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Drug Insight: Antioxidant Therapy in Inherited Ataxias

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Summary and Introduction

Summary

The inherited ataxias are a large, heterogeneous group of neurodegenerative disorders caused by a variety of gene mutations, the effects of which are exerted through different pathogenic mechanisms. Despite this diversity, oxidative stress seems to be a common factor in the pathogenesis of these disorders, indicating that antioxidants might be potential therapeutics for these currently incurable conditions. Some inherited ataxias, such as ataxia with vitamin E deficiency, are directly caused by defects in small-molecule antioxidants and might be treated by supplying the defective molecule. In most ataxias, however, oxidative stress has more-complex disease-specific causes and consequences, which must be better understood to enable effective treatments to be developed. Results from studies in cellular and animal models need to be brought to the clinic through rigorous trials. The rarity of each of these diseases can, however, make trial design and execution a very difficult task. Challenges include the development of validated clinical assessment tools and biomarkers, and the recruitment of a sufficient number of patients. Despite these obstacles, marked progress has been made in the case of Friedreich ataxia, a disease that has oxidative stress at the core of its pathogenesis. This condition seems to respond to idebenone, a coenzyme Q analog that has antioxidant and oxidative-phosphorylation-stimulating properties.

Introduction

The concept that oxidative damage contributes to the pathogenesis of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease and the inherited ataxias, has prompted attempts to treat these conditions with various antioxidants. The results have been largely negative or inconclusive, partly because the choice of the drug to be tested and the determination of its dosage were often based on an

incomplete knowledge of the relevant pathogenic mechanisms and pharmacological properties of the investigated molecule. Another confounding factor has been the poor design of trials, many of which have been uncontrolled and underpowered, with no clearly defined end point. This situation is changing, however, owing to progress in basic and clinical research and the convergence of these approaches in a translational research arena.

The brain detects and overcomes oxidative stress through a complex network of 'longevity assurance processes' integrated with the expression of genes termed 'vitagenes'. These vitagenes include genes encoding heat-shock proteins, which facilitate correct protein folding, and the gene encoding heme oxygenase-1, an inducible and redox-regulated enzyme that promotes cellular antioxidant defense.[1] Treatments that can activate this response are likely to be neuroprotective in a range of conditions. Various compounds are thought to have this property; for example, acetyl-L-carnitine induces heme oxygenase-1, in addition to heat-shock protein 70 and the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2, or SODM).[2]

In addition to understanding the general response to oxidative stress, it is now becoming possible to dissect the pathogenic mechanisms of different neurodegenerative diseases, the role that oxidative stress has in each case, and the specific pathways that are activated, thereby enabling disease-specific targets for antioxidant treatments to be identified. Knowledge of the basic and clinical pharmacological properties of antioxidant drugs is increasing, and, importantly, the design of clinical trials has improved substantially in recent years. Several advances, including the development and validation of rating scales and other tools (including biomarkers) to assess disease progression, functional capacity and quality of life, better characterization of the clinical features and natural history of neurodegenerative diseases, and the establishment of international consortia of investigators, are paving the way for appropriately designed, controlled and adequately powered studies, even for rare disorders. These efforts have been especially productive in the field of inherited ataxias, in particular Friedreich ataxia (FRDA). FRDA represents a prototypical disease in which oxidative damage directly derives from the primary molecular defect and lies at the core of the pathogenesis. This Review will focus first on the rationale for antioxidant treatment and on the results of clinical trials in FRDA, and then on the possible use of antioxidants for other inherited ataxias.

Pathogenesis of Friedreich Ataxia: The Role of Oxidative Stress

FRDA is an inherited recessive disorder characterized by progressive neurological disability and heart abnormalities. The first symptoms usually develop in childhood, but the age of onset varies, ranging from infancy to adulthood. Atrophy of sensory and cerebellar pathways causes ataxia, dysarthria, fixation instability, deep sensory loss and loss of tendon reflexes. Corticospinal degeneration leads to muscular weakness and extensor plantar responses. Hypertrophic cardiomyopathy can contribute to disability and cause premature death. Other common problems include kyphoscoliosis, pes cavus and, in 10% of patients, diabetes mellitus.[3]

FRDA is most commonly caused by a large expansion of an intronic GAA repeat in the *FXN* gene, which encodes the mitochondrial protein frataxin and has homologs in all eukaryotes and in Gram-negative bacteria.[4] The mutation results in decreased expression of *FXN*.

Experiments *in vitro* and in bacterial plasmids have shown that the pathological lengths of the GAA repeat can adopt a peculiar triple helical structure, involving strand exchanges between two repeat regions in the same or different DNA molecules.[5] This structure, known as 'sticky DNA', strongly inhibits transcription and might cause reduced expression of frataxin. In the eukaryotic nucleus, DNA is associated with proteins to form chromatin, and the expanded GAA repeat has been shown to silence the expression of frataxin by triggering chromatin condensation at the *FXN* gene.[6] It is currently unknown how this phenomenon relates to the structural properties of the repeat region, as demonstrated *in vitro* and in prokaryotic systems.

Frataxin is an essential protein in higher organisms. Yeast cells can survive without frataxin, but they progressively lose mitochondrial function and mitochondrial DNA.[7] An *Fxn* gene knockout is embryonic lethal in the mouse,[8] and a series of conditional knockout mouse models has been developed to circumvent this embryonic lethality. The first two models used Cre transgenes under the control of the muscle creatine kinase (*MCK*) and neuron-specific enolase (*NSE*) promoters to induce striated-muscle-restricted and neuron-restricted exon deletion, respectively.[9] Subsequently, conditional knockouts specific to liver, muscle or pancreatic β-cells were obtained.[10] In all these models, a total lack of frataxin resulted in the eventual demise of the targeted cells. The reason why frataxin is essential for survival, particularly during embryonic development, is still unknown, and it does not clearly emerge from our current knowledge of frataxin function (Figure 1). Even the mechanism of embryonic death is unclear, with no direct evidence of apoptosis.[8]

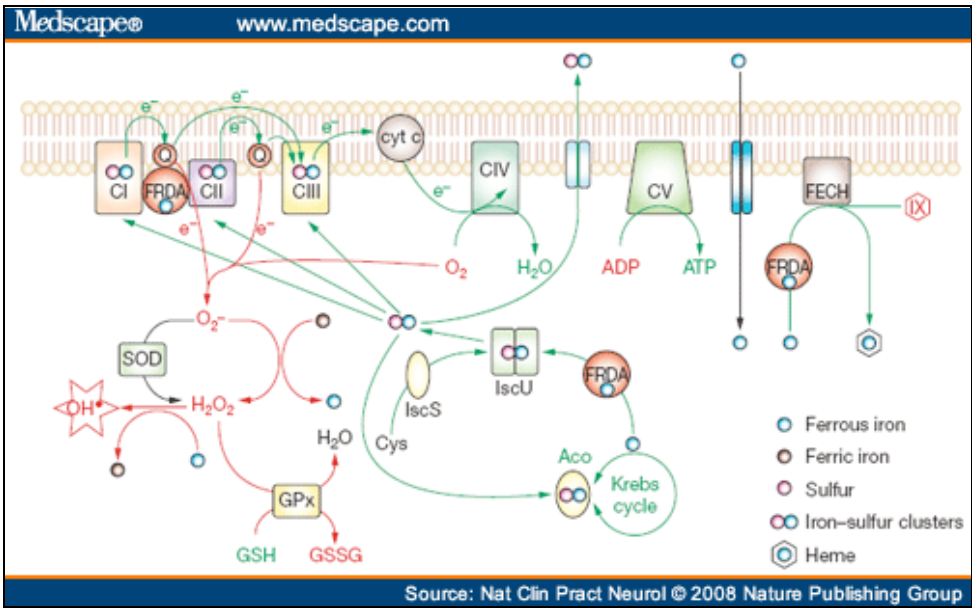

Figure 1.

Frataxin function and oxidative stress in Friedreich ataxia. Several of the postulated functions of frataxin are represented, including the provision of iron for iron–sulfur clusters and heme synthesis, and direct interaction with respiratory chain complexes. Green arrows and text indicate molecules and pathways that have decreased activity in frataxin deficiency; red arrows and text indicate molecules and pathways that have increased activity in frataxin deficiency. Abbreviations: Aco, aconitase; CI, respiratory

chain complex I; CII, respiratory chain complex II; CIII, respiratory chain complex III; CIV, respiratory chain complex IV; CV, respiratory chain complex V; Cys, cysteine; cyt c, cytochrome c; $e,^{1}$ electron; FECH, ferrochelatase; FRDA, frataxin; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; IscS, cysteine desulfurase; IscU, iron–sulfur cluster scaffold protein; IX, protoporphyrin IX; $OH\bullet$, hydroxyl radical; Q, coenzyme Q; SOD, superoxide dismutase.

Studies in yeast and mouse models and biochemical investigations indicate a role for frataxin in the assembly of iron–sulfur clusters (ISCs) in the mitochondrion.[11] This process takes place on the highly conserved scaffold protein IscU. ISCs are complexes of iron and sulfur atoms that serve as prosthetic groups for a series of enzymes with different functions, including energy metabolism (aconitase and complexes I, II and III of the respiratory chain), iron metabolism (iron-responsive protein I and ferrochelatase), purine synthesis and DNA repair. ISC-containing enzymes are localized in various cellular compartments, including the mitochondria, cytosol and nucleus. There is now ample evidence for dysfunction of ISC-containing enzymes in both mitochondria and extramitochondrial compartments as a consequence of frataxin deficiency.[12] Frataxin participates in the assembly of ISCs, possibly by making ferrous iron (Fe^{2+}) atoms available.[13] Similar to other defects in ISC synthesis, frataxin deficiency leads to iron accumulation in mitochondria through an as yet undiscovered mechanism.[14,15] In the yeast knockout model, loss of mitochondrial function and of mitochondrial DNA clearly depends on the amount of iron in the medium, indicating that iron accumulation is not just a secondary phenomenon.[14]

Frataxin-deficient yeast[7] and cultured cells from patients with FRDA[16] are highly sensitive to oxidants, in particular hydrogen peroxide (H_2O_2), probably because Fe^{2+} and H_2O_2 generate the highly toxic hydroxyl radical ($OH\bullet$) through the Fenton reaction. In mitochondria, H_2O_2 derives from the dismutation of superoxide (O_2^-) catalyzed by SOD2, but it can also be produced by a nonenzymatic reaction with ferric iron Fe^{3+}. In turn, O_2^- is generated by the direct addition to oxygen of electrons prematurely leaking from the respiratory chain. Normally, only a small percentage of the electrons carried by the respiratory chain leak prematurely, usually from the semiquinone form of coenzyme Q, but this leakage increases if the respiratory chain is defective. In frataxin deficiency, a vicious circle is established because of the inefficient use of iron for ISC synthesis. Reduced activity of the ISC-containing complexes I, II and III enhances the generation of $O_2,^{1}$ which is turned into H_2O_2 by SOD2 or Fe^{3+}. H_2O_2 is then partially eliminated by mitochondrial glutathione peroxidase, at the expense of the small-molecule antioxidant glutathione, but, in the presence of excess $Fe,^{1}$ it will also generate more toxic $OH\bullet$ species, causing further mitochondrial damage and respiratory chain dysfunction. In addition, a possible iron-independent mechanism of oxidative stress has recently been identified in frataxin-deficient yeast through the observation that signs of oxidative stress in these cells develop even in iron-depleted media, provided that the cells are cultured aerobically.[17]

Increased production of free radicals was directly demonstrated in cultured mouse cells engineered to produce reduced levels of frataxin.[18] Activation of stress pathways in frataxin-deficient cells further supports the pathogenic role of mitochondrial dysfunction and oxidative stress in FRDA. Studies on cultured PC12 cells—rat pheochromocytoma cells that can be differentiated into neurons by adding nerve growth factor—showed, in particular, increased expression and activity of the MAP kinase kinase 4–c-Jun-NH2-terminal kinase (MKK4–JNK) pathway, which might initially be a protective response but also eventually triggers apoptosis.[19] For reasons that are unclear, frataxin-deficient cells also seem to have a reduced ability to mobilize antioxidant defenses, in particular to induce SOD2 expression following mild exposure to oxidants.[20,21]

In patients with FRDA, oxidative stress is revealed by increased plasma levels of malondialdehyde (a lipid peroxidation product),[22] increased urinary 8-hydroxy-2'-deoxyguanosine (a marker of oxidative DNA damage),[23] decreased plasma free glutathione, and increased plasma glutathione *S*-transferase activity.[24] Intriguingly, no evidence of oxidative stress was obtained in studies of conditional knockout mouse models,[25] although the results might have been confounded by various factors, including the admixture of cells that contained normal frataxin with progressively disappearing cells that contained no frataxin, and the almost complete shut down of the respiratory chain in the frataxin-deficient cells.

Measures of Friedreich Ataxia

To study the natural history of FRDA and to perform clinical treatment trials, it is necessary to establish a measure of the severity of neurological dysfunction. It has, however, proved difficult to develop reliable rating scales owing to the complex clinical presentation of the disease, its variable age of onset and rate of progression, and the variety of neural systems that might be affected. The general ataxia scale ICARS (International Cooperative Ataxia Rating Scale) shows good inter-rater reliability and has been useful for the long-term follow-up of patients with ataxia. The ICARS was developed by a special ad hoc committee of the World Federation of Neurology, as part of an effort to produce a valid and reliable instrument that could be used in trials of new therapeutic agents in patients with different types of ataxia.[26] Scores vary from 0 (normal) to 100 (most severe ataxia). The ICARS incorporates various subscores, but clinical experience and validation tests have indicated that these are not as reliable and robust as the total score.

The ICARS was not tailored on the clinical spectrum of FRDA and has not been specifically validated in this disease. By contrast, the Friedreich Ataxia Rating Scale (FARS), a neurological rating scale specifically developed for FRDA, was validated in 2005.[27] The FARS includes assessments of stance, gait, upper and lower limb coordination, speech, proprioception and strength. In addition to the standard neurological examination, the FARS contains three quantitative performance measures and a component that assesses activities of daily living (ADL). Quantitative performance measures include the nine-hole peg test, a timed 25-foot walk and, as a test of dysarthria, a count of how many times the patient can repeat the syllables 'Pah-tah' in 10 s (PATA test). The addition to the FARS of low-contrast letter acuity has also been proposed as a way of globally evaluating visual function, because this test is sensitive to

both optic nerve damage and ocular movement abnormalities—in particular fixation instability—which are typical of FRDA.

Another clinical rating scale, the Scale for the Assessment and Rating of Ataxia (SARA), was recently validated in patients with dominant spinocerebellar ataxia, but it has not yet been evaluated in patients with FRDA.[28] The SARA was conceived to specifically measure ataxia in different disease contexts, even in the presence of other neurological impairments. Although the SARA inevitably misses some components of the complex picture of FRDA, it is likely to be a valuable tool for follow-up because of the major role of progressive ataxia in determining the functional impairment of patients with FRDA, even after they lose the ability to walk. The SARA is rapid (<15 min) and simple to administer, thereby helping to minimize patient fatigue, and it has very high inter-rater and test–retest reliability.

Imaging approaches have been proposed to provide surrogate markers for the neurodegenerative process in FRDA. MRI findings reflect the underlying neuropathology, and they include atrophy of the cervical spinal cord and no or only mild cerebellar atrophy; the latter condition is commonly seen only in advanced cases. This pattern is discrete and scarcely progressive,[3] so volumetric analysis does not seem a promising approach for follow-up. Diffusion-weighted MRI has provided some interesting preliminary results, with automated analysis revealing a progressively increasing apparent diffusion coefficient in the cerebellum. More-sophisticated diffusion-based MRI techniques, including tractography, might have additional potential to reveal the progressive degeneration of specific fiber systems, in particular the pyramidal tract, but the dying-back nature of this degeneration, consequently mostly involving fibers in the spinal cord, renders this approach difficult. Preliminary studies with proton magnetic resonance spectroscopy have also yielded encouraging results, showing a progressive decrease of the neuronal marker *N*-acetyl aspartate in the cerebellum. Quantitative evaluation of cardiomyopathy is also important. Heart hypertrophy can be assessed by ultrasound and also MRI. Functional parameters might be obtained by use of the same technologies. The best functional indicators for cardiomyopathy in FRDA have not yet been determined, but some parameters, such as strain rate and peak mitral angular velocity, seem to be of interest. Metabolic imaging of the heart by phosphorus magnetic resonance spectroscopy in patients with FRDA has clearly shown impaired ATP synthesis that responds to treatment,[29,30] but this technique has been implemented in only a few research-oriented centers.

Biochemical parameters, including the markers of oxidative stress mentioned in the previous section, are possible additional surrogate markers for FRDA. Microarray techniques are also beginning to be used to identify specific changes in gene expression in peripheral tissues—including blood cells—that might represent a signature of frataxin deficiency and respond to drug treatment.

Antioxidants in Friedreich Ataxia

Vitamin E

Early attempts to treat FRDA with antioxidants, before the *FXN* gene was discovered, were prompted by a generic assumption that oxidative stress contributes to neurodegeneration, as well as by the clinical resemblance between FRDA and vitamin E deficiency. Vitamin E is a

potent lipid-soluble antioxidant, deficiency of which can occur secondary to lipid malabsorption, as a result of lipoprotein hereditary disorders (such as abetalipoproteinemia), or as an isolated feature from a defect in liver α-tocopherol transfer protein, which catalyzes the binding of vitamin E to lipoproteins for transport in the blood. Clinically, affected individuals show a spinocerebellar syndrome and loss of proprioception similar to patients with FRDA, although some clinical differences, such as a nodding head tremor, pigmentary retinopathy and a lack of cardiomyopathy, are commonly observed.

Early small, open-label trials of vitamin E in FRDA did not show any therapeutic effect. More recently, but again in an open-label, uncontrolled study, vitamin E at a high dose (2,100 U/day) was given with coenzyme Q10 (400 mg/day) to 10 patients, who were subsequently followed up for more than 4 years.[30] The results were encouraging, as discussed below, but the relative roles of vitamin E and coenzyme Q10 could not be defined. Overall, because of the lack of controlled studies, the variable doses used and the association with other antioxidant medications, vitamin E has not been appropriately tested in FRDA, and no firm conclusions can yet be drawn about its safety and efficacy in this disorder.

N-acetyl Cysteine

N-acetyl cysteine (NAC) is a well-established mucolytic agent that has activity as a sulfhydryl group donor, stimulates glutathione synthesis and functions as a free-radical scavenger. The antioxidant and protective properties of NAC have been demonstrated in a number of cellular models, including some that have relevance to FRDA. On this basis, NAC has been proposed as a treatment for various conditions, including liver, kidney and lung diseases, and as a supportive treatment for HIV infection and cancer.[31] Currently, however, besides its mucolytic properties, it is only recognized as a treatment for toxic effects of paracetamol.

During the 1990s, particularly in the US, many patients with FRDA took NAC, often in combination with selenium and other antioxidants. This resulted in some anecdotal reports of possible improvement in strength and well-being, but also other reports of the drug having no effect. Doses have varied widely and no peer-reviewed articles on the subject can be found in a MEDLINE search. The lack of any controlled trial prevents conclusions from being drawn.

Interestingly, NAC had some protective effect in a cellular model that might be relevant to FRDA. The P19 embryonic carcinoma cell line has stem-cell-like properties and can be induced to differentiate into cells from all three embryonic layers. Retinoic acid treatment induces differentiation into cells resembling neurons and glia. P19 clones stably transfected with antisense frataxin constructs express significantly less frataxin protein than do wild-type cells. During retinoic-acid-induced neurogenesis, these antisense clones show increased production of reactive oxygen species and a striking rise in cell death. NAC treatment at this time has a partial effect—it decreases reactive oxygen species, but it only increases survival of glia-like cells that are not committed to neuronal lineages.[18]

Coenzyme Q Analogs

Idebenone is a short-chain coenzyme Q analog that can participate in electron transport through the respiratory chain. Idebenone is a substrate for complexes II and III,[32] but it marginally

inhibits complex I, strongly favoring succinate oxidation over NADH oxidation.[33] In its hydroquinone (reduced) form, idebenone is a potent antioxidant that can inhibit microsomal lipid peroxidation by ADP–Fe or organic hydroperoxides.[34] These properties prompted clinical trials of idebenone in Alzheimer's disease[35,36] and Huntington's disease,[37] but the results were disappointing, even—in the case of Alzheimer's disease—with doses of up to 1,080 mg/day.[36] Nevertheless, when the first reports emerged that frataxin deficiency causes mitochondrial iron accumulation and oxidative stress in the yeast model, idebenone was touted as a potential therapeutic for FRDA. In 1997, Rustin *et al*. used heart homogenates that they obtained from surgical specimens from patients with valvular stenosis to test the toxic effects of iron and how these effects might be counteracted by several antioxidants and chelators. These experiments showed that $Fe,^{1}$ but not $Fe,^{1}$ increased lipoperoxidation and decreased the activity of respiratory complex II. Ascorbate further increased lipoperoxidation by reducing Fe^{3+} to Fe^{2+}. Desferrioxamine protected complex II from iron injury, but the activity of the Krebs cycle enzyme aconitase was decreased by this treatment. Only idebenone protected complex II, lipids and aconitase from iron injury in the heart homogenates.

Encouraged by these *in vitro* findings, the same authors gave idebenone at a dose of 5 mg/kg/day to three patients (aged 11, 19 and 21 years) with FRDA and hypertrophic cardiomyopathy. In these patients, a decrease in left-ventricular mass index was observed between baseline and 4–9 months of idebenone treatment (patient 1 from 145 g to 114 g; patient 2 from 215 g to 151 g; patient 3 from 408 g to 279 g).[38] After this first pilot study, idebenone was tested at the same dose in several small, mostly uncontrolled, open-label trials. The results of these studies, including a randomized, placebo-controlled, double-blind trial conducted by Mariotti *et al*.,[39] were mostly—although not uniformly—suggestive of a positive effect on cardiomyopathy in FRDA, but no neurological benefit could be detected (Table 1).

The most comprehensive long-term prospective follow-up study of patients with FRDA treated with idebenone was published recently. The trial included 104 patients with FRDA, 88 of whom were treated with idebenone at a dose of 5 mg/kg/day and 16 of whom were given no treatment. Patients were assessed every 6 months over a median period of 5 years, with use of a systematic standardized protocol that evaluated neurological, cardiac and oculomotor functions. In this study, the total ICARS score worsened during follow-up, regardless of whether the patients were treated with idebenone, but there was a trend towards slower deterioration in those who were treated (1.93 ± 0.25 points per year, compared with 4.43 ± 1.56 points per year for untreated patients). Although cardiac hypertrophy decreased under treatment (-4.1 ± 1.5 g/m^{2} per year; $P = 0.008$), cardiac function did not improve, because the ejection fraction also decreased (–1.32 ± 0.29% per year; $P < 0.001$).[40]

In a UK study, 10 patients with FRDA were given coenzyme Q10 (400 mg/day) and vitamin E (2,100 units/day), as mentioned above.[30] After a follow-up of more than 4 years, neurological progression, as measured by the ICARS, seemed to be slower than in a cohort of historical controls, and phosphorus magnetic resonance spectroscopy of the heart revealed improved ATP synthesis that was sustained with long-term treatment.[30] These findings further support the use of lipid-soluble antioxidants in FRDA but, again, suffer from the lack of an appropriate trial

design and the small number of patients involved.

Despite the lack of rigorous, solid evidence of benefit and safety, the use of idebenone or coenzyme Q10 has rapidly diffused among FRDA patients. Idebenone is even reimbursed on a compassionate basis in several European countries. This increasing use demonstrates the urgent need for rigorous testing of the drug, but this has only recently been undertaken (see below).

The first study of idebenone in a mouse model of frataxin deficiency was published in 2004.[41] The model was a conditional knockout in which *Fxn* was deleted in heart and muscle, leading to a progressive, lethal cardiomyopathy. These mice show inactivation of the ISC-dependent enzymes, followed by time-dependent intramitochondrial iron accumulation. Idebenone at a dose of 90 mg/kg/day delayed the onset of cardiac disease, slowed disease progression and delayed death of frataxin-deficient animals by 1 week, but it did not correct the deficiency in ISC-dependent enzymes. Obviously, these results must be interpreted with care. First, although the benefit seems limited, it is important to stress that this animal model has no residual frataxin and develops a rapidly progressive, much more severe disease than do human patients. Second, the dose used was much higher than the dose commonly given to patients with FRDA.

Several clinical observations have highlighted the need to explore higher doses of idebenone in the clinic, including a few case reports of patients showing a decrease in heart hypertrophy only at doses of 10–15 mg/kg/day, and a study that found that the drug was undetectable in the cerebrospinal fluid of three out of five children treated with the usual 5 mg/kg/day dose.[42] Prompted by these observations, an open-label, phase IA dose-escalation trial, followed by an open-label, 1-month phase IB trial, was recently conducted at the NIH Clinical Center, Bethesda, MD.[43] The phase IA study included 78 subjects with FRDA (24 adults, 27 adolescents and 27 children), and the phase IB study included 15 subjects with FRDA (5 adults, 5 adolescents and 5 children). In the first phase of the study, the maximum permitted single dose of 75 mg/kg was achieved in all cohorts with no dose-limiting toxicity. Plasma levels of total idebenone were found to increase in proportion to the drug dose after a single dose of up to 55 mg/kg. In the second phase of the study, 14 out of 15 subjects with FRDA tolerated idebenone at a dose of 60 mg/kg/day given in three divided doses for 1 month, with only mild adverse events being observed. The pharmacokinetic parameters and half-life did not differ significantly between age cohorts. On the basis of these results, a placebo-controlled, double-blind phase II study was designed to further assess tolerability and to obtain initial efficacy data. This trial was conducted in 2006, also at the NIH Clinical Center, and the results on neurological function have recently been published.[44] Patients were aged between 9 and 18 years and were ambulatory. They were stratified according to the length of the GAA repeat, to ensure that the groups were comparable with regard to the proportions of subjects with repeat lengths over and under 800. Between 11 and 13 patients were randomized into each of three dose arms, the dose of idebenone being adjusted depending on whether body weight was above or below 45 kg (Table 2), or to placebo. The primary end point was the change in the level of the oxidative stress marker 8-hydroxy-2'-deoxyguanosine. Secondary end points evaluating tolerability included the type and frequency of adverse events and compliance with the dosing regimen. Secondary neurological end points included two ataxia rating scales (the ICARS and FARS) and an ADL evaluation with the ADL component of the FARS, in addition to the Fine Motor Control and Clinical Global Impression of Change scales. Cardiac parameters were also included as

secondary end points, but these data have not yet been communicated. Microarray studies were performed in peripheral blood lymphocytes, with the aim of detecting changes in gene expression related to the disease and to the drug response.

Surprisingly, no difference in the urinary levels of 8-hydroxy-2'-deoxyguanosine was found at baseline between control subjects and the enrolled patients with FRDA, contrasting with the previous study[23] that prompted the choice of this parameter as a primary end point. Perhaps unsurprisingly, no changes in the levels of 8-hydroxy-2'-deoxyguanosine were observed with treatment. Analysis of safety parameters further supported the excellent tolerability of idebenone, even at high doses. The main adverse event was transient leukopenia in a subject in the high-dose group. Although this adverse effect was fully reversible on drug withdrawal, it mandates regular monitoring of blood counts in individuals taking idebenone at higher doses. With regard to neurological efficacy, a trend towards improvement favoring the high and middle doses of idebenone versus placebo was observed for the total ICARS score. Statistical significance was achieved in a post hoc analysis that combined the middle and high doses and compared them with placebo (P = 0.047). In addition, a prespecified analysis that minimized floor and ceiling effects by excluding patients with very mild impairment (an ICARS baseline score of <10 points) and those with very severe impairment (individuals who used a wheelchair most of the time, corresponding to a baseline score of >54 points) showed a significant improvement with the middle (P = 0.009) and high (P = 0.002) doses. An interesting trend towards improvement was also seen in the ADL score for the middle (P = 0.035) and high (P = 0.083) doses. The total FARS score also showed a nonsignificant trend towards improvement with these doses. The other parameters gave less-consistent results, with no clear trend. Microarray analysis indicates that dose-related differences in gene expression might be identified in patients' lymphocytes and might even correlate with changes in the ICARS score, but these data are preliminary.

Currently, a phase III multicentric, placebo-controlled, double-blind 1-year trial using the same three doses of idebenone in children and adults with FRDA is in progress in Germany, Belgium, the UK, France and The Netherlands. Primary end points are the ICARS and FARS scores, and secondary end points include additional neurological, ADL, quality of life and cardiac parameters. The results are expected at the end of 2008. A shorter, 6-month phase III trial is planned in the US, starting in late 2007.

Finally, new coenzyme Q analogs are currently in the pipeline but have not yet been tested in clinical trials. One of these, mito-Q, is a molecule carrying a positively charged lipophilic triphenylphosphonium cation conjugated to a ubiquinone moiety. Mito-Q selectively accumulates in mitochondria by exploiting the mitochondrial transmembrane potential.[45] The disadvantage of this molecule is its inability to reduce complex III owing to the presence of the phosphonium group, meaning that it functions as an antioxidant but cannot interact with the respiratory chain in the same manner as idebenone. A phase II trial of mito-Q in patients with FRDA is planned in the near future.

Antioxidant Treatments in Other Ataxias

Oxidative damage is involved in the pathogenesis of inherited ataxias other than Friedreich ataxia, both recessive and dominant, so antioxidant treatment might also provide the basis for a therapeutic approach for these disorders.

Ataxia with Vitamin E Deficiency

The pathogenesis of the recessive ataxia AVED (ataxia with vitamin E deficiency) is directly related to impaired antioxidant defenses. AVED is a rare disorder with a symptomatology closely resembling that of FRDA. The condition is more common in individuals of Mediterranean or North African origin than in other populations. The biochemical hallmark of AVED is a very low plasma level of vitamin E (usually <3 mg/l) caused by a deficiency of liver α-tocopherol transfer protein, with normal lipid and lipoprotein profiles.[46] Vitamin E supplementation results in arrest of progression of neurological symptoms and signs and amelioration of established neurological abnormalities in a subset of patients. The response is optimal if therapy is started early in the course of the disease. The usual dose of the RRR stereoisomer of α-tocopherol needed to restore normal plasma levels of vitamin E in adults is between 1,000 mg and 2,400 mg in two daily doses, taken with fat-containing meals.[46] In children, the usual dose is 40 mg/kg/day. The plasma concentration of vitamin E should be measured regularly during therapy, especially in children—ideally, the plasma concentration should be in the high-normal range. Neurological signs do not develop if therapy is initiated in presymptomatic individuals, so all relatives, especially younger siblings of a proband, should be evaluated for vitamin E deficiency.

Ataxias Attributable to DNA Repair Defects

Ataxia teleangiectasia (AT), AT-like ataxia and ataxia with oculomotor apraxia (AOA) types 1 and 2 are all characterized by defective DNA repair, although the pathways involved are different. In AT and AT-like ataxia, the defective genes are primarily involved in the recognition and repair of double-strand breaks. In AOA1, the defective protein, aprataxin, participates in the single-strand-break repair complex following exposure to ionizing radiation or reactive oxygen species.[47] The defective protein in AOA2, senataxin, has both RNA and DNA helicase activity and also participates in a DNA repair pathway.[48] Neurons are nonrenewable cells, and, therefore, they need to effectively repair DNA damage, which would otherwise accumulate until incompatible with further survival. Oxidative stress is the primary cause of DNA damage in neurons and has a key role in the pathogenesis of AT,[49] creating a rationale for antioxidant treatment in this disorder. No rigorous clinical study has been performed to date, but some encouraging data have been obtained in cellular and animal models. In the AT mouse model, antioxidants reduced tumor formation and corrected the neurobehavioral phenotype.[50] The most extensively studied molecule in this model is NAC, but other compounds have also been effective, including tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl) and CTMIO (5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxyl),[50] but not vitamin E.[51]

Autosomal-dominant Spinocerebellar Ataxias

The autosomal-dominant spinocerebellar ataxias are a large and heterogeneous group of disorders, with varying etiologies and pathogenesis. The most common forms, however, share a single mutation mechanism: the expansion of a CAG repeat in a coding exon of the respective gene, resulting in an expanded polyglutamine (polyQ) tract in the encoded protein. The rationale for antioxidant treatment in these conditions is relatively tenuous. The pathogenesis of polyQ diseases involves a combination of specific mechanisms related to changes in the function and

interactions of the involved protein and a more general toxic effect of polyQ-containing proteins.[52] Evidence for mitochondrial dysfunction and increased oxidative stress has been found in several of these disorders, although the specific mechanisms might differ to some extent. Antioxidant trials, including those of vitamin E, coenzyme Q10 and idebenone, have been disappointing in Huntington's disease—another polyQ disorder—despite promising results in animal and cellular models.[53] Currently, it is reasonable to assume that appropriately chosen and sufficiently dosed antioxidants might contribute to the treatment of spinocerebellar ataxias, but additional basic and preclinical research is needed, in addition to adequate clinical trials.

Other Ataxias with Mitochondrial Dysfunction

Rare forms of inherited ataxia with mitochondrial dysfunction might also respond to antioxidant treatment. In ataxia with coenzyme Q deficiency, for example, respiratory chain and antioxidant defenses are directly impaired by the primary defect, and replacement therapy with coenzyme Q10 is reported to be effective at a dose of 150–300 mg/day.[54] Ataxia in the context of diseases attributable to mitochondrial DNA mutations that cause respiratory chain impairment might also benefit from antioxidant therapy, including NAC, coenzyme Q10 and vitamin E, although the clinical evidence remains anecdotal. Data in cellular models are encouraging; for example, the antioxidants NAC and dihydrolipoic acid have been effective at improving oxidative phosphorylation in a cellular model of NARP (neuropathy, ataxia and retinitis pigmentosa), an ataxic syndrome caused by a mutation in mitochondrial DNA.[55]

Conclusions

The inherited ataxias encompass a wide spectrum of disorders. Oxidative stress is likely to contribute to the pathogenesis of most, if not all, of these conditions, although its specific role and mechanisms vary between different diseases. FRDA has so far provided the best example of a disease in which oxidative damage occurs as a direct consequence of the molecular defect and lies at the root of the pathogenic process. Use of antioxidant treatment in FRDA is founded on increasingly solid knowledge of the disease process and on appropriately designed clinical trials. This is a remarkable accomplishment for a rare disorder, and it owes much to the establishment of large collaborations, involving basic and clinical scientists. The role of patients' advocate groups in supporting research and encouraging collaboration has been crucial, prompting pharmaceutical companies to sponsor clinical trials, despite the niche nature of the prospective market. Eventually, treatment guidelines will be supported by the same standards of evidence sought for more common disorders, rather than the anecdotal, empirical evidence usually available for rare diseases. Whether antioxidant treatment will turn out to be effective in other ataxias is less certain, although in some cases the rationale is strong. It is important that all efforts to evaluate these treatments maintain the highest methodological standards, despite the difficulties caused by the rarity of these diseases, as the story of FRDA has clearly shown.

Table 1. Clinical Studies of Idebenone in Friedreich Ataxia Carried Out Before the NIH phase I and II Trials.

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Study	Design	*n*	Idebenone dose (mg/kg/day)	Treatment period	Findings
Schulz *et al.* (2000)[56]	Open-label	8	5	8 weeks	Urinary 8-hydroxy-2'-deoxyguanosine decreased by ~20% ($P<0.05$)
Artuch *et al.* (2002)[57]	Open-label	9	5	1 year	Participants aged 11–19 years showed an improved ICARS score after 3, 6 and 12 months
Hausse *et al.* (2002)[58]	Open-label	38	5	6 months	Left ventricular mass index decreased by ~20% in half of the patients ($P<0.001$); ataxia not quantified
Rustin *et al.* (2002)[59]	Open-label	40	5	6 months	Left ventricular mass index decreased by ~20% in half of the patients ($P<0.001$)
Buyse *et al.* (2003)[60]	Open-label	8	5	1 year	Left ventricular mass index decreased by ~20% ($P<0.0001$); increased in ataxia (CAG scale)
Mariotti *et al.* (2003)[39]	Double-blind and placebo-controlled	29	5	1 year	Left ventricular mass index decreased by ~6% vs 11% increase in the placebo group ($P<0.01$); no neurological differences (ICARS)

Abbreviations: CAG, Cooperative Ataxia Group; ICARS, International Cooperative Ataxia Rating Scale.

Table 2. Doses of Idebenone used in the NIH Phase II Trial.[44]a

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Patient body weight (kg)	Low dose (mg/day)	Middle dose (mg/day)	High dose (mg/day)
≤45	180	450	1,350
>45	360	900	2,250

[a]Drug administered in three divided doses (e.g. 450 mg = 150 mg three times daily).

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Sidebar: Key Points

Oxidative stress is a key factor in the pathogenesis of inherited ataxias, but the mechanisms involved vary between different conditions, necessitating distinct therapeutic approaches

Friedreich ataxia (FRDA) is caused by deficiency of a mitochondrial protein, frataxin, which has direct antioxidant and iron-regulatory functions, making this disease a prime candidate for antioxidant therapy

Clinical rating scales and biomarkers for FRDA are rapidly being developed and are beginning to be used in controlled, double-blind clinical trials

Several antioxidants have been tested in FRDA, but placebo-controlled, double-blind trials have only been conducted with idebenone; a recent phase II trial provided very encouraging results regarding the safety and possible efficacy of this drug

A few rare forms of ataxia are directly attributable to the deficiency of small-molecule antioxidants—such as vitamin E and coenzyme Q10—for which replacement therapy is possible

There is a rationale for testing antioxidants in ataxias attributable to DNA repair defects, autosomal-dominant spinocerebellar ataxias and ataxias with primary mitochondrial dysfunction, but no controlled clinical trials have been conducted to date

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