Erythropoietin for acute multiple sclerosis in patients with optic neuritis as a first demyelination event

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ABSTRACT

الأهداف: التحقق من مدى سلامة، وتحمل، وفعالية العلاج باستخدام إريثروبويتنين (البؤتين الأحمر) بين المرضى المصابين بالتهاب العصب البصري وذلك كبداية لزوال الميالين.

الطريقة: أُجريت هذه الدراسة العشوائية المُعشاة من كلي الجانبين في جامعة شيراز للعلوم الطبية، شيراز، إيران خلال الفترة من مارس 2007م إلى يناير 2009م. لقد تراوح عمر المشاركين في الدراسة مابين 45–18 عاماً، وقد كانوا مصابين بالتهاب العصب البصري، بالإضافة إلى ظهور 3 آفات بؤرية في صور الأشعة المغناطيسية فلير وتي2، إلا أنهم لم يكونوا مصابين بالتصلب العصبي المتعدد المؤكد سريرياً. وقد قمنا بتقسيم المرضى عشوائياً إلى مجموعتين وهما: مجموعة الدراسة (5 مرضى) الذين عولجوا بميثيل بريدنيسولون عبر الوريد (1000 ملغ/24 ساعة) وإريثروبويتنين عبر الوريد (20,000 وحدة/24 ساعة) وذلك لمدة 5 أيام متتابعة، ومجموعة الشاهد (5 مرضى) التي عولجت بميثيل بريدنيسولون عبر الوريد بنفس الجرعة، بالإضافة إلى المادة الخاملة. وبعد ذلك قمنا بمتابعة المرضى في كلتي المجموعتين لمدة سنة واحدة، وعملنا مقارنة فيما بينهما وذلك على أساس مدى التقيد بالتجربة، وظهور الآثار الجانبية للعلاج، والفترة التي تحتاجها الحالة للتحول إلى التصلب العصبي المتعدد المؤكد سريرياً، وتغير نتائج الأشعة المغناطيسية.

النتائج: أشارت نتائج الدراسة إلى قدرة المجموعتين على تحمل العلاج، غير أن مريضاً واحداً من المرضى الذين عولجوا بالإريثروبويتنين قد أصيب لاحقاً بالتخثر الجيبي الدماغي، وظهور الأجسام المضادة للكرديوليبين. وكانت مواصفات مريض واحد من مجموعة الشاهد وليس مجموعة الدراسة تتماشى مع معايير ماكدونالدز للتصلب العصبي المتعدد خلال فترة المتابعة، بالمقابل لم يُصب أي من المرضى في كلتي المجموعتين بالتصلب العصبي المتعدد المؤكد سريرياً وذلك حسب معايير بوستر.

خاتمة: أظهرت الدراسة بأنه يمكن اعتبار العلاج بالإريثروبويتنين فعالاً، غير أنه يجب استخدامه مع أخذ الحيطة والحذر.

Objectives: To investigate the safety, tolerability, and short-term efficacy of treatment with erythropoietin in patients with optic neuritis as a first demyelination event.

Methods: We conducted this randomized double-blind pilot study in the Shiraz University of Medical Sciences, Shiraz, Iran, from March 2007 to January 2009. The participants were patients aged 18-45 years with optic neuritis and at least 3 hyperintense lesions on T2-weighted and FLAIR MRI, but no clinically definite multiple sclerosis (MS). They were randomized into 2 groups. The case group (5 patients) received intravenous methyl prednisolone (1000 mg/24 hours) and intravenous erythropoietin (20,000 unit/24 hours) for 5 consecutive days, and the control group (5 patients) received intravenous methyl prednisolone at the same dose as the case group, and a placebo. The groups were followed for one year and compared for adherence to protocol, adverse drug effects, mean duration of conversion to clinically definite MS, and MRI changes.

Results: All patients tolerated the protocol. One patient who received erythropoietin developed cerebral venous sinus thrombosis and anti-cardiolipin antibody positivity. One patient in the control group, but no patients in the case group, fulfilled the McDonald criteria for MS during the follow-up period, but none of the participants in either group developed clinically definite MS according to the Poser criteria.

Conclusion: Erythropoietin may be effective, but should be used with caution.

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Optic neuritis is one of the most frequent presentations of multiple sclerosis (MS).[1] The number of plaques in the brain in the first MRI study has been considered the most important prognostic factor for the evolution of MS. The 15-year risk of developing MS in the Optic Neuritis Treatment Trial (ONTT) was 25% with no lesions, but 75% with one or more lesions.[2] Intravenous methyl prednisolone (IVMP) may shorten the duration of neuritis manifestations, but has no significant effect on long-term improvement and does not prevent relapses in MS.[3] Erythropoietin (EPO) is an endogenous cytokine first characterized as a hematological factor able to stimulate the differentiation and proliferation of erythroid progenitor cells in response to hypoxia. This cytokine favors erythrocytosis by interfering with gene programs involved in normal apoptosis.[4] It was recently discovered that erythropoietin and its receptor (EPO-r) are also expressed by the CNS and increased in response to tissue damage.[5-7]

Herein, we report the safety, tolerability, and short-term efficacy of treatment with EPO in a pilot study of a small group of patients with optic neuritis as a first demyelination event.

Methods. The study was conducted at the Neurology and Ophthalmology Departments of Khalili Hospital and Motahhari Clinic, affiliated to Shiraz University of Medical Sciences in Shiraz, southern Iran, from March 2007 to January 2009. The inclusion criteria were age between 18 and 45 years, definite diagnosis of optic neuritis according to the ONTT criteria by a qualified neurologist and ophthalmologist, duration of symptoms between 24 and 72 hours, availability for at least one year, and the presence of at least 3 clinically silent periventricular, juxtacortical, or infratentorial hyperintense lesions at least 3 mm in diameter on T2-weighted and FLAIR images in brain MRI. Exclusion criteria were pregnancy, breastfeeding, clinically definite MS, acute disseminated encephalomyelitis (history of viral disease or vaccination in the previous 2 weeks), history of vasculitis or collagen vascular disease, sarcoidosis, Graves' disease, history of anterior ischemic optic neuropathy, history of diabetes mellitus, hypertension or heavy cigarette smoking, history of orbital trauma, history of any cancer, positive human immunodeficiency virus (HIV) or syphilis tests, any intraorbital infiltrative lesion, history of ethambutol, isoniazid, streptomycin, chloramphenicol, sulfonamide, or indomethacin use, history of lead or arsenic poisoning, history of chemotherapy or radiotherapy, self, or family history of Leber's optic neuropathy, history of severe ophthalmic disease such as central retinal vein occlusion, papillophlebitis, central serous retinopathy or chronic uveitis.

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The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. The study and possible outcomes were explained to all participants and written informed consents were obtained. The study was conducted according to the principles of the Helsinki Declaration.

Eligible patients who agreed to participate in the study were randomized into 2 groups (treatment or placebo) with computer-generated random number tables. Patients and all investigators including the qualified neurologist, ophthalmologist, radiologist, nurses, research assistants, and statisticians were unaware of which group each patient was allocated to. Each participant completed a questionnaire that included items on demographic data, a checklist of inclusion and exclusion criteria, and the results of basic laboratory tests, brain MRI, visual field (VF) and visual evoked potential (VEP) tests. A brain MRI was performed using a 1.5-Tesla unit (Philips Gyroscan Intera, Amsterdam, The Netherlands). The magnetic resonance sequences included axial and coronal (FSE) T2-weighted (TR/TE: 3631-4000/100-117), axial FLAIR (TR/TE/TI: 6000-6660/100-117/1200-2000), pre- and post-contrast axial, coronal and sagittal (SE) T1-weighted (TR/TE: 495-500/15-20) and in some patients sagittal proton density (TR/TE: 180/30) images. Section thickness was 5 mm. Gadolinium-based contrast material was used in first and/or follow-up MRI in 47 patients, and post-contrast axial, coronal, and sagittal T1 images were obtained. The contrast agent used in most patients was Magnevist (gadopentetate dimeglumine) or Omniscan (gadodiamide); in a few patients Dotarem (gadoterate meglumine) was used. The interval between contrast injection and imaging was 5 to 10 minutes.

The treatment regimen included IVMP for both case and control groups, intravenous EPO for the treatment group, and intravenous placebo for the control group. All participants received infusions of 1000 mg IVMP in 500 ml dextrose water 5% over 6 hours every day for 5 consecutive days. The treatment group received 20,000 units of recombinant human erythropoietin

(Eprex, Janssen-Cilag, Antwerp, Belgium) intravenously in 200 ml of saline solution over one hour every day for 5 consecutive days (simultaneously with IVMP). In the control group 200 ml saline solution as a sham drug was infused according to the same schedule as in the treatment group. The intervention lasted for 12 months.

The attending physicians and nurses meticulously monitored probable adverse effects of EPO including anaphylactic reactions, fever, flushing, convulsion, chest pain, pruritus, urticaria, hypertension, hypotension, and polycythemia during and after each infusion. Blood pressure was measured every 30 minutes during the first 6 hours after each infusion, then hourly for 24 hours. Complete blood count (CBC), blood glucose, and serum electrolytes were checked daily to screen for any undesirable changes. All patients were followed by the neurologist (new episodes, blood pressure, and abnormal laboratory data) and ophthalmologist weekly during the first month, every 2 weeks during months 3-6, and monthly during months 6-12. Follow-up brain and cervical MRI with contrast were carried out in months 6 and 12. Laboratory tests included a liver function test, CBC, blood urea nitrogen and creatinine every 2 weeks during the first 2 months, monthly during months 3-6, and every 2 months for the last 6 months. The primary endpoint was the mean period elapsed from the first demyelination episode to the diagnosis of clinically definite MS according to Poser's criteria.[8] The secondary endpoints were number of hyperintense lesions on T2WI, burden of lesions, number of enhancing lesions, and number of black holes.

The data in the tables are reported as the median and range. A probability value of less than 0.05 was considered significant for all statistical tests. The Mann-Whitney U test was used for comparison of the means. Statistical analyses were carried out with the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 13.0 software.

Results. The treatment group consisted of 5 patients (5 women, 0 men) with a mean age of 24.4±2.5 years. The control group consisted of 5 patients (5 women, 0 men) with a mean age of 25.2±2.3 years (p>0.05). Mean duration of symptoms was 22.4±0.9 hours in the treatment group and 20.3±0.7 hours in the control group (p>0.05). All patients adhered to the protocol and tolerated the intervention. No anaphylactic reactions occurred at the time of infusion. One patient in the treatment group developed pruritus. No flushing, convulsions, chest pain, urticaria, hypertensive crises, severe hypotension, or any other adverse drug effects were observed during the intervention period in either group. The frequency of side effects of IVMP such as hyperglycemia and leukocytosis did not differ significantly between cases and controls (p>0.05). As **Table 1** shows, there were no significant differences in mean blood pressure (mm Hg) or red blood cell (RBC) mass between the 2 groups.

Three months after the end of EPO administration, one patient in the treatment group developed headache, nausea, vomiting, visual obscurations, and bilateral papilledema, with an opening CSF pressure of 390 mm H_2O. Magnetic resonance angiography revealed thrombosis in both transverse and superior sagittal sinuses. The patient tested positive for anticardiolipin antibody (ACLA) (IgG ACLA: 39.6, IgM ACLA: 24.7) but not for antinuclear or anti-double stranded DNA antibody. The patient was not pregnant, not taking oral contraceptives, and had no polycythemia at that time. No protein C deficiency, protein S deficiency, antithrombin III deficiency, or activated protein C resistance was found. Heparin and a 3-month course of warfarin were started, and the patient recovered completely. No other attributable adverse drug reaction was seen during the one year follow-up period in patients treated with EPO or non-treated controls.

None of the participants developed clinically definite MS, so the primary endpoint could not be evaluated. As **Table 2** shows, there were no significant differences in the number of hyperintense lesions in T2WI, burden of lesions, number of enhancing lesions, or number of black holes between groups before, 6 months, and 12 months after the intervention. One patient in the control group, but no patients in the EPO-treated group had new enhancing lesions at the 12-month follow-up examination. In other words, one control participant but none of the participants in the treatment group

Table 1 - Median and 95% confidence interval (CI) of mean blood pressure (BP) and red blood cell (RBC) mass in patients treated with erythropoietin (EPO) and non-treated controls (non-EPO).

Parameters	Non-EPO median (95% CI)	EPO median (95% CI)	*P*-value
Mean BP (mm Hg)			
Baseline	73 (69.8-77.7)	73 (70.5-77.5)	0.916
Day 6	76 (72.8-78.8)	75 (72.6-78.6)	0.916
Month 12	73 (70.6-75.0)	73 (71-75)	0.831
RBC mass (10^6)			
Baseline	4.36 (4.23-4.55)	4.34 (4.23-4.53)	0.834
Day 6	4.37 (4.26-4.53)	4.36 (4.24-4.53)	0.754
Month 12	4.37 (4.23-4.56)	4.37 (4.22-4.53)	0.673

Table 2 - Median and 95% confidence interval (CI) of hyperintense lesions in T2 weighted imaging (T2WI), burden of lesions, number of enhancing lesions, and number of black holes in patients treated with erythropoietin (EPO) and non-treated controls (non-EPO).

Parameters	Non-EPO median (95% CI)	EPO median (95% CI)	*P*-value
No. of T2-WI hyperintense lesions			
Baseline	5 (3.7-5.5)	4 (3.6-5.6)	1.000
Month 6	4 (2.0-6.0)	4 (2.0-6.4)	0.831
Month 12	4 (2.5-6.3)	4 (1.8-6.6)	0.831
MRI burden			
Baseline	26 (17.7-29.9)	24 (20.9-26.3)	0.834
Month 6	14 (7.7-25.5)	18 (10.8-25.2)	0.673
Month 12	18 (5.9-26.1)	12 (3.0-28.2)	0.834
No. of enhancing lesions			
Baseline	0 (-0.4-0.7)	0 (-0.4-0.7)	1.000
Month 6	0 (0-0)	0 (0-0)	1.000
Month 12	0 (-0.4-0.7)	0 (0-0)	0.317
No. of black holes			
Baseline	0 (0-0)	0 (0-0)	1.000
Month 6	0 (0-0)	0 (0-0)	1.000
Month 12	0 (0-0)	0 (-0.4-0.7)	0.317

developed MS according to the McDonald criteria.[9] This difference was not statistically significant.

Discussion. This study was conducted to investigate the safety, tolerability, and short-term efficacy of treatment with EPO in patients with optic neuritis as a first demyelination event. We selected patients with more than 3 plaques to increase the chance of conversion to clinically definite MS in both treatment and control groups. There were no significant differences between groups in mean age, gender distribution, duration of symptoms of optic neuritis, and baseline MRI parameters. Erythropoietin was well tolerated and no adverse drug reactions were seen during the treatment period. The frequency of polycythemia and hypertension, 2 of the most worrisome adverse effects, did not differ significantly between groups. Nevertheless, one patient developed cerebral venous sinus thrombosis (CVST) and ACLA positivity. Although the primary endpoint could not be assessed because of the short follow-up period, the number of patients who developed new enhancing lesions was lower in patients treated with EPO.

The neurotrophic effects of EPO have been widely studied.[5-7] The presence of EPO receptors on brain capillary endothelial cells and astrocytic end feet may explain the capability of this cytokine to penetrate the blood-brain barrier.[9,10] Animal studies have shown that locally produced EPO might bind to neurons and increase their resistance to hypoxic stress.[11,12] As in erythrocytes, signaling in brain tissue occurs through the Bcl-2 family, especially Bcl-xL.[13] In a randomized clinical trial, recombinant human EPO yielded significantly better results in patients with ischemic stroke compared to placebo.[14]

Several studies have reported beneficial effects of EPO in experimental allergic encephalitis (EAE). Yuan et al[15] found that EPO treatment substantially reduced the acute clinical paralysis seen in EAE mice, and this improvement was accompanied by a large reduction in mononuclear cell infiltration, and downregulation of glial MHC class II expression within the inflamed CNS. Also in Diem et al's study,[16] combined EPO and high dose MP was more effective than monotherapy with EPO or MP alone in neuron and axon protection according to functional and histopathological investigations.[16]

In a pilot study, a high dose of EPO was effective in treating chronic progressive MS according to reduction in expanded disability status scale (EDSS), and of cognitive performance persisting for 3-6 months after cessation of EPO administration.[17] These findings lead us to consider recombinant human EPO as a therapeutic agent in the management of optic neuritis and its ability to delay the conversion of optic neuritis to clinically definite MS.

Although patients in our treatment group tolerated a relatively high dose well, one patient developed CVST. This patient had a high ACLA titer at the time of thrombosis, but not when optic neuritis was the only symptom. Whether this was a misdiagnosed case of antiphospholipid antibody syndrome (a known cause of CVST),[18] or she developed ACLA positivity and CVST due to EPO was a matter debate for us. We could find no other risk factor for CVST in this patient.[19] Although polycythemia has been reported to be associated with CVST,[20] the patient was not polycythemic. This is contrary to the only case report of CVST in the medical literature, a 37-year-old woman on peritoneal dialysis who was receiving epoetin-alpha and was polycythemic.[21] The chosen total dose of EPO was similar to Ehrenreich et al's study,[17] and proved to be well tolerated.

The major shortcomings of our study were the small sample size and short duration of follow-up. In addition, we were not able to measure serum and CSF levels of EPO, but it was previously shown that EPO can cross the blood-brain barrier. To rule out antiphospholipid antibody syndrome it would have been better to perform more sophisticated studies before the intervention.

In conclusion, the results of our pilot study showed that EPO, although it may be effective, should be used

with caution. Future larger size multicenter randomized clinical trials investigating the putative therapeutic role of EPO in different aspects of MS are recommended.

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