-beneficial shifts in iron stores, may make these variants unnecessary (see below).[119] Also, the safety and efficacy of these novel compounds in man need first to be confirmed in phase I studies.

ERYTHROPOIETIN AS NEUROPROTECTIVE/ NEUROREGENERATIVE TREATMENT STRATEGY FOR HUMAN BRAIN DISEASES: FIRST CLINICAL STUDIES

Although preclinical data on the use of rhEPO and EPO variants have been promising for over a decade now, translation to humans has been extremely slow and difficult due to expiring patent protection for EPO worldwide, as well as reluctant public funding for trials that use drugs already approved for other indications. Therefore, despite worldwide interest of clinicians and clinical scientists, there are only a few trials published to date that have investigated efficacy of EPO in human brain disease. Very recently, two safety studies have been concluded in very preterm infants and infants with extremely low birth weight.[120,121] In both studies, high-dose EPO was found well tolerated, causing no excess morbidity or mortality. A dose range of 1000 to 3000 IU/kg was found to yield neuroprotective serum levels.[121] Data on efficacy in these trials, however, are not yet available. Another study, including patients with subarachnoid hemorrhage, was recently published.[122] This study, unfortunately, had to be terminated prematurely due to poor recruitment efficiency. In this study, no conclusions on efficacy of EPO could be drawn. There are several studies just con-

cluded, still ongoing or planned worldwide, applying EPO for treatment of human nervous system diseases. These include: trials on neurotrauma, subarachnoid hemorrhage, spinal cord injury, cerebral malaria, optic neuritis, Friedreich's ataxia,[123] chemotherapy induced peripheral neuropathy, and diabetes-associated complications of the nervous system (for overview, see Table 2). To our knowledge, no EPO variants other than rhEPO itself have thus far been published in relation to the treatment of human nervous system diseases. The only studies published so far reporting on efficacy of rhEPO in human brain diseases are our own studies on ischemic stroke, schizophrenia and MS.[69,119,124]

Stroke

As early as 1997, we started the first proof-of-concept trial. This endeavor was based on our own preclinical work and the conviction that, having a compound in hand that in other indications had proven safe and well tolerated in millions of people, it was more than justified to rapidly translate our findings with EPO to man. This approach was shortly afterward supported by the first publications on rodent studies from other groups, showing beneficial effects of EPO in hypoxia/ischemia.[2,4,67] The "Göttingen EPO Stroke Study" comprised a safety and double-blind, placebo-controlled part.[69] We included only patients suffering from acute ischemic stroke in the MCA territory to closely mimic the experimental setting of MCA occlusion. The idea was also to provide an optimal basis for comparison of both clinical and imaging readouts in the study population. EPO was infused intravenously over 3 days daily at a dose of 33,333 IU to result in a total dose of 100,000 IU per patient. The first study drug application was performed as rapidly as possible after the onset of stroke, allowing for a time window to treatment of not more than 8 h. The second and third dose was given at 24 h and 48 h later, respectively. EPO was found to be well tolerated and safe in stroke patients. The dose applied led to an increase of CSF EPO concentrations in stroke patients (with a disturbed blood-brain barrier) of approximately 60 times the baseline level.[69] These findings encouraged continuation in form of a double-blind, placebo-controlled, randomized, proof-of-concept (phase IIb) study, which showed that EPO improved clinical outcome, indeed, and reduced evolution of lesion, as well as serum levels of the circulating glial damage marker, S100B, as compared to the placebo group.[69] Based on these very promising results, the German Multicenter EPO Stroke Trial (ClinicalTrials.gov Identifier: NCT00604630) was begun in February 2003, aiming at the inclusion of over 500 patients. The trial was performed in a multicenter setting within Germany, including university hospitals in Göttingen, Hannover, Bremen, Celle, Erlangen, Leipzig, Essen, Dresden, Braunschweig, Aachen, and Berlin. In the summer of 2008, the trial concluded. Of note is the fact that compared with the first EPO stroke study, the "stroke landscape" has changed due to approval of recombinant tissue plasminogen activator (rtPA) for treatment of ischemic stroke in Germany in the year 2000. As a result, a surprising amount of over 60% of the patients included in the German Multicenter EPO Stroke Trial received thrombolytic therapy. Therefore, for the currently running trial analysis, patients have to be divided into an rtPA and a non-rtPA population. At this point, the most important message may be that the results of the first trial have essentially been reproduced *(manuscript in preparation)*.

Schizophrenia

In the meanwhile, basic research of our group and also of ever-increasing numbers of other groups continued. While the original idea was to use EPO to target acute brain diseases, exploiting its potent anti-apoptotic action, more and more data have recently been accumulated that have found a high regenerative potential of EPO, ranging from neurotrophic, neurogenesis, plasticity-modulating to angiogenetic properties. Therefore, we moved on to study the effect of long-term, high-dose EPO treatment in a chronic brain disease, schizophrenia. In preparation of this study, we first tested the capability of EPO to penetrate an intact blood-brain barrier.[27] Using indium-111-labelled EPO, we delivered proof-of-principle that even in healthy subjects, peripherally applied EPO can accumulate within brain tissue. The accumulation of labelled compound within the brain, however, was increased in schizophrenic patients as compared to healthy individuals. This is most likely due to the higher density of EPOR expression in the brains of schizophrenics.[27] In support of our planned trial on EPO in schizophrenia, we obtained an improvement of cognitive performance in rodents on EPO.[27] Very recently, we discovered that EPO is able to enhance hippocampal long-term and short-term potentiation and neuronal plasticity, considered prerequisites for learning and memory processes in this brain area.[125] Most intriguingly, we found that EPO prevents the development of slowly progressing global brain atrophy in a mouse model of chronic neurodegeneration.[86] The fact that EPO reduced *in vitro* haloperidol-induced death of primary hippocampal neurons, further encouraged continuation of our plans to use this compound for treating schizophrenic patients.[27] From 2003 to 2005, we performed a double-blind, placebo-controlled multicenter trial (phase IIb) on EPO add-on treatment in chronic schizophrenic men displaying a defined cognitive deficit.[124] Participating centers included Göttingen, Kiel, Homburg, Cologne and Marburg. High-dose EPO (40,000 IU intravenously), applied over 12 weeks on a weekly basis, led to significant improvement of schizophrenia-relevant cognitive performance as compared to

Table 2. *Overview on Completed, Currently Running or Planned Studies Using Erythropoietin as Neuroprotective Compound*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
Göttingen EPO Stroke Study: Double-blind, placebo-controlled, randomized proof-of-principle study on rhEPO in acute ischemic stroke	Acute ischemic stroke (N=53)	3x EPO 33333U/d iv within 8h after onset: days 1-3 Comparator: placebo	IIb	H Ehrenreich Germany	Completed PMID: 12435860
Recombinant human erythropoietin therapy in critically ill patients: A dose response study; ISRCTN48523317	Critically ill patients with anemia (N=148)	EPO 40000U sc: weekly for 2-3 weeks + iron saccharate iv versus EPO 40000 sc: 3x weekly for 2-3 weeks + iron saccharate iv Comparator: iron saccharate iv	IIa	D Georgopoulos Greece	Completed PMID: 16277712
A double-blind, placebo-controlled, randomized, multicenter proof-of-principle phase IIb study on recombinant human erythropoietin in chronic schizophrenia	Chronic schizophrenia (N=39)	12x EPO 40000U iv weekly Comparator: placebo	IIb	H Ehrenreich Germany	Completed PMID: 17033631
Comparative pharmacokinetic and pharmacodynamic study of Epoetin alfa (Procrit®) in anemic critically ill patients randomized to one of six dose regimens for 15 days; NCT00210756	Critical illness (N=60)	3x EPO 40000U sc: weekly on days 1, 8, 15 versus 3x EPO 40000U iv: on days 1, 8, 15 versus 8x EPO 15000U sc: every other day on days 1, 3, 5, 7, 9, 11, 13, 15 versus 8x EPO 15000U iv: on days 1, 3, 5, 7, 9, 11, 13, 15 versus 2x EPO 40000U sc: on days 1, 3, then 6x EPO 15000U sc on days 5, 7, 9, 11, 13, 15 versus 2x EPO 40000U iv on days 1, 3, then 6x EPO 15000U sc on days 5, 7, 9, 11, 13, 15	IIa	Johnson&Johnson USA	Completed
An exploratory, open-label phase IIa study on recombinant human erythropoietin in chronic progressive multiple sclerosis	Chronic progressive multiple sclerosis (N=8)	Mpred 1g/d iv on days 1-3 + EPO 48000U iv on days 2-3, weekly for 12 weeks, bi-weekly for 12 weeks	IIa	H Ehrenreich Germany	Completed PMID: 17728357
A randomized, double-blind, placebo-controlled study to determine the efficacy and safety of Epoetin alfa in critically ill subjects; NCT00091910	Critically ill patients with anemia (N=1460)	3x EPO 40000U sc: on days 1, 8, 15 (if hemoglobin <12g/dl) + >150mg iron therapy on days 1-29 Comparator: placebo	III	Johnson&Johnson USA	Completed PMID: 17804841

(*Table continues*)

Table 2. *Continued*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
Friedreich's ataxia: Clinical pilot trial with recombinant human erythropoietin	Friedreich's ataxia (N=12)	EPO 5000U sc 3x weekly for 8 weeks	IIa	B Scheiber-Mojdehkar, Austria	Completed PMID: 17702040
A phase I/II trial of high-dose erythropoietin in extremely low birth weight infants: Pharmacokinetics and safety; IND 12656	Low birth weight infants (<1,000g) (N=60)	3x EPO 500U/kg iv: on days 1-3 versus 3x EPO 1000U/kg iv: on days 1-3 versus 3x EPO 2500U/kg iv: on days 1-3 Comparator: standard care	I II	SE Juul USA	Completed PMID: 18676557
Neurological effects of recombinant human erythropoietin in Friedreich's ataxia: A 6-month open-label clinical pilot study of safety and efficacy	Friedreich's ataxia (N=8)	EPO 2000U sc 3x weekly for 6 months	IIa	S Boesch Austria	Completed PMID: 18759345
The erythropoietin neuroprotective effect: Assessment in CABG surgery (TENPEAKS): A randomized, double-blind, placebo-controlled proof-of-concept clinical trial; NCT00336466	Cardiac surgery, brain injury (N=32)	3x EPO 125U/kg/d iv: pre-operative, day 1, 2 versus 3x EPO 250U/kg/d iv: pre-operative, day 1, 2 versus 3x EPO 500U/kg/d iv: pre-operative, day 1, 2 Comparator: placebo	IIb	D Zygun Canada	Completed
Effects of systemic erythropoietin therapy on cerebral autoregulation and incidence of delayed ischemic deficits in patients with aneurysmal subarachnoid hemorrhage; NCT00140010; ISRCTN30515245	Aneurysmal subarachnoid hemorrhage (N=80)	3x EPO 30000U iv: within 72h after SAH, then every other day Comparator: placebo	IIb	PJ Kirkpatrick Great Britain	Completed
Safety of beta-hCG + erythropoietin in acute stroke; NCT00362414	Acute stroke (N=12)	Beta-hCG 10000U iv: on days 1, 3, 5 + 3x EPO 30000U iv: on days 7, 8, 9	IIa	SC Cramer USA	Completed
German multicenter EPO stroke trial (phase II/III); NCT00604630	Acute ischemic stroke (N=522)	3x EPO 40000U/d iv within 6h after onset: days 1-3 Comparator: placebo	II III	H Ehrenreich Germany	Completed
A randomized, double-blind, placebo-controlled, multicenter, 18 week pilot study to investigate the neuroprotective effect of Procrit® (Epoetin alfa) on the development of peripheral neuropathy in patients receiving combination taxane and platinum-based chemotherapy for cancer; NCT00267007	Chemotherapy-induced peripheral neuropathy (N=120)	EPO 20000-60000U sc or iv injections (adjusted to hemoglobin) weekly up to 18 weeks Comparator: placebo	IIb	Johnson&Johnson USA	Completed

(*Table continues*)

Table 2. *Continued*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
Erythropoietin in patients with aneurysmal subarachnoid hemorrhage: A double-blind, randomized clinical trial	Aneurysmal subarachnoid hemorrhage (planned: N=262; N=73)	3x EPO 30000U iv: within 72h after SAH, then every other day Comparator: placebo	IIb	NV Olson Denmark	Terminated (slow enrolment) PMID: 17876497
Neuroprotective effect of high dose erythropoietin in very preterm infants; NCT00413946	Intracranial hemorrhage, periventricular leuko-malacia, cerebral palsy (IIa: N=45; IIb: N=420)	3x EPO 3000U/kg iv: at 3, 12-18, 36-42h after birth Comparator: placebo	IIa/b	HU Bucher Switzerland	Completed (IIa) Recruiting (IIb) PMID: 18676556
A randomized, multicenter pilot study to evaluate the efficacy and safety of Epoetin alfa (Procrit®) in the treatment of HIV-associated sensory neuropathy; NCT00528593	HIV infections, neuropathy (planned: N=46)	EPO every 3 weeks versus EPO every week	IIa	DB Clifford USA	Terminated (lack of enrolment)
A randomized, double-blind, placebo-controlled study to assess the effect of Epoetin alfa (Procrit®) on functional outcomes in anemic, critically ill, trauma subjects; NCT00210626	Anemia (planned: N=204)	EPO Comparator: placebo	IIb	Johnson&Johnson USA	Terminated (slow enrolment)
Recombinant human erythropoietin (r-HuEPO) in the prevention of neurologic sequelae from malignant spinal cord compression: A multi-center, placebo-controlled, phase II randomized study; NCT00220675	Nerve compression syndromes (N=7)	EPO Comparator: placebo	IIb	A Loblaw Canada	Terminated (insufficient accrual)
Single-center, open-label, sequential trial to test the efficacy, safety and tolerability of Epoetin alfa in patients with Friedreich's ataxia; NCT00631202	Friedreich's ataxia (N=10)	3x EPO: 1x 600U/kg sc, 1x 1200U sc after 1 month, 1x 2400U/kg sc after 3 months	IIa	A Filla Italy	Active, not recruiting
Phase II study of the effects of erythropoietin on neuronal cell death in traumatic brain injury patients; NCT00260052	Traumatic brain injury (N=86)	1x EPO 40000U iv: within 6h of injury Comparator: placebo	II III	R Nirula USA	Active, not recruiting
A phase IIb prospective, randomized, double-blind, placebo-controlled study of NTx™-265: Human Chorionic Gonadotropin (hCG) and Epoetin alfa (EPO) in acute ischemic stroke patients – REGENESIS CA; NCT00663416	Stroke (N=134)	3x hCG 10000U sc: on days 1, 3, 5 + 3x EPO 30000U iv: on days 7-9 Comparator: placebo sc: days 1, 3, 5 + placebo iv: days 7-9	IIb	MD Hill Canada	Active, not recruiting

(*Table continues*)

Table 2. *Continued*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
Randomized, double-blind, placebo-controlled, single-dose, dose-escalation study of the safety, tolerability, and pharmacokinetics of LuAA24493 in acute ischemic stroke; NCT00756249	Acute ischemic stroke (N=16)	1x LuAA24493 (CEPO) 0.005-50mcg/kg iv within 12-48h of symptom onset Comparator: placebo	I	H Lundbeck A/S Denmark	Active, not recruiting
The use of erythropoietin in the treatment of acute transverse myelitis	Acute transverse myelitis (N=30)	2x EPO 40000U: within 2 weeks of onset, 2 weeks later + 5-day 1g iv solumedrol daily + steroid taper Comparator: placebo + 5-day 1g iv solumedrol daily + steroid taper	I II	SC Keswani USA	Recruiting
Evaluation of functional and morphological retinal changes in the course of systemic Aranesp® treatment in patients with diabetes mellitus; NCT00704652	Diabetic retinopathy (N=40)	Diabetic patients with renal insufficiency in need of Darbepoetin substitution (target hemoglobin 10-12g/ml)	IIa	U Schmidt-Erfurth Austria	Recruiting
Phase II dose finding trial of prophylactic Darbepoetin alfa to improve outcomes from ischemic complications of surgery; NCT00647998	Spinal ischemia, stroke (N=40)	1x Darbepoetin 1-6.5mcg/kg iv injection pre-operative Comparator: historical controls	IIa	SR Messé USA	Recruiting
The use of erythropoietin in cardiac arrest victims: The impact on survival and neurological outcome; ISRCTN67856342	Cardiac arrest (N=200)	1x EPO 90000U iv injection in first 4min during CPR Comparator: standard care	IIa	S Grmec Slovenia	Recruiting
A prospective randomized, placebo-controlled study of the efficacy and safety of Darbepoetin alfa treatment in patients with severe traumatic brain injury; NCT00375869	Traumatic brain injury (N=15)	Darbepoetin Comparator: placebo	IIb	DJ Kutsogiannis Canada	Recruiting
Influence of G-CSF and EPO on associative learning and motor skills; NCT00298597	Chronic stroke, amyotrophic lateral sclerosis (N=180)	G-CSF sc + EPO Comparator: placebo	IIb	WR Schäbitz Germany	Recruiting
A randomized, masked, placebo-controlled study to assess the safety and efficacy of Darbepoetin alfa administered to preterm infants; NCT00334737	Infant, newborn (N=102)	Darbepoetin 10mics/kg sc: weekly for 10 weeks or until 35 completed weeks versus EPO 400U/kg sc: 3x weekly for 10 weeks or until 35 completed weeks Comparator: sham injection	IIb	RK Ohls, RD Christensen, S Wiedmeier, A Rosenberg USA	Recruiting

(*Table continues*)

Table 2. *Continued*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
Erythropoietin to enhance erection recovery in men following radical prostatectomy; NCT00737893	Prostate cancer, erectile dysfunction (N=100)	3x EPO 40000U sc: pre-operative, on day 1, 2 Comparator: placebo	IIb	AL Burnett USA	Recruiting
Evaluation of the tolerability and efficacy of erythropoietin (EPO) treatment in spinal shock: Comparative study versus methylprednisolone; NCT00561067	Spinal cord injury (N=100)	3x EPO 500U/kg: within 8h after injury, on day 1 (24h), day 2 (48h) Comparator: Mpred according to NASCIS III protocol	III	T Redaelli Italy	Recruiting
Double-blind, placebo-controlled study to determine the safety and efficacy of erythropoietin as an add-on therapy of methylprednisolone in subjects with acute optic neuritis (VISION PROTECT); NCT00355095	Optic neuritis (N=40)	3x EPO 33333U iv injection: on days 1-3 + 3x Mpred 1g/d iv: on days 1-3 Comparator: placebo + 3x Mpred 1g/d iv on days 1-3	IIb	R Diem Germany	Recruiting
Randomized trial of erythropoietin to prevent death from cerebral impairment during severe malaria; NCT00697164	Cerebral malaria (N=120)	3x EPO 1500U/kg: on days 1-3 + Quinine iv + oral ACT for 6 days Comparator: placebo	II III	S Picot, C Bernard Mali	Recruiting
High-dose erythropoietin in very low birth weight infants for the potential treatment of prematurity-related cerebral hemorrhagic-ischemic injury: A phase II safety/tolerability study; NCT00589953	Infant, premature brain injury, intraventricular hemorrhage, peri-ventricular leukomalacia (N=50)	7x EPO 400U/kg/d iv for 7 days versus 7x EPO 800U/kg/d iv for 7 days versus 7x EPO 1000U/kg/d iv for 7 days Comparator: placebo	IIb	A Sola USA	Recruiting
Relationship between clinical recovery and oxidative stress and inflammation following usage of erythropoietin in admitted traumatic patient in intensive care unit; NCT00622934	Multiple trauma (N=20)	3x EPO 300mg/kg on week 1 Comparator: placebo	IIb	M Mohammady Iran	Recruiting
Treatment with erythropoietin in patients with spinal trauma with neurological deficit, maximum tolerated dose study. TETRAM2; NCT00478517	Spinal trauma with neurological deficit (N=20)	1x EPO 600-2400U/kg (dose scale study)	I	T Lieutaud France	Recruiting
A double-blinded, placebo-controlled pilot study to evaluate the safety of treating patients with aneurysmal subarachnoid hemorrhage (SAH) with Epoetin alfa; NCT00626574	Subarachnoid hemorrhage (N=20)	3x EPO 40000U iv injections: within 36h of SAH/ before clipping, on day 1 (after 24h), day 2 (48h) Comparator: placebo	IIb	EM Camporesi USA	Recruiting

(*Table continues*)

Table 2. *Continued*

Official Title; Registration ID	Condition	Treatment	Phase	Contact	Status
High dose erythropoietin neuroprotection for neonatal cardiac surgery; NCT00513240	Congenital heart surgery (N=240)	3x EPO 1000U/kg iv: 12-72h pre-operative, intra-operative after CPB, post-operative (24h) Comparator: placebo	I II	DB Andropoulos USA	Recruiting
A phase IIb prospective, randomized, double-blind, placebo-controlled study of NTx™-265: Human Chorionic Gonadotropin (hCG) and Epoetin alfa (EPO) in acute ischemic stroke patients – REGENESIS US; NCT00715364	Stroke (N=30)	3x hCG 385μg sc: on days 1, 3, 5 + 3x EPO 30000U iv: on days 7-9 Comparator: placebo sc: days 1, 3, 5 + placebo iv: days 7-9	IIb	SC Cramer USA	Not yet recruiting
High dose erythropoietin for neonates with asphyxia; NCT00491413	Asphyxia (N=15)	1x high-dose EPO within 6h	I	RD Christensen USA	Not yet recruiting
Neonatal erythropoietin in asphyxiated term newborns: A phase I trial; NCT00719407	Hypoxic-ischemic encephalopathy (N=26)	3x EPO 1000U/kg/d versus 3x EPO 2500U/kg/d versus 3x EPO 5000U/kg/d	I	YW Wu USA	Not yet recruiting
The effect of intravenous erythropoietin treatment on cognition during hypoglycemia in patients with type 1 diabetes; NCT00615368	Type 1 diabetes, hypoglycemia (N=12)	1x 40000U EPO iv injection 6 days before induction of experimental hypoglycaemia Comparator: placebo	IIb	PL Kristensen Denmark	Not yet recruiting
Double-blind, randomized, placebo-controlled multicenter trial to determine the efficacy of recombinant human erythropoietin as neuro-protective add-on treatment in first-episode schizophrenia (EPO-S)	First-episode schizophrenia (N=120)	Flupentixol + 12x EPO 48000U iv weekly, 6x bi-weekly for 12 weeks Comparator: flupentixol + placebo	IIb	H Ehrenreich Germany	In preparation
Recombinant human erythropoietin as neuroprotective/-regenerative treatment of chronic progressive multiple sclerosis (EPO-MS): A double-blind, randomized, placebo-controlled multicenter phase IIb trial	Chronic progressive multiple sclerosis (N=152)	Mpred 1g/d iv: on days 1-3 + EPO 48000U iv on days 2-3 (versus 16000U versus 32000U), weekly for 12 weeks, bi-weekly for 12 weeks (year 1) + *all-to-verum switch* (year 2) Comparator: Mpred 1d iv days 1-3 + placebo (year 1)	IIb	H Ehrenreich Germany	In preparation

Data collected mainly from registers: www.clinicaltrials.gov (Clinical Trials Registration Systems of the National Institutes of Health), the U.S. Food and Drug Administration (FDA), and the U.S. Department of Health and Human Services, and www.controlled-trials.com/isrctn/ (International Standard Randomized Controlled Trial Number Register).
IND = registration ID of the U.S. FDA device exemption approval program; PMID = PubMed Identifier; ACT = artemisinin-based combination therapy; CABG = coronary artery bypass grafting; CPR = cardiopulmonary resuscitation; EPO = erythropoietin; G-CSF = granulocyte-colony stimulating factor; h = hour(s); iv = intravenous; Mpred = methylprednisolone; SAH = subarachnoid hemorrhage; sc = subcutaneous; SAH = subarachnoid hemorrhage.

placebo-treated patients.[124] In addition to the stepwise improvement in cognitive performance, we found that EPO was able to delay progression of cortical gray matter loss in chronic schizophrenic patients, as determined by most comprehensive, voxel-based morphometrical MRI analysis *(manuscript in preparation)*. In this respect, EPO-induced prevention of brain atrophy, as observed in rodent studies,[86] could be nicely reproduced in man. In contrast, within the 3 months observation period, no EPO effect was noted on psychopathology (PANSS ratings) or psychosocial outcome parameters in the schizophrenic population. Taken together, the fact that EPO is the first compound ever that appears to exert a beneficial effect on cognition and reduction of cortical gray matter loss in schizophrenia should stimulate further work along these lines. A treatment trial including patients with a first episode of schizophrenic psychosis is presently being planned, as are further studies with chronic schizophrenic patients.

Multiple sclerosis

Encouraged by the neuroprotective/neuroregenerative effects of EPO seen in our human trials, and the increasing number of positive rodent studies on EAE and related conditions,[33,67,91–96] we initiated another investigator-driven, exploratory, open-label study (phase IIa) addressing patients suffering from either primary or secondary chronic progressive MS.[119] The main objectives of this study were: 1) to evaluate safety of long-term, high-dose intravenous EPO treatment in MS; and 2) to collect first evidence of potential efficacy of EPO on the most MS-relevant clinical outcome parameters. The study design comprised a 6-week lead-in period set up to obtain a clean estimate of baseline performance, a 12-week treatment phase with weekly intravenous applications of EPO, followed by another 12-week treatment phase with bi-weekly EPO, and a 24-week post-treatment period. The individual study duration lasted for up to 1 year in total. Despite including only a small number of subjects, we were able to demonstrate that chronic progressive MS patients improved considerably with respect to motor function and cognition on high-dose, long-term EPO treatment. As compared to the efficacious 48,000 IU dose, "low-dose" treatment (8000 IU) did not yield any significant effects. Interestingly, two drug-naïve Parkinson patients, who also received high-dose EPO, did not improve over the 12-week weekly treatment period. Motor and cognitive improvement in high-dose MS patients were clearly visible already after the 12-week weekly treatment phase, remained stable over the following bi-weekly EPO period, and essentially persisted over the EPO-free follow-up time. Figure 3 illustrates the course of performance of high-dose EPO MS patients in selected tests, reflecting cognitive, as well as fine motor improvement. The hematocrit did not increase in parallel, but rather slightly decreased over the observation time in these patients. In the whole study, there were no safety concerns, no study drug-related adverse events reported or observed, and a surprisingly low need of

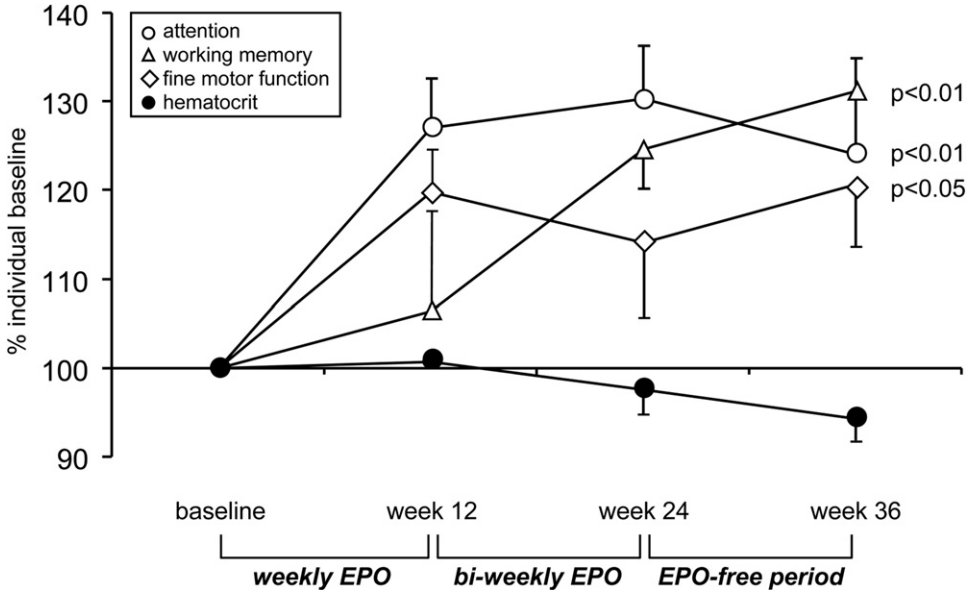

FIG. 3. Lasting improvement of cognitive and motor functions in the absence of hematocrit increase in five patients with chronic progressive multiple sclerosis receiving high-dose erythropoietin (EPO) treatment. Attention (Visual Scanning—critical trials), working memory (Trail Making test, part B) and fine motor coordination (MacQuarrie Tapping test) show significant improvement over baseline that persists even with cessation of EPO treatment. The mean of two baseline measurements was set to 100% to provide a reliable baseline value and was used for calculating individual improvement over time. Mean change of all patients at denoted time-points of follow-up with treatment or during the treatment-free period is expressed as % individual baseline. Respective hematocrit values are presented in parallel. Mean ± standard error of the mean. Statistical analysis: Friedmann test.

blood lettings. We interpret the infrequent requirement of blood lettings in MS patients as a relative hyporesponsiveness of the hematopoietic system to EPO, as known in other diseases, characterized by a systemic latent inflammatory condition.[126] Therefore, EPO may well be the compound of choice for the indication MS where derivatives, harbouring neuroprotective, but no hematopoietic properties, may be unnecessary.

Precautions for the use of EPO in nervous system indications

The use of EPO as a neuroprotective/neuroregenerative treatment strategy in human brain disease, in particular when considering the high dose of EPO required to obtain sufficient levels within the brain in situations of widely intact blood-brain barrier, requires meticulous and comprehensive safety management. EPO in these indications will never be a *"let go treatment."* A close follow-up of patients at all times is mandatory, including clinical as well as laboratory examination combined with each and every EPO application.

Red blood cell parameters and iron

Hematocrit (hemoglobin) has to stay within clearly defined limits during EPO treatment. Even though the necessity of blood lettings in schizophrenics, and particularly in MS patients was very low, it is still of outmost importance to carefully follow each and every individual and to strictly initiate blood letting whenever the hematocrit increases over 48% in females and over 50% in males. Note that *no* iron substitution is allowed *at any time*. Iron would induce a push of red blood cell production, definitely undesirable in nervous system indications. Such a push may be helpful when EPO is used for treating anemia, but never in the treatment of brain diseases. In our studies, we actually exploit the fact that the need for blood lettings will undergo rapid self-limitation in the absence of iron substitution. Because EPO treatment leads to temporary shifts in iron stores, leading to laboratory readouts, similar to true iron deficiency, the patients themselves and all potentially involved physicians have to be made aware that iron substitution is not necessary and may be even damaging. With cessation of EPO treatment, most iron parameters will rapidly return to normal levels.[119,124] Interestingly, the temporary reduction in iron availability caused by EPO might even provide an additional benefit in chronic progressive MS in which iron has even been seen as an inflammation-supporting agent, and iron chelators have been proposed for treating this condition.[127]

Thrombocytes

Platelet counts have to be carefully and continuously monitored at all times of EPO treatment and even in the weeks thereafter. Patients with thrombocyte counts distinctly above the normal range have to be excluded at any time, be it on inclusion or during treatment with EPO. Although appreciable stimulation of thrombocytes occurs in only few patients, EPO is known to also act on megakaryocytes.[128] FIG. 4 illustrates the case of a male patient where, starting from normal levels, EPO treatment induced an increase in hematocrit/hemoglobin

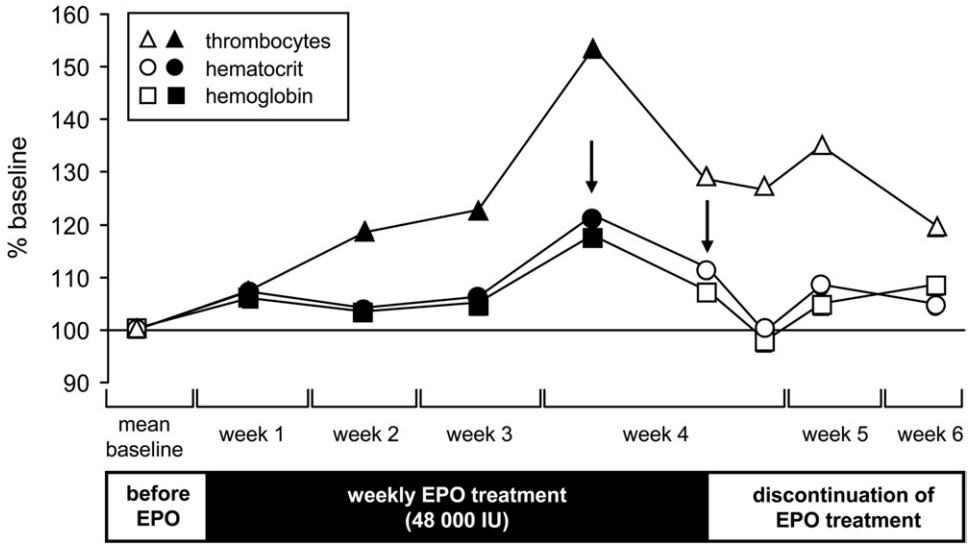

FIG. 4. Course of hematocrit, hemoglobin, and thrombocytes in a 44-year-old, male patient, suffering from progressive supranuclear palsy, with high-dose weekly erythropoietin (EPO) treatment. At week 4, a first blood letting (arrow) had to be performed, due to an increase in hematocrit over the tolerable limit of 50%. Three days later, a second blood letting (arrow) corrected the still increased hematocrit back to baseline values. Hemoglobin levels behaved identically. In contrast, thrombocytes had also reached numbers distinctly above the normal limit at week 4, but did not respond to the two blood lettings with return to baseline level. Therefore, treatment with EPO was discontinued. The mean of two baseline measurements was set to 100% and was used for calculating individual course of laboratory values over time (expressed as percentage of baseline).

above the limit of 50% at week 4 of high-dose EPO treatment. Therefore, blood letting was performed in this patient twice (FIG. 4, see arrows) within 1 week, until complete normalization of hematocrit/hemoglobin was achieved. In this patient, thrombocyte counts had gradually increased to reach a level above normal at the time point of first blood letting. In contrast to hematocrit/hemoglobin, however, blood letting did not lead to return of platelet counts to the individual baseline level. Therefore, this patient had to discontinue treatment with EPO to avoid any risk of thromboembolic complications. In addition, the patient received acetyl salicylic acid for 4 weeks (i.e., until platelets had returned to normal). Importantly, patients with past thromboembolic complications have to be strictly excluded. Exsiccosis has to be prevented at any time. Immobility poses a great risk for thromboembolic complications, and therefore patients who are prone to bed should not be included in a chronic treatment study. Furthermore, patients with cardiovascular disease or cardiovascular risk factors (e.g., diabetes, severe therapy-resistant hypertension, smoking, or contraceptive medication) have to be excluded.

Blood pressure

EPO is known to potentially induce increases in blood pressure in susceptible patients (e.g., patients with renal failure).[129,130] Although no such increases have been clearly noted in any of the patients in our studies, blood pressure monitoring is absolutely essential and increases in blood pressure with EPO treatment have to be either pharmacologically controlled or should be considered as an exclusion criterion.

Tumors

Although the exact effects of EPO on tumor growth are still unclear and may not be the same in all kinds of tumors, patients suffering from any kind of malignancy, whether treated or untreated, should be strictly excluded from EPO treatment in the indications discussed here.[131–135]

EPO antibody formation

Like all proteins, EPO has the potential to induce antibody formation.[74,75] However, in contrast to e.g. insulin, this apparently occurs very rarely. In fact, a temporary problem of neutralizing antibody formation induced by EPO has recently been resolved. For several years, EPO antibody formation was observed more frequently with certain EPO preparations in which obvious components of packaging material (rubber stopper) acted as "Freud adjuvant." On changing of the packaging procedure and careful adherence to the cooling chain, no further reports on EPO antibody formation became publicly available. Nevertheless, antibody determination should be part of an EPO treatment program and should be performed before starting and after terminating EPO treatment in all patients with neurological or psychiatric indications. Importantly, EPO antibody formation has to be suspected at any time when reticulocyte counts drop below the normal range.

CONCLUSION

The promising results of clinical trials and exploratory clinical studies will hopefully encourage not only clinical scientists but also industry and public sponsors to perform further trials. Treatment of neuropsychiatric patients with EPO outside of studies can not be recommended at this time. In any case, meticulous adverse event monitoring and safety management, as well as most careful observation of potential contraindications, is absolutely mandatory. Even with future approval of EPO for nervous system indications, these safety rules should never be neglected; otherwise, a potentially highly beneficial drug will soon acquire a negative reputation.

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