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Clinical Trial

Erythropoietin Therapy for Acute Stroke Is Both Safe and Beneficial

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Abstract

Background: Erythropoietin (EPO) and its receptor play a major role in embryonic brain, are weakly expressed in normal postnatal/adult brain and up-regulated upon metabolic stress. EPO protects neurons from hypoxic/ischemic injury. The objective of this trial is to study the safety and efficacy of recombinant human EPO (rhEPO) for treatment of ischemic stroke in man.
Materials and Methods: The trial consisted of a safety part and an efficacy part. In the safety study, 13 patients received rhEPO intravenously (3.3×10^4 IU/50 ml/30 min) once daily for the first 3 days after stroke. In the double-blind randomized proof-of-concept trial, 40 patients received either rhEPO or saline. Inclusion criteria were age <80 years, ischemic stroke within the middle cerebral artery territory confirmed by diffusion-weighted MRI, symptom onset <8 hr before drug administration, and deficits on stroke scales. The study endpoints were functional outcome at day 30 (Barthel Index, modified Rankin scale), NIH and Scandinavian stroke scales, evolution of infarct size (sequential MRI evaluation using diffusion-weighted [DWI] and fluid-attenuated inversion recovery sequences [FLAIR]) and the damage marker S100ß.
Results: No safety concerns were identified. Cerebrospinal fluid EPO increased to 60–100 times that of nontreated patients, proving that intravenously administered rhEPO reaches the brain. In the efficacy trial, patients received rhEPO within 5 hr of onset of symptoms (median, range 2:40–7:55). Admission neurologic scores and serum S100β concentrations were strong predictors of outcome. Analysis of covariance controlled for these two variables indicated that rhEPO treatment was associated with an improvement in follow-up and outcome scales. A strong trend for reduction in infarct size in rhEPO patients as compared to controls was observed by MRI.
Conclusion: Intravenous high-dose rhEPO is well tolerated in acute ischemic stroke and associated with an improvement in clinical outcome at 1 month. A larger scale clinical trial is warranted.

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Introduction

Stroke remains a leading cause of death and disability throughout the world. Currently, thrombolysis using recombinant tissue plasminogen activator (rTPA) is the only pharmacologic treatment with

acceptable side effects shown to effectively limit neurologic damage in acute stroke (1). Use of rTPA is mainly restricted to those patients who present less than 3 hr after the onset of a nonhemorrhagic stroke, leaving >90% of stroke patients without specific treatment. Any successful effort to develop a widely applicable treatment of stroke, complementary and/or alternative to rTPA, would therefore be of major clinical importance.

Although, apart from clot-dissolving strategies, a variety of agents targeting different aspects of stroke pathology appear effective in animal models, successful translation of any to the treatment of human stroke has proven elusive. Recently, attention has focused on potential therapeutic roles for endogenous brain proteins possessing neuroprotective properties. Erythropoietin (EPO), a member of the growth hormone/prolactin cytokine family (2), is one promising candidate. Although EPO was first characterized by and is now widely known for its role as a hematopoietic hormone (3), the detection of EPO and its receptor (EPOR) in rodent and human brain tissue (4–7) as well as in cultured neurons (8,9) and astrocytes (6,9,10) expanded the search for other biological roles of EPO. EPO gene expression in most tissues, including the brain, is oxygen-dependent and regulated by hypoxia-inducible factor-1 (3). We have recently observed dramatic changes in the expression of EPO and its receptor localized to regions within and around infarcts in human brain following ischemic/hypoxic injury (11). Similar observations have been obtained in animal models (12). EPO is also neurotrophic in model systems and marked changes in EPO gene expression are a prominent feature of normal brain development (5).

Several research groups have reported that EPO added to neuronal cultures protects against hypoxic and glutamic acid toxicity (7,8,12) and when directly administered into the brain reduces neurologic dysfunction in rodent models of stroke (4,12–15). Furthermore, neutralization of endogenous brain EPO potentiates ischemic brain injury (14), confirming a pivotal role for the endogenous EPO system in neuronal survival after ischemia. Finally, inflammatory cells (16,17) have been shown to express EPOR; thus, EPO may play important roles in modulating the inflammatory response to injury.

We recently showed that EPO does not require intrathecal administration to provide effective neuroprotection; systemically delivered recombinant human (rh) EPO reduces infarct volume by about 75% in a rodent model of middle cerebral artery (MCA) stroke (4). The biological basis of this neuroprotection includes the inhibition of neuronal apoptosis within potentially viable peri-infarct region (penumbra) (15). In this rodent model, rhEPO is neuroprotective when administered 24 hr before arterial occlusion as well as up to 3 hr afterwards. Partial efficacy is seen as late as 6 hr following the induction of ischemia.

Over the past decade, rhEPO has been administered to millions of patients, many with associated serious medical conditions, as a highly efficacious and safe treatment for anemia. Considering these preclinical and clinical data, rhEPO is an attractive candidate to evaluate as a therapy for human stroke. The aims of the present study were to determine whether the use of rhEPO in acute stroke is safe and whether administration of rhEPO in human stroke is beneficial over a 30-day follow-up period. We find that rhEPO administered intravenously at high doses to patients with documented acute stroke within the MCA territory is well tolerated and safe. Further, in a double-blind study, rhEPO administered intravenously within 8 hr of the onset of ischemic symptoms significantly hastens improvement of neurologic function and ameliorates stroke-related disability assessed at 30 days.

Patients and Methods

The study protocol was approved by the Ethical Committee of the Georg-August-University of Goettingen, Germany. Since December 1998, a total of 53 patients have been included in this first study evaluating the use of rhEPO in treating human stroke. The trial consisted of two consecutive parts. After inclusion of 13 patients in the safety study with open-label rhEPO administration (erythropoietin-ß; Roche, Grenzach-Wyhlen, Germany), the study was switched to a double-blind placebo-controlled proof-of-concept trial. Study endpoints were sequential neurologic scoring using the NIH and Scandinavian stroke scales (SSS), clinical outcome ratings at day 30 as assessed by Barthel index and modified Rankin scale, lesion size at days 1, 3, and 18 as determined by MRI and the serum marker of brain injury, S100β (18,19). Additional clinical variables collected on days 1, 3, 7, 18, and 30 included serum EPO, hematocrit, hemoglobin, leukocyte and thrombocyte counts, partial prothrombin time (PTT), C-reactive protein (CRP), ferritin, transferrin, iron, electrolytes, glucose, blood urea nitrogen (BUN), and creatinine.

Inclusion Criteria

To facilitate determination of the imaging endpoints (a comparative measurement of affected brain area by MRI) only patients with an ischemic stroke in the MCA were accepted. Inclusion further required a definitive temporal onset of symptoms, allowing for rhEPO infusion within 8 hr, as well as neurologic deficits using the stroke scales. A cranial computerized tomography (CT) scan was performed to first exclude any kind of hemorrhage. An acute stroke was confirmed using diffusion-weighted MRI (DWI), whereas fluid-attenuated inversion

recovery (FLAIR) sequences should essentially be free of fresh infarct signs and rule out a recent infarct in the same territory. Enrollment was limited to patients less than 80 years old, and informed consent by patient, entitled relatives, or an independent physician was required.

Exclusion Criteria

Exclusion criteria were defined in part to facilitate evaluation of the study by homogenizing the sample population, as well as in consideration of the literature on rhEPO application in other indications. They included any contraindication to MRI, unclear onset of clinical signs, quickly resolving neurologic symptoms, coma or pre-coma, previous infarction within the same arterial territory, brain trauma or surgery within the last 4 weeks, subarachnoid or intracerebral hemorrhage, intracranial neoplasia, septic embolism, endocarditis, malignant hypertension, florid malignancy, myeloproliferative disorder, polycythemia, allergy against rhEPO, serum creatinine >3 mg/dl, hyperkalemia, follow-up for 30 days not guaranteed, or recent participation in other therapeutic trials.

Safety Study

The safety study goals were to provide data to assess whether intravenously administered rhEPO was safe in the setting of an acute stroke and, further, whether rhEPO crosses the blood–brain barrier into the brain. Characteristics of the 13 patients enrolled in the safety study are summarized in Table 1. Cerebrospinal fluid (CSF) was obtained from four safety study patients with stroke in the MCA territory to whom rhEPO was administered as an intravenous infusion of 3.3×10^4 IU/50 ml/30 min on 3 consecutive days after stroke (total dose 100,000 IU), starting within 8 hr of initial stroke symptoms. The chosen dosage had been among the highest doses tested in clinical trials at the time the study was designed and proven to be well tolerated (20). CSF sampling was performed on day 2, at 6.4 ± 5.2 (mean ± SEM) hr after the second infusion. For comparison, CSF was also obtained from two patients (a 68-year-old man, and a 74-year-old woman) of the intensive care unit who had an acute stroke in the MCA territory (36 and 48 hr ago), happened to have a lumbar puncture for diagnostic reasons but were not in the EPO study and had not received rhEPO, and from 6 neurologic disease controls (essentially healthy patients undergoing a diagnostic evaluation for migraine or lower back pain and who also did not receive rhEPO).

Double-Blind Proof-of-Concept Study

Forty patients were enrolled in the double-blind proof-of-concept study. For blinding, a pharmacist

Table 1. Baseline characteristics of the patients in the two parts of the study

	Part I: Safety rhEPO (n = 13)	Part II: Double-Blind		
		rhEPO (n = 21)	Placebo (n = 19)	f
Age (years; median, range)	68(21–78)	68(39–80)	63(49–79)	n.s.
Sex (male/female)	8/5	15/6	13/6	n.s.
Hemisphere (left/right)	5/8	11/10	10/9	n.s.
Stroke subtype				
Cardioembolic	6	9	10	n.s.
Small vessel occlusive	4	3	3	n.s.
Large vessel occlusive	1	5	4	n.s.
Other	2	4	2	n.s.
ASA pretreatment	7	5	9	n.s.
Stroke scales				
SSS (median, range)	31(9–46)	30(8–52)	30(6–54)	n.s.
NIHSS (median, range)	12(4–27)	11(3–26)	11(1–28)	n.s.
Imaging				
DWI (cm^3; mean ± SEM)	—	40.1 ± 11.8	35.4 ± 8.0	n.s.
FLAIR (cm^3; mean ± SEM)	—	14.1 ± 8.5	3.8 ± 1.3	n.s.
Mean arterial blood pressure (mm Hg; median, range)	122(109–145)	127(91–150)	130(100–177)	n.s.
CRP (U/I; mean ± SEM)	4.3 ± 0.8	13.3 ± 6.6	9.0 ± 4.6	n.s.
Glucose (mg%; mean ± SEM)	146.7 ± 19.9	123.5 ± 8.7	137.2 ± 14.0	n.s.
Time to treatment (h:min) (median, range)	6:20(3:30–8:00)	5:00(2:40–7:55)	4:45(3:20–7:45)	n.s.

prepared and numbered identical vials containing either saline (0.9% NaCl) or EPO reconstituted in saline. The vials were randomly assigned to patients upon enrollment with the contents of each vial known only by the pharmacist. None of the clinicians performing neurologic or imaging analyses had access to any of the serum laboratory data obtained (platelet or reticulocyte counts) during the study. Serum EPO levels were analyzed only after unblinding the study. Study groups turned out to be highly comparable with respect to baseline characteristics, with no significant differences in age, sex, hemisphere involved, NIHSS, SSS scores or DWI lesion size upon enrollment (Table 1). Time to treatment was slightly better as compared to the safety study, due to improvements in enrollment management, but did not differ significantly between treatment groups, nor did pretreatment of patients with aspirin or presumed stroke etiology differ. In addition, pre-existing risk factors as determined by medical history were not different in the two groups. Also, blood pressure upon admission, glucose, and C-reactive protein levels in serum were comparable.

Study Design

A history and physical examination were obtained for every stroke patient screened for participation in the study, followed by a routine laboratory evaluation and cranial CT scan before being referred to MRI. Following initial MRI, the final decision upon inclusion was made based on the criteria delineated. Intravenous infusion of rhEPO (3.3×10^4 IU/50 ml/30 min) was started immediately thereafter, in all cases within the first 8 hr after onset of symptoms (day 1). EPO was administered again 24 and 48 hr later to provide a cumulative dose of 100,000 IU of rhEPO for each stroke patient. For the control arm of the study, saline (0.9% NaCl) was used as placebo. MRI data were also obtained on days 3 and 18. Neurologic scoring and laboratory tests were performed on days 1 (before treatment), 3, 7, 18, and 30 following stroke onset. For determination of EPO in serum, an additional blood sample was taken 3 hr after each rhEPO infusion. Blood pressure was measured on days 1, 2, and 3 on the left arm with the patient maintained in a 30° head up tilt immediately before and 5, 15, 30, 60, 120, and 180 minutes after the start of the rhEPO infusion.

S100ß Assay

Serum concentrations of S100ß, a glial marker of brain damage (18), were determined using the LIAISON-Sangtec-S100 assay (Byk-Sangtec Diagnostica, Diezenbach, Germany). The sensitivity of the assay is 0.02 μg/l. Normal serum concentrations of S100ß are <0.12 μg/l.

Erythropoietin ELISA

Concentrations of EPO were measured in serum (efficacy study) and in paired samples of CSF and serum (safety study) using a commercially available ELISA kit (R&D Systems, Wiesbaden, Germany) according to the manufacturer's protocol. Serum samples of the double-blind study were not analyzed before unblinding of the study. CSF from control subjects was concentrated by drying 2 ml of CSF overnight under nitrogen, and the sample rediluted with the sample diluent (included in the kit) to the final volume of 120 μl. This procedure improved the sensitivity of the kit allowing measurement of EPO in normal CSF (lower limit of detection <0.2 mU/ml). For determination of fractional recovery, paired samples ($n = 3$) of CSF and CSF spiked with 20 μl of the 50 mU/ml rhEPO standard (included in the kit) were dried, rediluted, and measured in parallel with a recovery of 95%, 112%, and 102%.

MRI Evaluation of Lesion Size

MRI data were obtained on days 1, 3, and 18. During each session, DWI and FLAIR images were produced, consisting of 16 and 20 comparable horizontal sections, respectively. To determine the lesion size and its evolution over time, a standard volumetric procedure was performed using the EasyVision software (CT/MR Release 2.1 Level 5, Philips Medical Systems, Best, The Netherlands) on a Sun workstation.

Data Analysis

Primary endpoints evaluated were neurologic scores (NIH and SSS) and functional outcome at day 30 (Barthel's and modified Rankin). Secondary endpoints were time-dependent changes in the neurologic scores and serum marker of injury (S100β) over 30 days, as well as infarct size at days 3 and 18 as assessed by DWI and FLAIR. Several considerations guided the approach to the data analysis. First, the wide range of severity of stroke at enrollment (NIHSS range of 1–28) contributed a major share of the data variance because the neurologic score is the major determinant of both outcome and lesion size, as has been reported by many studies (21,22). Second, early evaluation of infarct size can be accurately obtained by use of MRI and serum markers which increase by day 3 (22,23). Of these, S100β, a product of activated astroglia and oligodendrocytes, is a marker released in proportion to the size of the infarct, with a clear biological relevance to inflammation and injury (18,24). Further, S100β has been shown to predict neurobehavioral outcome after stroke (19). Multiple regression analysis was performed with the outcome measures serving as dependent variables, and treatment as the independent variable, with admission stroke score and S100β concentration on day 3 as covariates in the analysis. Inclusion of other covariates such as age or time to treatment did not change the results of the analysis. Ordinal data (Barthel and modified Rankin) were analyzed using logistical methods. Furthermore, Mann-Whitney U-test, Fisher exact probability test, or independent Student's *t*-test were used where

indicated. A percentile distribution analysis, originally devised for the quantitative evaluation of cerebral infarct volume (25), was applied both to evolution of the lesion size (DWI) and to the NIHSS. Briefly, the scores within each treatment group were ranked in an ascending order and the ranks were converted into percentiles. Individual scores were displayed as a function of the percentile rank within their group, whereas scores of each group were matched according to their corresponding percentile rank. This allows a quantitative display of individual data points, revealing the spectrum of change between groups. Three patients died during the course of the study. None of the deaths was related to treatment. They occurred much later in the course of post-stroke complications. In the clinical analysis (NIHSS, SSS, Barthel, and Rankin), dead patients received the worst possible score, which actually tends to worsen the results of the study (Figs. 2 and 3). In the percentile distribution analysis (Figs. 1 and 5) and the imaging analysis (Fig. 4), patients were censored at death since individual data points are presented or required.

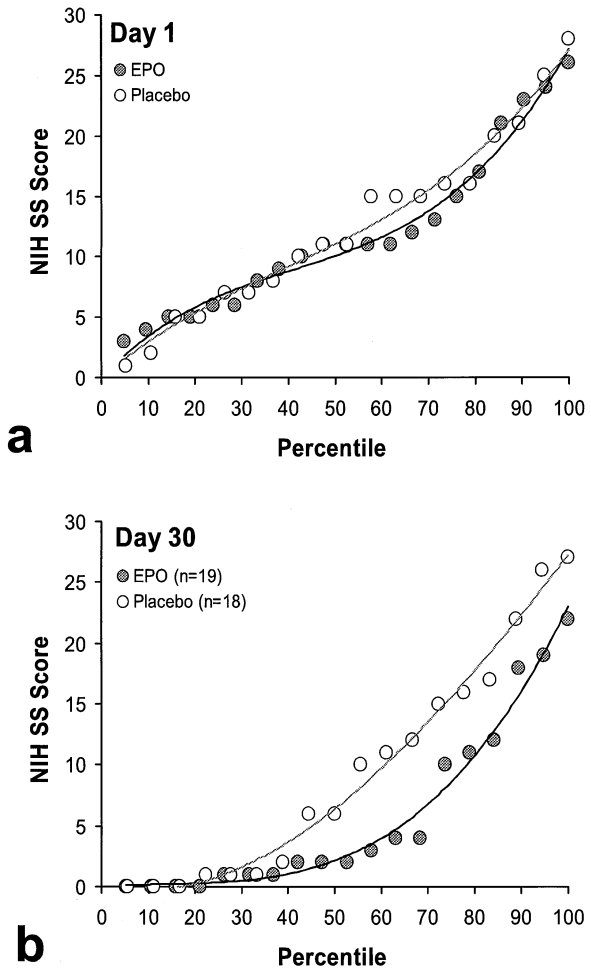

Fig. 1. Percentile distribution analysis of NIH stroke scale for placebo and rhEPO-treated patients (A) upon admission (baseline); (B) at day 30 after treatment. Note the remarkable dissociation of curves over time for moderately to severely affected patients.

Results

Of the 320 stroke patients screened for participation in the trial, less than 20% ($n = 53$) fulfilled the enrollment criteria. The most common reasons for exclusion were time to treatment longer than 8 hr, infarcts outside the MCA territory, contraindications to MRI, older than 80 years, or quickly resolving neurologic symptoms consistent with a transient ischemic attack. About half of the patients enrolled had a cardioembolic etiology (Table 1).

Safety Study

Baseline characteristics of patients studied are summarized in Table 1. Intravenous infusion of rhEPO (3.3×10^4 IU/50 ml/30 min) was started within the first 8 hr (median 6 hr 20 min; range 3 hr 30 min to 8 hr) after onset of symptoms (day 1) and was readministered 24 hr and 48 hr later (days 2 and 3). Serum levels of rhEPO obtained 3 hr after each rhEPO infusion rose to a mean ± SD of 4132 + 561, 4794 ± 1183, and 5649 ± 903 mU/ml on days 1, 2, and 3, respectively, which was not associated with any changes in blood pressure. The hematocrit, hemoglobin, and red blood cell counts remained stable throughout the 30-day observation period. Simultaneous samples of the CSF of patients receiving rhEPO contained EPO at a concentration more than 60 times greater (17.1 ± 5.6 mU/ml, $n = 4$) than samples obtained from two nontreated stroke patients (0.7 mU/ml and 0.3 mU/ml) or patients with other neurologic conditions (0.3 ± 0.06 mU/ml, $n = 6$) ($p < 0.01$).

Double-Blind Proof-of-Concept Study

Figure 1A illustrates that the study groups were highly comparable in terms of baseline stroke severity. In the efficacy study, three deaths occurred, one within the placebo arm (day 6, malignant brain edema as a consequence of progressive stroke resulting in total infarction of the left MCA territory) and two within the rhEPO arm (day 17 and day 27 from pneumonia, sepsis, multi-organ failure). Time to drug infusion was 5 hr (range 2 hr 40 min to 7 hr 55 min) for rhEPO and 4 hr 45 min (range 3 hr 20 min to 7 hr 45 min) for placebo. The use of intravenous heparin during the course of the study was not different between the two groups (placebo, 11/19; rhEPO, 11/21). Other admission values, including S100β and stroke scores, were not significantly different (Table 1).

Serum EPO concentrations obtained for the first nine rhEPO-treated patients rose to a mean of 5148 + 1094 mU/ml (Table 2) and were not associated with blood pressure changes. Follow-up neurologic scorings obtained on days 3, 7, 18, and 30, using the NIH

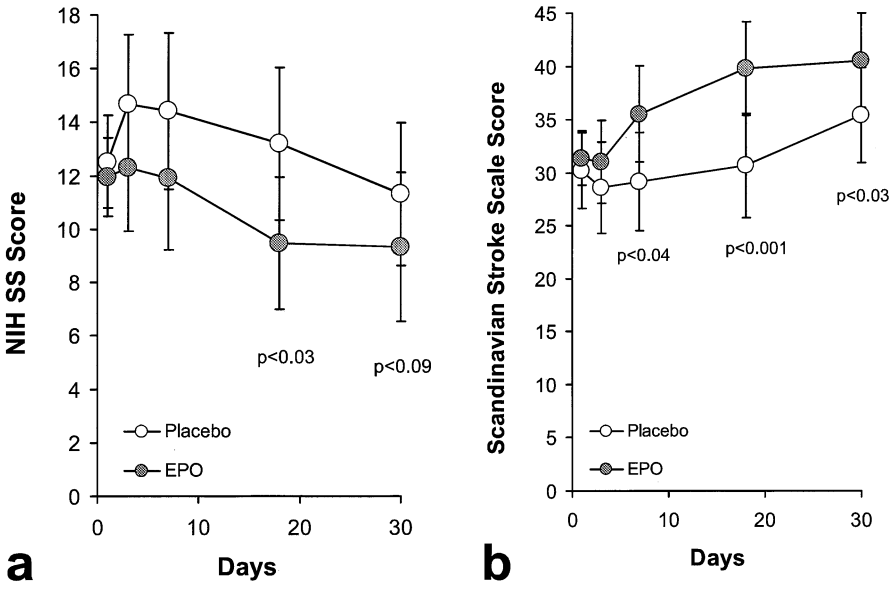

Fig. 2. Functional recovery of patients in the double-blind proof-of-concept trial. (A) NIH stroke scale. **(B)** Scandinavian stroke scale. Data represent mean ± SEM ($n = 19$ for placebo, $n = 21$ for rhEPO). p-Values according to multiple regression analysis. Dead patients received the worst possible score.

and Scandinavian stroke scales, expectedly agreed well. Nevertheless, because of inherent differences in the rating of severely affected comatose patients, the relationship between NIHSS score and SSS score is not linear: In the NIHSS, motor function is scored in all extremities, whereas in the SSS only motor function of the affected side is rated. This explains the slightly different results obtained with the two scales (Fig. 2 A and B). In fact, the rhEPO-treated patients improved earlier with the largest difference between the treatment groups occurring on day 18 (score differences of 9 in the SSS and 4 in the NIHSS; Fig. 2 A and B). The Scandinavian stroke scale data showed an earlier improvement, with a significant difference evident by day 7. The better course of the rhEPO-treated patients continued throughout the remaining study period. A generally similar pattern was obtained using the NIHSS, except that a significant improvement was only observed on day 18 ($p < 0.03$) and was maintained to day 30 ($p < 0.09$). The diminished separation between the treatment groups in both stroke scales at day 30 appears to depend on an improvement in the placebo arm scores (Fig. 2 A and B). Percentile distribution analysis of the NIHSS illustrates a clear separation between treatment groups at day 30 (Fig. 1B).

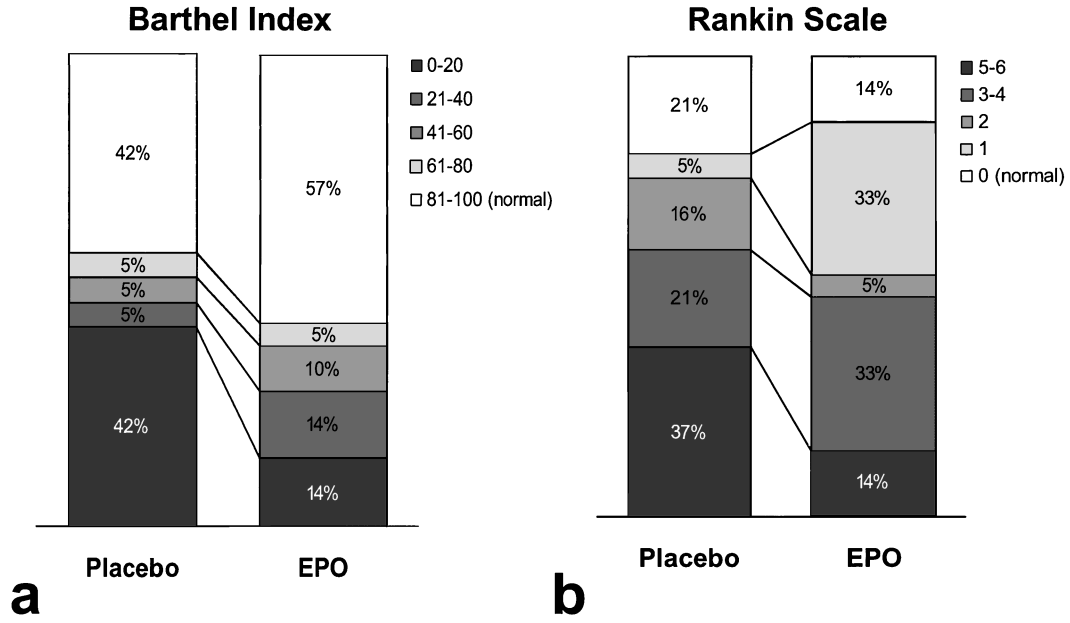

Fig. 3. Clinical outcome of patients in the double-blind proof-of-concept trial. (A) Barthel index (rhEPO versus placebo $p < 0.05$). **(B)** Modified Rankin scale (rhEPO versus placebo $p < 0.07$) on day 30. Dead patients received the worst possible score.

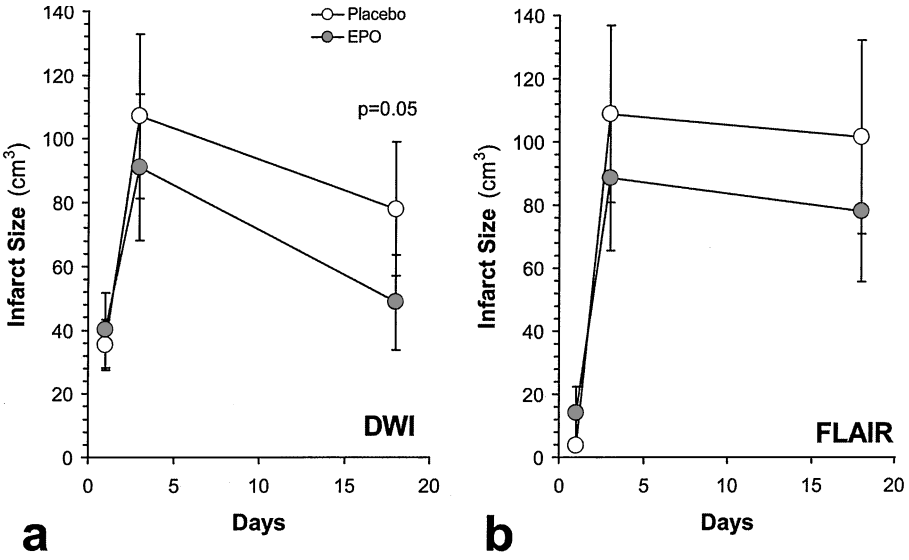

Fig.4 Evolution of lesion size of patients in the efficacy trial. (A) DWI. **(B)** FLAIR. Data represent mean ± SEM. *p*-Value according to multiple regression analysis. Dead patients have been censored.

The subgroup of mildly affected patients is, however, likely to have a good recovery independent of the treatment arm. The clinical outcome evaluation performed using the Barthel index also showed a significantly superior outcome after rhEPO treatment ($p < 0.05$). As shown in Figure 3A, at 1 month 42%, of the placebo patients were severely affected (score <20), compared to 14% of the rhEPO-treated patients. Further, the percentage of patients with a good outcome (score >80) was 57% in the rhEPO group compared to 42% in the placebo group. Similar results were obtained for the modified Rankin scale with 37% of the placebo patients severely affected (score 5–6) compared to 14% of the rhEPO patients (Fig. 3B). The results of the modified Rankin evaluation, however, failed to reach statistical significance ($p < 0.07$).

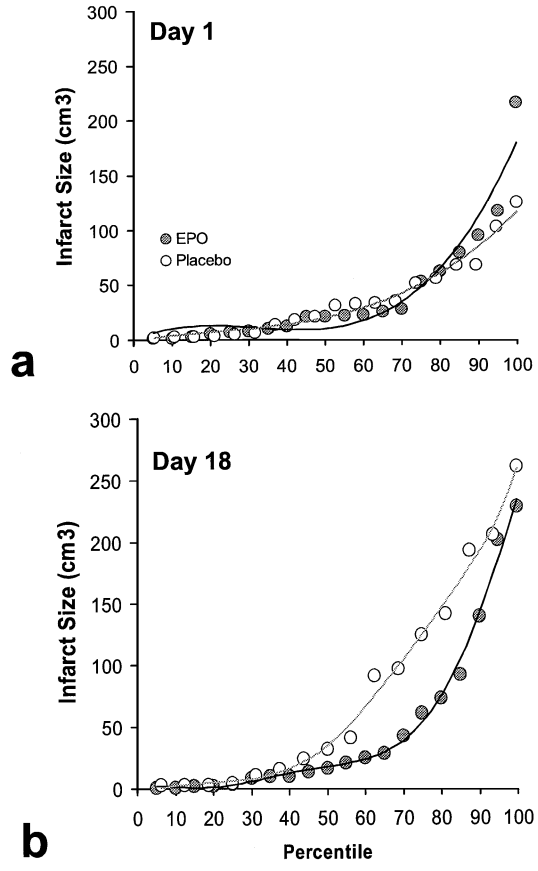

Fig. 5. Percentile distribution analysis of DWI for placebo and rhEPO-treated patients **(A)** upon admission (baseline); **(B)** at day 18 after treatment. Note the remarkable dissociation of curves over time for moderately to severely affected patients.

Serial MRI data are presented in Figure 4. A superior course in rhEPO versus placebo patients was evident in the DWI on day 18 ($p = 0.05$). Inspection of the percentile distribution analysis of the DWI data (Fig. 5) shows a distinct dissociation between groups in respect to evolution of lesion volume. The serum marker of infarct damage, S100β, was identical in both groups on admission to the trial and increased after time of infarct, peaking by day 7. The rhEPO group peaked at a lower level and earlier than the placebo group and thereafter returned to within the normal range sooner (Fig. 6). At day 30, serum S100β of the rhEPO-treated group had normalized, whereas those of the placebo treated group remained elevated ($p < 0.05$).

Selected follow-up laboratory parameters are presented in Table 2. The hematocrit and red blood cell counts did not increase to levels exceeding the normal range in rhEPO patients. There was only a tendency toward higher levels of these parameters in the rhEPO-treated group as compared to controls. In contrast, the hematocrit of placebo-treated patients fell slightly. As expected, the reticulocyte count increased to 29.2 ± 10.0% in rhEPO-treated patients, peaking at day 7 and normalizing thereafter (data not shown). Thrombocyte counts showed comparable increases by day 18 in both treatment groups.

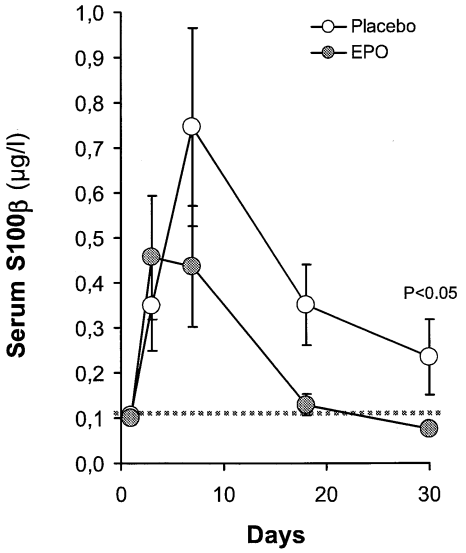

Fig. 6. Time course of serum S100β levels in placebo and rhEPO-treated patients over the 30-day follow-up period. Data represent mean ± SEM. Mann-Whitney U-test. The dashed line denotes normal serum concentration.

Discussion

Although a number of therapeutic trials for stroke have been undertaken in the past decade, no broadly applicable, safe, and efficacious treatment has been identified (26–28). The present data support a novel approach to neuroprotection of cerebral ischemic injury by use of rhEPO, clinically one of the best-characterized and well-tolerated therapeutics. The clear-cut results of this trial supporting a significantly better recovery of rhEPO-treated versus placebo-treated patients with respect to the clinical endpoints of stroke and outcome scales, coupled with a faster normalization in the circulating serum marker of injury, S100β, and a more favorable outcome in MRI data, compelled us to report these results despite the relatively small number of patients studied thus far.

It is important to note that only one patient recruited into this study could be administered rhEPO within the critical 3 hr time window found for effective rTPA therapy (1), which is, apart from aspirin, the only currently approved protective treatment for acute stroke. The 3-hr time window is likely of critical relevance for rhEPO as well, because programmed cell death, one target of rhEPO therapy (15), is initiated within 3 hr after infarction (29). We have evaluated this issue in detail using a rodent model of MCA stroke, for which administration of a single dose of rhEPO 3 hr after occlusion is as effective as when given at occlusion, but is only 50% as active if delayed to 6 hr. By 9 hr after occlusion, rhEPO is without neuroprotective benefit in rats (4). In retrospect, not much can be said about a potential influence of a late inclusion time point in the present study. In the double-blind (efficacy) part, the time to treatment was much shorter than the designated 8-hr time window in most patients. In fact, only two patients in each group were included after 6 hr of onset of symptoms. Although time course and sequence of pathophysiologic events contributing to the evolution of ischemic lesion may be somewhat different in man and rat, it is a distinct possibility that patients administered rhEPO within 3 hr of stroke onset would experience an even better outcome than observed in the present study.

Although a variety of in vivo and in vitro studies show that EPO is also protective of excessive (neurotoxic) excitation (4,14), the protection observed here appears to mainly target later events (e.g., apoptosis or inflammation subsequent to brain injury) (4). In fact, rhEPO will likely provide significant protection only within the penumbra and not within the ischemic core. Pathologic/regenerative processes of longer latency than examined in this study may also be beneficially affected (15,30). Whether the more rapid improvement noted in the rhEPO treatment group will be maintained over longer periods of follow-up will need testing by conducting additional clinical trials. Prerequisite for the use of peripherally applied rhEPO in stroke was to demonstrate the availability of this large (34,000-dalton) molecule in the brain after intravenous infusion. The results of the safety study confirmed that intravenous administration of a total of 100,000 IU of rhEPO after a documented stroke led to a 60- to 100-fold increase in CSF EPO levels. Interestingly, our rat experiments suggest that movement of EPO into the CSF does not even require a breakdown of the blood–brain barrier: Peak concentrations of EPO in CSF from the cisterna magna after intraperitoneal injection amount to 0.5–1% that of serum at around 3.5 hr (unpublished observations).

The use of the neurochemical serum marker, S100β, provides valuable information concerning nervous tissue injury. Although the biological function of S100β is not entirely clear, its release into the circulation is associated with secretion by reactive astrocytes and functional disturbances of membrane integrity (31). The normalization of S100β after 30 days for the rhEPO arm but not the placebo arm may suggest that the inflammatory processes and/or blood–brain barrier integrity improve more rapidly or completely after rhEPO administration.

For statistical analysis of the data, it was necessary to control for the admission stroke score as well as the S100β levels at day 3, as both have been shown to be strong predictors of outcome (19,21). Because the admission stroke scores and day 3 S100β serum levels were similar in both patient groups, a similar outcome would be predicted, in contrast with the findings of this study. The results of the percentile distribution analysis of NIHSS and DWI data (Figs. 1 and 5) do not only demonstrate a clear dissociation of curves upon the respective later time point but also suggest that the predominant effect of

Table 2. Follow-up parameters in part II of the study (double-blind)

	Day 1		Day 3		Day 7		Day 18		Day 30	
	rhEPO	Placebo	rhEPO	Placebo	rhEPO	Placebo	rhEPO	Placebo	rhEPO	Placebo
Hematocrit (%)	43.10 ± 0.90	42.70 ± 1.39	41.70 ± 1.00	40.20 ± 1.27	41.40 ± 1.46	38.00 ± 1.91	41.80 ± 1.23	38.60 ± 1.61	42.00 ± 1.06	40.50 ± 1.25
Red blood cell count ($10^6/\mu l$)	4.80 ± 0.12	4.70 ± 0.16	4.60 ± 0.11	4.40 ± 0.13	4.50 ± 0.16	4.20 ± 0.22	4.60 ± 0.15	4.20 ± 0.19	4.70 ± 0.14	4.40 ± 0.16
Thrombocyte count ($\times 10^3/\mu l$)	226.20 ± 14.12	213.50 ± 11.69	218.00 ± 15.56	218.90 ± 14.14	243.20 ± 19.78	239.40 ± 13.17	344.50 ± 26.61	362.30 ± 39.46	246.40 ± 19.62	282.10 ± 20.76
Serum EPO* (U/l)	10.60 ± 1.58	12.00 ± 1.09	5148.40 ± 1094.64	19.50 ± 2.75	24.40 ± 4.37	31.60 ± 12.59	8.00 ± 1.00	28.50 ± 11.73	14.90 ± 3.56	21.00 ± 5.64

*Determined in the first nine patients of each group only.

EPO is within the moderately to severely affected patients.

Several design factors are likely responsible for the relative success of this small trial. The rigorous enrollment criteria for this study—selection of patients suffering from infarcts of the MCA territory only (for comparability of clinical and imaging data)—led to the inclusion in our center of less than 20% of the stroke patients, at the price of extending the duration of the study. Another advance of the present trial, and unique thus far (despite recent recommendations of MRI for early diagnosis of stroke [32]), are the three MRI sessions, on days 1 (emergency MRI), 3, and 18 (follow-up MRIs). These guarantee not only the exclusion of patients with transitory ischemic attacks (no signs in DWI) but also a more objective estimation of the real time window (DWI positive and FLAIR still essentially negative as inclusion criteria). On the other hand, at least one factor may have decreased the discriminative power of this study: the median severity of stroke upon admission was less than that in the only successful large scale trial to date (NIHSS of 11 versus 14) (1), whereas it was identical to the ECAAS II study (33). The enrollment of mildly affected patients seemingly reduced the magnitude of the beneficial effect in our study (Fig. 1B). It will therefore have to be considered to define a NIHSS cutoff (presumably >5) for inclusion of patients in follow-up studies.

The results of this study are fully consistent with the efficacy of rhEPO observed in animal models of ischemic brain injury. The clear results obtained using a small group of patients with moderately severe strokes suggest that the effects of rhEPO are robust. Thus, a formal clinical efficacy trial will not likely require an excessively large enrollment. The availability of an apparently safe agent that can be given hours after the onset of ischemia will need confirmation by a larger trial with longer follow-up. Confirmation of these findings will then provide the basis for a generally applicable therapy for acute ischemic stroke.

Stroke therapy with rhEPO is not primarily aiming at re-opening of the feeding artery. This is the domain of rTPA and related substances. The neuroprotective approach using EPO is aimed at protecting potentially viable brain tissue from the spreading of death signals. EPO will in the future be of great value to add to the effect of clot-dissolving strategies and may even reduce reperfusion injury. The fact that a beneficial action can already be demonstrated in a pure neuroprotective study should actually be even more encouraging. Undoubtedly, a successful stroke therapy in the future will have to be a combined therapy. The present work prepares a hopeful ground for the neuroprotective part of this strategy. In other words, EPO is not a competitor of but complement to rTPA. In cases with contraindication to lysis (still >90% of strokes), EPO alone will provide an alternative.

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