Recombinant human erythropoietin: novel strategies for neuroprotective/neuro-regenerative treatment of multiple sclerosis

Therapeutic Advances in Neurological Disorders
(2008) 1(3) 193–206
DOI: 10.1177/1756285608098422

Claudia Bartels, Kira Späte, Henning Krampe and Hannelore Ehrenreich

***Abstract*:** Treatment of multiple sclerosis (MS) is still unsatisfactory and essentially non-existing for the progressive course of the disease. Recombinant human erythropoietin (EPO) may be a promising neuroprotective/neuroregenerative treatment of MS. In the nervous system, EPO acts anti-apoptotic, antioxidative, anti-inflammatory, neurotrophic and plasticity-modulating. Beneficial effects have been shown in animal models of various neurological and psychiatric diseases, including different models of experimental autoimmune encephalomyelitis. EPO is also effective in human brain disease, as shown in double-blind placebo-controlled clinical studies on ischemic stroke and chronic schizophrenia. An exploratory study on chronic progressive MS yielded lasting improvement in motor and cognitive performance upon high-dose long-term EPO treatment.

***Keywords*:** hematopoietic growth factor, chronic progressive multiple sclerosis (MS), motor function, cognition, experimental autoimmune encephalomyelitis (EAE), clinical trial, neuro-degeneration, erythropoietin receptor (EPOR)

Introduction

With a prevalence of more than 100 per 100,000 in Northern and Central Europe (annual incidence of 3.5–5 per 100,000 in Germany), and a disease onset predominantly in individuals aged between 20 and 40 years, multiple sclerosis (MS) is the most common cause of neurological disability in young and middle-aged adults, and of premature retirement in 33% of affected subjects [Flachenecker *et al*. 2008, 2005; Pugliatti *et al*. 2006]. MS is etiologically as well as pathogenetically heterogeneous and despite intensive research efforts still poorly understood.

To date, MS is not curable and even worse there are no means of stopping the disease process lastingly. Immunomodulatory, immunosuppressive and anti-inflammatory treatment approaches have at best led to a reduction of the relapse rate and temporary improvement of clinical severity in relapsing-remitting MS, but it is still unclear if newer compounds such as natalizumab or rituximab will deliver any persisting amelioration of the clinical syndrome [Confavreux and Vukusic, 2006; Feldmann and Steinman, 2005; Giovannoni, 2004; Hohlfeld and Wekerle, 2004; Noseworthy, 2003; Wingerchuk and Noseworthy, 2002]. In chronic progressive MS, treatment approaches are essentially all ineffective. Importantly, no neuroprotective/neuroregenerative strategy has been developed so far for MS despite the fact that disease progression is mainly driven by neurodegenerative pathological changes [e.g. Confavreux and Vukusic, 2006; Hauser and Oksenberg, 2006; Compston and Coles, 2002]. Therefore, identification of an efficient *add-on* treatment targeting axonal repair and remyelination, in addition to a perhaps disease subtype-oriented and more effective immunomodulatory therapy, will be the pivotal challenge for MS therapy research over the next decade [Hauser and Oksenberg, 2006; Rovaris *et al*. 2006; Brück, 2005; Giovannoni, 2004; Compston and Coles, 2002].

Erythropoietin, a hematopoietic growth factor with potent neuroprotective/neuro-regenerative properties in the nervous system

Erythropoietin (EPO) is a hematopoietic growth factor that has long been known to be produced in kidney and fetal liver, and has more recently been described to be expressed in the brain [Masuda *et al*. 1994, 1993; Konishi *et al*. 1993].

Correspondence to:
Hannelore Ehrenreich
Division of Clinical Neuroscience, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany,
ehrenreich@em.mpg.de

Claudia Bartels
Kira Späte
Henning Krampe
Division of Clinical Neuroscience, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany

The EPO system plays an important role during normal brain development, where it is associated with physiological apoptosis as well as the production and differentiation of neuronal precursor cells [Knabe *et al.* 2005, 2004; Shingo *et al.* 2001; Juul *et al.* 1998]. Postnatal and, even more pronounced during adulthood, the EPO system is downregulated, resulting in low-level expression of EPO and EPO receptor (EPOR) in the normal adult brain [Ehrenreich *et al.* 2005, 2004; Marti *et al.* 1996]. In situations of distress, ranging from metabolic to inflammatory, from ischemic to neurodegenerative conditions, EPO/EPOR appear to act as part of an endogenous neuroprotective 'stand by' system. In these situations, EPO and/or EPOR are strongly upregulated [Ehrenreich *et al.* 2004; Eid *et al.* 2004; Chung *et al.* 2004; Sirén *et al.* 2001b]. Interestingly, extraction of endogenous EPO during experimental stroke by intracerebroventricular application of soluble EPOR was found to induce a dramatic increase in ischemic damage [Sakanaka *et al.* 1998]. Knock-out of the brain EPOR, in turn, provokes higher rates of neuronal apoptosis and enhanced vulnerability to hypoxia [Yu *et al.* 2002]. Reduced concentrations of EPO in the cerebrospinal fluid (CSF) in amyotrophic lateral sclerosis (ALS) may point to a relative deficiency of endogenous EPO production in neurodegenerative disease. A more efficient extraction of any free molecule of EPO by brain tissue due to high reactive EPOR expression would perhaps explain this phenomenon [Brettschneider *et al.* 2006].

As in other organs, expression of the brain EPO system appears to be mainly stimulated via low tissue oxygen concentration. In this condition, hypoxia-inducible factor-1 (HIF-1) is activated, leading to increased production of EPO and EPOR together with various other hypoxia-inducible genes, for example vascular endothelial growth factor (VEGF) [Jelkmann, 2007; Ehrenreich *et al.* 2005; Beleslin-Cokic *et al.* 2004; Sharp and Bernaudin, 2004; Chikuma *et al.* 2000; Lewczuk *et al.* 2000; Bauer and Kurtz, 1989].

EPO acts on cells of the nervous system by binding to its specific receptor, EPOR, which belongs to the cytokine type-1 receptor super-family [Jelkmann, 2007, 1992; Fisher, 2003]. Dimerizing of EPOR upon ligand binding leads to autophosphorylation of the receptor-associated Janus tyrosine kinase 2 and activation of distal signal transduction cascades: phosphatidylinositol-3-kinase (PI3-K)/Akt (protein kinase-B), RAS/mitogen-activated protein kinases (MAPK), signal transducers and activators of transcription-5 (STAT-5), as well as NF-κB-dependent transcription [Byts *et al.* 2006; Park *et al.* 2006; Kilic *et al.* 2005; Digicaylioglu and Lipton, 2001; Sirén *et al.* 2001a]. Up to now, it is unclear whether EPOR in the nervous system consists of a homodimer, homopolymer or, as claimed by Brines and coworkers, is composed of an EPOR monomer, dimerizing with the so-called beta-common receptor (βCR), to form a heterodimer [Tsai *et al.* 2006; Brines *et al.* 2004; Livnah *et al.* 1999; Jubinsky *et al.* 1997]. Among others, a modified brain EPOR could explain why certain EPO analogs, for example carbamylated erythropoietin (CEPO), still possess the neuroprotective properties of EPO but are essentially devoid of its hematopoietic effects [Leist *et al.* 2004]. The group of nonhematopoietic EPO analogs/derivatives or EPOR stimulants may provide interesting future pharmacological tools for long-term treatment of brain diseases [for review see Jelkmann, 2008].

EPO has been described both in cell culture work and animal models of neurological and psychiatric diseases to exert potent anti-apoptotic, anti-inflammatory, and antioxidative properties. In addition, its neurotrophic, axon-protective, angiogenetic and neurogenetic properties make EPO an attractive candidate for neuroprotection/neuroregeneration. It stimulates axonal sprouting and synaptogenesis under certain circumstances and modulates plasticity [Adamcio *et al.* 2008; Byts *et al.* 2008; Jelkmann, 2007; Erbayraktar *et al.* 2006; Kaiser *et al.* 2006; Tsai *et al.* 2006; Diem *et al.* 2005; Bianchi *et al.* 2004; Chang *et al.* 2004; Csete *et al.* 2004; Kertesz *et al.* 2004; Keswani *et al.* 2004; Sättler *et al.* 2004; Weishaupt *et al.* 2004; Villa *et al.* 2003; Bocker-Meffert *et al.* 2002; Celik *et al.* 2002; Genc *et al.* 2002; Gorio *et al.* 2002; Grasso *et al.* 2002; Grimm *et al.* 2002; Springborg *et al.* 2002; Campana and Myers, 2001; Genc *et al.* 2001; Shingo *et al.* 2001; Sirén *et al.* 2001a; Brines *et al.* 2000; Bernaudin *et al.* 1999; Sadamoto *et al.* 1998; Sakanaka *et al.* 1998; Konishi *et al.* 1993]. Interestingly, EPO has very potent effects on learning and memory-associated readouts of hippocampal neuronal plasticity (e.g. long-term and short-term potentiation) [Adamcio *et al.* 2008].

This unusual assortment of properties, likely derived from a prominent multifaceted role of the EPO system during brain development, may explain the broad neuroprotective action of EPO

in animal models of diseases as different as hypoxia/ischemia, traumatic brain or spinal cord injury, subarachnoid hemorrhage or epilepsy, retina degeneration, peripheral neuropathies and radiation-induced brain damage [Jelkmann, 2007; Erbayraktar *et al.* 2006; Sirén *et al.* 2006; Tsai *et al.* 2006; Bianchi *et al.* 2004; Chang *et al.* 2004; Keswani *et al.* 2004; Weishaupt *et al.* 2004; Villa *et al.* 2003; Bocker-Meffert *et al.* 2002; Celik *et al.* 2002; Gorio *et al.* 2002; Grasso *et al.* 2002; Grimm *et al.* 2002; Springborg *et al.* 2002; Campana and Myers, 2001; Sirén *et al.* 2001a; Brines *et al.* 2000; Bernaudin *et al.* 1999; Sadamoto *et al.* 1998; Sakanaka *et al.* 1998]. Furthermore, reports on beneficial effects of EPO exist in animal models of Parkinson's disease [Csete *et al.* 2004; Genc *et al.* 2002, 2001] ALS [Grignaschi *et al.* 2007; Grunfeld *et al.* 2007; Koh *et al.* 2007], or cerebral malaria [Wiese *et al.* 2008; Kaiser *et al.* 2006]. Interestingly, regarding the neurological consequences of cerebral malaria, the amount of endogenous EPO has recently been found to play an important role for disease severity of children, once more pointing to the importance of EPO as an endogenous neuroprotective system [Casals-Pascual *et al.* 2008].

Both *in vitro* and *in vivo*, EPO has been shown to have a bell-shaped dose–response curve regarding its neuroprotective properties [Ehrenreich *et al.* 2005; Weishaupt *et al.* 2004; Sakanaka *et al.* 1998]. This bell-shaped curve makes the prediction of the adequate dose for optimal translation from animal models to man difficult. Perhaps the most suitable determinant of the 'right dose' is the amount of EPO reaching the brain via the blood–brain barrier. While EPO, given systemically at high doses, can indeed penetrate an intact blood–brain barrier sufficiently to exert neuroprotective effects (0.1–1% of the peripherally applied dose), the exact mechanism of penetration of this over 30,000 Dalton molecular weight protein via the blood–brain barrier is still unclear [Xenocostas *et al.* 2005; Banks *et al.* 2004; Ehrenreich *et al.* 2004; Martinez-Estrada *et al.* 2003; Brines *et al.* 2000].

Taken together, EPO possesses a broad spectrum of properties suitable to address several of the pathophysiological mechanisms involved in neuropsychiatric diseases. Since many of these mechanisms also play a major role in MS, it was most logical to explore the potential of EPO in relevant MS animal models.

Effects of EPO in animal models of neuroinflammatory disease

With respect to the clinical application of EPO as neuroprotective/neuroregenerative treatment in MS, several preclinical studies have shown potential efficacy of EPO as modulator of disease severity, using experimental autoimmune encephalomyelitis (EAE) as an animal model of MS (see Table 1 for overview).

Among the available animal models, various different EAE models most suitably resemble different basic mechanisms and specific features of the histopathology and neurobiology of MS and therefore provide relevant and established tools to investigate emerging therapeutic approaches. EAE in rodents can be induced; for example, via active immunization with central nervous system tissue, myelin oligodendrocyte glycoprotein (MOG), myelin or myelin basic protein (MBP), and results in a high incidence of disease with a reproducible clinical course [Gold *et al.* 2006].

In a pivotal paper, screening the effect of EPO in various different animal models of neurological and psychiatric diseases, Brines and colleagues noted that the onset of acute MBP-induced EAE was delayed upon EPO and that the severity of symptoms was significantly reduced [Brines *et al.* 2000]. The same group showed later that EPO has an effect on the inflammatory component of EAE, with delayed increase in tumor necrosis factor and blunted elevation of IL-6, together with reduced gliosis and cell infiltration in the spinal cord of EPO-treated EAE rats [Agnello *et al.* 2002]. Because EAE in the Lewis rat is considered a short-term transient monophasic disease model, another model was subsequently used by Li and coworkers that apparently shares more clinical and neuropathological features with MS [Li *et al.* 2004]. In the progressive model of MOG-induced EAE in mice, a reduction of symptom severity was found even upon EPO treatment start after disease onset. EPO blocked axonal injury, demyelination and blood–brain barrier leakage, as well as reduced glial expression of major histocompatibility complex (MHC) class II, interpreted as modulation by EPO of inflammation in EAE [Li *et al.* 2004]. In a MOG-induced optic neuritis model in Brown Norway rats, improved survival and function of retinal ganglion cells in EPO-treated animals were described [Sättler *et al.* 2004]. In the same model, a combined treatment

Table 1. Effects of erythropoietin (EPO) in rodent models of experimental autoimmune encephalomyelitis (EAE).

Reference	Model	Readouts	Beneficial EPO effects on	No EPO effect on	Remarks
Brines *et al.* 2000	Lewis rats MBP – acute model	Clinical rating	Delayed symptom onset and reduced symptom severity	—	EPO (5000 IU/kg/day i.p.) from day 3 after MBP for 15 days
Agnello *et al.* 2002	Lewis rats MBP – acute model	Clinical rating Histology TNF + IL-6 bioassays	Reduced gliosis and cell infiltration in spinal cord; TNF increase delayed and IL-6 increase reduced	—	EPO (500–5000 IU/kg/day i.p.) from day 3 after MBP for 15 days
Li *et al.* 2004	C57BL/6 mice MOG – chronic progressive model	Clinical rating Histology	Reduction of symptom severity, axonal injury, demyelination, blood-brain-barrier leakage and glial expression of MHC II in spinal cord	—	EPO (range 0.5–5000 IU/kg/day i.p.) from 36 to 48 h after symptom onset for 14 days
Sättler *et al.* 2004	Brown Norway rats MOG – hyperacute model of optic neuritis	Clinical rating Electrophysiology Histology	Increased survival of retinal ganglion cells and improvement in electroretinogram	No change of clinical outcome, demyelination, inflammatory infiltration, axon density, optic nerve function	Research into intracellular neuroprotective pathways; EPO (2000–10000 IU/kg/day i.p.) for 1 or 8 days after MOG
Diem *et al.* 2005	Brown Norway rats MOG – hyperacute model of optic neuritis	Clinical rating Electrophysiology Histology	Increased survival of retinal ganglion cells and improvement in electroretinogram	No significant change of clinical outcome, demyelination, inflammatory infiltration, axon density and function of optical nerve	Combined treatment of methylprednisolone (20 mg/kg/day i.p.) on day 1–3 and EPO (5000 IU/kg/day i.p.) for 8 days after MOG revealed best neuron and axon protection
Zhang *et al.* 2005	SJL/J mice Myelin PLP – relapsing-remitting model	Clinical rating Histology	Reduction of symptoms, demyelination and inflammatory infiltration; increase in oligodendrocyte progenitors and BDNF positive cells in brain	Relapses despite EPO-treatment	EPO (5000 IU/kg/day i.p.) for 7 days after symptom onset
Savino *et al.* 2006	C57BL/6 mice MOG – chronic progressive model	Clinical rating Histology Cytokine expression	Reduction of clinical symptoms and cytokine expression in spinal cord and peripheral mononuclear cells	—	Investigation also of non-hematopoietic EPO derivatives (CEPO, ASIALO EPO); EPO (0.5 or 50 μg/kg i.p.) for up to 29 days; three times per week: 3 days after MOG or directly after symptom onset or 15 days after symptom onset
Yuan *et al.* 2008	C57BL/6 mice MOG – chronic progressive model	FACS of EAE-derived cells Cytokine levels of T-cell cultures Histology	Modulation of immune balance in periphery and spinal cord; blocking of T-cell proliferation and dendritic cell expansion in lymph nodes	—	EPO (5000 IU/kg/day i.v. for 3 days + 1000 IU/kg/day i.v. for the next 3 days) after MOG or until 7 days post-MOG

MBP, myelin basic protein; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; myelin PLP, myelin proteolipid protein; BDNF, brain-derived neurotrophic factor.

of EPO and methylprednisolone was superior to each component alone in targeting the inflammatory as well as the neurodegenerative aspects of optic neuritis [Diem *et al.* 2005]. Finally, EPO was found to increase oligodendrocyte progenitor cell proliferation and to elevate brain-derived neurotrophic factor (BDNF)-expressing cells in a relapsing-remitting proteolipid protein (PLP) induced murine EAE model [Zhang *et al.* 2005]. EPO as well as nonhematopoietic EPO derivatives, CEPO [Leist *et al.* 2004] and ASIALO EPO [Erbayraktar *et al.* 2003], decreased production of inflammatory cytokines in spinal cord and peripheral lymphocytes in a chronic MOG-induced mouse EAE model [Savino *et al.* 2006]. Very recently, Yuan and coworkers focused on the immunological aspects of MOG-induced EAE in mice and found that EPO induces substantial long-term tissue protection in the host through signaling to several critical subsets of immune cells that reside in the peripheral lymphatic system as well as through modulating their corresponding cytokines [Yuan *et al.* 2008].

To summarize, various different groups showed with different EAE models and different treatment schedules that EPO reduces clinical severity, improves electrophysiological and histological readouts of EAE, and reduces inflammatory cytokine expression. These stimulating results from preclinical MS models obtained by ourselves and others, together with the beneficial effects of EPO in our human studies in stroke and schizophrenia, have strongly motivated us to perform a first exploratory study on EPO in chronic progressive MS (see below).

Human studies on EPO effects in brain disease

Stroke

Before performing exploratory work in human MS patients using high-dose EPO treatment, we collected evidence of beneficial effects of EPO in other human brain diseases. The first study of its kind was initiated in 1997 and included patients suffering from acute ischemic stroke in the territory of the middle cerebral artery [Ehrenreich *et al.* 2002]. This study was set up as a small proof-of-concept (phase IIb) trial and comprised a safety and a double-blind placebo-controlled part. EPO was applied over 3 days daily at a dose of 33,000 IU as intravenous infusion to result in a total dose of 100,000 IU. The first application was performed as rapidly as possible after the onset of stroke with a time window of maximally 8 h. The second and third dose was given 24 h and 48 h later, respectively. EPO was well tolerated in this group of patients and led to an improvement compared with the placebo group with respect to clinical outcome, imaging results and serum levels of the circulating glial damage marker S100B. Based on these very encouraging results, the German Multicenter EPO Stroke Trial (ClinicalTrial.gov Identifier: NCT00604630) was started in 2003, and aimed to include over 500 patients. This trial, performed in a multicenter setting including Göttingen, Hanover, Bremen, Celle, Erlangen, Leipzig, Essen, Dresden, Braunschweig, Aachen and Berlin has been concluded in summer 2008, as expected. As compared with the first EPO stroke trial, the 'entire stroke landscape' has changed due to the approval of recombinant tissue plasminogen activator (rtPA) for treatment of stroke patients, which took place in Germany in 2000 [Weimar *et al.* 2006]. As a result, over 60% in total of the patients included in this trial received thrombolytic therapy. Therefore, for analysis of a neuroprotective EPO effect, patients will be divided into an rtPA and a non-rtPA group.

Schizophrenia

After the promising results of short-term high-dose EPO treatment in human stroke as a classic example of an acute brain disease, we investigated the effect of long-term high-dose EPO treatment in chronic brain diseases. In the first disease that we chose, schizophrenia, neuroprotection was an important challenge to meet. In preparation of this trial, we had to first test the capability of EPO to penetrate an intact blood–brain barrier [Ehrenreich *et al.* 2004]. We did not only prove that EPO enters the brain in healthy rats, achieving a peak level in the CSF after 3.5h of intraperitoneal injection, but also demonstrated, using indium 111-labeled EPO, that even in healthy human subjects, EPO enriched within brain tissue [Ehrenreich et al. 2004]. The accumulation of labeled compound within the brain, however, was higher in schizophrenic patients as compared with healthy controls, most likely explained by a higher density of EPOR expression in frontal cortex and hippocampus. Importantly, EPO is able to improve cognitive functioning in mice and to enhance hippocampal long-term potentiation and various features of neuronal plasticity, essential for learning and memory processes

[Ehrenreich *et al.* 2004, Adamcio *et al.* 2008]. Intriguingly, we found that EPO prevents the development of slowly progressing global brain atrophy in a mouse model of chronic neurodegeneration [Sirén *et al.* 2006]. Furthermore, EPO reduces haloperidol-induced cell death in primary hippocampal neuronal cultures [Ehrenreich *et al.* 2004].

Based on these grounds, we performed a double-blind placebo-controlled multicenter trial on EPO *add-on* treatment in chronic schizophrenic men [Ehrenreich *et al.* 2007b]. Participating centers were Göttingen, Kiel, Homburg, Cologne and Marburg. Treatment over 12 weeks with high-dose weekly (40,000 IU intravenously) EPO led to significant improvement of cognitive performance compared to placebo controls [Ehrenreich *et al.* 2007b]. Employing voxel-based morphometrical magnetic resonance imaging (MRI) analysis, we obtained first evidence that EPO treatment of chronic schizophrenic patients delays progressive cortical gray matter loss (manuscript in preparation), similar to the prevention by EPO of brain atrophy, observed in our mouse model of chronic neurodegeneration [Sirén *et al.* 2006]. In contrast, we did not see any effect on psychopathology or psychosocial outcome parameters within the 3-month observation time of the human study [Ehrenreich *et al.* 2007b]. The fact that EPO is the first compound ever that appears to exert a beneficial effect on cognition in schizophrenia will definitely encourage further work along these lines. A treatment trial including patients with a first episode of a schizophrenic psychosis has been initiated.

Multiple sclerosis

Encouraged by the manifold neuroprotective effects of EPO in experimental models of MS [Chopp *et al.* 2008, Yuan *et al.* 2008; Savino *et al.* 2006; Diem *et al.* 2005; Zhang *et al.* 2005; Li *et al.* 2004; Sättler *et al.* 2004; Agnello *et al.* 2002; Brines *et al.* 2000] as well as by the success of our human trials on stroke and schizophrenia [Ehrenreich *et al.* 2007b; Ehrenreich *et al.* 2002], we initiated an investigator-driven, exploratory open label study (phase IIa) addressing patients suffering from either primary or secondary chronic progressive MS [Ehrenreich *et al.* 2007a]. The main objectives of this study were as follows: (1) evaluation of the safety of long-term high-dose intravenous EPO treatment in MS, and (2) collecting first evidence of potential efficacy of EPO on clinical outcome parameters most relevant for MS. We included a total of eight MS patients [five randomly assigned to high-dose (48,000 IU), three to low-dose (8,000 IU) EPO treatment] and, as disease controls, two drug-naive Parkinson's disease patients also receiving high-dose EPO. The study design comprised a 6-week lead-in phase, a 12-week treatment phase with weekly intravenous applications of EPO, another 12-week treatment phase with biweekly EPO application and a 24-week post-treatment phase (with an individual study duration of up to 1 year in total). After 12 weeks of weekly EPO treatment, a decision was made for each individual patient on continuing or terminating study participation. Qualification for study continuation required that the patient had improved upon treatment in at least three independent test items (e.g. walking distance, fine motor and bladder function). It turned out that all high-dose EPO patients fulfilled the criteria of study continuation whereas none of the low-dose patients did. Clinical and electrophysiological improvement of motor function, reflected even by a reduction in the Expanded Disability Status Score (EDSS), and improvement of cognitive performance, in particular working memory, was found upon high-dose EPO treatment in MS patients, persisting for 3–6 months after cessation of EPO application. In contrast, low-dose EPO MS patients as well as drug-naive Parkinson's disease patients receiving high-dose EPO did not improve in any of the parameters tested.

In this small exploratory trial there were no safety concerns, no study drug-related adverse events reported or observed, and a surprisingly low need for blood lettings. The infrequent requirement of blood lettings in MS upon EPO is interesting since it might reflect a relative hyporesponsiveness of the hematopoietic system to EPO as shown in other conditions of systemic latent inflammatory disease [Kwack and Balakrishnan 2006]. Altered cytokine patterns in chronic MS may well modulate the bone marrow response causing an 'EPO nonresponder profile'. Since clear diagnosis of flaring inflammation in MS and thus the prediction of imminent disease progression are essentially impossible up to now, we suggest developing the degree of hematopoietic response of MS patients to EPO as a biological readout of occult inflammation. Estimation of required additional immunosuppression may be derived

from such a test. Most interestingly, the hyporesponsiveness of the hematopoietic system as observed in our group of high-dose EPO MS patients supports the notion that the therapeutic efficacy of EPO in MS is not simply related to improved oxygen supply via increased red blood cell mass (Figure 1).

In addition to the above described results of our exploratory phase IIa trial in chronic progressive MS, we have accumulated further evidence of beneficial effects of EPO in MS over the years, based on long-term follow up of individual patients. In Figure 2, the effect of high-dose long-term EPO treatment on strength and fine motor function of paretic upper extremities is illustrated. The data were obtained from a single patient with relapsing-remitting MS who presented with considerable residual dysfunction of the sensory system (up to level C8/Th1) and severe bilateral pareses of both upper extremities, mainly affecting his hands. Despite treatment with corticosteroids, plasmapheresis, mitoxantrone and glatiramer acetate (the latter continuing over the EPO treatment period), no improvement of his situation was observed. The clinical state had been stable over half a year before EPO treatment was initiated. As illustrated in Figure 2, the EPO-free lead-in period shows a slight training effect on strength and endurance as well as on fine motor performance. Upon weekly and biweekly EPO treatment, a dramatic further improvement became obvious that persisted over the EPO-free follow-up period, similar to the results obtained in maximum walking distance in the exploratory chronic progressive MS trial [Ehrenreich *et al.* 2007a].

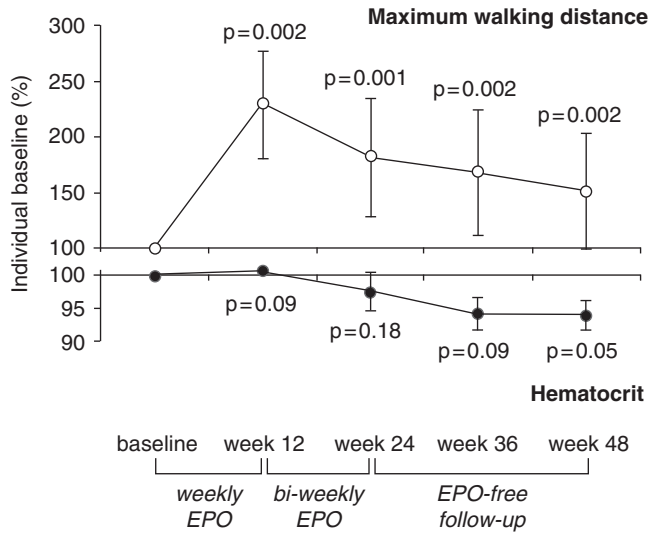

Figure 1. Course of maximum walking distance and of hematocrit in five nonanemic, chronic progressive multiple sclerosis patients receiving high-dose erythropoietin (EPO). The mean of all available measurements of maximum walking distance of each patient obtained during the whole lead-in period was set to 100% (to provide a reliable baseline value) and used for calculating individual improvement over time. Mean change of all patients at denoted timepoints of follow-up upon treatment or during the treatment-free period was calculated from percentage individual baseline. Respective hematocrit values are presented in parallel. Note the difference in scales. The slight decrease in hematocrit was explained by strong encouragement of patients to adhere to high fluid intake. Mean ± s.e.m given. Statistical analysis: Friedman test.

Management of hematopoietic effects and prevention of potential side effects of high-dose EPO treatment in MS

Employing EPO as neuroprotective/neuroregenerative treatment of chronic progressive MS (particularly when using the high dose of EPO required to obtain sufficient EPO levels within the brain in a situation of predominantly intact blood–brain barrier), requires careful and comprehensive safety management. A most elaborated follow-up at all times is mandatory, including clinical examination as well as routine laboratory screening attached to each EPO application. The hematocrit (hemoglobin) has to stay within clearly defined limits. Although the necessity of blood letting in our patients was very low due to the above-described reduced response of the hematopoietic system to EPO, it is still of the utmost importance to carefully follow each and every individual and strictly initiate blood lettings in a situation of hematocrit increase over 48% in females and over 50% in males. Along the same lines, *no* iron substitution is allowed at any time. Iron substitution, as performed in studies targeting anemia, pushes hematopoiesis and therefore would definitely induce an undesired side-effect in non-anemic patients suffering from diseases of the nervous system. Indeed, we make use of the observation that the need for blood lettings will not perpetuate but undergo self-limitation in the absence of iron substitution. Interestingly, the fact that EPO treatment leads to temporary shifts in iron stores as delineated in our exploratory trial in chronic progressive MS [Ehrenreich *et al.* 2007a], might provide an additional beneficial effect in chronic progressive MS patients. Accelerated and intensified integration of iron into new red blood cells and thereby withdrawal of iron from its stores, leads to a picture similar to that of true iron deficiency. This picture is

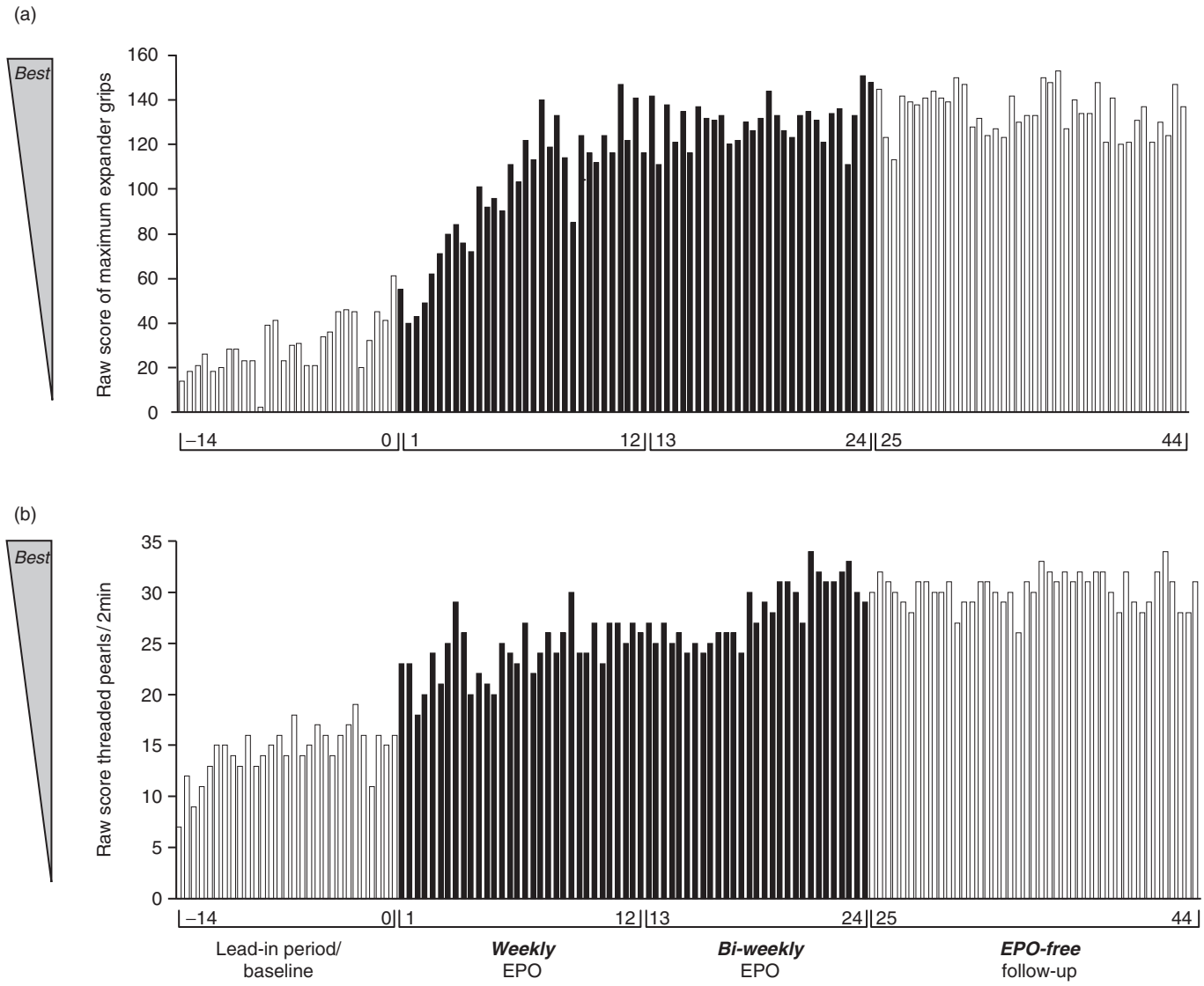

Figure 2. Case report: Follow-up of parameters of motor function upon high-dose erythropoietin (EPO) treatment in a right-handed multiple sclerosis (MS) patient with pareses of both upper extremities. (a) motor strength/endurance of the left (nondominant, more severely affected) hand, presented as number of maximum expander grips for every test timepoint; (b) fine motor performance of the left hand, presented as number of threaded pearls over 2 min for every test timepoint. Improvement of the right hand in both tests was similar. Light columns on the left (−14 to 0 weeks) show performance during lead-in/baseline period. Dark columns (1 to 24 weeks) show performance during active-treatment phase. Light columns (25 to 44 weeks) show performance during EPO-free follow-up.

corrected after termination of EPO treatment without iron substitution. In the absence of appreciable blood loss and upon normal nutrition, iron deficiency will not occur. Binding more and more of the freely available iron, on the other hand, might additionally reduce inflammatory processes in MS and thereby contribute to beneficial effects of high-dose long-term EPO treatment. In fact, iron chelators have been proposed for treatment of MS [Lynch *et al.* 2000]. Disturbed iron metabolism has been described in MS patients [Sfagos *et al.* 2005] and iron-deficient mice fail to develop EAE [Weilbach *et al.* 2004; Grant *et al.* 2003]. Taken together, the temporary relative iron depletion induced by EPO might add to the panel of neuroprotective effects of this growth factor.

Thrombocyte (platelet) counts have also to be carefully and constantly observed. Patients with thrombocytosis have to be excluded at any time, be it upon inclusion or during treatment with

EPO, which in some patients can lead to a pronounced increase in circulating platelets above normal limits. Risk patients with past thromboembolic complications have to be strictly excluded. Exsiccosis has to be prevented at any time. Immobility is contraindicated, precluding the use of this treatment in patients who are prone to bed. Furthermore, patients with cardiovascular disease or cardiovascular risk factors (diabetes, therapy-resistant hypertension, smoking, contraceptive medication) have to be excluded. Potential side-effects of EPO include increases in blood pressure as observed in some patients suffering from anemia due to renal failure [Miyashita *et al.* 2004; Quaschning *et al.* 2003; Vaziri *et al.* 1995; Carlini *et al.* 1993a; 1993b]. Although in our studies no such increases have been noted in any of our patients, blood pressure monitoring is essential and increases in blood pressure upon EPO treatment have to either be pharmacologically counteracted or should lead to exclusion of patients.

Although the exact effects of EPO on tumor growth are still unclear, patients suffering from any kind of malignancy, treated or untreated, should be strictly excluded [for review see (Jelkmann *et al.* 2008)].

The temporary problem of antibody formation upon EPO has essentially resolved due to changes in EPO preparations/packaging introduced by manufacturers and more careful adherence to the 'cooling chain' upon EPO transportation and storage. Nevertheless, antibody determinations should be performed before EPO treatment in all MS patients due to the known strong autoimmune predisposition in this population. Furthermore, potential formation of EPO antibodies has to be controlled at any time when reticulocyte counts below the normal limit are observed, and at the end of each treatment period.

To conclude, results of further proof-of-concept trials on EPO in MS should be awaited before treatment of MS patients with EPO can be recommended. In any case, careful risk and side-effect management is mandatory. Even upon potential future approval of EPO for the indication MS, these safety rules have to be carefully followed. High-dose EPO, although very well tolerated, will never be a '*laissez-faire*' treatment in any of the neuropsychiatric patient populations.

Planning a proof-of-concept (phase IIb) trial on EPO in chronic progressive MS

Based on the auspicious results obtained in our exploratory study on high-dose long-term EPO treatment in chronic progressive MS [Ehrenreich *et al.* 2007a], a pivotal trial for proof-of-concept and potential preparation of approval of EPO for treatment of MS is under planning. The design of the trial resembles in many ways the design used for the exploratory study (Figure 3). In this double-blind, placebo-controlled, randomized

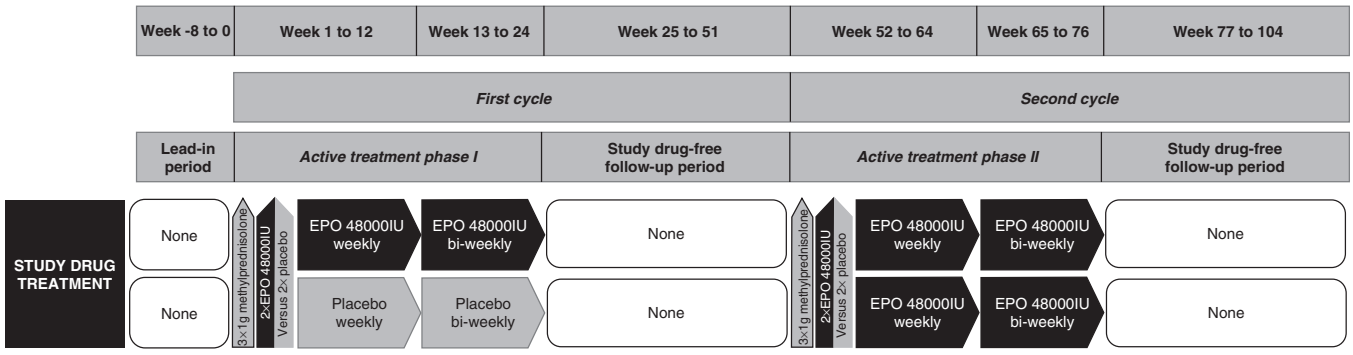

Figure 3. Investigational drug treatment design of 'recombinant human erythropoietin as a neuroprotective/-regenerative treatment of chronic progressive multiple sclerosis (EPO-MS): a double-blind, placebo-controlled, randomized phase IIb trial'. Patients start with an 8-week lead-in period, allowing extensive, repeated rating of baseline performance (estimation of fluctuations) ended by a short, dense 'start-up assessment' (including clinical, neurophysiological, neuroophthalmological, MRI, cognitive, laboratory assessments). Treatment initiation with 3×1000 mg methylprednisolone given on 3 consecutive days, and EPO (48,000 IU; in additional dose-finding treatment arms alternative dosing with 32,000 and 16,000 IU, respectively) *vs* placebo on days 2 and 3 of methylprednisolone treatment follows. Patients then receive EPO/placebo weekly for 12 weeks, biweekly for another 12 weeks and undergo a 6-month post-treatment observation period with visits every 6 weeks. In the second year all patients are switched to verum following the same schedule until individual study end after 26 months ($2 + 12 + 12$).

proof-of-concept (phase IIb) trial, EDSS will be used as a primary outcome measure, supplemented by motor and cognitive parameters as well as readouts of neurophysiology, neuro-ophthalmology and MRI as secondary outcome measures. The total duration of this study will extend to 3–4 years with an individual duration of two years, comprising two EPO treatment cycles. The second EPO cycle is planned as 'all patients switch to verum'.

Concluding remarks

Development of EPO, EPO derivatives/analogs, EPO mimetics or EPO inducers (all together EPO variants), for improving motor function or cognitive performance in patients suffering from chronic progressive MS or other neuropsychiatric diseases, is a novel and promising field of clinical neuroscience that is still in its infancy. It will not only require further human trials but also a return to rodent studies designed to better understand the mechanisms of action of EPO. These mechanisms in turn will help to design even better treatment schedules; for example, with respect to timing of applications, duration and combination therapies to just name a few, and perhaps also to work on new EPO mimetics. Main result of the translational EPO work in the field of neuroscience at this point is that the concept of neuroprotection works in man and that its introduction into clinical practice is feasible.

Conflict of interest statement/ acknowledgment

The clinical studies reported here have been funded by the Max-Planck-Society (MPG) and by research grants from Johnson & Johnson – Ortho Biotech (Stroke). Basic research received grant support predominantly from MPG, the German Research Foundation (DFG-CMPB), Johnson & Johnson – Ortho Biotech, Lundbeck, and Roche.

References

Adamcio, B., Sargin, D., Stradomska, A., Medrihan, L., Gertler, C., Theis, F. *et al.* (2008) Erythropoietin enhances hippocampal long-term potentiation and memory. *BMC Biol* 6: 37.

Agnello, D., Bigini, P., Villa, P., Mennini, T., Cerami, A., Brines, M.L. *et al.* (2002) Erythropoietin exerts an anti-inflammatory effect on the CNS in a model of experimental autoimmune encephalomyelitis. *Brain Res* 952: 128–134.

Banks, W.A., Jumbe, N.L., Farrell, C.L., Niehoff, M.L. and Heatherington, A.C. (2004) passage of erythropoietic agents across the blood–brain barrier: a comparison of human and murine erythropoietin and the analog darbepoetin alfa. *Eur J Pharmacol* 505: 93–101.

Bauer, C. and Kurtz, A. (1989) Oxygen sensing in the kidney and its relation to erythropoietin production. *Annu Rev Physiol* 51: 845–856.

Beleslin-Cokic, B.B., Cokic, V.P., Yu, X., Weksler, B.B., Schechter, A.N. and Noguchi, C.T. (2004) Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. *Blood* 104: 2073–2080.

Bernaudin, M., Marti, H.H., Roussel, S., Divoux, D., Nouvelot, A., Mackenzie, E.T. *et al.* (1999) A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *J Cereb Blood Flow Metab* 19: 643–651.

Bianchi, R., Buyukakilli, B., Brines, M., Savino, C., Cavaletti, G., Oggioni, N. *et al.* (2004) Erythropoietin both protects from and reverses experimental diabetic neuropathy. *Proc Natl Acad Sci USA* 101: 823–828.

Bocker-Meffert, S., Rosenstiel, P., Rohl, C., Warneke, N., Held-Feindt, J., Sievers, J. *et al.* (2002) Erythropoietin and VEGF promote neural outgrowth from retinal explants in postnatal rats. *Invest Ophthalmol Vis Sci* 43: 2021–2026.

Brettschneider, J., Widl, K., Ehrenreich, H., Riepe, M. and Tumani, H. (2006) Erythropoietin in the cerebrospinal fluid in neurodegenerative diseases. *Neurosci Lett* 404: 347–351.

Brines, M., Grasso, G., Fiordaliso, F., Sfacteria, A., Ghezzi, P., Fratelli, M. *et al.* (2004) Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. *Proc Natl Acad Sci USA* 101: 14907–14912.

Brines, M.L., Ghezzi, P., Keenan, S., Agnello, D., De Lanerolle, N.C., Cerami, C. *et al.* (2000) Erythropoietin crosses the blood–brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci USA* 97: 10526–10531.

Brück, W. (2005) Clinical implications of neuropathological findings in multiple sclerosis. *J Neurol* 252(Suppl. 3): iii10–iii14.

Byts, N., Samoylenko, A., Fasshauer, T., Ivanisevic, M., Hennighausen, L., Ehrenreich, H. *et al.* (2008) Essential role for Stat5 in the neurotrophic but not in the neuroprotective effect of erythropoietin. *Cell Death Differ* 15: 783–792.

Byts, N., Samoylenko, A., Woldt, H., Ehrenreich, H. and Siren, A.L. (2006) Cell type specific signalling by hematopoietic growth factors in neural cells. *Neurochem Res* 31: 1219–1230.

Campana, W.M. and Myers, R.R. (2001) Erythropoietin and erythropoietin receptors in the

peripheral nervous system: changes after nerve injury. *Faseb J* 15: 1804–1806.

Carlini, R., Obialo, C.I. and Rothstein, M. (1993a) Intravenous erythropoietin (rhuepo) administration increases plasma endothelin and blood pressure in hemodialysis patients. *Am J Hypertens* 6: 103–107.

Carlini, R.G., Dusso, A.S., Obialo, C.I., Alvarez, U.M. and Rothstein, M. (1993b) Recombinant human erythropoietin (rhuepo) increases endothelin-1 release by endothelial cells. *Kidney Int* 43: 1010–1014.

Casals-Pascual, C., Idro, R., Gicheru, N., Gwer, S., Kitsao, B., Gitau, E. *et al.* (2008) High levels of erythropoietin are associated with protection against neurological sequelae in african children with cerebral malaria. *Proc Natl Acad Sci USA* 105: 2634–2639.

Celik, M., Gokmen, N., Erbayraktar, S., Akhisaroglu, M., Konakc, S., Ulukus, C. *et al.* (2002) Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. *Proc Natl Acad Sci USA* 99: 2258–2263.

Chang, K.H., Tam, M. and Stevenson, M.M. (2004) Modulation of the course and outcome of blood-stage malaria by erythropoietin-induced reticulocytosis. *J Infect Dis* 189: 735–743.

Chikuma, M., Masuda, S., Kobayashi, T., Nagao, M. and Sasaki, R. (2000) Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus. *Am J Physiol Endocrinol Metab* 279: 1242–1248.

Chopp, M., Li, Y. and Zhang, J. (2008) Plasticity and remodeling of brain. *J Neurol Sci* 265: 97–101.

Chung, Y.H., Joo, K.M., Kim, Y.S., Lee, K.H., Lee, W.B. and Cha, C.I. (2004) Enhanced Expression of erythropoietin in the central nervous system of Sod1(G93a) transgenic mice. *Brain Res* 1016: 272–280.

Compston, A. and Coles, A. (2002) Multiple sclerosis. *Lancet* 359: 1221–1231.

Confavreux, C. and Vukusic, S. (2006) Accumulation of irreversible disability in multiple sclerosis: from epidemiology to treatment. *Clin Neurol Neurosurg* 108: 327–332.

Csete, M., Rodriguez, L., Wilcox, M. and Chadalavada, S. (2004) Erythropoietin receptor is expressed on adult rat dopaminergic neurons and erythropoietin is neurotrophic in cultured dopaminergic neuroblasts. *Neurosci Lett* 359: 124–126.

Diem, R., Sattler, M.B., Merkler, D., Demmer, I., Maier, K., Stadelmann, C. *et al.* (2005) Combined therapy with methylprednisolone and erythropoietin in a model of multiple sclerosis. *Brain* 128: 375–385.

Digicaylioglu, M. and Lipton, S.A. (2001) Erythropoietin-mediated neuroprotection involves cross-talk between JAK2 and NF-kappab signalling cascades. *Nature* 412: 641–647.

Ehrenreich, H., Degner, D., Meller, J., Brines, M., Behe, M., Hasselblatt, M. *et al.* (2004) Erythropoietin: a candidate compound for neuroprotection in schizophrenia. *Molecular Psychiatry* 9: 42–54.

Ehrenreich, H., Fischer, B., Norra, C., Schellenberger, F., Stender, N., Stiefel, M. *et al.* (2007a) Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis. *Brain* 130: 2577–2588.

Ehrenreich, H., Hasselblatt, M., Dembowski, C., Cepek, L., Lewczuk, P., Stiefel, M. *et al.* (2002) Erythropoietin therapy for acute stroke is both safe and beneficial. *Molecular Medicine* 8: 495–505.

Ehrenreich, H., Hasselblatt, M., Knerlich, F., Von Ahsen, N., Jacob, S., Sperling, S. *et al.* (2005) A hematopoietic growth factor, thrombopoietin, has a proapoptotic role in the brain. *Proc Natl Acad Sci USA* 102: 862–867.

Ehrenreich, H., Hinze-Selch, D., Stawick, S., Aust, C., Knolle-Veentjer, S., Wilms, S. *et al.* (2007b) Improvement of cognitive functions in chronic schizophrenic patients by recombinant human erythropoietin. *Molecular Psychiatry* 12: 206–220.

Eid, T., Brines, M.L., Cerami, A., Spencer, D.D., Kim, J.H., Schweitzer, J.S. *et al.* (2004) Increased expression of erythropoietin receptor on blood vessels in the human epileptogenic hippocampus with sclerosis. *J Neuropathol Exp Neurol* 63: 73–83.

Erbayraktar, S., De Lanerolle, N., De Lotbiniere, A., Knisely, J.P., Erbayraktar, Z., Yilmaz, O. *et al.* (2006) Carbamylated erythropoietin reduces radiosurgically-induced brain injury. *Mol Med* 12: 74–80.

Erbayraktar, S., Grasso, G., Sfacteria, A., Xie, Q.W., Coleman, T., Kreilgaard, M. *et al.* (2003) Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. *Proc Natl Acad Sci USA* 100: 6741–6746.

Feldmann, M. and Steinman, L. (2005) Design of effective immunotherapy for human autoimmunity. *Nature* 435: 612–619.

Fisher, J.W. (2003) Erythropoietin: physiology and pharmacology update. *Exp Biol Med* 228: 1–14.

Flachenecker, P., Stuke, K., Elias, W., Freidel, M., Haas, J., Pitschnau-Michel, D. *et al.* (2008) Multiple-Sklerose-Register in Deutschland: Ausweitung des Projektes 2005/2006. *Deutsches Ärzteblatt* 105: 113–119.

Flachenecker, P., Zettl, U.K., Gotze, U., Haas, J., Schimrigk, S., Elias, W. *et al.* (2005) [Ms registry in Germany – design and first results of the pilot phase]. *Nervenarzt* 76: 967–975.

Genc, S., Akhisaroglu, M., Kuralay, F. and Genc, K. (2002) Erythropoietin restores glutathione peroxidase activity in 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine-induced neurotoxicity in C57bl mice and stimulates murine astroglial glutathione peroxidase production in vitro. *Neurosci Lett* 321: 73–76.

Genc, S., Kuralay, F., Genc, K., Akhisaroglu, M., Fadiloglu, S., Yorukoglu, K. *et al.* (2001) Erythropoietin exerts neuroprotection in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57/Bl mice via increasing nitric oxide production. *Neurosci Lett* 298: 139–141.

Giovannoni, G. (2004) Management of secondary-progressive multiple sclerosis. *CNS Drugs* 18: 653–669.

Gold, R., Linington, C. and Lassmann, H. (2006) Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 129: 1953–1971.

Gorio, A., Gokmen, N., Erbayraktar, S., Yilmaz, O., Madaschi, L., Cichetti, C. *et al.* (2002) Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. *Proc Natl Acad Sci USA* 99: 9450–9455.

Grant, S.M., Wiesinger, J.A., Beard, J.L. and Cantorna, M.T. (2003) Iron-deficient mice fail to develop autoimmune encephalomyelitis. *J Nutr* 133: 2635–2638.

Grasso, G., Buemi, M., Alafaci, C., Sfacteria, A., Passalacqua, M., Sturiale, A. *et al.* (2002) Beneficial effects of systemic administration of recombinant human erythropoietin in rabbits subjected to subarachnoid hemorrhage. *Proc Natl Acad Sci USA* 99: 5627–5631.

Grignaschi, G., Zennaro, E., Tortarolo, M., Calvaresi, N. and Bendotti, C. (2007) Erythropoietin does not preserve motor neurons in a mouse model of familial ALS. *Amyotroph Lateral Scler* 8: 31–35.

Grimm, C., Wenzel, A., Groszer, M., Mayser, H., Seeliger, M., Samardzija, M. *et al.* (2002) HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. *Nat Med* 8: 718–724.

Grunfeld, J.F., Barhum, Y., Blondheim, N., Rabey, J.M., Melamed, E. and Offen, D. (2007) Erythropoietin delays disease onset in an amyotrophic lateral sclerosis model. *Exp Neurol* 204: 260–263.

Hauser, S.L. and Oksenberg, J.R. (2006) The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron* 52: 61–76.

Hohlfeld, R. and Wekerle, H. (2004) Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy: from pipe dreams to (therapeutic) pipelines. *Proc Natl Acad Sci USA* 101(Suppl. 2): 14599–14606.

Jelkmann, W. (1992) Erythropoietin: structure, control of production, and function. *Physiol Rev* 72: 449–489.

Jelkmann, W. (2007) Erythropoietin after a century of research: younger than ever. *Eur J Haematol* 78: 183–205.

Jelkmann, W. (2008) Developments in the therapeutic use of erythropoiesis stimulating agents. *Br J Haematol* 141: 287–297.

Jelkmann, W., Bohlius, J., Hallek, M. and Sytkowski, A.J. (2008) The erythropoietin receptor in normal and cancer tissues. *Crit Rev Oncol Hematol* 67: 39–61.

Jubinsky, P.T., Krijanovski, O.I., Nathan, D.G., Tavernier, J. and Sieff, C.A. (1997) The beta chain of the interleukin-3 receptor functionally associates with the erythropoietin receptor. *Blood* 90: 1867–1873.

Juul, S.E., Anderson, D.K., Li, Y. and Christensen, R.D. (1998) Erythropoietin and erythropoietin receptor in the developing human central nervous system. *Pediatr Res* 43: 40–49.

Kaiser, K., Texier, A., Ferrandiz, J., Buguet, A., Meiller, A., Latour, C. *et al.* (2006) Recombinant human erythropoietin prevents the death of mice during cerebral malaria. *J Infect Dis* 193: 987–995.

Kertesz, N., Wu, J., Chen, T.H., Sucov, H.M. and Wu, H. (2004) The role of erythropoietin in regulating angiogenesis. *Dev Biol* 276: 101–110.

Keswani, S.C., Buldanlioglu, U., Fischer, A., Reed, N., Polley, M., Liang, H. *et al.* (2004) A novel endogenous erythropoietin mediated pathway prevents axonal degeneration. *Ann Neurol* 56: 815–826.

Kilic, E., Kilic, U., Soliz, J., Bassetti, C.L., Gassmann, M. and Hermann, D.M. (2005) Brain-derived erythropoietin protects from focal cerebral ischemia by dual activation of ERK-1/-2 and AKT pathways. *Faseb J* 19: 2026–2028.

Knabe, W., Knerlich, F., Washausen, S., Kietzmann, T., Siren, A.L., Brunnett, G. *et al.* (2004) Expression patterns of erythropoietin and its receptor in the developing midbrain. *Anat Embryol (Berl)* 207: 503–512.

Knabe, W., Siren, A.L., Ehrenreich, H. and Kuhn, H.J. (2005) Expression patterns of erythropoietin and its receptor in the developing spinal cord and dorsal root ganglia. *Anat Embryol (Berl)* 210: 209–219.

Koh, S.H., Kim, Y., Kim, H.Y., Cho, G.W., Kim, K.S. and Kim, S.H. (2007) Recombinant human erythropoietin suppresses symptom onset and progression of G93a-Sod1 mouse model of ALS by preventing motor neuron death and inflammation. *Eur J Neurosci* 25: 1923–1930.

Konishi, Y., Chui, D.H., Hirose, H., Kunishita, T. and Tabira, T. (1993) Trophic effect of erythropoietin and other hematopoietic factors on central cholinergic neurons in vitro and in vivo. *Brain Res* 609: 29–35.

Kwack, C. and Balakrishnan, V.S. (2006) Managing erythropoietin hyporesponsiveness. *Semin Dial* 19: 146–151.

Leist, M., Ghezzi, P., Grasso, G., Bianchi, R., Villa, P., Fratelli, M. *et al.* (2004) Derivatives of erythropoietin

that are tissue protective but not erythropoietic. *Science* 305: 239–242.

Lewczuk, P., Hasselblatt, M., Kamrowski-Kruck, H., Heyer, A., Unzicker, C., Siren, A.L. *et al.* (2000) Survival of hippocampal neurons in culture upon hypoxia: effect of erythropoietin. *Neuroreport* 11: 3485–3488.

Li, W., Maeda, Y., Yuan, R.R., Elkabes, S., Cook, S. and Dowling, P. (2004) Beneficial effect of erythropoietin on experimental allergic encephalomyelitis. *Ann Neurol* 56: 767–777.

Livnah, O., Stura, E.A., Middleton, S.A., Johnson, D.L., Jolliffe, L.K. and Wilson, I.A. (1999) Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. *Science* 283: 987–990.

Lynch, S.G., Fonseca, T. and Levine, S.M. (2000) A multiple course trial of desferrioxamine in chronic progressive multiple sclerosis. *Cell Mol Biol (Noisy-le-grand)* 46: 865–869.

Marti, H.H., Wenger, R.H., Rivas, L.A., Straumann, U., Digicaylioglu, M., Henn, V. *et al.* (1996) Erythropoietin gene expression in human, monkey and murine brain. *Eur J Neurosci* 8: 666–676.

Martinez-Estrada, O.M., Rodriguez-Millan, E., Gonzalez-De Vicente, E., Reina, M., Vilaro, S. and Fabre, M. (2003) Erythropoietin protects the in vitro blood-brain barrier against vegf-induced permeability. *Eur J Neurosci* 18: 2538–2544.

Masuda, S., Nagao, M., Takahata, K., Konishi, Y., Gallyas, F., Tabira, T. *et al.* (1993) Functional erythropoietin receptor of the cells with neural characteristics. Comparison with receptor properties of erythroid cells. *J Biol Chem* 268: 11208–11216.

Masuda, S., Okano, M., Yamagishi, K., Nagao, M., Ueda, M. and Sasaki, R. (1994) A Novel site of erythropoietin production. Oxygen-dependent production in cultured rat astrocytes. *J Biol Chem* 269: 19488–19493.

Miyashita, K., Tojo, A., Kimura, K., Goto, A., Omata, M., Nishiyama, K. *et al.* (2004) Blood pressure response to erythropoietin injection in hemodialysis and predialysis patients. *Hypertens Res* 27: 79–84.

Noseworthy, J.H. (2003) Management of multiple sclerosis: current trials and future options. *Curr Opin Neurol* 16: 289–297.

Park, M.H., Lee, S.M., Lee, J.W., Son, D.J., Moon, D.C., Yoon, D.Y. *et al.* (2006) ERK-mediated production of neurotrophic factors by astrocytes promotes neuronal stem cell differentiation by erythropoietin. *Biochem Biophys Res Commun* 339: 1021–1028.

Pugliatti, M., Rosati, G., Carton, H., Riise, T., Drulovic, J., Vecsei, L. *et al.* (2006) The epidemiology of multiple sclerosis in Europe. *Eur J Neurol* 13: 700–722.

Quaschning, T., Ruschitzka, F., Stallmach, T., Shaw, S., Morawietz, H., Goettsch, W. *et al.* (2003) Erythropoietin-induced excessive erythrocytosis activates the tissue endothelin system in mice. *Faseb J* 17: 259–261.

Rovaris, M., Confavreux, C., Furlan, R., Kappos, L., Comi, G. and Filippi, M. (2006) Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol* 5: 343–354.

Sadamoto, Y., Igase, K., Sakanaka, M., Sato, K., Otsuka, H., Sakaki, S. *et al.* (1998) Erythropoietin prevents place navigation disability and cortical infarction in rats with permanent occlusion of the middle cerebral artery. *Biochem Biophys Res Commun* 253: 26–32.

Sakanaka, M., Wen, T.C., Matsuda, S., Masuda, S., Morishita, E., Nagao, M. *et al.* (1998) In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci USA* 95: 4635–4640.

Sättler, M.B., Merkler, D., Maier, K., Stadelmann, C., Ehrenreich, H., Bahr, M. *et al.* (2004) Neuroprotective effects and intracellular signaling pathways of erythropoietin in a rat model of multiple sclerosis. *Cell Death Differ* 11(Suppl. 2): S181–192.

Savino, C., Pedotti, R., Baggi, F., Ubiali, F., Gallo, B., Nava, S. *et al.* (2006) Delayed administration of erythropoietin and its non-erythropoietic derivatives ameliorates chronic murine autoimmune encephalomyelitis. *J Neuroimmunol* 172: 27–37.

Sfagos, C., Makis, A.C., Chaidos, A., Hatzimichael, E.C., Dalamaga, A., Kosma, K. *et al.* (2005) Serum ferritin, transferrin and soluble transferrin receptor levels in multiple sclerosis patients. *Mult Scler* 11: 272–275.

Sharp, F.R. and Bernaudin, M. (2004) Hif1 and oxygen sensing in the brain. *Nat Rev Neurosci* 5: 437–448.

Shingo, T., Sorokan, S.T., Shimazaki, T. and Weiss, S. (2001) Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. *J Neurosci* 21: 9733–9743.

Sirén, A.L., Fratelli, M., Brines, M., Goemans, C., Casagrande, S., Lewczuk, P. *et al.* (2001a) Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA* 98: 4044–4049.

Sirén, A.L., Knerlich, F., Poser, W., Gleiter, C.H., Brück, W. and Ehrenreich, H. (2001b) Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. *Acta Neuropathol* 101: 271–276.

Sirén, A.L., Radyushkin, K., Boretius, S., Kämmer, D., Riechers, C.-C., Natt, O. *et al.* (2006) Global brain atrophy after unilateral parietal lesion and its prevention by erythropoietin. *Brain* 129: 480–489.

Springborg, J.B., Ma, X., Rochat, P., Knudsen, G.M., Amtorp, O., Paulson, O.B. *et al.* (2002) A single

subcutaneous bolus of erythropoietin normalizes cerebral blood flow autoregulation after subarachnoid haemorrhage in rats. *Br J Pharmacol* 135: 823–829.

Tsai, P.T., Ohab, J.J., Kertesz, N., Groszer, M., Matter, C., Gao, J. *et al.* (2006) A critical role of erythropoietin receptor in neurogenesis and post--stroke recovery. *J Neurosci* 26: 1269–1274.

Vaziri, N.D., Zhou, X.J., Smith, J., Oveisi, F., Baldwin, K. and Purdy, R.E. (1995) In vivo and in vitro pressor effects of erythropoietin in rats. *Am J Physiol* 269: F838–845.

Villa, P., Bigini, P., Mennini, T., Agnello, D., Laragione, T., Cagnotto, A. *et al.* (2003) Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. *J Exp Med* 198: 971–975.

Weilbach, F.X., Chan, A., Toyka, K.V. and Gold, R. (2004) The cardioprotector dexrazoxane augments therapeutic efficacy of mitoxantrone in experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 135: 49–55.

Weimar, C., Kraywinkel, K., Maschke, M. and Diener, H.C. (2006) Intravenous thrombolysis in German stroke units before and after regulatory approval of recombinant tissue plasminogen activator. *Cerebrovasc Dis* 22: 429–431.

Weishaupt, J.H., Rohde, G., Polking, E., Siren, A.L., Ehrenreich, H. and Bahr, M. (2004) Effect of erythropoietin axotomy-induced apoptosis in rat retinal ganglion cells. *Invest Ophthalmol Vis Sci* 45: 1514–1522.

Wiese, L., Hempel, C., Penkowa, M., Kirkby, N. and Kurtzhals, J.A. (2008) Recombinant human erythropoietin increases survival and reduces neuronal apoptosis in a murine model of cerebral malaria. *Malar J* 7: 3.

Wingerchuk, D.M. and Noseworthy, J.H. (2002) Randomized controlled trials to assess therapies for multiple sclerosis. *Neurology* 58: S40–48.

Xenocostas, A., Cheung, W.K., Farrell, F., Zakszewski, C., Kelley, M., Lutynski, A. *et al.* (2005) The pharmacokinetics of erythropoietin in the cerebrospinal fluid after intravenous administration of recombinant human erythropoietin. *Eur J Clin Pharmacol* 61: 189–195.

Yu, X., Shacka, J.J., Eells, J.B., Suarez-Quian, C., Przygodzki, R.M., Beleslin-Cokic, B. *et al.* (2002) Erythropoietin receptor signalling is required for normal brain development. *Development* 129: 505–516.

Yuan, R., Maeda, Y., Li, W., Lu, W., Cook, S. and Dowling, P. (2008) Erythropoietin: a potent inducer of peripheral immuno/inflammatory modulation in autoimmune EAE. *PLoS ONE* 3: e1924.

Zhang, J., Li, Y., Cui, Y., Chen, J., Lu, M., Elias, S.B. *et al.* (2005) Erythropoietin Treatment Improves Neurological Functional Recovery in EAE Mice. *Brain Res* 1034: 34–39.