Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomised, placebo-controlled trial

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Summary

Background Friedreich's ataxia (FA) is a progressive, multisystem, degenerative disorder caused by a reduction in frataxin. Loss of frataxin results in mitochondrial dysfunction and oxidative damage in patients and model systems. Previous studies have indicated that the antioxidant idebenone (5 mg/kg daily) reduces cardiac hypertrophy, but definite improvement in neurological function has not been shown.

Methods 48 genetically confirmed FA patients, aged 9–17 years, were enrolled in a 6-month, randomised, double-blind, placebo-controlled study. The patients received placebo or one of three doses of idebenone (~5 mg/kg, 15 mg/kg, and 45 mg/kg), stratified by body weight. The primary endpoint was change from baseline in urinary 8-hydroxy-2′-deoxyguanosine (8OH2′dG), a marker of oxidative DNA damage. Secondary endpoints included changes in the international cooperative ataxia rating scale (ICARS), the FA rating scale (FARS), and a survey of activities of daily living (ADL). This study is registered with ClinicalTrials.gov, number NCT00229632.

Findings Idebenone was generally well tolerated with similar numbers of adverse events in each group. One child receiving high-dose idebenone developed neutropenia after 6 months, which resolved after discontinuation of treatment. 8OH2′dG concentrations were not increased, and did not significantly change with idebenone treatment. Whereas an overall analysis did not show a significant difference in ICARS, FARS, or ADL total scores, there were indications of a dose-dependent response in the ICARS score. A second, pre-specified analysis, excluding patients who required wheelchair assistance, showed a significant improvement in ICARS (Bonferroni p=0·03) and suggested a dose-related response in ICARS, FARS, and ADL scores.

Interpretation Treatment with higher doses of idebenone was generally well tolerated and associated with improvement in neurological function and ADL in patients with FA. The degree of improvement correlated with the dose of idebenone, suggesting that higher doses may be necessary to have a beneficial effect on neurological function.

Introduction

Friedreich's ataxia (FA) is the most common hereditary ataxia in Europe and North America, and affects approximately 1 in 40 000 individuals.[1] The condition is characterised by progressive gait and limb ataxia, dysarthria, areflexia, loss of vibratory and position sense, and distal extremity weakness. Additional features include hypertrophic cardiomyopathy, scoliosis, and diabetes.[2] FA is inherited in an autosomal recessive pattern, with a homozygous expansion of a GAA trinucleotide repeat in the first intron of the frataxin gene as the causative mutation in 97% of cases. The expansion causes decreased transcription of the gene and consequent reduction in the concentration of the gene product, frataxin, which is a mitochondrial protein.[3] Loss of frataxin has a deleterious effect on mitochondrial function. In FA patients' cells and in model systems, there is a loss of iron-sulphur proteins, including the respiratory chain complexes I, II, III, and aconitase, which results in reduced ATP generation, as confirmed in patients by magnetic resonance spectroscopy.[4] In addition, mitochondria become overloaded with iron, leading to the formation of reactive oxygen species through Fenton chemistry, as indicated by increased concentrations of markers of oxidative damage in blood and urine samples from FA patients.[5,6] Thus, mitochondrial dysfunction and oxidative tissue damage may both contribute to FA pathogenesis.

On the basis of these findings, use of lipid-soluble antioxidants has been explored as a potential treatment. Idebenone, a short-chain benzoquinone structurally related to coenzyme Q10, is a potent antioxidant and electron carrier.[7] Clinical studies in FA patients with idebenone treatment at a daily dose of 5 mg/kg have shown reduction in oxidative stress markers and cardiac hypertrophy,[6,8–10] but not in neurological function. One small, open-label trial in young FA patients found an improvement in overall neurological function that was related to plasma idebenone concentrations.[11] This suggests that treatment may be effective if given early in the disease course, and that improvement may be dependent on higher doses of idebenone. This suggestion is supported by the finding in two other trials that patients who still had cardiac hypertrophy after treatment with 5 mg/kg idebenone daily subsequently responded when the dose was increased to 10 mg/kg daily.[8,9] In phase 1 dose-escalation and tolerability trials in FA patients, we found that idebenone at 60 mg/kg daily was well tolerated over a 1-month period in children, adolescents, and adults with FA, with no clinically significant adverse events or laboratory abnormalities.[12] We therefore did a

double-blind, placebo-controlled study to examine the safety and feasibility of higher doses of idebenone in young FA patients.

Methods

Patients

Patients with FA were enrolled between October, 2005, and February, 2006, with follow-up occurring between April and August, 2006, at the National Institutes of Health (NIH) Clinical Center (Bethesda, MD, USA). Eligible participants were able to walk 7·5 m with or without an assistance device, were genetically confirmed, were neurologically symptomatic, aged 9–17 years, and weighed 30–80 kg. The participants were not exposed to idebenone, coenzyme Q10, or other dietary supplements for a period of at least 1 month before enrolment. Exclusion criteria included hypersensitivity to idebenone or coenzyme Q10, serious concurrent illness, and laboratory abnormalities as defined by the following: platelet count, white blood cell count, or haemoglobin below the lower limit of normal, based on the paediatric reference range provided by the testing laboratory; alkaline phosphatase, bilirubin, or serum transaminases greater than 1·5 times the upper limit of normal; or creatinine greater than 1·5 times the upper limit of normal. Young women of childbearing potential had to have a negative pregnancy test before entry into the study and agreed to use two reliable methods of birth control for the duration of the study if sexually active. Informed consent and assent was obtained before enrolment. The study was done with the approval of the National Institute of Neurological Disorders and Stroke (NINDS) Institutional Review Board, and an independent data and safety monitoring board oversaw study conduct and safety issues.

Study drug and dosing

Idebenone was assigned investigational new drug number 62 926 by the US Food and Drug Administration (FDA), and a copy of the trial protocol was sent to the agency before beginning the trial. This trial was registered with ClinicalTrials.gov (NCT00229632). Before study initiation, a list of randomisation numbers and corresponding treatment numbers was computer generated by a third party (Hesperion Ltd, Allschwil, Switzerland). The randomisation assignment was stratified by weight (≤45 kg or >45 kg) to maintain the dose range, and by the shorter GAA repeat length (≤800 or >800) to control for disease progression. Patients and investigators were blinded to the allocated study treatment. The treatment assignments were maintained by the third party and only made available after the trial was complete and the database finalised. 6-month supplies of idebenone (60 mg and 150 mg tablets) or matching placebo were provided by Santhera Pharmaceuticals (Liestal, Switzerland) in pre-packaged kits marked with the appropriate treatment number. The kits were maintained and dispensed by the NIH Clinical Center Pharmacy Department to eligible patients. The total dose of idebenone was stratified by body weight (≤45 kg or >45 kg): low dose (180 mg or 360 mg), intermediate dose (450 mg or 900 mg), or high dose (1350 mg or 2250 mg). Thus, the low-dose treatment was 4–8 mg/kg, intermediate dose was 10–20 mg/kg, and high dose was 30–50 mg/kg. The total daily dose was divided, and patients were instructed to take the study drug three times a day with food. Compliance was monitored by entries in a daily drug log and by pill counts, and by assessment of serum concentration of total idebenone at the end of the study.

Objectives

The primary endpoint for the study was the change from baseline to 6-month follow-up in 8-hydroxy-2′-deoxyguanosine (8OH2′dG), a marker of oxidative DNA damage.[13] Secondary endpoints included safety and tolerability based on the number, type, and severity of adverse events, and absolute and percentage change from baseline in scores derived from the international cooperative ataxia rating scale (ICARS),[14] the FA rating scale (FARS),[15] and a survey of activities of daily living (ADL) developed with the FARS. Other secondary endpoints in this study included cardiac, exercise, and gene-expression analyses, the results of which are to be reported elsewhere.

Statistical analysis

A sample size of seven patients per treatment group (total 28) was calculated to have 90% power to detect a difference in concentrations of the oxidative marker 8OH2′dG between the placebo and treatment groups with a two-sided α level of 5%. This calculation was based on a one-way ANOVA model with a four-level factor (corresponding to four treatment groups) by use of previously reported results that indicated a 2·6 times increased concentration of 8OH2′dG in FA patients relative to healthy controls, with a 20% reduction in levels (SD 18%) after 8 weeks of treatment with 5 mg/kg idebenone daily.[6] On the basis of these data, we hypothesised changes in 8OH2′dG concentrations of 0%, 13%, 27%, and 40% for patient treated with placebo, low-dose, intermediate-dose, and high-dose idebenone, respectively. Given the uncertainties underlying this analysis, the phenotypic variation in the secondary neurological endpoints, and allowing for a dropout rate of up to 25%, we enrolled 12 patients per treatment group.

All statistical analyses were done on the total patient population by use of a null hypothesis of no difference between groups, with a two-sided significance level of 5%. Analysis was also done on a pre-specified subgroup (10<ICARS<54) because of perceived limitations in the performance-based scales in the less ambulatory, wheelchair-using (but not dependent) patients. Both groups were analysed by ANCOVA, using the baseline

values and genetic stratification as covariates, and by the Jonckheere trend test, a non-parametric procedure specifically designed to detect differences arising from ordered treatments (eg, dose levels).[16] ANCOVA assumptions about the normality and heterogeneity of residuals, and lack of interaction between covariates and the treatment group factor were examined and found to be tenable by use of the Shapiro-Wilk test and models that explicitly examined possible interactions. Adjusted p values are also reported by use of a Bonferroni correction for multiple comparisons made with the three specified neurological and functional secondary endpoints; the adjustments were obtained by multiplying the nominal unadjusted p values by 3.

For the primary endpoint (oxidative marker 8OH2′dG) and a secondary endpoint that reached significance (ICARS), a supplemental, descriptive analysis was done using pairwise comparisons of the changes over time seen in the placebo group with those changes in each of the three drug groups. The comparisons were conceptually similar to a set of two-group *t*-tests of differences, but were based on contrasts derived from the ANCOVA model, and hence took into account the baseline and genetic covariates. In addition to each estimated difference between groups, 95% CIs and uncorrected and Bonferroni-corrected p values are given. The uncorrected p values and associated 95% CIs are not adjusted for multiple comparisons and thus should be interpreted as descriptive information rather than directly assessing significant differences among pairs. The Bonferroni adjustment takes into account there are three comparisons of the placebo to the active dose.

No interim analyses were planned or done. Power analyses were done using version 4.0 of nQuery software (Statistical Solutions, Saugus, MA, USA). The Jonckheere tests were done using version 6 of StatXact software (Cytel Inc., Cambridge, MA, USA) and reported two-sided p values. The ANCOVA results were done using version 2.4 of the R statistical program (http://cran.r-project.org).

Study conduct

This trial was done as a prospective, randomised, double-blind, placebo-controlled, single-site study. It consisted of a 2-day baseline assessment followed by a 6-month treatment period and a 2-day follow-up assessment. During the 6-month treatment period, the patients underwent monthly laboratory safety testing, and a physical examination and electrocardiogram by local primary-care physicians after the third month. During the baseline and follow-up visits, patients underwent laboratory testing, electrocardiograms, and physical examinations. All safety laboratory samples were processed by the NIH Clinical Center Department of Laboratory Medicine and were interpreted by the principal investigator. Plasma, serum, and urine samples collected from the patients for biomarker analysis were processed and stored at –80°C. After all patients had completed the initial visit, coded urine samples were shipped on dry ice to ESA Laboratories (Chelmsford, MA, USA) for 8OH2′dG analysis. The urinary concentration of 8OH2′dG was measured by carbon column-based liquid chromatography with electrochemical detection and expressed as a ratio to urinary creatinine.[6,17]

A parent or guardian completed the ADL questionnaire at the beginning of the examination session. The patients were assessed using the ICARS and FARS scales merged into a single, 1 h examination to ensure accuracy and

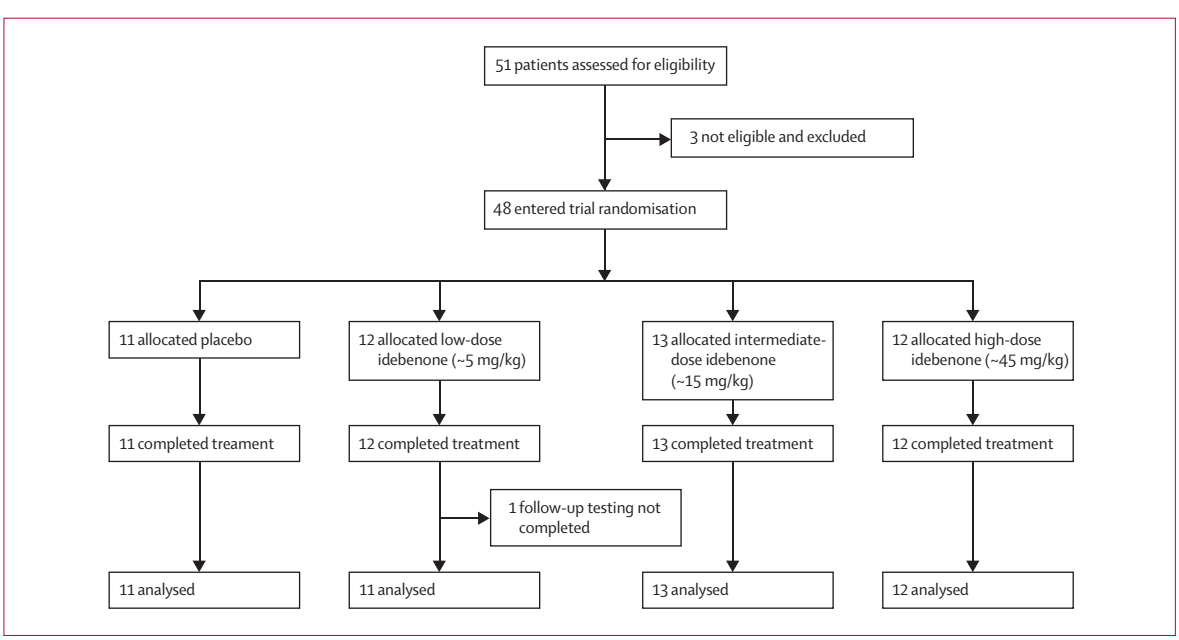

Figure 1: **Trial profile**

	Age (years)	Sex (male)	GAA repeat length (n)		Age at diagnosis (years)	FA stage	ADL score	ICARS score	FARS score
			Allele 1	Allele 2					
Total study population									
Placebo (n=11)	12·9 (2·3)	6 (54%)	801 (82)	921 (326)	9·7 (2·5)	2·7 (1·0)	12·2 (4·3)	34·1 (14·0)	47·0 (17·0)
Low dose (n=11)	14·3 (2·2)	4 (36%)	760 (220)	1055 (194)	9·1 (3·3)	3·1 (1·1)	15·6 (4·7)	46·4 (15·7)	57·8 (17·6)
Intermediate dose (n=13)	13·3 (3·1)	6 (46%)	776 (177)	924 (332)	10·1 (1·9)	2·8 (1·1)	14·5 (4·8)	39·7 (14·0)	49·8 (15·7)
High dose (n=12)	13·2 (2·1)	8 (67%)	764 (113)	934 (184)	9·7 (2·4)	2·8 (1·0)	12·6 (4·1)	41·3 (12·4)	51·3 (17·5)
All patients (n=47)	13·4 (2·4)	24 (51%)	775 (153)	957 (267)	9·8 (2·3)	2·8 (1·0)	13·7 (4·5)	40·4 (14·2)	51·4 (16·8)
Subset group (10<ICARS score<54)									
Placebo (n=8)	13·4 (2·4)	5 (63%)	803 (91)	873 (336)	10·3 (3·1)	2·6 (0·7)	12·0 (3·0)	32·6 (4·5)	44·8 (9·8)
Low dose (n=6)	13·2 (2·3)	3 (50%)	656 (213)	1000 (188)	11·3 (1·3)	2·3 (0·4)	12·6 (3·9)	33·8 (7·4)	43·9 (10·4)
Intermediate dose (n=10)	12·4 (2·9)	3 (30%)	782 (202)	891 (348)	9·9 (2·7)	2·4 (0·8)	13·0 (4·4)	33·9 (9·7)	43·4 (11·2)
High dose (n=9)	12·7 (1·9)	5 (56%)	785 (125)	968 (168)	10·7 (2·1)	2·3 (0·6)	11·6 (4·2)	35·7 (8·5)	43·2 (11·0)
All patients (n=33)	12·8 (2·3)	16 (48%)	765 (165)	927 (280)	10·4 (2·4)	2·4 (0·7)	12·3 (3·8)	34·1 (7·7)	43·2 (10·2)

Data are means (SD) or number (%), unless otherwise indicated. Friedreich's ataxia (FA) stage and FA rating scale (FARS) scores are based on Subramony et al,[15] and the international cooperative ataxia rating scale (ICARS) score is based on Trouillas et al.[14] ADL=Activities of daily living.

Table 1: **Baseline characteristics and demographics of study groups**

minimise confounding by fatigue. The total scores of these scales have been shown to be reliable and valid instruments for assessing FA disease status.[14,15] A single rater, a nurse practitioner trained to do the examinations and blinded to the treatment groups, did all baseline and follow-up assessments. All testing manoeuvres at baseline and follow-up were done at the same time of day, in the same order, and in the same setting to minimise variability. Data were entered directly into paper case-report forms. The data were then entered into electronic databases for analysis. Another investigator audited the data to ensure accuracy.

For this study, adverse events were graded as mild, moderate, or severe, based on the degree of discomfort or impairment. Serious adverse events were defined according to the FDA Safety Information and Adverse Event Reporting Program (http://www.fda.gov/medwatch/). The patient and their parent(s) or legal guardian(s) were instructed to contact the investigator immediately (through a 24 h paging system) if the patient had any signs or symptoms perceived as serious during the period from the first study procedure to 1 month after the last dose of study medication. Less serious adverse events were recorded in the patient's diary and reviewed monthly by an investigator. The nature, severity, and potential relation to study drug of each adverse event were recorded in the case-report form and regularly reviewed in summary format by the data and safety monitoring board.

Role of the funding source

This study was supported entirely by intramural research funds from NINDS, NIH, USA. There was no other funding source, and the investigators had sole discretion over study design, collection, analysis, and interpretation of data, writing of the report, and decision to submit it for publication.

Results

48 participants met the eligibility criteria, gave informed assent with parent or guardian consent, and were randomly assigned to one of the four treatment groups (figure 1). The overall baseline characteristics of the treatment groups were balanced in both the total population and the subset cohort (table 1). There were no protocol deviations and no drug discontinuations, although one patient in the low-dose group could not undergo follow-up testing because of intercurrent illness and was excluded for the final analysis of ICARS and FARS (these data were not imputed), but was included in the primary endpoint analysis and ADL assessment. Compliance rates based on diary entries for the placebo, low-dose, intermediate-dose, and high-dose groups were

	Placebo (n=11)	Low dose (n=12)	Intermediate dose (n=13)	High dose (n=12)
Anxiety	0	2 (16%)	2 (15%)	0
Chest pain	2 (18%)	1 (8%)	2 (15%)	1 (8%)
Decreased concentration	0	0	2 (15%)	0
Diarrhoea	0	2 (16%)	3 (23%)	0
Dyspepsia	1 (9%)	2 (16%)	2 (15%)	1 (8%)
Epistaxis	2 (18%)	0	0	0
Fever	0	3 (25%)	0	0
Gastroenteritis	1 (9%)	3 (25%)	5 (38%)	2 (16%)
Headache	4 (36%)	5 (42%)	5 (38%)	6 (50%)
Myalgia	4 (36%)	4 (33%)	5 (38%)	2 (16%)
Muscle sprain	0	1 (8%)	0	2 (16%)
Nausea	2 (18%)	2 (16%)	3 (23%)	1 (8%)
Pharyngitis	0	2 (16%)	2 (15%)	0
Respiratory tract infection	5 (45%)	7 (58%)	6 (46%)	6 (50%)
Total patients with any event	58 (22%)	80 (31%)	72 (28%)	48 (19%)

*≥10% for any group.

Table 2: **Most common adverse events***

94%, 89%, 91%, and 91%, respectively (91% overall), whereas on the basis of residual pill counts, compliance was 94%, 99%, 95%, and 97%, respectively (96% overall).

There were a total of 258 adverse events recorded during this study: 238 were graded as mild, 18 were moderate, and two were severe and serious enough to result in hospital admission. One serious adverse event (chest pain) occurred in a patient receiving placebo and the other (nausea, vomiting, and dehydration) in a patient in the low-dose group 3 weeks after study completion and was judged to be unrelated. Only one adverse event was deemed to be possibly related to the study drug. This was the development of neutropenia (absolute neutrophil count 736×10⁶/L) in a male patient in the high-dose group after 6 months of treatment, which resolved within 1 week of drug discontinuation. There were no deaths in this study and no safety concerns with respect to vital signs, laboratory testing (except for the neutropenia noted), or electrocardiograms.

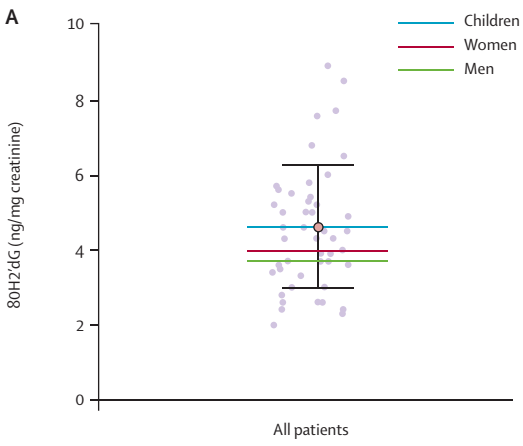

***Figure 2*: Urinary 8OH2′dG concentrations and change after treatment**
A: Baseline concentrations of 8-hydroxy-2′-deoxyguanosine (8OH2′dG) in urine, as analysed by use of carbon column-based liquid chromatography with electrochemical detection and normalised to urinary creatinine. Individual values are shown, with mean (±SD). Mean reference controls for children, adult women, and adult men are also shown.[17] B: Mean (±SEM) absolute changes in the concentrations of urinary 8OH2′dG after 6 months of treatment with either placebo, low-dose, intermediate-dose, or high-dose idebenone are shown.

	Difference in change between group and placebo (95% CI)	p	
		Uncorrected	Bonferroni
Low dose	0·44 (−0·20 to 1·08)	0·17	0·52
Intermediate dose	0·25 (−0·38 to 0·88)	0·42	1·00
High dose	0·33 (−0·32 to 0·99)	0·31	0·93

p values and 95% CIs were derived using an ANCOVA model applied to the entire study sample.

***Table 3*: Estimated differences in 8OH2′dG change among groups**

Overall, there was a relatively balanced distribution of adverse events, with 58 in the placebo group (22%), 80 in the low-dose group (31%), 72 in the intermediate-dose group (28%), and 48 in the high-dose group (19%). Table 2 shows the most frequently reported adverse events (occurring in >10% of patients in any treatment group). Adverse events were also assessed to determine whether they were more often associated with active drug and higher drug concentrations. The analysis combined the results across the different strata (different categories) into a single chi-squared statistic using a generalised Cochran-Mantel-Haenszel approach. This approach combines results of different adverse events and assumes that the reporting of events is done independently. The results indicate that there is not an increasing incidence of adverse events in the four dose groups (p=0·77). If the groups are collapsed into only two categories (placebo and active drug), the results are still not significant (p=0·24).

Two batches of samples (48 baseline and 48 follow-up) were analysed by ESA Laboratories for creatinine and 8OH2′dG by use of carbon column-based liquid chromatography with electrochemical detection.[17] At baseline, the mean (±SD) of the concentration of urinary 8OH2′dG for all 48 patients was 4·5±1·6 ng/mg creatinine (range 2·0–8·9 ng/mg) as compared to the mean of 10·4 ng/mg previously reported in FA patients (figure 2).[6] This level is mildly increased in comparison to the controls in the previous study (3·5 ng/mg) and comparable to the means of controls studied during the development of this assay (3·7–4·6 ng/mg).[17] Although we did not measure matched controls in our study, the results indicate that this metabolite failed to measure oxidative stress (if present) in our study population. After 6 months of treatment (figure 2), the mean (±SD) concentrations of urinary 8OH2′dG were reduced in all groups (−1·51±1·3 ng/mg) with no significant difference between the treatment groups (p=0·57, by ANCOVA). Table 3 shows differences between the placebo and each active drug group for 8OH2′dG. The results reinforce the non-significant p value associated with the ANCOVA *F*-test, in that no comparison is suggestive of a difference. The use of the baseline metabolite concentration as a covariate has the effect of reducing the magnitude of differences seen in the raw data and in figure 2.

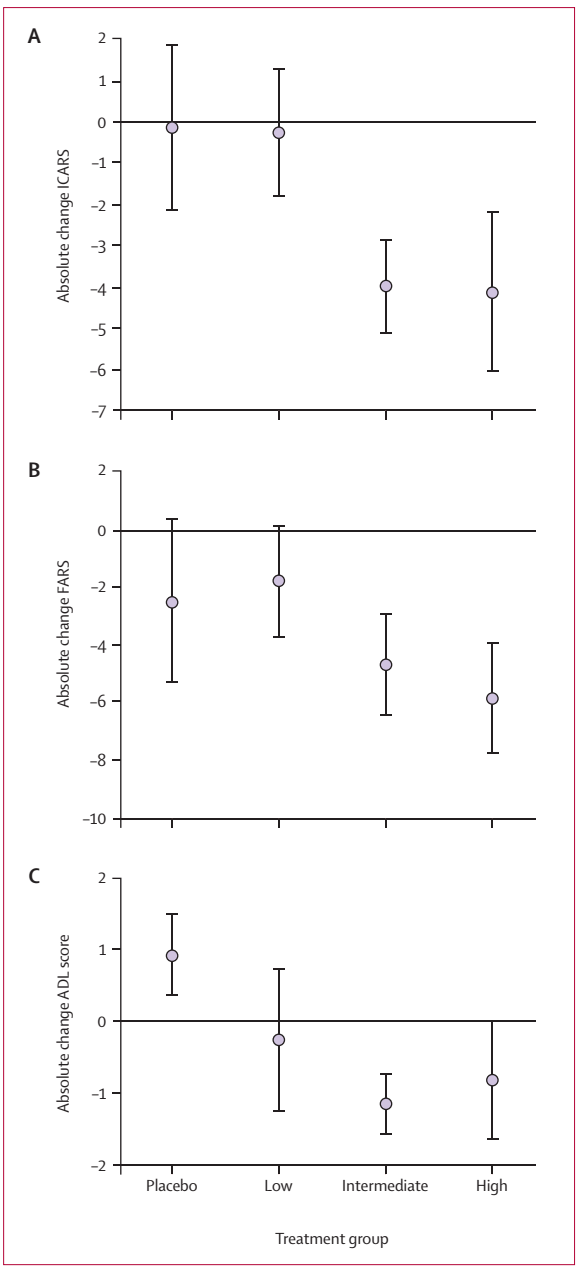

Figure 3: **Neurological and functional change by treatment group in the total study population**
There was no overall significant change between treatment groups by ANCOVA in (A) international cooperative ataxia rating scale (ICARS, p=0·17), (B) Friedreich's ataxia rating scale (FARS, p=0·47), or (C) activities of daily living (ADL, p=0·22) scores. There were indications of a dose-dependent improvement in ICARS (p=0·03), but not FARS (p=0·14) or ADL (p=0·16) scores, by use of the Jonckheere trend test. Means (±SEM) are shown.

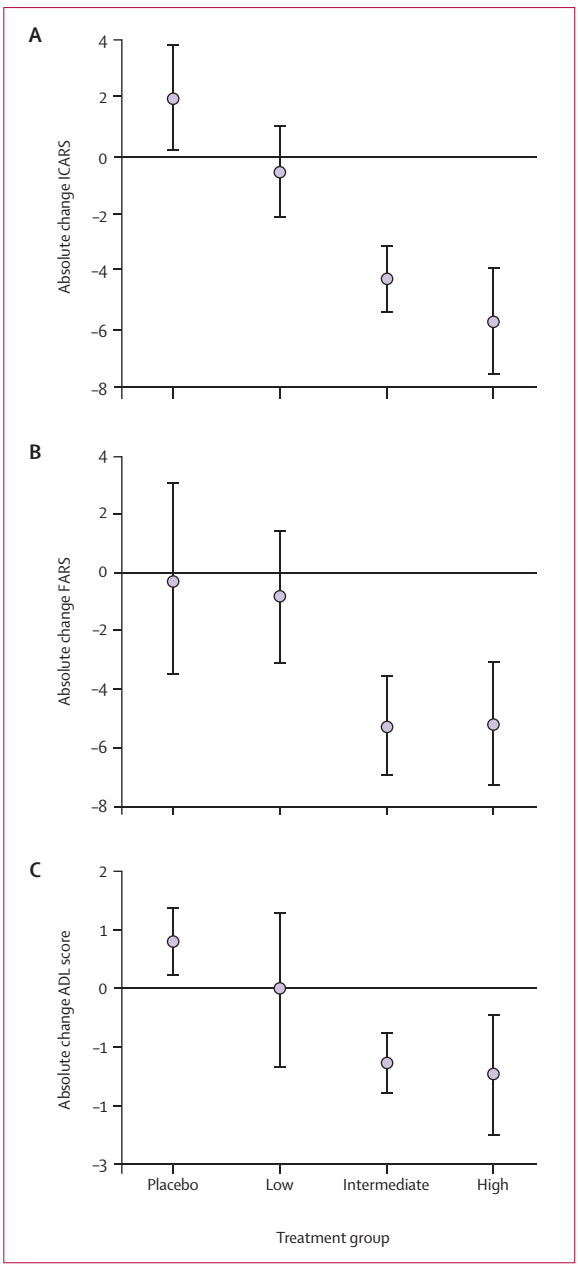

Figure 4: **Neurological and functional change by treatment group in the ambulatory subgroup**
There was a significant change between treatment groups by ANCOVA (p=0·03) and by the Jonckheere test (p=0·006) in (A) international cooperative ataxia rating scale (ICARS), but not in (B) Friedreich's ataxia rating scale (FARS, p=0·93 and p=0·12, respectively), or (C) activities of daily living (ADL, p=0·57 and p=0·15, respectively) scores (p values adjusted for multiple comparisons). Means (±SEM) are shown.

A paired *t*-test of the placebo group values suggests that a batch effect was present (t=4·81, df=10, p=0·0007) if the baseline and 6-month samples are compared, and was due to the separate analysis of the samples. However, this effect should be seen in samples from all groups and would not have obscured the hypothesised drug effect had it been present (ie, an ANCOVA model would still have been able to detect a greater decline in the higher-dose groups).

Neurological function of patients was assessed at the beginning and end of the study by use of ICARS and FARS by a single rater to reduce interrater variability. In

addition, an ADL survey that captures the patient's ability to perform various daily tasks was completed by the parent(s) or guardian(s).[15] The ICARS range is 0–100 points, FARS is 0–117, and the ADL survey is 0–36, with the higher scores reflecting a greater degree of disability. The baseline scores for each treatment group are shown in table 1. After 6 months of treatment, there was no significant difference among the groups in the degree of change from baseline in ICARS (p=0·17), FARS (p=0·47), or ADL (p=0·22) by ANCOVA (figure 3). There was an indication of dose-dependent improvement in ICARS (p=0·03) but not FARS (p=0·14) or ADL (p=0·16) on the Jonckheere trend test.

Because of limitations in the scales, the clinical assessors reasoned that a ceiling effect may be encountered in wheelchair-using patients (FA stage ≥4),[15] and a floor effect for the one symptom-free individual. Therefore, a second, pre-specified analysis was done on patients with ICARS scores more than 10 but less than 54. The mean scores for this subgroup are more homogeneous, indicating less variability in the disability level (table 1). Analysis of this subgroup showed an improvement in ICARS (p=0·01), but not in FARS (p=0·31) or ADL (p=0·19) by ANCOVA (figure 4). The evidence for dose-dependent change became stronger for ICARS (p=0·002), FARS (p=0·04), and ADL (p=0·05) on the Jonckheere test. By use of a Bonferroni correction for multiple comparisons, the change in ICARS is significant, with an adjusted p value of 0·03 by ANCOVA and 0·006 with the Jonckheere test. Table 4 shows pairwise differences in the ICARS score between the placebo and each active drug group. The results suggest that the placebo versus high-dose groups and placebo versus intermediate-dose comparisons are responsible for the overall significance of the ANCOVA *F* statistic.

	Difference in change between group and placebo (95% CI)	p	
		Uncorrected	Bonferroni
Low dose	1·99 (−3·57 to 7·54)	0·47	1·00
Intermediate dose	6·24 (1·60 to 10·89)	0·010	0·03
High dose	7·76 (2·96 to 12·56)	0·003	0·010

p values and 95% CIs were derived using an ANCOVA model applied to the subset sample.

Table 4: **Estimated differences in ICARS change among groups**

Discussion

This study examined the safety and efficacy of three doses of idebenone compared to placebo in young, ambulatory FA patients. The primary aim of this study was to determine whether idebenone treatment decreases urinary 8OH2′dG, a marker of oxidative stress,[6] in a dose-dependent manner. Since urinary 8OH2′dG was previously reported to be raised in FA patients, decreased after low-dose idebenone treatment, and detection of a change required a small sample size, its use seemed an attractive method of assessing the biological effects of higher doses of idebenone in FA patients. However, in our patient population, we did not observe the increase in urinary 8OH2′dG concentrations that had been previously reported,[6] and although some of the values were greater than the reference mean, they were within the range reported in the development of the assay.[17] Therefore, that we also did not observe a change in the concentrations of this marker after idebenone treatment is not surprising. The reduction in concentrations in all groups after treatment was likely to be due to batch variation. In addition, we could find no correlation between the pre-treatment concentrations of 8OH2′dG and neurological impairment (ICARS and FARS scores), function level (ADL score), age, disease duration, or GAA length (data not shown), indicating that 8OH2′dG may not be a reliable biomarker for this disease, at least for the age range of patients we studied. Other markers of oxidative stress have been investigated, including malondialdehyde and free glutathione, but these markers either did not change or had not been previously investigated with idebenone treatment and therefore could not be used here to determine sample size.[5,18,19] Only malondialdehyde has been reported to correlate with disease severity,[19] but another study failed to find this correlation, possibly due to a smaller number of patients.[5] Whereas oxidative stress may play a role in FA pathogenesis, measuring whole-body markers of oxidative stress may be limited for various reasons: (1) the small subset of affected cells (neurons) relative to the total body pool, (2) the limited diffusion of markers across the blood-brain barrier, or (3) the need for certain stressed conditions to produce an increased concentration. Overall, it may be worth exploring other markers of oxidative stress to either validate or help identify useful biomarkers for FA.

We also examined the effects of idebenone on neurological function and the patients' ability to do specific ADLs. A small open-label study and a double-blind placebo-controlled study did not observe changes in neurological function in FA patients after low-dose idebenone treatment for 1 year.[10,20] However, the primary endpoint for these trials was cardiac function, and the overall functional state of the study population was very heterogeneous, making assessments in a slowly progressive condition difficult. One small, open-label trial reported neurological benefit in young, ambulatory patients that correlated with the serum concentrations of idebenone.[11] These observations suggested that higher doses of idebenone may be required and that the greatest benefit may be seen in those who were in earlier stages of the disease. We recently had completed a dose-escalation and tolerability study of idebenone and found that, although patients tolerated up to 75 mg/kg, the dose-proportional increase in serum concentrations

diminished beyond 55 mg/kg, and at around those concentrations, urine discoloration was occasionally seen due to drug metabolites, which may have confounded blinding.[12,21] We therefore chose to use a fixed dosing regimen that approximated the standard low-dose of idebenone at 5 mg/kg daily and two higher doses of 15 mg/kg and 45 mg/kg daily.

We observed an indication of dose-dependent improvement in total ICARS scores in the total study population. Changes in eye movements and speech made the greatest contribution to the overall change in score, but indications of change were seen in all subscores. Although the pattern of change seemed similar with the FARS scores, the results did not show the same trend as with ICARS; this difference is likely to be due to differences in the type and weighting of subscores and the degree of linearity of each scale. Examination of the baseline data revealed a plateau in the ICARS scores above a score of 54, which included patients who used wheelchairs for most activities but who were not completely wheelchair bound. Based on this observation, a second analysis plan that excluded patients with ICARS scores above 54 at baseline and one patient who was symptom free was prespecified before the database was finalised and the assignment code revealed. The population scores of this subgroup were more homogeneous (table 1), and the analysis showed more differences among treatment groups in ICARS, FARS, and ADL scores, but with less variance.

Based on the structure of this study, there are several sources of error that need to be acknowledged when interpreting these results. First, the scales and survey all have components that are inherently subjective, which may amplify rater variability. In addition, weighting of subscores may also exaggerate the response reported by each scale. There are also effort and fatigue issues that can be compounded by the patient's psychological state. Finally, the number of outcome measures could lead to systematic error. Although we tried to minimise as many of the sources as possible by having only one rater for all of testing, by doing all the tests in the same order at the same time of day, and by integrating the testing so that each scale's components were tested at the same time, each of these components could have introduced sources of bias into our results.

The slowing of the progression of disease has been widely held as the most that could be expected of a treatment for FA. If these results we report here are confirmed, then improvement in neurological function may be possible for FA patients, particularly those treated early in the course of the disease. The overall change in ICARS and FARS scores at higher doses was modest (~4–6 points). However, when considered relative to the baseline score, the change was 10–17%, which is likely to have been clinically meaningful. This inference is supported by the corresponding change in the ADL scores. Whether function would improve in patients treated for longer than 6 months, how much ultimate benefit (if any) would be seen, how long treatment would maintain these effects, and whether and how much later-stage patients might benefit from the drug also remain to be determined.

The exact mechanism of idebenone's effect in FA is unclear. In the absence of a detectable effect based on the hypothesised mechanism (ie, reduced oxidative stress), a non-specific effect of idebenone cannot be excluded. Although idebenone was developed as an antioxidant, it also functions as an electron transport carrier, like coenzyme Q, and has been reported to have various other effects, including stimulation of nerve-growth-factor production and blockade of voltage-sensitive calcium channels.[7] In the murine conditional-knockout model of FA, oxidative stress is not observed, but idebenone nonetheless exerts effects on cardiac measures and increases life span.[22] Idebenone also exerts antioxidant effects and enhances viability of FA fibroblasts in vitro under stressed conditions.[23] Further examination of idebenone in vitro may help us to better understand its mechanism of action and provide a template for other, more potent, drugs.

With regard to safety, there were similar types and frequencies of reported adverse events between placebo and the idebenone treatment groups. A statistical analysis did not reveal an increasing incidence of adverse events across the four dose groups or active drug (the three treatment groups) compared to placebo. In addition, all adverse events were graded as either unrelated, unlikely, or possible, with the exception of neutropenia, which was graded as probable. The occurrence of neutropenia in one patient who was receiving high-dose treatment seemed to be an idiosyncratic drug reaction, which occurred after 6 months of treatment and resolved shortly after discontinuation of the drug. Review of post-marketing data from when the drug was prescribed in Japan revealed 10 cases of neutropenia in approximately 8 million treated individuals. This suggests that neutropenia may be a rare side-effect of idebenone treatment, although it is unclear whether this effect will occur more often at higher doses. Careful monitoring for neutropenia would be advisable in future trials of high-dose treatment to ascertain the risk and association with dose.

Overall, the findings of this study suggest that idebenone at higher doses than previously tested may offer neurological benefits to FA patients and improve their functional capacity. Although this study is not definitive, it helps set the parameters of future phase III trials, including selection of the target population, dose level, safety monitoring, and outcome measures. If the findings of this study are validated, then safe and effective treatment for FA may become a reality. Moreover, high-dose idebenone treatment may be explored in diseases in which oxidative stress and bioenergetic defects are believed to play a part, including neurodegenerative disorders, such as Huntington's disease and Alzheimer's disease, in which only low doses have previously been studied.

Contributors

NADP was involved in study concept and design, study conduct, analysis of data, and drafting of the manuscript. AB was involved in study conduct. NJ was involved in study concept and design, and analysis of data. KHF was involved in study concept and design, and drafting of the manuscript. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest

We have no conflicts of interest.

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