

Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study

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Summary

Background Friedreich's ataxia is caused by a deficiency of frataxin, a protein involved in regulation of mitochondrial iron content. We have reported a combined deficiency of a Krebs-cycle enzyme, aconitase, and three mitochondrial respiratory-chain complexes in endomyocardial biopsy samples from patients with this disorder. All four enzymes share iron-sulphur cluster-containing proteins that are damaged by iron overload through generation of oxygen free radicals. We used an in-vitro system to elucidate the mechanism of iron-induced injury and to test the protective effects of various substances. On the basis of these results, we assessed the effect of idebenone (a free-radical scavenger) in three patients with Friedreich's ataxia.

Methods Heart homogenates from patients with valvular stenosis were tested for respiratory-chain complex II activity, lipoperoxidation, and aconitase activity by spectrophotometric assays, in the presence of reduced iron (Fe^{2+}), oxidised iron (Fe^{3+}), desferrioxamine, ascorbic acid, and idebenone. The Friedreich's ataxia patients (aged 11 years, 19 years, and 21 years) underwent ultrasonographic heart measurements at baseline and after 4–9 months of idebenone (5 mg/kg daily).

Findings Fe^{2+} (but not Fe^{3+}) decreased complex II activity and increased lipoperoxidation in heart homogenate. Addition of ascorbate or desferrioxamine increased some of the iron-induced adverse effects. Idebenone protected against these effects. In the three patients, left-ventricular mass index decreased from baseline to 4–9 months of idebenone treatment (patient 1, 145 g to 114 g; patient 2, 215 g to 151 g; patient 3, 408 g to 279 g).

Interpretation Our in-vitro data suggest that both iron chelators and antioxidant drugs that may reduce iron are potentially harmful in patients with Friedreich's ataxia. Conversely, our preliminary findings in patients suggest that idebenone protects heart muscle from iron-induced injury.

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Introduction

Friedreich's ataxia is a degenerative disease with autosomal recessive inheritance characterised by progressive limb and gait ataxia, areflexia and pyramidal signs in the legs, and hypertrophic cardiomyopathy;^{1,2} the incidence is 1 per 30 000 livebirths. The disease gene encodes a ubiquitous mitochondrial protein called frataxin.^{3–9} Friedreich's ataxia is primarily caused by a GAA repeat expansion in the first intron of the frataxin gene.⁴

We have previously reported a combined deficiency of a Krebs-cycle enzyme, aconitase, and three mitochondrial respiratory-chain complexes (complexes I to III), in endomyocardial biopsy samples from patients with Friedreich's ataxia.¹⁰ All four enzymes share iron-sulphur cluster-containing proteins (ISP), which are extremely sensitive to injury by oxygen free radicals.¹¹ Both heart tissue from patients with Friedreich's ataxia and yeast strains encoding a deleted frataxin-gene counterpart, accumulate intracellular iron;^{7,8,12} we therefore postulated that alteration of ISP might result from iron overload through generation of oxygen free radicals in Friedreich's ataxia.¹⁰

Idebenone, a short-chain quinone analogue acts as a potent free-radical scavenger. We investigated whether this drug can protect mitochondrial enzymes from iron-induced injury in vitro. Since the drug has no known side-effects,^{13,14} we then investigated its effects in three patients with Friedreich's ataxia.

Patients and methods

The patients treated were an 11-year-old girl, and two young adults (aged 19 and 21 years). Informed consent was given. The diagnosis of Friedreich's ataxia was confirmed by detection of a trinucleotide-repeat expansion in the first intron of the frataxin gene (patient 1, expansion of 2.6 kb and 5.0 kb; patient 2 2.8 kb

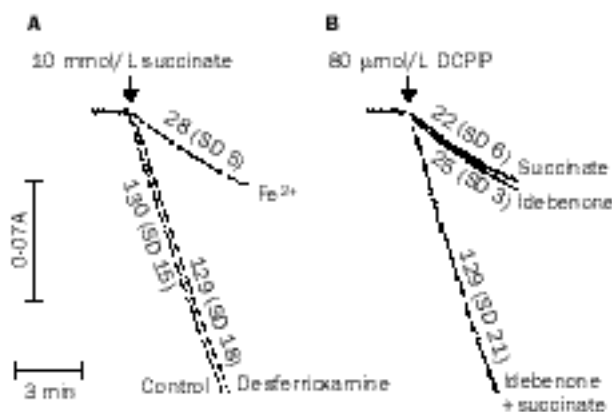


Figure 1: Effects of various substances on complex II in human heart homogenates

A: loss of complex II activity in presence of Fe^{2+} compared with control and full protection of complex II activity by 250 μmol/L desferrioxamine. B: protection of complex II by idebenone (60 μmol/L) in the presence of succinate (10 μmol/L); either succinate or oxidised idebenone alone was not protective.

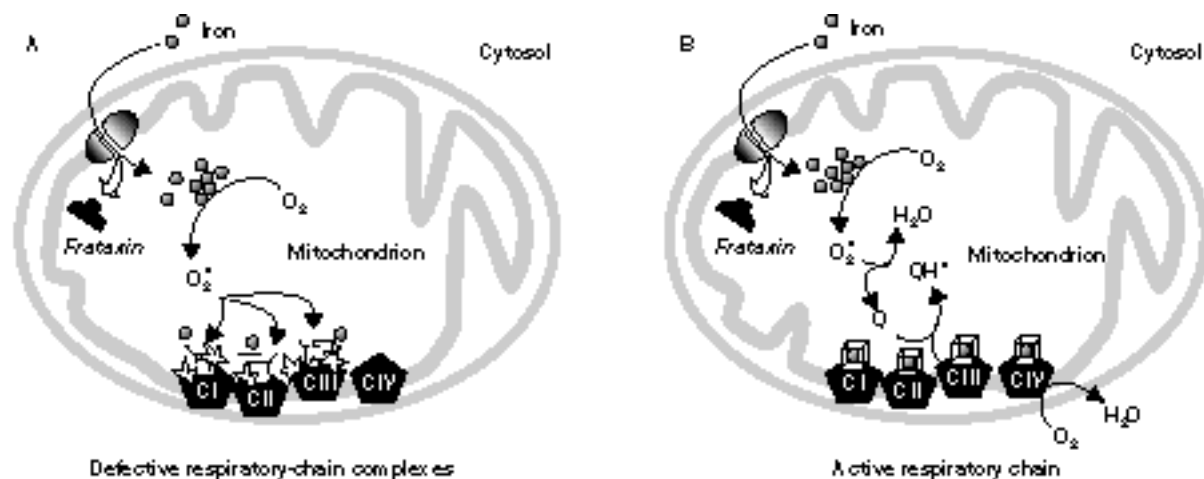


Figure 2: Effects of various substances on lipid peroxidation in human heart homogenates

A: lipoperoxidation in presence of Fe²⁺ or Fe³⁺ plus ascorbic acid.

B: protection of membrane lipids by idebenone plus succinate; either succinate or idebenone alone was not protective.

and 3.5 kb; patient 3 3.1 kb and 4.6 kb; controls below 75 bp). For molecular analyses, the total DNA extracted from leucocytes was amplified by PCR with previously designed conditions and primers.¹⁰ The patients received idebenone (5 mg/kg daily, in three doses) for 4–9 months. Heart measurements were made by the same two ultrasonographers with the same ultrasonographic scanner (Acuson XP128, Mountain View, CA, USA), in the M-mode, on parasternal, longitudinal, and transversal views. The scan was repeated three times on each view, with no substantial variations recorded.

Samples of human heart (10 mg) were obtained surgically with parental consent from three patients (10–15 years of age) with valvular stenosis and no evidence of mitochondrial deficiency. Homogenates were immediately prepared on ice,¹⁵ filtered through a 90 µm nylon net, sampled, and frozen at -80°C for the enzyme studies. Complex II activity in freeze-thaw heart homogenates was measured by an assay of dichlorophenolindophenol (DCPIP) quinone reductase activity.¹⁵ Aconitase activity, corresponding to the combined mitochondrial and cytosolic enzyme activities, was measured by spectrophotometry as aconitate accumulation at 240 nm in the presence of citrate.¹⁰ Idebenone oxidation/reduction status was measured by ultraviolet spectrophotometry (extinction coefficient 12.5 (nmol per L)⁻¹ cm⁻¹ at 275 nm; band-pass 2 nm). All measurements were done with a DW2000 Aminco Spectrophotometer (SLM Instruments, Urbana, IL, USA).¹⁵ Cis-parinaric acid was used as a fluorescent probe for lipoperoxidation¹⁶ in a LS540B Spectrofluorimeter (Perkin-Elmer, Beaconsfield, UK; excitation 318 nm, 5 nm band-pass; emission 410 nm, 5 nm band-pass).

Results

There was a rapid loss of complex II activity (more than 75%) in the presence of reduced iron (5 nmol/L/Fe²⁺; figure 1) but no loss of activity was observed when oxidised iron (Fe³⁺) was substituted for Fe²⁺ (not shown). Idebenone efficiently protected complex II activity against iron-induced injury when reduced in the respiratory chain by succinate (figure 1). In the absence of succinate, idebenone remained oxidised and was ineffective as was

succinate alone. Reduced iron also triggered a rapid membrane lipoperoxidation (figure 2). Similarly, the reduction of oxidised iron by ascorbic acid caused lipoperoxidation in our system (figure 2). Idebenone protected membrane lipids against iron-induced injury, again only in the presence of succinate.

Desferrioxamine, a widely used iron chelator that is known to remove membrane-bound iron, fully protected complex II from iron injury (figure 1). By contrast, in the presence of desferrioxamine, reduced iron decreased the activity of aconitase (basal rate 75 nmol min⁻¹ mg⁻¹ [SD 8]; desferrioxamine alone 73 nmol min⁻¹ mg⁻¹ [SD 11]; Fe²⁺ alone 75 nmol min⁻¹ mg⁻¹ [SD 11] desferrioxamine plus Fe²⁺ 12 nmol min⁻¹ mg⁻¹ [SD 2]). This soluble enzyme of the Krebs cycle also controls cellular iron homeostasis.¹⁷ Thus, iron bound to membranes did not affect soluble enzymes but chelation of the iron by desferrioxamine induced a rapid loss of aconitase activity. Our in-vitro findings suggest caution about desferrioxamine administration in Friedreich's ataxia, because this drug could exacerbate toxic effects of iron on Krebs-cycle enzymes in heart and possibly other organs affected by the disease.

Treatment of the patients with idebenone for 4–9 months was accompanied by substantial decreases in septal thickness, left-ventricular wall thickness, and left-ventricular mass index (table). In addition, in patient 1 the shortening fraction was substantially improved; left-ventricular outflow obstruction decreased (from 40 mm Hg to 10 mm Hg gradient pressure, based on doppler flow) so β-blocker treatment could be discontinued in this patient. Preliminary data on the neurological consequences of short-term treatment suggest that ataxia and deep-tendon reflexes did not change after 4–9 months. However, strength and delicate movements (eg, handwriting) improved, according to parents and teachers.

Parameters	Patient 1			Patient 2			Patient 3		
	Baseline	9 months	Change (%)	Baseline	4 months	Change (%)	Baseline	4 months	Change (%)
Septal thickness (cm)	1.74	1.12	-36	1.51	1.02	-32	1.50	1.03	-31
LV posterior-wall thickness (cm)*	1.18	1.04	-12	1.07	0.86	-20	1.41	1.30	-8
Shortening fraction (%)	47	60	28	37	34	-8	37	38	0
LV mass index (g)	145	114	-21	215	151	-30	408	279	-32

LV=left-ventricular. *Diastole.

Heart measurements by ultrasonography at baseline and after 4–9 months of idebenone treatment

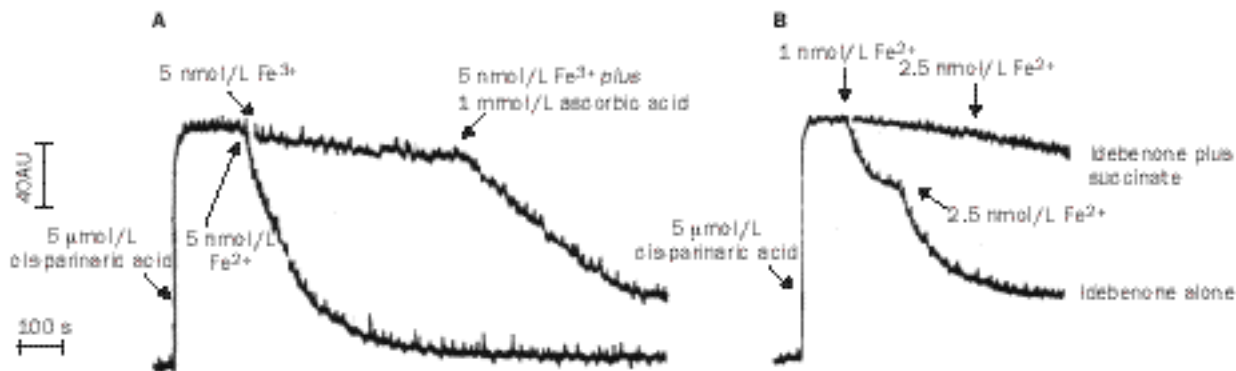


Figure 3: Mechanism of iron toxicity and postulated effect of idebenone in Friedreich's ataxia

A: lack of frataxin leads to mitochondrial iron overload with generation of oxygen free radicals, which damage ISP of respiratory-chain enzymes.

B: idebenone (I), when reduced by the respiratory chain (OH), acts as a free-radical scavenger, enabling reconstitution of ISP and subsequent reactivation of respiratory chain.

Discussion

Friedreich's ataxia has been linked to a deficiency of frataxin, a protein that probably acts as a regulator of iron transport into the mitochondria.⁵⁻⁹ We have previously hypothesised¹⁰ that the disease stems from a mitochondrial iron overload, which triggers the generation of oxygen free radicals. These radicals are toxic for the ISP of the mitochondrial respiratory chain and the Krebs cycle (figure 3).

We have developed an experimental system that mimics the iron-induced damage observed in heart biopsy material from patients with Friedreich's ataxia.¹⁰ In this study iron triggered rapid membrane lipoperoxidation and caused loss of activity in membrane-anchored respiratory enzymes (complex II) in control heart homogenates in vitro. The oxidation/reduction status was important: reduction of Fe³⁺ by ascorbic acid caused cell injury in our system. For this reason, reducing agents (ascorbic acid and glutathione) should be regarded as harmful drugs in patients with iron overload, for these drugs may reduce free iron in vivo. In patients with iron overload ascorbate led to increased peroxidation (triggered by the ascorbate/iron salt mixture).¹⁸ Similarly, although desferrioxamine protected complex II from iron injury, the drug caused pronounced loss of aconitase activity in the presence of Fe²⁺ in our system. Based on these results, we suggest that patients with Friedreich's ataxia should not be given water-soluble chelators or antioxidant drugs that may reduce iron.

Idebenone, a short-chain quinone known to cross cell membranes readily (including the blood-brain barrier),^{13,14} protected heart homogenate from iron-induced injury. 4-9 months idebenone administration to three patients with Friedreich's ataxia was associated with a substantial decrease in myocardial hypertrophy. Randomised studies are a wider way to see whether these results can be confirmed and clarify whether idebenone also improves neurological signs, particularly hand clumsiness, slurred voice, muscle weakness, tremor, gait ataxia, and asynergia or dysmetria.

Contributors

P Rustin devised and initiated the biochemical experiments, which were carried out with K Chantrel-Groussard. J-C von Kleist-Retzow and A Munnich were involved in the initiation of the idebenone treatment and the follow-up of the patients. D Sidi was responsible for the heart measurements. A Rötig was in charge of the molecular studies and characterised the GAA triplet expansion in the frataxin gene of the Friedreich's ataxia patients included in this study, and was also involved in the overall conception of this work. P Rustin and A Munnich wrote the paper.

Acknowledgments

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References

- Geoffroy G, Barbeau A, Breton G, et al. Clinical description and roentgenologic evaluation of patients with Friedreich's ataxia. *Can J Neurol Sci* 1976; **3**: 279-86.
- Harding A. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain* 1981; **104**: 598-620.
- Chamberlain S, Shaw J, Wallis J, et al. Genetic homogeneity of the Friedreich ataxia locus on chromosome 9. *Am J Hum Genet* 1989; **44**: 518-21.
- Campuzano V, Montermini L, Molto MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 1996; **271**: 1423-27.
- Koutnikova H, Campuzano V, Foury F, et al. Studies of human, mouse and yeast homologue indicate a mitochondrial function for the frataxin. *Nat Genet* 1997; **16**: 345-51.
- Priller J, Scherzer CR, Faber PW, McDouals ME, Young AB. Frataxin gene of Friedreich's ataxia is targeted to mitochondria. *Ann Neurol* 1997; **42**: 265-69.
- Babcock M, de Silva D, Oaks R, et al. Regulation of mitochondrial iron accumulation by Yfh 1p, a putative homolog of frataxin. *Science* 1997; **276**: 1709-12.
- Foury F, Cazzalini O. Detection of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. *FEBS Lett* 1997; **411**: 373-77.
- Wilson RB, Roof DM. Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nat Genet* 1997; **16**: 352-57.
- Rötig A, de Lonlay P, Chretien D, et al. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet* 1997; **17**: 215-17.
- Gardner PR, Nguyen DDH, White CW. Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells in rat lungs. *Proc Natl Acad Sci USA* 1994; **91**: 12248-52.
- Sanchez-Casis G, Côté M, Barbeau A. Pathology of the heart in Friedreich's ataxia: review of the literature and report of one case. *Can J Neurol Sci* 1977; **3**: 349-54.
- Gillis JC, Benfield P, McTavish D. Idebenone. *Drugs Aging* 1994; **5**: 133-52.
- Nz-Nagy I. Chemistry, toxicology, pharmacology and pharmacokinetics of idebenone; a review. *Arch Gerontol Geriatr* 1990; **11**: 177-86.
- Rustin P, Chretien D, Bourgeron T, et al. Biochemical and molecular investigations in respiratory chain deficiencies. *Clin Chim Acta* 1994; **228**: 35-51.
- García-Ruiz C, Colell A, Morales A, Kaplowitz N, Fernandez-Checa JC. Role of oxidative stress generated from the mitochondrial electron transport chain and mitochondrial glutathione status in loss of mitochondrial function and activation of transcription factor nuclear factor-KB: studies with isolated mitochondria and rat hepatocytes. *Mol Pharmacol* 1995; **48**: 825-35.
- Hentze MW, Kühn LC. Molecular control of vertebrate iron metabolism: mRNA based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proc Natl Acad Sci USA* 1996; **93**: 8175-82.
- Nienhuis AW. Vitamin C and iron. *N Engl J Med* 1981; **304**: 170-71.