

Exploring recombinant human erythropoietin in chronic progressive multiple sclerosis

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The neurodegenerative aspects of chronic progressive multiple sclerosis (MS) have received increasing attention in recent years, since anti-inflammatory and immunosuppressive treatment strategies have largely failed. However, successful neuroprotection and/or neuroregeneration in MS have not been demonstrated yet. Encouraged by the multifaceted neuroprotective effects of recombinant human erythropoietin (rhEPO) in experimental models, we performed an investigator-driven, exploratory open label study (phase I/IIa) in patients with chronic progressive MS. Main study objectives were (i) evaluating safety of long-term high-dose intravenous rhEPO treatment in MS, and (ii) collecting first evidence of potential efficacy on clinical outcome parameters. Eight MS patients, five randomly assigned to high-dose (48 000 IU), three to low-dose (8000 IU) rhEPO treatment, and, as disease controls, two drug-naïve Parkinson patients (receiving 48 000 IU) were followed over up to 48 weeks: A 6-week lead-in phase, a 12-week treatment phase with weekly EPO, another 12-week treatment phase with bi-weekly EPO, and a 24-week post-treatment phase. Clinical and electrophysiological improvement of motor function, reflected by a reduction in expanded disability status scale (EDSS), and of cognitive performance was found upon high-dose EPO treatment in MS patients, persisting for three to six months after cessation of EPO application. In contrast, low-dose EPO MS patients and drug-naïve Parkinson patients did not improve in any of the parameters tested. There were no adverse events, no safety concerns and a surprisingly low need of blood-lettings. This first pilot study demonstrates the necessity and feasibility of controlled trials using high-dose rhEPO in chronic progressive MS.

Keywords: recombinant human erythropoietin; EPO; primary and secondary progressive multiple sclerosis; neuroprotection; neuroregeneration; neuropsychology; expanded disability status scale (EDSS); walking distance

Abbreviations: EDSS = expanded disability status scale; EPO = erythropoietin; MCV = Mean corpuscular volume; MCH = mean corpuscular haemoglobin; MEP = motor evoked potential; MRI = magnetic resonance imaging; MS = multiple sclerosis

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Introduction

Understanding and treatment of the progressive phase of multiple sclerosis (MS), which is characterized by the steady accumulation of neurological disability, is far from being satisfactory. Progression is most likely driven by the high prevalence of neurodegenerative compared with inflammatory pathological changes, explaining the limited long-term efficacy of current anti-inflammatory and immunosuppressive treatment strategies. It seems that once the cascade of events leading to neuronal and axonal loss is established, even an effective suppression of inflammation fails to

protect from clinical disease progression. The development of *add-on* treatments targeting axonal repair and remyelination and/or the slowing of disease progression through neuroprotection/neuroregeneration remains therefore the most important challenge and goal in clinical management of chronic progressive MS (Compston and Coles, 2002; Brück, 2005; Hauser and Oksenberg, 2006; Rovaris *et al.*, 2006).

Among potential candidate compounds for neuroprotection/neuroregeneration in progressive MS, erythropoietin

(EPO) appears to be a very promising agent. Originally known as a haematopoietic growth factor, EPO has been found in recent years to be part of a highly potent endogenous neuroprotective system in the brain (Ehrenreich *et al.*, 2004a; Juul, 2004; Brines and Cerami, 2005; Jelkmann, 2005; Hasselblatt *et al.*, 2006). As such, it possesses a number of properties suitable to address several of the pathophysiological mechanisms involved in progressive MS. EPO acts in an anti-apoptotic and anti-oxidative fashion, promotes neurite outgrowth and axonal repair, neurogenesis and angiogenesis (Konishi *et al.*, 1993; Campana and Myers, 2001; Shingo *et al.*, 2001; Siren *et al.*, 2001; Celik *et al.*, 2002; Kertesz *et al.*, 2004; Keswani *et al.*, 2004). These properties could be demonstrated, both *in vitro* and *in vivo*, using a variety of different animal models of diseases of the nervous system, including hypoxia/ischaemia, neurotrauma, spinal cord injury, epilepsy and Parkinson's disease (Sakanaka *et al.*, 1998; Bernaudin *et al.*, 1999; Brines *et al.*, 2000; Genc *et al.*, 2001; Siren *et al.*, 2006). Importantly, we could show that EPO can prevent global brain atrophy in a mouse model of chronic neurodegeneration (Siren *et al.*, 2006). Several groups, working on experimental autoimmune encephalitis (EAE), an animal model of MS (Gold *et al.*, 2006), could demonstrate significant beneficial effects of EPO treatment on clinical symptoms, proinflammatory cytokine expression, blood-brain-barrier integrity, electrophysiological and histological findings (Agnello *et al.*, 2002; Li *et al.*, 2004; Sattler *et al.*, 2004; Diem *et al.*, 2005; Zhang *et al.*, 2005; Savino *et al.*, 2006). *In vitro* studies further support these findings (Avasarala and Konduru, 2005; Genc *et al.*, 2006). Encouraging results on the neuroprotective/neurotrophic efficacy of EPO *in man* have been obtained from our recent treatment trials in stroke patients (Ehrenreich *et al.*, 2002) and in patients with chronic schizophrenia (Ehrenreich *et al.*, 2007). These trials underline that the translational step from rodents to man for EPO is realistic.

The present investigation has been designed as an exploratory open-label study (phase I/IIa) in preparation for a double blind proof-of-concept (phase II) trial on EPO treatment in chronic progressive MS. The main study objectives were: (i) to evaluate safety of long-term high-dose EPO treatment in chronic progressive MS, and (ii) to collect first evidence of potential efficacy with respect to a variety of clinical parameters, including walking distance, Expanded Disability Status Scale (EDSS; Kurtzke, 1983), fine motor function and cognition. A careful and comprehensive individual follow-up of a small number of patients during a 6-week *lead-in* phase, a 12–24 week treatment phase and a 24-week post-treatment phase should allow to consolidate plans for a consecutive phase II trial. Further, this exploratory setting should help in determining outcome parameters and estimating the number of patients required for a future phase II trial. It should deliver information about dosing of EPO, necessary duration of treatment in order to see improvement, and effect of EPO

treatment-free periods on maintaining status. Finally, effects found in chronic progressive MS should be compared to effects observed in drug-naïve patients suffering from another degenerative disease, Morbus Parkinson.

Materials and methods

Patients and procedures

Following announcement of the present exploratory study ('EPO MS study') to the local ethical committee, a total of eight patients suffering from chronic progressive MS (primary or secondary), who had previously failed on disease-modifying drugs, and, as disease control, two patients with Morbus Parkinson were included (Table 1). Written informed consent was obtained after comprehensive and repeated information of the respective patient in the absence and in the presence of selected relatives or close advisors (mostly physicians). Patients were fully aware that they participated in a purely experimental study, set up to evaluate safety and gain first impressions on efficacy of EPO in chronic progressive MS.

The study was designed as an exploratory open-label study employing two different doses of recombinant human (rh) EPO. The high dose (48 000 IU of EPO α , ERYPO^R, Janssen-Cilag, Germany) was selected as presumably effective dose, based on our previous trials in stroke (Ehrenreich *et al.*, 2002) and in schizophrenia patients (Ehrenreich *et al.*, 2007), whereas the low dose (8000 IU of EPO α) lies in the middle to upper dose range of anaemia treatment (e.g. Eschbach *et al.*, 1987) and was explored for the first time in a neurological indication. Of the eight MS patients, five received high-dose rhEPO, whereas three received low-dose rhEPO. Dosing was randomly assigned. Patients were unaware of the dose being 'high' or 'low'. The two drug-naïve Parkinson patients received high-dose rhEPO (48 000 IU of EPO α).

An overview of the study design including tests and follow-up parameters is presented in Fig. 1. After baseline examination and confirmation of inclusion/exclusion criteria, patients entered a *lead-in* phase of six weeks duration, where they were asked to regularly score or test their performance in a number of items, either specifically reflecting their individual handicaps or important for general well-being and health. For that, every patient received an individually tailored questionnaire, which should provide insight into the longitudinally evaluated baseline performance as well as, later, the treatment and post-treatment follow-up period.

Following the *lead-in* period, the treatment period was initiated. It started with a one-week inpatient setting, which allowed a comprehensive examination of the patient including MRI of brain and spinal cord, neurological, neuropsychological, electrophysiological, urological, ophthalmological examination and routine laboratory analyses (including EPO-antibody testing). Patients were then started on high-dose prednisolone (1000 mg/100 ml over 30 min intravenously) in order to create a comparable immunosuppression as a starting point of neuroprotective therapy (Milligan *et al.*, 1987). One day later, a second infusion of prednisolone was given, followed by the first infusion of EPO (48 000 or 8000 IU of EPO α , respectively, in 50 ml of 0.9% sodium chloride over 15 min intravenously). The third treatment day included prednisolone followed by EPO. Parkinson patients did not receive prednisolone, but were otherwise treated

Table 1 Patient characteristics

Treatment group	Patient ID	Age (years)	Sex	Education total (years)	Premorbid intelligence quotient ^a	Disease duration (years)	Disease subtype	Optic nerve involvement ^b	Leading symptoms	EDSS upon inclusion	Walking distance upon inclusion
MS high-dose (N = 5)	1	34	Female	19.5	118	9.2	PPMS	None	Tetraspastic (mainly legs/right side), slight ataxia	5.5	88 m
	2	42	Male	22	112	9.7	SPMS	None	Ataxia, dysarthria, urinary dysfunction, mild cognitive deficits, fatigue, inadequate affect	6.0	140 m (with crutch)
	3	53	Male	20.5	136	19.3	PPMS	Bilateral	Tetraspastic (mainly legs/left side), urinary/bowel dysfunction, slight fatigue	6.5	25 m (with walker)
	4	44	Male	20.5	112	12.1	PPMS	Bilateral	Tetraspastic (mainly arms/right side), ataxia, respiratory insufficiency (muscle weakness)	5.5	110m
	5	45	Female	20	143	14.8	SPMS	None	Tetraspastic, ataxia of upper limbs, urinary/bowel dysfunction, fatigue, dysthymia	6.0	77 m (intermittent assistance)
		43.6 (6.8)	m/f: 3/2	20.5 (0.9)	124.2 (14.4)	13.0 (4.1)	3 × PPMS 2 × SPMS			5.9 (0.4)	88.0 (42.7)
MS low-dose (N = 3)	6	38	Male	20	130	9.5	PPMS	Unilateral	Ataxia, paraparesis, severe sensory dysfunction, urinary dysfunction, slight dysarthria	4.5	381 m
	7	42	Male	23	107	18.3	SPMS	Bilateral	Tetraspastic (mainly legs), urinary dysfunction, cognitive deficits, INO, dysarthria	6.5	54 m (with walker)
	8	63	Female	23	136	10.4	SPMS	None	Paresis (mainly legs/right side), muscle cramps, urinary dysfunction, dysarthria	6.0	118 m (with cane)
		47.7 (13.4)	m/f: 2/1	22.0 (1.7)	124.3 (15.3)	12.7 (4.8)	1 × PPMS 2 × SPMS			5.7 (1.0)	184.3 (173.3)
Treatment group	Patient ID	Age (years)	Sex	Education total (years)	Premorbid intelligence quotient^a	Disease duration (years)	Disease subtype	–	Leading symptoms	UPDRS upon inclusion	–
Parkinson high-dose (N = 2)	9	43	Male	24	145	1.8	Idiopathic	–	Tremor (mainly right side), rigidity, bradykinesia, micrographia, mild cognitive deficits, dysarthria	30	–
	10	73	Female	18	118	0.7	Idiopathic	–	Tremor (mainly legs), bradykinesia, impaired fine motor function, fatigue, cognitive dysfunction	20	–

Means (SD) presented for treatment groups 'MS high-dose' and 'MS low-dose'. PPMS = primary progressive multiple sclerosis; SPMS = secondary progressive multiple sclerosis; EDSS = expanded disability status scale; UPDRS = unified parkinson's disease rating scale; INO = internuclear ophthalmoplegia. ^aPremorbid intelligence quotient based on results of the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B). ^bState of ophthalmological examination and VEPs (visual evoked potentials) upon study entry which remained unchanged during follow-up.

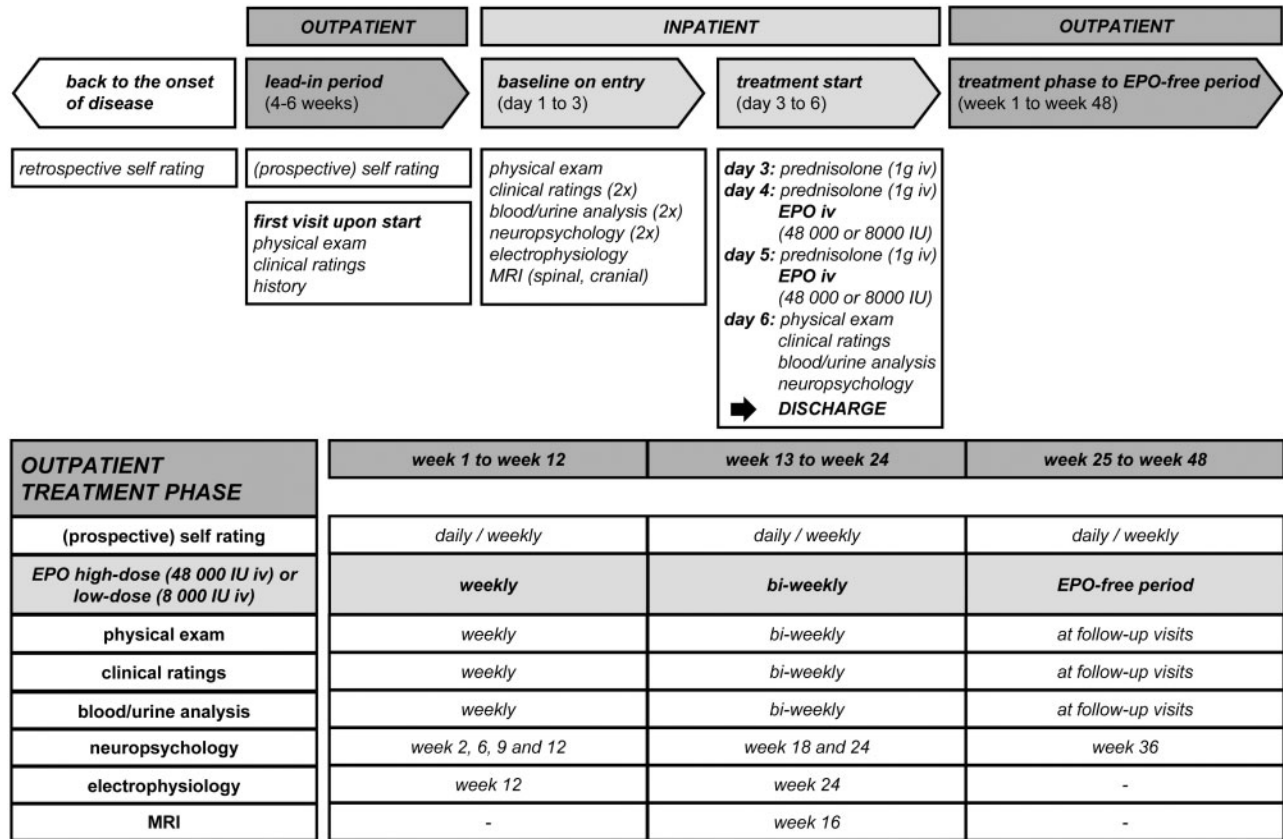


Fig. 1 Design of the EPO MS exploratory study. Low-dose EPO treatment was only performed until week 12.

identically. One day later, patients were discharged and asked to return weekly to the clinic for application of the study drug, documentation of clinical state, performance rating, monitoring of adverse events and safety, including measurement of blood pressure and routine laboratory workup. Blood-letting (350–450 ml) was performed if the haematocrit exceeded 50% in male or 48% in female patients on two consecutive weeks. During the 1-week inpatient phase, the 12–24 week outpatient treatment phase, as well as the 24-week post-treatment phase, patients were asked to continue regular (daily/weekly/monthly) self-rating using their individually tailored questionnaire.

Neuropsychological baseline and follow-up testing of patients included the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B; Lehl, 1999), for determination of the premorbid intelligence quotient, four subtests (Information, Similarities, Picture Completion, Block Design) of the revised German version of the Wechsler Adult Intelligence Scale (HAWIE-R; Tewes, 1991), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998), subtests Visual Scanning, Working Memory and Alertness of the computer-assisted battery for attention testing (TAP; Zimmermann and Fimm, 1995), subtest Letter Number Sequencing of the Wechsler Memory Scale – Revised (WMS-R; Wechsler, 1998), Wisconsin Card Sorting Test – 64 Card Version (WCST-64; Kongs et al., 2000) and the Trail Making Test (Reitan, 1958).

MRI of brain and spinal cord was conducted on a Siemens 1.5T scanner (scans before and after gadolinium; for cranial imaging transverse, coronal and sagittal T1-weighted sequences, T2-weighted TSE- and TIRM-sequences; for spinal imaging sagittal and transverse T1-weighted TIRM- and TSE-sequences, T2-weighted TSE-sequences; slice thickness: 6 mm (19 slices, cranial), 3 mm (15 slices, cervical); 4 mm (11 slices, thoracic); semi-automated volumetrical analyses carried out, in a blinded fashion, with Centricity Radiology RA 1000, General Electrics, in T2-weighted images). Electrophysiology wherever technically possible (motor evoked potentials: MEPs), maximum walking distance, fine motor assessment using the 9-hole peg test (Cutter et al., 1999; Rudick et al., 2001), as well as MacQuarrie Tapping and Dotting tests (MacQuarrie, 1925, 1953) were performed.

Inclusion and exclusion criteria

An overview of all eight MS patients and the two Parkinson patients is provided in Table 1. MS patients had to be in a chronic progressive state (either primary or secondary) of their disease. They had to be free of other severe psychiatric or neurological disorders. A minimum of measurable walking distance was required. Patients were not allowed to smoke or to take sex steroid hormones to avoid a potential additional vascular risk on top of their relative immobility. Two patients quit smoking several weeks before the lead-in phase, another patient stopped taking

sex steroid medication (in accordance with her gynaecologist). All medication had to be documented. Start of any MS-related novel medication during the study was not allowed nor was any kind of iron substitution. Previous medication in all MS patients included corticosteroids, intrathecal prednisolone, beta interferon, copaxone, mitoxantrone, cyclophosphamide, iv-immunoglobulins, deoxyspergualin and riluzole. Treatment was terminated at least half a year before EPO treatment due to lack of efficacy. All patients had shown distinct progression of their disease in the past year.

'Consilium': decision on study continuation after 12 weeks

After 12 weeks of weekly EPO treatment, a so-called *consilium* took place, integrating all test results and observations of all participating parties, the patient, potential relatives or family members, an independent MS specialist, members of the clinical neuroscience team, a clinical neurophysiologist, a neuroradiologist, and a physiotherapist. Continuation of treatment was only recommended if a patient had clearly improved performance in at least three independent items, previously identified to be affected in this particular individual, e.g. walking distance, cognition and bladder function.

Statistical analysis

All numerical results are presented as mean \pm SD. For analysis, individual mean baseline performance in each of the items of interest was set to 100% in order to reach comparable baseline values among patients. Individual change during follow-up upon treatment or during the treatment-free period was expressed in % individual baseline. This way, patients could be compared and intra-group comparisons investigating the course of various variables in the high-dose EPO MS group, including all testing time points from baseline on, could be performed using the Friedman test of SPSS 14.0 for Windows. This special nonparametric procedure for comparing repeated measures data with small sample sizes can be used for metric as well as ordered categorical data. Supplementary Tables 2 and 3 additionally provide significance values based on analyses of raw scores. Inter- and intra-group comparisons of MRI data were carried out using the nonparametric Mann–Whitney–U-test (independent and dependent). Statistical significance was set to 0.05 for all analyses.

Results

Study participation

All 10 patients participated in the study until the *consilium* took place after 12 weeks of weekly EPO treatment. At that time point, only the five high-dose MS patients met criteria for continuation, whereas all three low-dose EPO MS patients as well as the two high-dose EPO Parkinson patients did not show sufficient improvement that would have justified continuation according to our *consilium* criteria for beneficial treatment response. The five high-dose EPO MS patients stayed on continuous treatment for another 12 weeks to

receive intravenous high-dose EPO now once every other week. After this second treatment period, there was a 24-week treatment-free follow-up period for all high-dose EPO MS patients. One of these patients could be followed over another EPO treatment cycle.

Safety

There were no adverse events reported or observed in any of the patients at any time. One Parkinson patient complained about being tired for approximately two to three days after each EPO infusion. All other patients (high-dose as well as low-dose EPO MS and Parkinson) reported on feeling physically stronger, less tired, more optimistic, more enduring. They did not report that their sleep was in any way affected. Quality of life self-rating during that time stayed stable (data not shown). No relapses were observed in any of the MS patients during the study period. Mean changes in blood cell counts and iron parameters during and after EPO treatment are illustrated in Fig. 2. Original data (mean \pm SD) of high-dose and low-dose MS patients and of Parkinson patients are presented in Supplementary Table 1. The response of haemoglobin, haematocrit and erythrocytes to EPO in MS patients was surprisingly low. Of the high-dose EPO MS group, a total of only five blood-lettings were necessary during the whole treatment phase (three times in one patient and once in two patients; see Supplementary Table 1). In the low-dose EPO group, no blood-letting had to be performed. The two Parkinson patients responded stronger to EPO and both required blood-letting. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) declined similarly strongly in all high-dose patients. The iron parameters showed the expected pattern, more pronounced upon high-dose and less upon low-dose EPO treatment: distinct decrease in serum ferritin levels, paralleled by increases in serum transferrin and, particularly, in soluble transferrin receptor (Fig. 2; Supplementary Table 1). Platelet counts increased in all patients at approximately the same rate but stayed essentially within the normal range. Whereas there was no measurable change in CRP, erythrocyte sedimentation rate tended to decrease in all patients over time of EPO treatment. All patients were EPO-antibody negative at baseline and none of the patients had developed EPO antibodies by the end of the treatment period. No appreciable change in blood pressure upon EPO treatment was observed in any of the patients (data not shown).

Motor function

All patients in the high-dose EPO MS group showed a significant improvement in their maximum walking distance over time as compared to baseline, which became first apparent after three weeks, gradually improved during the

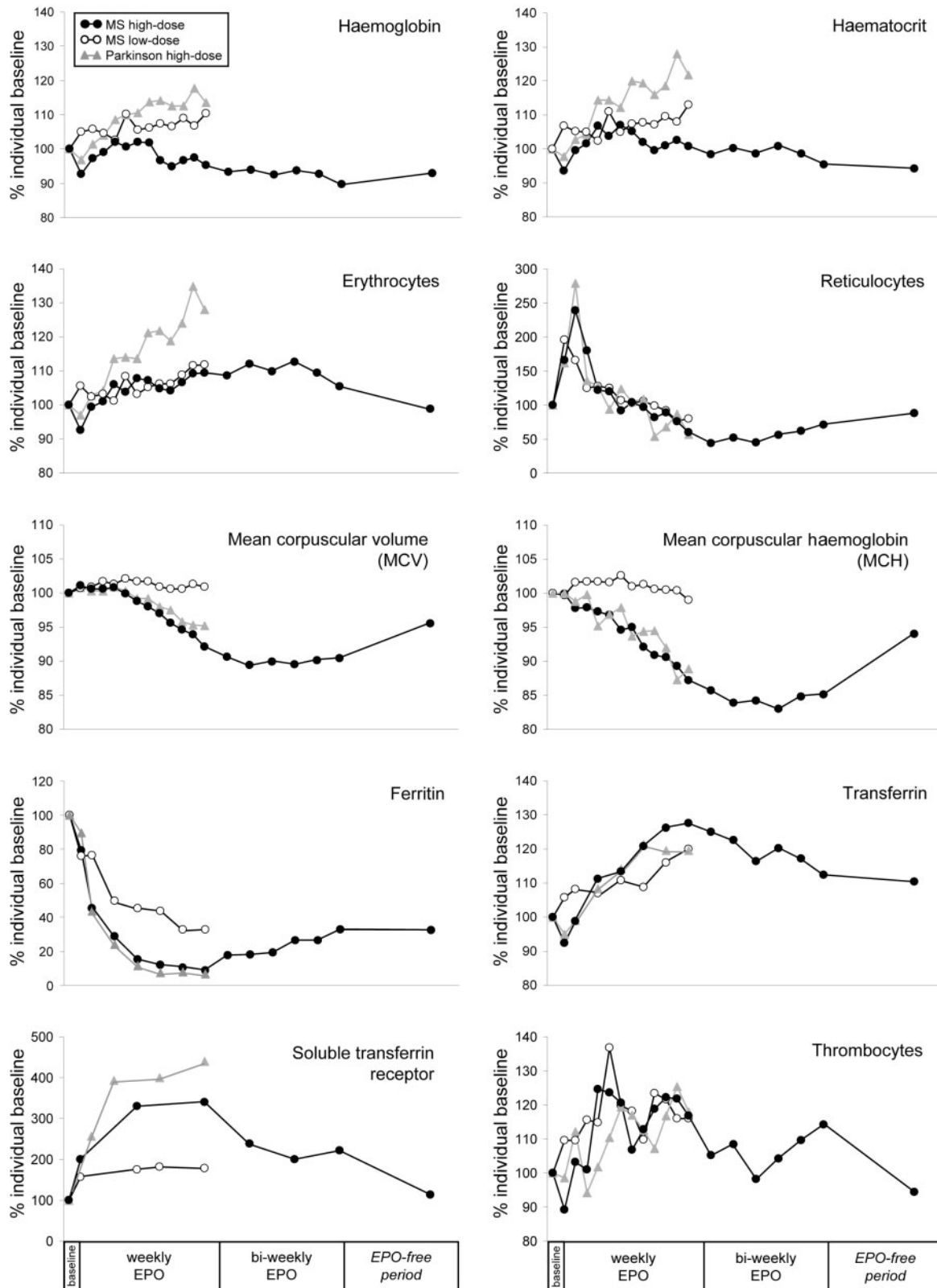


Fig. 2 Changes in blood cell counts and iron parameters during and after EPO treatment. The mean of two baseline values of each patient was set to 100% for each of the laboratory parameters and used for calculating individual change over time. Mean change of all patients within each group during follow-up upon treatment or during the treatment-free period is expressed in % baseline. Low-dose EPO MS patients and Parkinson patients were only followed until week 12. Filled circles: high-dose EPO MS patients (N = 5); open circles: low-dose EPO MS patients (N = 3); grey triangles: high-dose EPO Parkinson patients (N = 2).

treatment phase to reach a plateau at around eight weeks and still persisted after cessation of EPO treatment (Fig. 3A and B; Supplementary Table 2). The increase in maximum walking distance in high-dose EPO MS patients resulted in a reduction of the EDSS (Fig. 3C) and was paralleled in patients, who could be followed electrophysiologically, by a reduction in the central motor conduction time (left Tibialis MEP; Fig. 3D, Supplementary Table 2). In contrast, neither low-dose EPO MS patients nor Parkinson patients, according to the Unified Parkinson's Disease Rating Score (Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003; Fahn and Elton, 1987), displayed any measurable beneficial effect on walking distance/gait. Fig. 3B illustrates the course of the maximum walking distance, both supervised and self-measurements, over more than one year in one of the high-dose EPO MS patients who underwent two EPO treatment periods. The trend line from lead-in to the end of the observation period clearly directs upwards, with no change after switch to biweekly application. The EPO treatment break did not provoke any loss of the gained function, underlining that the effect of EPO is long lasting. Also, results of supervised ratings and self-ratings were quite consistent.

Fine motor performance in MacQuarrie Tapping and Dotting tests also showed improvement in high-dose EPO MS patients only (Fig. 4A and B), whereas in the 9-hole peg test, there was no significant beneficial effect of EPO treatment in any group and no group difference (Fig. 4C; Supplementary Table 2). Medianus MEP stayed stable over time (Supplementary Table 2). Bladder function was initially reported to be affected in three of the five high-dose and all three low-dose EPO MS patients. Subjective rating by the patients yielded distinct improvement only upon high-dose EPO treatment (data not shown). One MS patient with respiratory insufficiency due to muscle weakness showed improvement in lung function (vital capacity and forced vital capacity) upon high-dose EPO treatment.

Cognitive functions

Premorbid intelligence as measured at baseline with the MWT-B was almost identical in all MS patients (Table 1). In contrast, estimation of the current intelligence using HAWIE-R showed a higher variability (high-dose EPO MS: 132.8 ± 15.4 ; low-dose EPO MS: 119.3 ± 14.7). High-dose EPO MS patients displayed a clear improvement in cognitive tests related to executive functioning, i.e. Trail Making – Part B, WMS-R Letter Number Sequencing, Visual Scanning, RBANS Coding and Working Memory and psychomotor speed (Trail Making – Part A), which was absent both in low-dose MS and Parkinson patients (Fig. 5, Supplementary Table 3). This improvement remained stable even during the EPO treatment break. In contrast, parameters of learning and memory (see Supplementary Table 3 for the most relevant items) remained essentially

unchanged on a high performance level, consistent with a ceiling effect.

Other outcome and follow-up parameters

Analysis of MRI data did not uncover changes upon EPO treatment. Volumetrical analysis of total brain (excluding cerebellum, brain stem and ventricles) as well as of ventricles did not yield differences among high and low-dose MS patients upon study entry (1195.8 ± 65.6 ml versus 1013.6 ± 189.7 ml, $P=0.4$; and 48.3 ± 17.6 ml versus 46.4 ± 12.8 ml, $P=0.857$). Follow-up of the high-dose patients after three months (3.6 ± 1.1 months) showed no change in total brain or ventricle volume compared to baseline (baseline versus 3 months: 1195.8 ± 65.6 ml versus 1152.4 ± 47.7 ml, $P=0.317$; and 48.3 ± 17.6 ml versus 47.9 ± 17.8 ml, $P=1.0$). Serum levels of the glial damage marker S100B did not reveal consistent changes in any of the patients upon treatment (Supplementary Table 1).

Discussion

We present here the results of an open label study, designed to explore safety and potential beneficial effects of long-term EPO treatment in chronic progressive MS. In this study, there were no adverse events, no safety concerns and an astonishingly low need of blood-lettings. These findings of the safety part of this pilot trial may be encouraging for future studies but will certainly not make careful monitoring of patients' safety parameters dispensable. Using an intra-individual follow-up design, we found significant clinical and a tendency of electrophysiological improvement of motor function in chronic progressive MS upon high-dose EPO treatment, also reflected by a reduction in EDSS. To our knowledge, improvement of this score has not yet been observed in any chronic progressive MS trial, although the non-blinded nature of our study limits the value of these findings. Of course, in MS patients with a high disability, EDSS only reflects walking ability and completely disregards cognition or function of upper limbs. Therefore, we included a comprehensive set of data measuring cognitive functions. Indeed, cognitive performance was also improved in high-dose EPO MS patients. Similarly, in a recent double-blind proof-of-concept (phase II) study, we had demonstrated cognitive improvement in chronic schizophrenic patients upon high-dose EPO treatment over 12 weeks (Ehrenreich *et al.*, 2007).

We do not think that these beneficial effects on motor function and cognition are simply explained by improved mood since an improvement in general well-being and mood was observed in all patients, independent of the dose, and became evident already after the first infusion of EPO. In contrast to mood and general well-being, there was no clear beneficial effect of low-dose EPO treatment in MS patients on any of the other parameters tested and no measurable effect of high-dose EPO treatment in the two

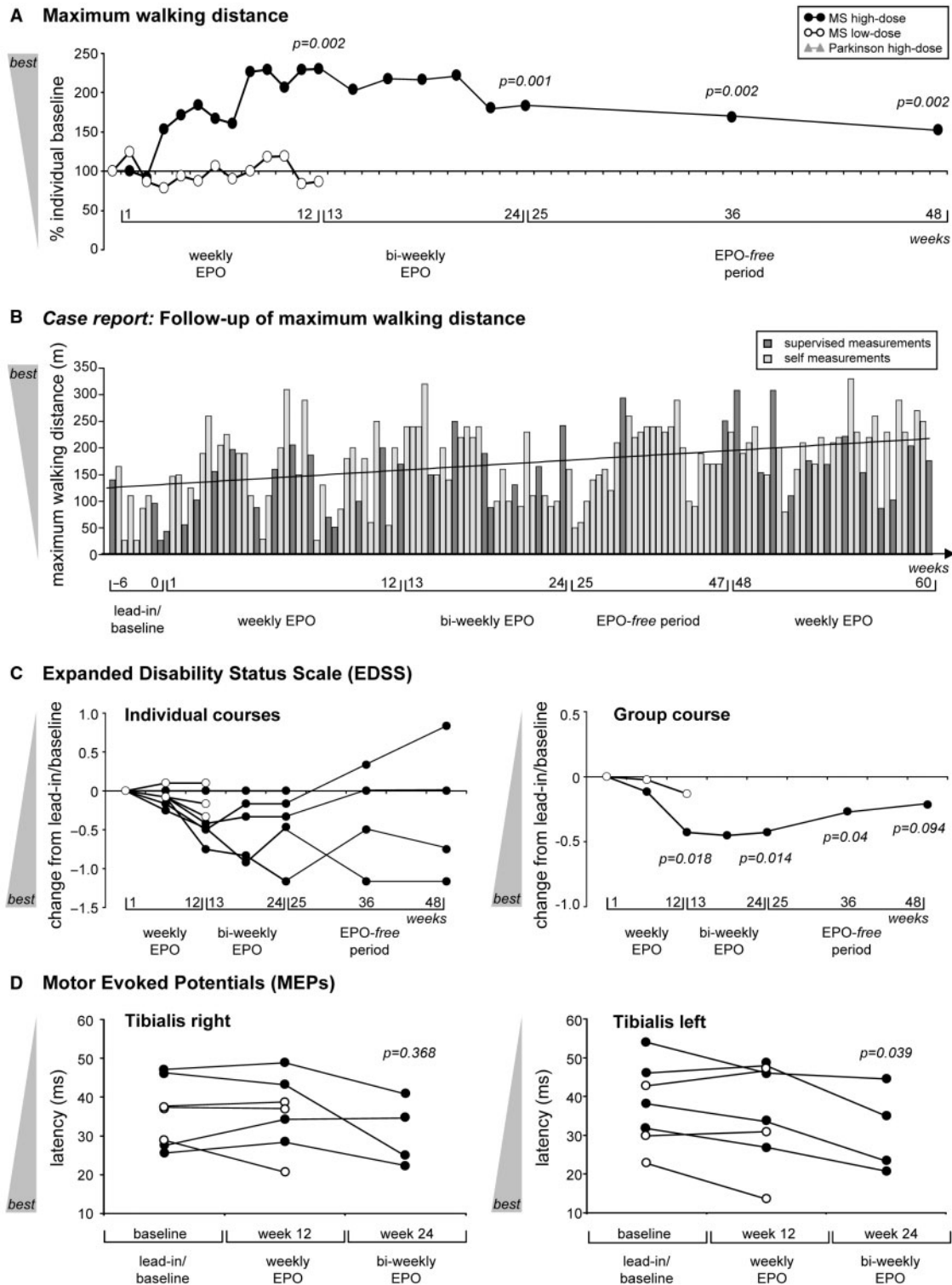


Fig. 3 Changes in parameters of motor function upon EPO treatment. **(A)** The mean of all available baseline values of maximum walking distance of each patient obtained during the whole lead-in period was set to 100% and used for calculating individual change over time. Mean change of all patients within each group during follow-up upon treatment or during the treatment-free period is expressed in % baseline. **(B)** Follow-up of maximum walking distance of one high-dose EPO MS patient over a total of 60 weeks, including two EPO treatment periods, is presented as raw data for every test time point. The trend line illustrates the improvement over time. **(C)** For presenting the course of EDSS scores during the study, mean individual baseline was set to 0 and subsequent values denote change of EDSS score during follow-up of individual MS patients (left panel) or of mean EDSS score of the groups (right panel). For determining individual EDSS change over time, the mean value of consecutive ratings over six week follow-up periods was used. **(D)** Intra-individual course of central motor conduction time (Tibialis MEP, original data; MS high-dose patients $N=4$, due to methodological problems

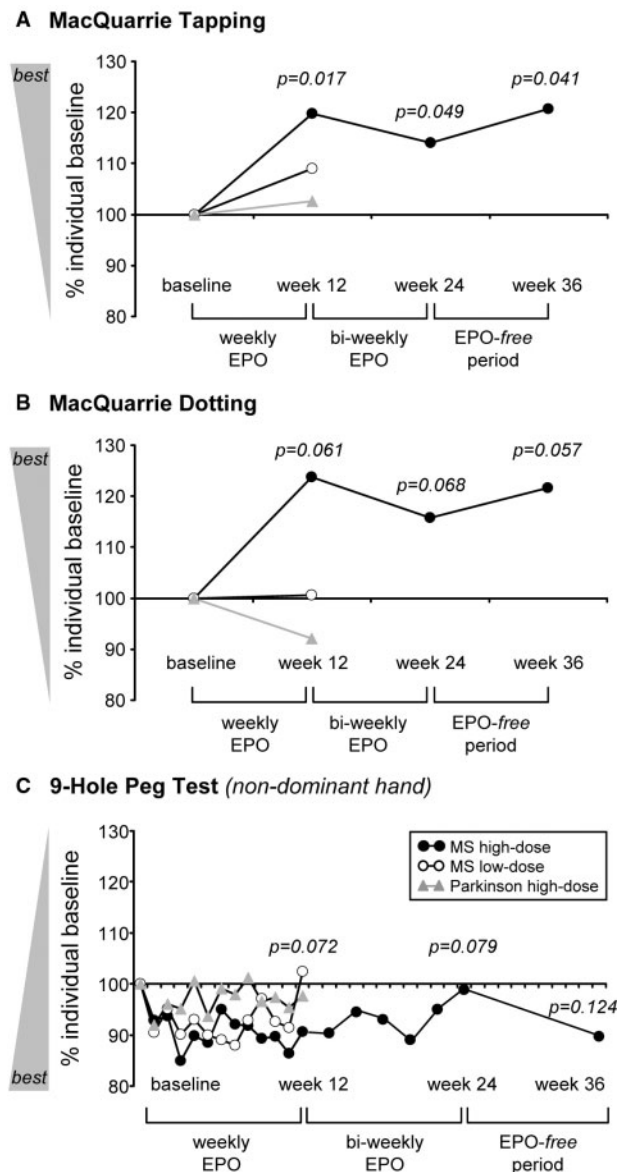


Fig. 4 Changes in parameters of fine motor coordination upon EPO treatment. **(A)** Change of performance in MacQuarrie Tapping test, **(B)** MacQuarrie Dotting test and **(C)** 9-hole peg test, is expressed as % individual baseline. The mean of all available baseline values of each patient was set to 100% for each of these parameters and used for calculating individual change over time. Mean change of all patients within each group during follow-up upon treatment or during the treatment-free period is expressed in % baseline. Low-dose EPO MS patients and Parkinson patients were only followed until week 12. Filled circles: high-dose EPO MS patients ($N = 5$); open circles: low-dose EPO MS patients ($N = 3$); grey triangles: high-dose EPO Parkinson patients ($N = 2$). Significance values refer to the high-dose EPO MS patients only and denote changes of the respective parameter from baseline to the end of each treatment period, including all testing time points. Statistical analysis: Friedman test.

drug-naïve Parkinson patients. These latter findings may also exclude a pure placebo effect to explain the improvement found in high-dose EPO MS patients.

The improvement was kept during EPO reduction and even after complete cessation of EPO treatment over a follow-up time of 24 weeks, pointing to a regenerative effect mediated by EPO rather than a temporary and short-lived action. These results may suggest an interval treatment in future studies. It remains to be determined, however, whether continuation of EPO treatment in MS patients after a treatment-free interval will lead to further improvement, or at least contribute to maintaining the improved status and to slowing of progression. The observations in one of our patients who could be followed over more than one year and two EPO treatment cycles, would support the assumption that the effect of EPO is a long-lasting one.

Taken together, we were able to provide first evidence that EPO may show an effect on the clinical course of chronic progressive MS, acting via as yet undetermined mechanisms that improve function, enhance regeneration and/or slow deterioration. In line with our observations, encouraging data had been obtained previously by different groups showing beneficial effects of EPO in rodent studies of EAE (Agnello *et al.*, 2002; Li *et al.*, 2004; Sattler *et al.*, 2004; Diem *et al.*, 2005; Zhang *et al.*, 2005; Savino *et al.*, 2006). In these animal models, various other agents have also been found to improve outcome (Gold *et al.*, 2006). From a translational point of view, however, EPO is the first compound that apparently leads to an improvement in EDSS and a tendency of improvement in central motor conduction time in a cohort of chronic progressive MS patients.

Of note is the fact that *all* MS patients, high-dose as well as low-dose EPO received the same three-day high-dose corticosteroid infusion to create an immunologically comparable starting point of long-term EPO therapy (Milligan *et al.*, 1987). Although no persisting beneficial effects of steroid treatment *per se* would be expected (but rather a potential rebound effect, i.e. a relapse of the disease after discontinuation of therapy—Agnello *et al.*, 2002), the lasting clinical improvement exclusively in the high-dose EPO MS group makes a steroid effect solely explaining this improvement very unlikely. Interestingly, however, we could demonstrate in a murine EAE model that a combination of EPO and corticosteroids is superior to each treatment on its own (Diem *et al.*, 2005). Along the same lines, Agnello and coworkers (2002) found EPO to exert its effect by a mechanism different from steroids, underlining the potential synergism of the two drugs. Conversely, in a spinal cord injury model, the

in one patient during baseline measurement). Low-dose EPO MS patients and Parkinson patients were only followed until week 12. Filled circles: high-dose EPO MS patients ($N = 5$); open circles: low-dose EPO MS patients ($N = 3$); grey triangles: high-dose EPO Parkinson patients ($N = 2$). Significance values refer to the high-dose EPO MS patients only and denote changes of the respective parameter from baseline to the end of each treatment period, including all testing time points. Statistical analysis: Friedman test.

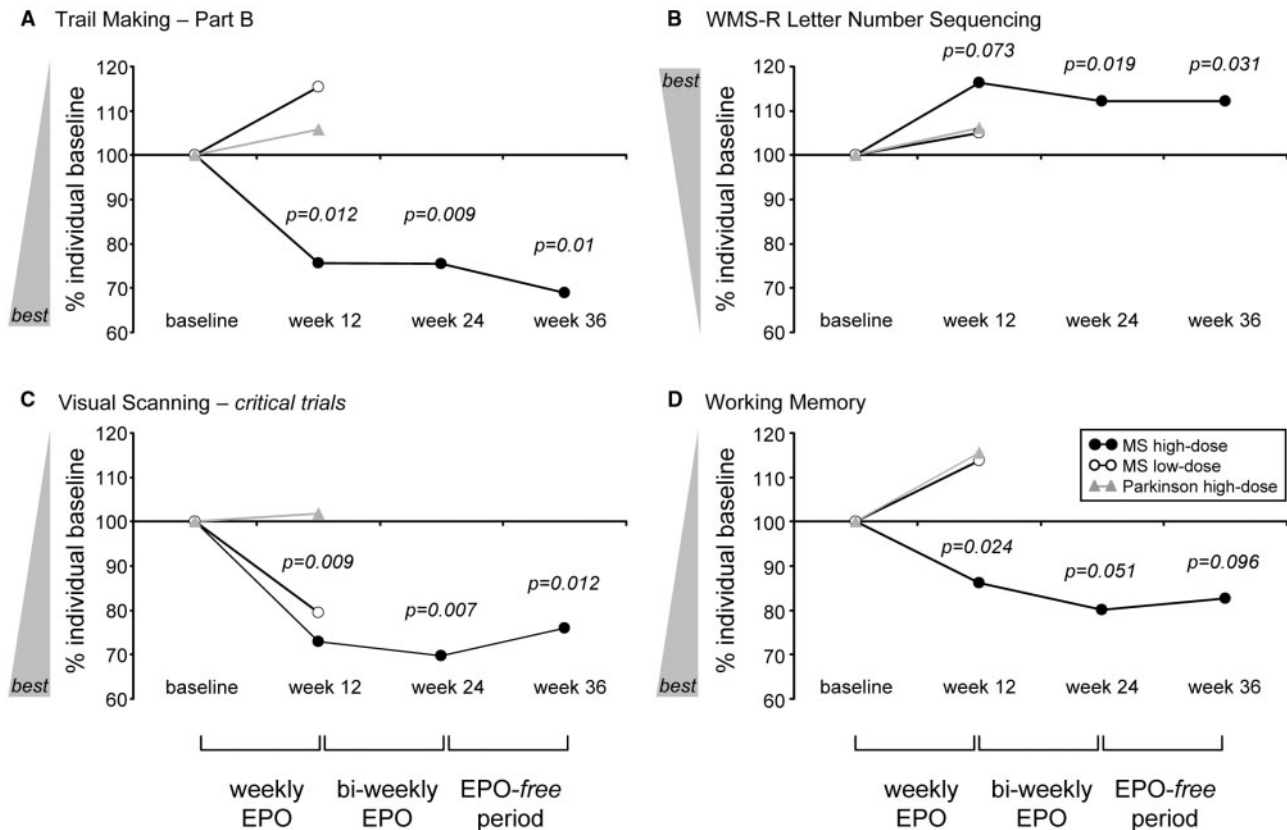


Fig. 5 Changes in cognitive function tests upon EPO treatment. **(A)** Decrease of reaction time in Trail Making – Part B. **(B)** Improvement of performance on WMS-R Letter Number Sequencing. **(C and D)** Reduction of reaction time in TAP subtests Visual Scanning – critical trials and Working Memory in the high-dose EPO MS group ($N = 5$). Low-dose EPO MS and Parkinson patients fail to show improvement. The mean of two baseline values of each patient was set to 100% for each of the cognitive parameters and used for calculating individual change over time. Mean change of all patients within each group during follow-up upon treatment or during the treatment-free period is expressed in % baseline. Low-dose EPO MS patients and Parkinson patients were only followed until week 12. Filled circles: high-dose EPO MS patients ($N = 5$); open circles: low-dose EPO MS patients ($N = 3$); grey triangles: high-dose EPO Parkinson patients ($N = 2$). Significance values refer to the high-dose EPO MS patients only and denote changes of the respective parameter from baseline to the end of each treatment period, including all testing time points. Statistical analysis: Friedman test.

neuroreparative effects of EPO were suspected to be neutralized by glucocorticoids (Gorio *et al.*, 2005). In this regard, however, the obviously different pathologies underlying EAE versus injury, have to be considered.

Regarding the mechanism of action of EPO on motor and cognitive performance in chronic progressive MS, the observed gradual improvement, first visible after a latency of several weeks, and, in particular, its stability over several months of EPO reduction and EPO treatment-free interval, may suggest a morphological rather than a purely functional and transient effect. This assumption may be further supported by the observed improvement of the central motor conduction time. This improvement is particularly striking and difficult to reconcile with the known spectrum of EPO functions. Remyelination by EPO has not yet been demonstrated, and effects of EPO on stimulating oligodendrocytes to remyelinate axons are presently under investigation in our laboratory. Interestingly, axon protecting properties of EPO were already shown in the peripheral nervous system (Keswani *et al.*, 2004). Using conventional

MRI, including volumetrical analysis of the brain, however, we could not yet find any clear-cut changes over time, which is not too surprising in chronic progressive MS and such a small group and short observation time.

Whereas the obvious failure of low-dose EPO to lead to an appreciable improvement in MS patients might be explained by an insufficient concentration of EPO achieved in the central nervous system, it is unclear why the Parkinson patients did not have any measurable benefit. EPO penetrates through an intact blood-brain-barrier, at least upon high-dose peripheral application (Brines *et al.*, 2000; Banks *et al.*, 2004; Ehrenreich *et al.*, 2004b; Xenocostas *et al.*, 2005), should therefore have reached the brain also in the two Parkinson patients in amounts sufficient to exert neurotrophic effects. In this disease, however, symptoms become usually overt when 70% of neurons in the substantia nigra are degenerated (Koller, 1992). Neurotrophic effects on the remaining neuronal population may not lead to rapid clinical improvement or require longer time periods of treatment and follow-up.

The infrequent requirement of blood-lettings in MS patients, consistent with a relative hyporesponsiveness of the haematopoietic system to EPO (Kwack and Balakrishnan, 2006), might be due to a systemic latent inflammatory condition altering cytokine patterns that modulate the bone marrow response to EPO. This proposed latent inflammatory condition cannot be easily diagnosed with routine laboratory parameters of inflammation. Here, the response of the haematopoietic system to EPO might even serve in future studies of MS as an indicator of occult inflammation, and thus requirement of additional immunosuppression. Moreover, the hyporesponsiveness of the haematopoietic system, in contrast to the nervous system, to EPO in MS supports the notion that the observed therapeutic efficacy in MS is not simply due to improved oxygen supply via increased red blood cell mass.

EPO treatment leads to temporary shifts in iron stores as delineated here by several determinants of iron metabolism. 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 corrected after termination of EPO treatment without extra 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 the beneficial effect of high-dose long-term EPO treatment. In fact, iron chelators have been proposed for treatment of MS (e.g. desferrioxamine; Lynch *et al.*, 2000). Interestingly, disturbed iron metabolism has been described in MS patients (Sfagos *et al.*, 2005) and iron-deficient mice fail to develop autoimmune encephalomyelitis (Grant *et al.*, 2003; Weilbach *et al.*, 2004).

Being aware that our explorative study included only a small number of patients and no placebo group, we believe that the encouraging results with high-dose EPO, contrasted by the lack of efficacy of low-dose EPO in MS patients (with maintained blinding of patients to the EPO dosage) and the non-responsiveness of high-dose EPO treatment in Parkinson patients, deliver some pivotal information for designing a phase II trial. Importantly, long-term high-dose EPO treatment in MS was found to be safe and well tolerated. Based on our results we propose a double-blind, placebo-controlled proof-of-concept trial on high-dose (48 000 IU weekly) EPO treatment in chronic progressive MS. As a primary outcome measure, the EDSS should be applied, and supplemented by motor and cognitive parameters as well as neurophysiology and MR imaging as secondary outcome measures. The total duration of this study should extend to three years with an individual duration of two years (comprising at least two EPO treatment cycles). We hope that the present work will soon lead to an urgently warranted phase II trial.

Supplementary material

Supplementary material is available at *Brain* online.

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