Epoetin Alfa Increases Frataxin Production in Friedreich's Ataxia Without Affecting Hematocrit

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ABSTRACT

Objective of the study was to test the efficacy, safety, and tolerability of two single doses of Epoetin alfa in patients with Friedreich's ataxia. Ten patients were treated subcutaneously with 600 IU/kg for the first dose, and 3 months later with 1200 IU/kg. Epoetin alfa had no acute effect on frataxin, whereas a delayed and sustained increase in frataxin was evident at 3 months after the first dose (+35%; $P < 0.05$), and up to 6 months after the second dose (+54%; $P < 0.001$). The treatment was well tolerated and did not affect hematocrit, cardiac function, and neurological scale. Single high dose of Epoetin alfa can produce a considerably larger and sustained effect when compared with low doses and repeated administration schemes previously adopted. In addition, no hemoglobin increase was observed, and none of our patients required phlebotomy, indicating lack of erythropoietic effect of single high dose of erythropoietin. © 2010 *Movement* Disorder Society

Key Words: Friedreich's ataxia; Epoetin alfa; erythropoietin; frataxin; iron

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Friedreich's ataxia (FA) is an autosomal recessive ataxia[1] caused by a trinucleotide GAA expansion in the first intron of the *FXN* gene.[2] The gene encodes for a 210aa mitochondrial protein called frataxin, whose mRNA and protein levels are severely reduced in FA.[3] It has been suggested that frataxin is involved in iron–sulphur cluster and heme biogenesis, iron binding/storage, and chaperone activity.[4–6]

Erythropoietin (EPO) is a glycoprotein that acts as a main regulator for erythropoiesis. Evidence suggests that both EPO and its receptor are expressed in the nervous tissue,[7,8] and neuroprotective effects have been shown in animal models of cerebral ischemic damage.[9,10]

EPO increases frataxin levels in cultured human lymphocytes from FA patients.[11] However, frataxin protein increase is not preceded by mRNA increase, suggesting that a post-transcriptional mechanism is involved.[12] Two phase II clinical trials have tested EPO in FA patients. In the first, 12 patients were treated with 5,000 IU of Epoetin beta thrice a week (TIW) for 8 weeks. This resulted in a 27% frataxin increase in peripheral blood mononuclear cells (PBMCs), reduction in oxidative stress markers, and a small clinical improvement.[13] The same group of investigators performed a second trial in which Epoetin beta was given to 8 patients at a dose of 2,000 IU TIW for 6 months. The results largely confirmed those of the first trial. However, 4 patients (50%) required repeated phlebotomy.[14]

Patients and Methods

Study Design

We designed an open-label, phase IIa clinical trial to test the efficacy of two subcutaneous single high dose of Epoetin alfa (Eprex, Janssen-Cilag, Milano, Italy). The lower dose was 600 IU/kg body weight (BW) (max 40,000 IU) and the higher dose was 1200 IU/kg (max 80,000 IU). Doses were chosen based on the previous pharmacokinetics studies[15] and on the intent to reproduce a serum EPO concentration similar to previous in vitro experiments.[11] A 3-month washout was programmed between the doses. Primary endpoint of the study was the change from baseline in frataxin levels in PBMCs at 24, 48, 96 hours, 7, 15, 30, and 60 days. Secondary endpoints were safetyand tolerability measured with clinical scale, echocardiography, and laboratory parameters. The local Ethics Committee approved the clinical trial, and it was registered at www.clinicaltrials.gov, NCT00631202.

Patients

Ten patients were enrolled in the study after giving informed consent (Table 1); 1 patient withdrew his consent 30 days after the first administration and was

Table 1. Patient characteristics

Patient	Age	Sex	Age at onset	Disease duration	GAA1	GAA2	ICARS baseline	ICARS frataxin peak*
1	19	M	8	11	709	830	56	61
2	29	F	23	6	800	1000	36	39
3	30	F	18	12	600	1267	58	56
4	25	M	14	11	958	958	51	48
5	43	M	23	20	200	421	25	25
6	22	F	11	11	489	1022	39	36
7	22	M	15	7	734	934	49	52
8	19	M	9	10	752	1041	66	–
9	31	F	15	16	355	561	68	78
10	40	F	28	12	580	780	55	45
Mean	29 ± 8.2		17.2 ± 6.4	11.8 ± 4.2	602.8 ± 232	863.7 ± 253.9	48.6 ± 13.1	48.9 ± 15.4

Mean ± SD for the 9 patients who completed the study.
*ICARS performed 6 months after the second Epoetin alfa administration (month 9 in Fig. 1).
GAA1, triplet repeat number in the minor allele; GAA2, triplet repeat number in the major allele; ICARS, International Cooperative Ataxia Rating Scale.[15]

not considered for endpoint evaluation. Inclusion criteria were clinical and molecular diagnosis of FA (18–50 years of age). Exclusion criteria were idebenone treatment, wheelchair use, renal, hepatic or hematological disease, positive thrombosis history, hypertension, acute disease, pregnancy, and breastfeeding.

Quantitative Analysis of Frataxin and EPO

PBMCs were extracted from 15 mL of ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood using Leucosep tubes (Greiner Bio-one, Germany). PBMCs were lysed, and total protein was measured using the bicinchoninic acid (BCA) assay. About 7.125 μg of each protein extract was analyzed in duplicate with lateral-flow immunoassay[16] and calibrated using frataxin protein standard (kit and Hamamatsu ICA-1000 scanner; Mitosciences, Eugene, OR). Preliminary test showed an intra-assay and interassay coefficient of variability both <5%. Data from 31 carriers and 19 control individuals were analyzed in parallel. Serum concentrations of EPO were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Secondary Outcomes

Laboratory parameters were monitored at screening, baseline, and 7, 15, 30, and 60 days after each administration and comprised hematology, urine examination, coagulation, and a serum routine biochemistry with iron, ferritin and transferrin determination. Adverse events and blood pressure were monitored at each visit. Electrocardiograms were performed at screening and 30 days after each administration. The International Cooperative Ataxia Rating Scale (ICARS)[17] was measured at baseline, 7 and 30 days after each administration, and 6 months after the last dose.

Conventional M-mode, two-dimensional imaging, and quantitative regional strain and strain rate were used to evaluate cardiac morphology and function. Echocardiography was performed at baseline and 6 months after the second EPO administration.

Statistical Analysis

Statistical analysis for continuous variables was conducted by two-way ANOVA for repeated measures. P values of less than 0.05 were considered statistically significant. Posthoc analysis was performed with the Dunnett's test to compare basal levels with different time points (*Graphpad Prism 5.0c*).

Results

Frataxin

We did not observe significant variations of frataxin in the 24 hours to 60 days interval, either after the first or the second EPO administration. Peaks were observed 96 hours after the first dose (mean increase 8.9%; $P = 0.47$) and 48 hours after the second (16.3%; $P = 0.34$). Surprisingly, when analyzing frataxin levels throughout the study, we found a slow and sustained increase (Fig. 1). Basal frataxin was 12.7 ± 3 pg/μg total protein and rose to 17.1 ± 5.8 pg/μg (35% relative increase from baseline; $P < 0.05$) 3 months after the first Epoetin alfa administration. After the second injection, frataxin increased to 18.3 ± 7 pg/μg (44% relative increase compared with baseline; $P < 0.01$) 2 months later. Given this unexpected delayed effect of Epoetin alfa, we decided to extend the observation period. At 6 months from the second administration, we found frataxin to be 19.5 ± 5.4 pg/μg (54% relative increase from baseline; range 14–144; $P < 0.001$). At 12 months, frataxin values were no longer significantly higher than baseline (16.1 ± 9 pg/μg; $P > 0.05$).

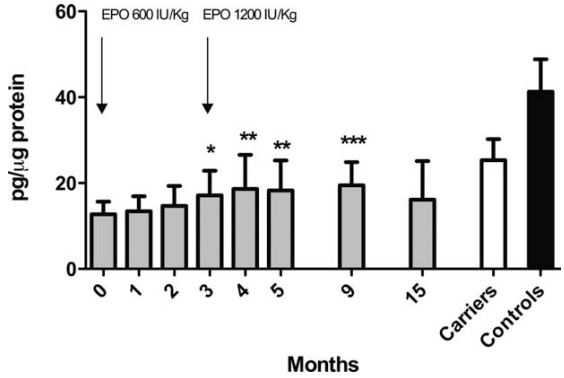

FIG. 1. Frataxin levels after two single doses of Epoetin alfa. Frataxin levels are shown as pg/μg of total protein extract from the patients who completed the study (n = 9, gray bars), carriers (white bar; n = 31; 25.3 ± 4.9 pg/μg total protein), and healthy controls (black bar; n = 19; 41.3 ± 7.5 pg/μg total protein). All values are mean ± standard deviation (statistical significance compared with baseline = $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Serum EPO

Twenty-four hours after the first administration, serum EPO increased from 11.2 ± 8.6 mIU/mL at baseline to 1153 ± 354 mIU/mL (Supporting Information Figure). EPO then decreased to basal levels 7 days after injection. The second injection produced similar results (19.7 ± 19.6 and 3343 ± 657 mIU/mL).

Iron

Total iron decreased from 75.0 ± 39.1 μg/dl at baseline to 44.4 ± 24.4 μg/dl ($P < 0.05$) at 7 days and returned to 81.1 ± 31.5 μg/dl at 30 days. The second injection produced similar results (83.6 ± 46.5, 31.0 ± 14.4, 76.8 ± 34.3 μg/dl; $P < 0.01$). Ferritin decreased from 104.9 ± 98.7 ng/mL at baseline to 18.5 ± 12.4 ng/mL ($P < 0.001$) at 7 days after the first dose and returned to 73.4 ± 79.2 ng/mL at 30 days. The higher dose caused similar results (70.3 ± 90.2, 30.5 ± 41.4, 62.1 ± 77.6 ng/mL, $P < 0.01$). Transferrin remained stable for the same time points (first dose 250.1 ± 58.68, 273.4 ± 48.5, 244.6 ± 58.8 mg/dl; second dose 285.1 ± 53.0, 273.8 ± 64.9, 288.1 ± 52.3 mg/dl, P = NS). Mean transferrin saturation decreased by 44.6% ($P < 0.01$) 7 days after the first and 63.2% ($P < 0.001$) 7 days after the second dose, when compared with baseline.

Secondary Outcomes

A total of 10 grade 1 adverse events were recorded after the lower dose and two after the higher dose (myalgia, flu-like syndrome, hypotension, nausea, itching and reaction at injection site, headache, nocturnal sweating, and extremities warm feeling). Blood pressure and electrocardiograms showed no change during the study. Hematocrit, erythrocytes, hemoglobin, and all other laboratory safety parameters were not influenced by treatment. Echocardiographic measures showed no change from baseline. As a prototype of myocardial contractility, the left ventricle strain was −19.9 ± 5.1% at basal and −18.5 ± 6.7% at 6 months (P = NS). ICARS was unchanged during the study (Table 1; baseline 48.6 ± 13.1 vs. 48.9 ± 15.4 at frataxin peak, P = NS).

Discussion

In vivo, our study did not replicate the previous in vitro findings of an acute increase in frataxin after EPO stimulation. Although the lack of statistical significance of the early increase we observed may be because of the small sample size, any clinical significance of such a small change is likely to be limited. In contrast, an unexpected delayed effect of Epoetin alfa on frataxin levels in PBMCs was observed 3 months after 600 IU/kg of Epoetin alfa (35% increase). The second injection caused an additional increase in frataxin up to 54% above baseline. A carry-over effect cannot be excluded and could be responsible of the observed effect. Interestingly, the increase was evident after serum EPO returned to basal levels, suggesting that a direct stimulation of the EPO receptor is not involved in the delayed effect. Previous clinical trials[13,14] were designed with continuous low dose administration, and the interval between EPO administration and frataxin increase could not be assessed. We demonstrate that single high dose of EPO can produce a considerably larger and sustained effect when compared with low TIW doses. In addition, no hemoglobin increase was observed, and none of our patients required phlebotomy, indicating lack of erythropoietic effect of single high dose of EPO. In contrast, our study failed to demonstrate a clinical improvement that was reported in the past trials. This could be explained by a lower sensitivity of ICARS, compared with Friedreich Ataxia Rating Scale (FARS) or the scale for the assessment and rating of ataxia (SARA) scale, or by the presence of a learning effect of repeated measuring in the previous trials.

Exact effect of EPO on frataxin is unknown. EPO administration is known to reduce circulating hepcidin,[18] and as a consequence ferroportin inhibition is released and iron stores are reduced.[19] In this study, EPO reduced transferrin saturation, indicating iron redistribution from peripheral tissues to the bone marrow. The absence of a clear hematopoietic effect remains unclear. Perhaps, very high single EPO doses may have a very strong and rapid relocating effect on iron, but repeated dosing may be necessary to obtain a hematopoietic effect. This iron relocating effect may be a clue to explain the delay in frataxin increase. The sustained effect of EPO remains obscure, and long-lasting post-transcriptional effects cannot be excluded.

In conclusion, it is possible to achieve a considerable increase in frataxin using a very simple administration scheme of Epoetin alfa with no hematological side effects. In the absence of a control group, the present data should still be regarded as preliminary until a

randomized, placebo-controlled trial, has been performed. ■

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Somatosensory Temporal Discrimination Tested in Patients Receiving Botulinum Toxin Injection for Cervical Dystonia

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ABSTRACT

We designed this study to find out more about the relationship between the sensory effects of Botulinum toxin type A (BTX) and the clinical benefits of BTX therapy in patients with cervical dystonia (CD). In 24 patients with CD, we tested sensory temporal discrimination (STD) in the affected and two unaffected body regions (neck, hand, and eye) before and 1 month after BTX injection. In 8 out of the 24 patients with CD, STDT values were tested bilaterally in the three body regions before, 1 and 2 months after BTX injection. As expected, STD testing disclosed altered STD threshold values in all three body regions tested (affected and unaffected by dystonic spasms) in patients with CD. STD threshold values remained unchanged at all time points of the follow-up in all CD patients. The lack of BTX-induced effects on STD thresholds suggests that STD recruits neural structures uninvolved in muscle spindle afferent activation. © 2010 *Movement* Disorder Society

Key Words: botulinum toxin; cervical dystonia; somatosensory temporal discrimination

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