A Randomized, Double-Blind, Phase 2 Study of Erythropoietin in Optic Neuritis

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Objective: Based on findings in animal models of autoimmune optic nerve inflammation, we have assessed the safety and efficacy of erythropoietin in patients presenting with a first episode of optic neuritis.
Methods: Patients with optic neuritis who attended the University Hospitals of Homburg/Saar, Göttingen, or Hamburg (Germany) were included in this double-blind, placebo-controlled, phase 2 study (ClinicalTrials.gov, NCT00355095). They were randomly assigned to groups receiving either 33,000IU recombinant human erythropoietin intravenously daily for 3 days or placebo as an add-on therapy to methylprednisolone. The primary outcome parameter was change in retinal nerve fiber layer (RNFL) thickness after 16 weeks. Secondary outcome parameters included optic nerve atrophy as assessed by magnetic resonance imaging, and changes in visual acuity, visual field, and visual evoked potentials (VEPs).
Results: Forty patients were assigned to the treatment groups (21/19 erythropoietin/placebo). Safety monitoring revealed no relevant issues. Thirty-seven patients (20/17 erythropoietin/placebo) were analyzed for the primary endpoint according to the intention-to-treat protocol. RNFL thinning was less apparent after erythropoietin treatment. Thickness of the RNFL decreased by a median of 7.5μm by week 16 (mean $\pm$ standard deviation, 10.55 $\pm$ 17.54μm) compared to a median of 16.0μm (22.65 $\pm$ 29.18μm) in the placebo group ($p = 0.0357$). Decrease in retrobulbar diameter of the optic nerve was smaller in the erythropoietin group ($p = 0.0112$). VEP latencies at week 16 were shorter in erythropoietin-treated patients than in the placebo group ($p = 0.0011$). Testing of visual functions revealed trends toward an improved outcome after erythropoietin treatment.
Interpretation: These results give the first indications that erythropoietin might be neuroprotective in optic neuritis.
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Neurodegeneration in multiple sclerosis (MS) is the dominant structural correlate of permanent disability.[1] It is currently not effectively treated because available disease-modifying therapies have only limited neuroprotective properties.[2] Optic neuritis, among the most common first manifestations of MS,[3] offers several advantages to test neuroprotective agents, as it represents a homogenous disease where neurodegeneration occurs rapidly and to a predictable extent. After an episode of optic neuritis, atrophy of the retinal nerve fiber layer (RNFL) can be detected.[4,5] In addition, following an episode of optic neuritis, the optic nerve undergoes significant atrophy.[6] A further advantage of using optic neuritis as a model disease for inflammation-induced

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neurodegeneration lies in the compartmentalization of the affected anatomical structures, which can be imaged separately by optical coherence tomography (OCT) and magnetic resonance imaging (MRI). OCT in particular allows the imaging of the proximal axon fibers of retinal ganglion cells (RGCs), a subpopulation of central nervous system neurons, without the presence of confounding myelin sheaths.[7] Additionally, optic neuritis allows the combination of imaging with functional and electrophysiological measurements.[3]

Methylprednisolone pulse therapy is the standard treatment for acute optic neuritis. Although it accelerates visual recovery, it does not influence visual outcome, lesion length, or atrophy of the optic nerve.[3] In an animal model of optic neuritis, methylprednisolone increased RGC degeneration by inhibition of an endogenous neurotrophin-dependent pathway.[8] Data from animal models of optic neuritis and MS generally indicate that the downstream mechanisms of neurodegeneration involve pathways regulated by neurotrophins and that neurotrophic factors exert a variety of beneficial effects.[9–11] Erythropoietin has shown neurotrophinlike properties in various models of brain injury such as experimental ischemia, trauma, epilepsy, and MS,[12,13] and in contrast to classical neurotrophins can be applied systemically. In an animal model of optic neuritis, erythropoietin was particularly effective when given in combination with methylprednisolone.[14] Based on these findings, we assessed the safety and efficacy of erythropoietin given as an add-on to methylprednisolone in patients presenting with their first episode of autoimmune optic neuritis.

Patients and Methods

Patients

Patients between 18 and 50 years of age with a first episode of optic neuritis and visual acuity decreased to $\leq$0.5 (decimal system) were eligible to be included in this double-blind, placebo-controlled, parallel group study. Please note that a decimal value of 0.5 corresponds to a logarithmic (log) visual acuity of -0.3, a minimum angle of resolution (MAR) of 2.0, a logMAR value of 0.3, or a Snellen equivalent of 6/12 (with meters used instead of feet). Onset of symptoms had to be within 10 days prior to inclusion and had to be accompanied by typical clinical features and visual evoked potential (VEP) findings.[3] Diagnosis was always confirmed by an ophthalmologist. Patients were not eligible if they had a history of optic neuritis or any ocular disease (affected or nonaffected eye), visual acuity of $<$1.0 in the nonaffected eye, significant hyperopia, myopia, or astigmatism. Additional exclusion criteria consisted of pregnancy, lactation period, elevated blood pressure, thrombotic events, malignancy, seizures, or any disease interfering with the use of corticosteroids or the ability to undergo MRI. Treatment with corticosteroids or erythropoietin within 30 days prior to inclusion was not allowed, nor was use of any immunosuppressive or immunomodulatory pretreatments. The study was approved and monitored by the ethics committee of the University of Göttingen (as the central ethics committee) and the German Federal Institute for Drugs and Medical Devices, Bonn, and is registered with ClinicalTrials.gov (NCT00355095) and with EUDRACT (2005-005592-14). All participants gave written informed consent prior to screening.

Randomization

Patients were randomly assigned (1:1) to erythropoietin or placebo via the SAS (Cary, NC) software RANBIN stratified according to center (University Clinic Homburg/Saar, Göttingen, or Hamburg, Germany). Medication was prepared by the pharmacist with a confidential randomization number, which was matched to the patient's number to assign patients either to erythropoietin or to placebo. Treating and evaluating physicians, as well as patients, remained masked to treatment (details on procedures to ensure blinding are given in the Supplementary Material).

Procedures

Thirty-three thousand international units of recombinant human erythropoietin (Epoetin-alpha, ErypoFS; Janssen-Cilag, Berchem, Belgium) were applied as an intravenous (i.v.) injection once daily on 3 consecutive days.[15] Placebo consisted of 0.9% NaCl solution of identical appearance. Study medication was given by the treating neurologist after application of methylprednisolone (1000mg i.v. per day; Urbason solubile forte; Aventis Pharma, Mumbai, India). OCT, MRI, VEP recordings, and assessments of visual acuity, visual field parameters, and neurological status were done at baseline (prior to treatment) and again at weeks 1, 4, 8, and 16, except for the week 1 visit, which did not include OCT or MRI (Supplementary Table).

The primary outcome parameter was reduction of RNFL thickness from baseline to week 16. Secondary outcome parameters consisted of differences in RNFL thickness at week 16 compared to the contralateral eye or compared between the treatment groups. Further secondary outcome parameters consisted of changes in optic nerve diameter from baseline to week 16 as measured by MRI, recovery of visual acuity and visual field, and changes in latencies and amplitudes of VEPs over 16 weeks as well as comparisons of the week 16 results. Safety assessment included monitoring of blood pressure, electrocardiography, adverse events, and analysis of blood parameters.

Method descriptions for OCT, MRI, VEP recordings, perimetry, and ophthalmological examinations are given in the Supplementary Material.

Statistical Analysis

A sample size of 40 patients was chosen to provide an initial assessment of efficacy. The sample size estimation was based on data on RNFL thickness, which ranges around 100μm (standard deviation [SD], $\sim$15μm) in healthy subjects and decreases by 15 to 25% within 6 months after optic neuritis,[4,16] and on

findings in an animal model of optic neuritis demonstrating a reduction of RGC death by 50% after erythropoietin treatment.[14] Efficacy was analyzed using an adaptive design[17] with $\alpha = 0.0116$ (Bonferroni adjusted). The decision to continue or stop patient recruitment after inclusion of 40 patients was made according to the results of this primary analysis. Group medians and corresponding 0.9884 confidence intervals (CIs) were estimated using SAS 9.2, including the procedures PROC-UNIVARIATE and CIPCTLDF. Patients who underwent baseline and week 16 assessments were analyzed per protocol. The intention-to-treat (ITT) analysis included all patients who had baseline assessment and at least 1 follow-up. In a total of 5 patients (3 erythropoietin, 2 placebo), missing values were replaced by the last observation carried forward (LOCF) method. Patients were stratified according to center and sex. All primary and secondary outcome parameters were further analyzed by exploratory nonparametric factorial analysis using the SAS macro F1_LD_F1[18] and the Wilcoxon–Mann–Whitney (WMW) test. Additional exploratory analyses were performed using Student *t* test with assessment of 95% CIs. Furthermore, we performed an analysis of covariance (ANCOVA) to test whether baseline differences may have any effects on the results of the primary outcome parameter. For each parameter, the difference between baseline and week 16 was assessed and compared between the groups. Additionally, we compared absolute values at week 16, and differences between the affected and nonaffected eye as well as Pearson coefficients to determine any correlation between the various outcome parameters. Two patients who developed contralateral optic neuritis during the follow-up period were excluded from intraindividual comparison.

Results

Patient Disposition and Clinical Course

Forty participants were randomly assigned to receive erythropoietin (n = 21) or placebo (n = 19) in addition to methylprednisolone pulse therapy after screening of 97 patients with first episode of optic neuritis (Fig 1). Table 1 gives an overview of the most frequent reasons for noninclusion, indicating that recruitment of patients with sufficiently decreased visual acuity was the most challenging inclusion criteria. The baseline characteristics of both groups did not differ significantly (Table 2); however, a *p* value of 0.0756 (*t* test) was found upon comparing VEP latencies, indicating a strong trend toward a baseline imbalance. Three of the patients reported a prior episode of neurological symptoms (other than visual impairment), suggestive of MS. A further 5 patients had a second relapse with new neurological findings during the 16-week follow-up period and were diagnosed with MS, 3 of whom had received erythropoietin and 2 placebo. In 2 of those 5 patients (both erythropoietin), the relapse consisted of contralateral optic neuritis. An additional 5 patients (all receiving placebo) did not experience a second clinical episode but fulfilled the McDonald criteria for the diagnosis of MS[19] on the basis of MRI changes.

Retinal Nerve Fiber Layer Changes

Two patients (1 per group) were lost to follow-up, and an additional 4 (2 per group) were lost to final OCT follow-up (although secondary outcome parameters were assessed), leaving 34 who had OCT at week 16 (18 erythropoietin, 16 placebo). After LOCF substitution of values, which was performed in a total of 5 patients, the ITT analysis of the primary outcome parameter included 20 patients in the erythropoietin group and 17 in the placebo group (see Fig 1). Four of those patients whose data were substituted by LOCF (2 erythropoietin, 2 placebo) had complete data except from the final visit. However, in 1 patient, missing values were replaced for the last 2 visits. To disclose any distorting effect of applying LOCF in this patient, we performed additional analyses of all outcome parameters excluding this patient. These analyses revealed nearly identical results (data not shown) when compared to analyses of the total ITT patient population. In the total ITT population, change in RNFL thickness of the affected eye over 16 weeks was smaller than in the placebo group (Fig 2, Table 3). The primary comparison of the medians revealed a *p* value of <0.5, and therefore, according to the adaptive design of the study, would have allowed continuation of patient recruitment. However, the decision was made to terminate the study, as the results from nonparametric analysis indicated efficacy ($p = 0.0357$, WMW test). The per protocol analysis revealed similar results to those of the ITT population, with a median decrease of 7μm (mean $\pm$ SD, $10.35 \pm 18.89\mu$m) in the erythropoietin group (n = 17) and 17μm (23.60 ± 31.06) in the placebo group ($p = 0.046$, WMW test; n = 15; mean difference, 13.2471μm; 95% CI, -5.0637 to 31.5578), indicating that dropout of patients had no significant influence on the results of the study. Efficacy of the erythropoietin treatment with respect to the primary outcome was also seen after performing an ANCOVA adjusted for baseline parameters. After adjustment for baseline VEP latency (a factor imbalanced at baseline) and RNFL thickness, the *p* value of the primary outcome parameter comparison was 0.0022. In the respective covariate test, which assesses the influence baseline VEP latency exerts on the primary outcome parameter, a *p* value of 0.3678 was obtained, indicating a weak or even nonexistent effect of this covariate.

The preservation of RNFL after erythropoietin treatment was similar in females and males. Changes in RNFL thickness did not clearly correlate with any of the secondary outcome parameters (data not shown). RNFL thickness of the nonaffected eye did not decrease over

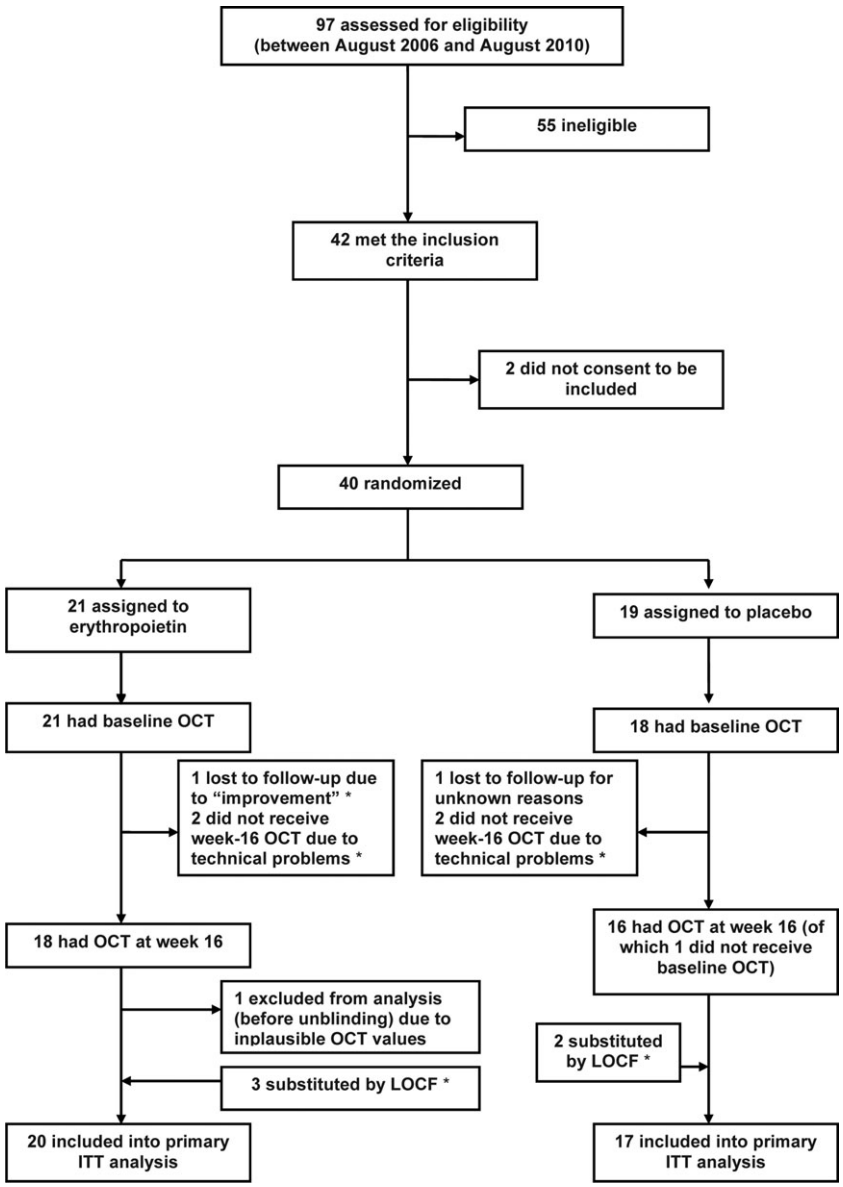

FIGURE 1: Trial profile. Final patient numbers correspond to those included in the intention-to-treat (ITT) analysis of the primary outcome parameter. Asterisks indicate patients who underwent last observation carried forward (LOCF) substitution of week 16 optical coherence tomography (OCT) values.

the observation period (see Fig 2). When performing an intraindividual comparison, RNFL thickness of the affected eye at week 16 was decreased to a median of 3.5μm compared to the nonaffected eye in erythropoietin-treated patients (mean $\pm$ SD, 4.39 $\pm$ 12.98) versus 11μm (11.56 $\pm$ 9.36) in patients of the placebo group ($p = 0.0555$, WMW test; ITT). At baseline, RNFL thickness of the affected eye was higher than that of the nonaffected eye but, in both groups, it returned to healthy levels until approximately week 6 or 7 (see Fig 2B). The initial RNFL swelling was seen to a similar extent in both treatment groups, with a median difference of 7μm in both erythropoietin- and placebo-treated patients. However, respective SDs were different between the groups (mean $\pm$ SD, 10.00 $\pm$ 11.40μm for erythropoietin; 10.82 $\pm$ 26.82μm for placebo), indicating a higher variability of baseline RNFL thickness in the placebo-treated group of patients. This is also reflected in Figure 3, which gives an overview of RNFL thickness values of each single patient in both treatment groups. To estimate the influence extreme baseline RNFL values exert on the primary outcome parameter, we performed an additional analysis excluding the single placebo-treated patient who showed RNFL swelling of $>$100μm (when compared to the contralateral eye; see Fig 3). Exclusion of this patient resulted in a p value of 0.049 (WMW test, ITT population).

TABLE 1: Reasons for Noninclusion of Patients with First Episode of Autoimmune Optic Neuritis

Condition	Patients, No.
Visual acuity >0.5 (decimal system)	31
Onset of symptoms >10 days ago	11
Visual acuity <1.0 in contralateral eye	9
Severe hyperopia, myopia, or astigmatism	3
Concomitant ocular disease	3
Elevated blood pressure	3
History of thrombosis	2
History of malignancy	2
Concomitant acute infectious disease	2
No consent obtained	2
Others	6

A total of 97 patients were screened during a time period of 49 months. Patients were considered for inclusion into the study after diagnosis of optic neuritis (first episode) was made by an independent neurologist and was confirmed by an independent ophthalmologist. Conditions that inhibited study inclusion are listed if they occurred in a minimum of 2 patients. In some cases, >1 condition occurred in a single patient.

Optic Nerve Changes

Retrobulbar diameter of the affected optic nerve as assessed by MRI remained almost stable in the erythropoietin-treated group of patients but decreased in the placebo group (see Table 3). Again, a similar result was seen in the groups included into the per protocol analysis (n = 16 each). Whereas optic nerve diameters did not significantly change in the erythropoietin group (median change, 0.0mm), a median decrease of 0.1mm (mean ± SD, 0.17 ± 0.19mm) was detected in the placebo group. The mean difference was 0.1625mm with a 95% CI of 0.00743 to 0.3176. At baseline, both groups showed increased optic nerve diameters when compared to the contralateral eye, indicating inflammatory edema. Optic nerve diameter was increased by 9.76% when compared to the healthy side in the patient group assigned to erythropoietin treatment, and by 15.87% in the group assigned to placebo. Optic nerve lesion size was not different between the groups at any of the time points. Prechiasmatic optic nerve diameters and those within the optic canal did not change over time in any of the groups, and retrobulbar diameters of the nonaffected eyes also remained unchanged (data not shown).

Latencies and Amplitudes from VEPs

VEP latencies at week 16 were shorter in the erythropoietin group when compared to placebo treatment (median, 113.15 milliseconds; mean ± SD, 115.64 ± 9.79 milliseconds for erythropoietin, n = 20, vs 126.90 milliseconds and 132.73 ± 17.00 milliseconds for placebo, n = 19; $p = 0.0011$, WMW test, ITT analysis). However, as summarized in Table 2, VEP latencies at baseline were already shorter in the erythropoietin group. Therefore, the median difference from baseline to week 16 in erythropoietin-treated patients was not significantly different compared to the placebo group (ITT analysis; see Table 3). Conversely, intraindividual comparison (nonaffected vs affected eye) at week 16 showed differences between the treatment groups, with a median difference in latency of 6.55 milliseconds (8.51 ± 9.48) in erythropoietin-treated patients and a median difference of 20.70 milliseconds (25.59 ± 15.91) in the placebo group ($p = 0.0004$, WMW test). Amplitudes from VEP recordings recovered to a similar extent in both treatment groups (see Table 3). The per protocol analysis of VEP latencies and amplitudes revealed results similar to those of the ITT analysis (data not shown).

Visual Acuity and Visual Field

Recovery of visual acuity and visual field perception did not show significant differences between the treatment groups, although some trends toward better outcome in erythropoietin-treated patients were observed. Mean visual acuity (±SD) at week 16 was 0.96 ± 0.26 in erythropoietin-treated patients (n = 21) and 0.85 ± 0.25 in the placebo group (n = 18; ITT analysis; $p = 0.1013$, WMW test). The increase in visual acuity over 16 weeks as well as the decrease in volume of scotoma in the 2 groups is given in Table 3.

Safety

The majority of recorded adverse events consisted of side effects associated with methylprednisolone therapy, such as hot flushes, facial flushing, mood changes, or hyperglycemia, and did not occur more frequently in erythropoietin-treated patients (data not shown). Five patients complained of headache during the treatment period, 4 of whom had received erythropoietin. Four serious adverse events (SAEs) were recorded throughout the study but were judged not to be related to the study medication (headache after lumbar puncture; worsening of optic neuritis and later contralateral optic neuritis in a single patient; contralateral optic neuritis in another patient). Both cases of contralateral optic neuritis fulfilled the criteria of an MS relapse and were counted as being 2 of the 5 clinical relapses that occurred during this

TABLE 2: Baseline Characteristics

Characteristic	Erythropoietin, n = 21	Placebo, n = 19	*t* Test
Age, yr	32.52 (±7.07), 31 {24–46}	34.74 (±7.10), 35 {23–47}	—
Women	14 [67%]	13 [68 %]	—
Days after onset of symptoms	4.52 (±1.69), 4 {2–8}	5.47 (±2.20), 6 {2–9}	—
Visual acuity, decimal system	0.24 (±0.18), 0.3 {0.0–0.5}	0.21 (±0.19), 0.2 {0.0–0.5}	0.5927
RNFL thickness, µm[a]	103.50 (±15.61), 99 {82–137}	106.44 (±27.4), 102.5 {74–193}	0.6916
Optic nerve diameter, retrobulbar, mm[b]	3.93 (±0.62), 3.9 {3.0–5.5}	3.97 (±0.65), 3.9 {3.1–5.1}	0.8300
VEP latency, ms	139.88 (±28.58), 137.1 {99.9–170.0}	153.54 (±17.31), 156.6 {117.0–170.0}	0.0756
VEP amplitude, µV	3.16 (±3.36), 2.4 {0–10.7}	2.68 (±3.08), 2.5 {0–11.0}	0.6486
Volume of scotoma, dBgrad^{2}[b]	25,575.16 (±23,050.70), 17,818.0 {90–65,942}	26,128.73 (±20,629.36), 21,546.0 {2,438–70,304}	0.9419
Blood pressure, systolic, mmHg[c]	121.79 (±14.73), 120 (100–150)	119.67 (±11.37), 119.5 (100–140)	—
Blood pressure, diastolic, mmHg[c]	73.37 (±9.98), 70 (60–90)	74.44 (±8.33), 78 (60–87)	—
Hemoglobin, g/dl, males	15.37 (±1.02), 15.3 {13.6–17.1}	14.75 (±0.69), 14.5 {14.2–15.8}	—
Hemoglobin, g/dl, females	13.76 (±1.28), 14.2 {10.5–15.1}	13.80 (±0.86), 13.8 {11.9–15.4}	—

Data are mean (±standard deviation), number [%], or median {minimum–maximum range}. Primary and secondary outcome parameters at baseline were compared by using *t* test. Respective *p* values are indicated.
[a]Data available for 20 patients in the erythropoietin group and 18 patients in the placebo group.
[b]Data available for 18 patients in the erythropoietin group and 16 patients in the placebo group.
[c]Data available for 19 patients in the erythropoietin group and 18 patients in the placebo group.
RNFL = retinal nerve fiber layer; VEP = visual evoked potential; dB=decibel.

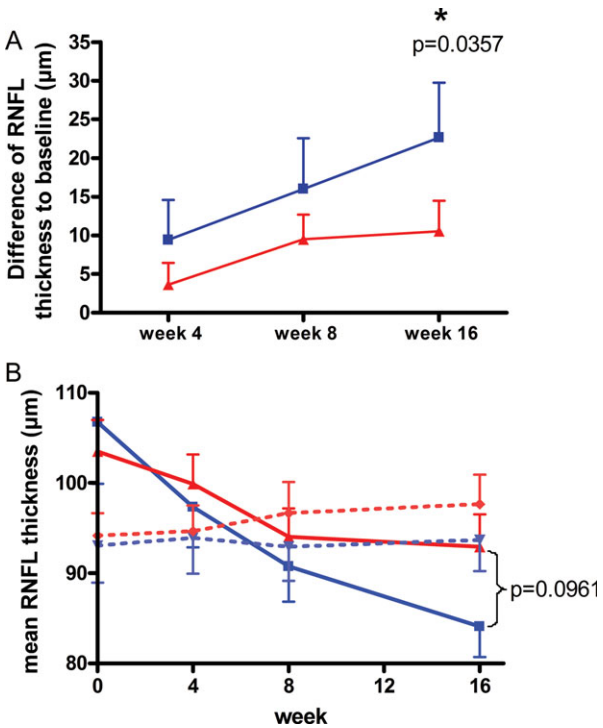

FIGURE 2: Intention-to-treat analysis of the primary outcome parameter. (A) Comparison of differences of mean retinal nerve fiber layer (RNFL) thickness to baseline. (B) Mean RNFL thickness at the different time points analyzed in relation to RNFL thickness of the contralateral healthy eye. Red line = erythropoietin, affected eye; blue line = placebo, affected eye; dashed red line = erythropoietin, healthy eye; dashed blue line = placebo, healthy eye; Bars = standard error of the mean.

study (see Patient Disposition and Clinical Course). All patients with recorded SAEs were treated with erythropoietin. Hemoglobin values showed a transient increase at week 1 in the erythropoietin-treated group that went back to baseline levels 4 weeks after erythropoietin treatment (Fig 4). Blood pressure after treatment with erythropoietin remained stable during the treatment period and did not differ from values after methylprednisolone application alone (Table 4).

Discussion

In this proof-of-concept trial, we have tested whether erythropoietin exerts any influence on RNFL thinning and optic nerve changes in acute optic neuritis when given as an add-on treatment to methylprednisolone. The rationale for choosing erythropoietin comes from observations of neuroprotective effects in animal models of experimental optic neuritis[13,14] and beneficial effects on clinical parameters in progressive MS in addition to a good safety profile.[20] Nonparametric analyses of primary and secondary outcome parameters suggested that the use of erythropoietin was associated with a smaller degree of RNFL thinning. Additionally, retrobulbar optic nerve diameters only slightly decreased, in contrast to those of patients who received methylprednisolone alone, and some of the electrophysiological parameters were improved. These findings are in accordance with previous observations in a rodent model of optic neuritis[14] and suggest that erythropoietin might counteract neurodegeneration in an acute autoimmune inflammatory setting, although additional effects on resolution of edema cannot be fully excluded.

Monitoring RNFL thickness has been proposed as an accurate method of quantifying neurodegeneration in MS and assessing the efficacy of neuroprotective therapies.[5,21] The extent of RNFL thinning observed in our placebo-treated group is in accordance with data from meta-analysis of RNFL changes after optic neuritis.[21] In this analysis, including 12 independent studies and 2,063 tested eyes, the mean decrease in RNFL thickness was 20.38μm when compared to the eyes of healthy controls. In our study, by longitudinal intraindividual follow-up, we observed a mean decrease of 22.65μm over a 16-week time period in the placebo group, which, however, had received methylprednisolone treatment. Although steroid pulse therapy is part of the standard German treatment of acute MS relapses that include severe optic neuritis,[22] it is controversial whether it exerts beneficial or furthermore, neuroprotective effects.[3] Whereas a recent publication showed RNFL protection after steroid therapy in optic neuritis associated with neuromyelitis optica,[23] a comparison of our data with results from former studies does not point in this direction; however, we are aware that conclusions drawn from interstudy comparisons must be treated with caution. With respect to the kinetics of RNFL atrophy, it has been shown previously that the first changes can be detected at a mean of 1.6 months after the onset of symptoms. It has further been shown that the mean time to a 90% loss (in relation to the total amount of RNFL atrophy) was 2.38 months, and that RNFL changes seen >3 months after disease onset cannot be distinguished from background noise.[5] From these observations, it can be concluded that the follow-up period chosen for our study was of sufficient length at least with respect to the primary outcome parameter. In our study, a slight reduction of RNFL thickness (~6.6% in the erythropoietin group; ~8.7% in the placebo group) was detectable already at week 4, which, however, might reflect a resolution of the initial RNFL edema. By week 8, the time course of RNFL thinning was similar in both groups but stabilized in erythropoietin-treated patients with no further decline between weeks 8 and 16. From this observation and from prior observations in animal models,[13,14] it can be speculated

TABLE 3: Intention-to-Treat Comparison for Rates of Change in Outcome Measures over the 16-Week Observation Period (Baseline − Week 16)

Measure	Erythropoietin	Placebo	*p*, WMW Test	Difference (95% CI)
RNFL thickness, μm[a,b]	10.55 [±17.54], 7.5 {1.5 to 14.5}	22.65 [±29.18], 16.0 {8.0 to 20.0}	0.0357	12.097 (−4.5571 to 28.7512)
Retrobulbar optic Nerve diameter, mm[a,c]	0.01 [±0.24], 0.00 {−0.1 to 0.1}	0.16 [±0.19], 0.1 {0.1 to 0.2}	0.0112	0.1585 (0.00813 to 0.3088)
VEP latencies, ms[a,d]	25.82 [±25.16], 26.25 {−0.4 to 48.35}	19.88 [±16.61], 15.60 {9.6 to 30.60}	0.572	−5.9317 (−20.1313 to 8.2680)
VEP amplitudes, μV[d,e]	−3.94 [±4.25], 3.95 {−6.75 to −1.75}	−3.34 [±3.00], 2.90 {−4.5 to −1.8}	0.6075	−0.5988 (−1.8996 to 3.0973)
Visual acuity, decimal system[e,f]	−0.72 [±0.27], 0.70 {−0.9 to −0.6}	−0.63 [±0.28], 0.62 {−0.84 to −0.5}	0.29	−0.0945 (−0.0831 to 0.2721)
Volume of scotoma, dBgrad[2a,g]	24,934.39 [±23,525.20], 18,878 {1,632 to 50,676}	23,146.50 [±18,905.18], 21,442 {10,776 to 35,022}	0.9827	−1,787.9 (18,124.5 to 14,548.7)

Data are mean [±SD] or median {Q1–Q3 range}.
[a]Parameters showed decrease over time.
[b]Data available for 20 patients in the erythropoietin group and 17 patients in the placebo group.
[c]Data available for 17 patients in the erythropoietin group and 16 patients in the placebo group.
[d]Data available for 20 patients in the erythropoietin group and 19 patients in the placebo group.
[e]Parameters showed increase over time.
[f]Data available for 21 patients in the erythropoietin group and 18 patients in the placebo group.
[g]Data available for 15 patients in the erythropoietin group and 14 patients in the placebo group.
CI = confidence interval; RNFL = retinal nerve fiber layer; VEP = visual evoked potential; WMW = Wilcoxon–Mann–Whitney; dB=decibel.

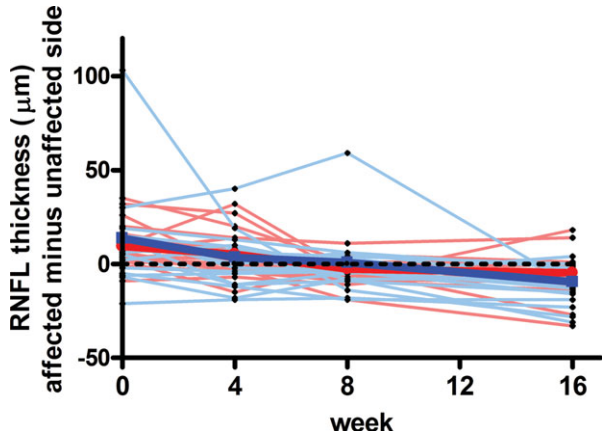

FIGURE 3: Affected minus unaffected retinal nerve fiber layer (RNFL) thickness in each single patient. The dashed line represents the level at which the affected and unaffected eye have the same value. Red line = erythropoietin, mean RNFL thickness; light red lines = erythropoietin, RNFL thickness in each single patient; blue line = placebo, mean RNFL thickness; light blue lines = placebo, RNFL thickness in each single patient.

that neurodegeneration in optic neuritis might have an initial acute period that is difficult to treat successfully, followed by a subacute period that can be counteracted by the activation of neuroprotective pathways. However, in view of the values for baseline RNFL thickness in both treatment groups and, in particular, in view of the different SDs at baseline, we cannot exclude that the effect we saw with erythropoietin is biased by the higher variability of values in the placebo group of patients. This concern is supported by the result of a primary outcome parameter comparison after exclusion of 1 placebo-treated patient with a high amount of baseline RNFL swelling. This additional analysis revealed a *p* value close to the border of no longer being significant, arguing for larger sample sizes to be included into future neuroprotection trials of this type. In principle, there are 2 ways higher RNFL baseline values in single patients of the placebo group could confound the results. First, greater swelling with more severe optic neuritis could be associated with more ultimate true RNFL atrophy, and this could exaggerate the effect of treatment. Second, the resolution of enhanced axoedema may artificially exaggerate the interpretation of RNFL loss.

In contrast to OCT, which allows visualization of the RNFL without the presence of confounding myelin, MRI gives information on more global aspects of optic neuritis. In accordance with previous reports,[6] we observed optic nerve edema in both patient groups during acute optic neuritis. The further decline in retrobulbar optic nerve diameter was much more apparent in the placebo-treated patient group, which could be interpreted as the result of increased levels of axonal damage. However, these potentially indirect signs of Wallerian degeneration have been observed in previous studies at later time points during follow-up periods of up to a year.[6] Theoretically, the minimal decrease in optic nerve diameters we observed in erythropoietin-treated patients could also reflect a slower resolution of optic nerve edema, although this would contradict our findings of improved electrophysiological parameters of the optic nerve and the observed trends toward better visual acuity. Additionally, erythropoietin is generally considered to have anti-inflammatory and blood–brain barrier (BBB)-protecting properties.[24,25] However, in a recent animal study on cerebral ischemia, it was proposed that erythropoietin might increase BBB disturbances when combined with tissue-plasminogen activator.[26] More defined side effects compounding the use of erythropoietin and its structural variants consist of increases in red blood cell and thrombocyte counts, elevations of blood pressure, and thromboembolic complications.[25] Because the risk of these events occurring might especially be increased if erythropoietin is combined with methylprednisolone, a combination so far only described in single patients with hematopoietic disease therapy,[27,28] we have excluded patients with vascular risk factors from study

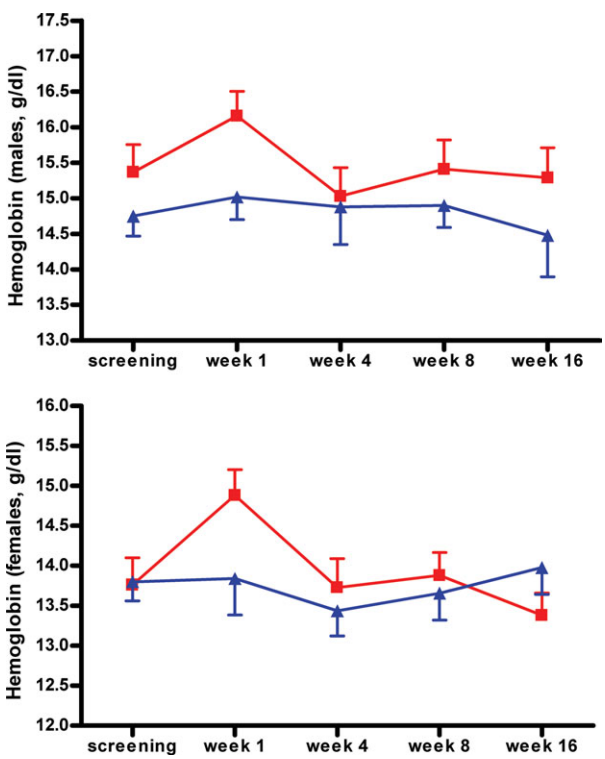

FIGURE 4: Hemoglobin values in males and females. Red lines = erythropoietin; blue lines = placebo; bars = standard error of the mean.

TABLE 4: Blood Pressure during Treatment Days

Blood Pressure	Erythropoietin	Placebo
Systolic 1 hour before treatment[a]	123.0 (±11.9), 121.7 [113–133.3]	121.1 (±12.0), 120.0 [110–129.7]
Diastolic 1 hour before treatment[a]	75.5 (±9.5), 73.3 [72.8–78.5]	71.0 (±5.7), 72.5 [68.3–75.7]
Systolic 1 hour after end of treatment[b]	125.5 (±14.4), 121.7 [113.3–136.7]	125.6 (±11.7), 123.3 [118.0–133.3]
Diastolic 1 hour after end of treatment[b]	74.4 (±7.9), 73.3 [68.3–80.0]	72.0 (±6.9), 70.0 [66.7–76.7]
Systolic 4 hours after end of treatment[c]	129.1 (±19.3), 127.2 [111.7–141.7]	123.8 (±10.8), 122.5 [120.0–126.7]
Diastolic 4 hours after end of treatment[c]	76.4 (±9.5), 75.0 [70.0–78.8]	73.9 (±5.3), 73.8 [71.7–76.7]

Data are mean mmHg (±standard deviation) or median mmHg [Q1–Q3 range] from all treatment days.
[a]Data available for 20 patients in the erythropoietin group and 19 patients in the placebo group.
[b]Data available for 21 patients in the erythropoietin group and 19 patients in the placebo group.
[c]Data available for 20 patients in the erythropoietin group and 18 patients in the placebo group.

participation. Although monitoring of blood cell counts and blood pressure did not disclose any critical elevations, it is not unlikely that these problems could occur during routine treatment of patients other than the young and concomitant disease-free patients included in clinical studies. Other exclusion criteria of our study such as history of malignancy or epilepsy are also explained by the spectrum of known or assumed undesired effects of erythropoietin.[25] Additionally, a rare but potentially fatal side effect of erythropoietin consists of pure red cell aplasia, a disease condition most likely caused by neutralizing antibodies induced by repetitive exposure to subcutaneously but eventually also intravenously applied erythropoietin formulations.[29] Particularly when considering the application of erythropoietin at multiple doses during the course of MS, the potential clinical benefit of the drug must be carefully balanced against these safety risks.

Although we have observed some trends toward better visual function in erythropoietin-treated patients, changes in functional parameters were not significantly different between the treatment groups. We did not assess low-contrast letter acuity, which has recently been reported to detect even subtle visual impairment,[30,31] or measures of color and motion vision,[32,33] which might be very useful functional parameters to include in neuroprotection trials. With the methods we have used, we saw visual acuity and visual field recovering rapidly and to a large extent in both groups, which is characteristic for optic neuritis, at least if it occurs as a first episode.[3] However, recent data from OCT imaging revealed that irrespective of good functional recovery, RNFL degeneration reaches significant levels.[5] From animal models, it is known that approximately 50% of RGCs must degenerate before visual acuity measurably decreases.[34] In humans, it has been shown that the degree of RNFL atrophy correlates with functional outcome and that below a threshold of 75μm, recovery of visual function remains incomplete.[16] In both patient groups, mean RNFL thickness at week 16 was above this threshold, a result in accordance with the good functional recovery that was observed. Nevertheless, it appears likely that patients with RNFL degeneration following a first episode of optic neuritis have a high risk of developing further deficits during the ongoing course of MS or after a second episode of optic neuritis, due to a decreased capacity for functional compensation. Moreover, the finding that a certain amount of RNFL thinning occurs during MS independently of manifest optic neuritis[35,36] implies that neuroprotective intervention at later time points during disease might be less effective, at least in terms of preservation of visual functions. These aspects argue for the early implementation of neuroprotective therapies despite the finding that good functional compensation is often to be expected in clinically isolated syndromes or acute relapses during the initial years after the diagnosis of MS.

In conclusion, this is, to the best of our knowledge, the first trial assessing a potentially neuroprotective agent in patients with acute optic neuritis. To allow more definite interpretation of the results in future studies, certain aspects of the design may be improved. First, a longer follow-up duration could be helpful to allow tissue loss to be studied with greater confidence that any edema had fully resolved, especially if MRI outcome parameters

have been included. Second, inclusion of suitable functional outcome parameters such as low contrast vision might help to detect even subtle differences in visual function. Additionally, if relatively small numbers of patients are included, baseline imbalances should be disclosed, and analyses should be adjusted by inclusion of covariates.

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Authorship

K.-W.S. and K.H. contributed equally to the study.

Potential Conflicts of Interest

K.-W.S.: speaking honoraria, UCB Schwarz; travel expenses, Biogen Idec, Bayer Healthcare, TEVA. K.H.: travel expenses, Biogen Idec, Bayer Healthcare, TEVA. M.B.S.: speaking honoraria, Merck Serono, Bayer Healthcare; travel expenses, Merck Serono, TEVA. C.H.: consultancy, Biogen Idec; grants/grants pending, Merck Serono, Novartis, TEVA; speaking honoraria, Merck Serono, Novartis, TEVA; travel expenses, Biogen Idec, Merck Serono, Bayer Healthcare, TEVA, Novartis. M.B.: grants/grants pending, German Research Foundation. R.D.: consultancy, Synthon B.V.; grants/grants pending, German Research Foundation, Hertie Foundation; travel expenses, Biogen Idec.

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