Free Radical Research, 2002 VOL. 36 (4), pp. 461–466

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healthsciences

Mitochondrial Dysfunction in Friedreich's Ataxia: From Pathogenesis to Treatment Perspectives

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Accepted by Professor H. Sies

(Received 21 June 2001; In revised form 12 September 2001)

Friedreich's ataxia (FRDA), the most common inherited ataxia, is an autosomal recessive degenerative disorder caused by a GAA triplet expansion or point mutations in the *FRDA* gene on chromosome 9q13. The FRDA gene product, frataxin, is a widely expressed mitochondrial protein, which is severely reduced in FRDA patients. The demonstration that deficit of frataxin in FRDA is associated with mitochondrial iron accumulation, increased sensitivity to oxidative stress, deficit of respiratory chain complex activities and *in vivo* impairment of cardiac and skeletal muscle tissue energy metabolism, has established FRDA as a "new" nuclear encoded mitochondrial disease. Pilot studies have shown the potential effect of antioxidant therapy based on idebenone or coenzyme Q_{10} plus Vitamin E administration in this condition and provide a strong rationale for designing larger randomized clinical trials.

Keywords: Friedreich's ataxia; Genetics; Mitochondria; Oxidative stress; Iron metabolism; Cardiomyopathy

CLINICAL, GENETIC AND PATHOPHYSIOLOGICAL FEATURES OF FRIEDREICH'S ATAXIA

Friedreich's ataxia (FRDA) is the commonest form of inherited ataxia with a frequency of one in 50,000 live births. FRDA is an autosomal recessive degenerative disorder characterized clinically by onset before the age of 25 of progressive gait and limb ataxia, cerebellar dysarthria, loss of limb deep tendon reflexes and position and vibration sense, spasticity and extensor plantar responses.[1,2] Neuropathology in FRDA is characterized by early degeneration of large sensory neurons in the dorsal root ganglia, followed by degeneration of sensory posterior columns, spinal–cerebellar tracts, cortical–spinal motor tracts and atrophy of the large sensory fibers in peripheral nerves. Electrocardiographic or echocardiographic signs of hypertrophic cardiomyopathy are present in a large proportion of FRDA patients and cardiac causes are the most common cause of premature death.[1,2] Left ventricular histological abnormalities consist of myocyte hypertrophy, diffuse fibrosis and focal myocardial necrosis.[3–5] Scoliosis and pes cavus are found in about two thirds and diabetes or glucose intolerance in one third[1,2] of the patients.

The causative mutation of FRDA is an abnormally expanded GAA triplet repeat in the first intron of the *FRDA* gene on chromosome 9q13.[6] Ninety-seven percent of FRDA patients are homozygous for the GAA expansion, the remainder carrying a repeat expansion in one FRDA allele and a point mutation in the other.[2,6] The size of the GAA expansion in FRDA patients ranges from about 100 repeats to 1700,[2,6] normal chromosomes having between 8 and

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/10715760290021324

22 repeats.[6] The expression of a number of symptoms/signs in FRDA is dependent upon the length of the GAA repeat expansion in the smaller allele.[2] In particular, the age at onset correlates negatively[2,7,8] and the rate of progression of the disease positively with the number of GAA repeats in the smaller allele.[2] The frequency and severity of hypertrophic cardiomyopathy increases with the size of the GAA expansion in the smaller allele.[2,7,9,10]

Mutations in the FRDA gene, either GAA expansions or point mutations, result in reduced expression of a protein called frataxin[11] which has been shown to be localized to mitochondria.[11–14] In normal subjects, the highest level of expression of the FRDA gene has been found in the heart and spinal cord, intermediate levels in the cerebellum, liver, skeletal muscle and pancreas and very little in the cerebral cortex.[6] The amount of residual frataxin in lymphoblastoid cell lines from FRDA patients correlates with the GAA expansion size in the smaller allele[11] and likely represents the molecular basis of the relationship between GAA expansion size and phenotypic expression of the disease.[2]

Although the function of frataxin is still unknown, there is evidence that this protein is involved in the regulation of mitochondrial iron export[15] and is associated with a specific deficiency of iron–sulfur (Fe–S) proteins.[16,17] Studies of yeast disrupted for yeast frataxin homologue (*YFH*1) gene have demonstrated that lack of the yeast frataxin homologue leads to a severe defect of mitochondrial respiration, loss of mitochondrial DNA, intra-mitochondrial iron accumulation and increased sensitivity to oxidative stress.[12,13,18,19] Iron is a highly reactive species and is potentially toxic by virtue of its capacity to produce free oxygen radicals via iron-catalyzed Fenton chemistry. Excessive production of free radicals would damage lipids, proteins and mtDNA. Accumulation of iron in the heart in FRDA was first demonstrated more than 20 years ago[20,21] but only recently was it shown that the positive iron staining pattern in cardiac tissue from FRDA patients was granular in appearance and had a distribution consistent with mitochondrial iron accumulation.[17] In the same study iron accumulation was also found in hepatocytes and the spleen of FRDA patients. In cardiac muscle samples from FRDA patients collected by biopsy or at post-mortem, reduced activities of respiratory chain complexes, I, II and III and of the Krebs cycle enzyme aconitase have been found.[16,17] These enzymes all contain iron–sulphur (Fe–S) clusters, and therefore frataxin could play a role in mitochondrial iron metabolism and the formation of Fe–S centers, or, alternatively, these enzymes may be targeted because of their particular sensitivity to damage by oxygen free radicals.[16] In fibroblast lines from FRDA patients mitochondrial iron was increased by 40–50 percent[22,23] while total cellular iron content was unchanged.[23] Residual low levels of frataxin expression in human cells are likely to be responsible for the substantially less marked iron overload found in human tissues compared to that found in *YFH*1 knockouts.[13,19] Fibroblasts from FRDA patients do, however, show an increased sensitivity to H_2O_2 and iron stress.[22]

IN VIVO TISSUE ENERGY METABOLISM IN FRIEDREICH'S ATAXIA PATIENTS

Tissue oxidative metabolism can be assessed *in vivo* using phosphorus magnetic resonance spectroscopy (^{31}P-MRS). ^{31}P-MRS is a non invasive technique that detects phosphorus-containing compounds and cytosolic pH. The major compounds detectable are ATP, phosphocreatine (PCr) and inorganic phosphate (Pi). Free (metabolically active) [ADP], the major regulator of the oxidative phosphorylation, can be calculated from the MRS data using the creatine kinase equilibrium expression.[24] ^{31}P-MRS cardiac and skeletal muscle studies of nine FRDA patients (age range 16–40 years) and 10 healthy volunteers (age range 22–41) were performed.

Cardiac Muscle

Cardiac ^{31}P-MRS enables the *in vivo* measurement of PCr to ATP ratio, which has been shown by ^{31}P-MRS and conventional biochemistry to be a good measure of the energetic state of cardiac muscle.[25,26] Patients lay prone in the magnet and standard spin-echo MRI was used to position the heart in the center of the magnet. Cardiac ^{31}P spectra were collected using a 7 cm circular surface coil placed below the chest. Data were acquired using a slice selective one-dimensional spectroscopic imaging technique which separately localizes signal from the chest wall and myocardium.[27] *Trans*-thoracic echocardiography was used to measure posterior wall and septal thickness.

Figure 1 reports cardiac PCr/ATP in nine FRDA patients, five with and four without left ventricular hypertrophy (LVH), but all with normal cardiac function.[28] Cardiac PCr to ATP ratio was significantly reduced not only in FRDA patients with LVH (1.42 ± 0.61 vs. 2.40 ± 0.14 in controls, $p < 0.001$) but was also reduced to about half of the mean normal value in the subgroup of 4 FRDA patients with normal echocardiography (1.24 ± 0.57, $p < 0.001$).[28] PCr is a central metabolite in the biochemical pathways that supply high-energy phosphates for muscle contraction. Through the creatine kinase reaction PCr is in equilibrium with ADP.[29] Thus, if we assume that total creatine is unchanged, low PCr

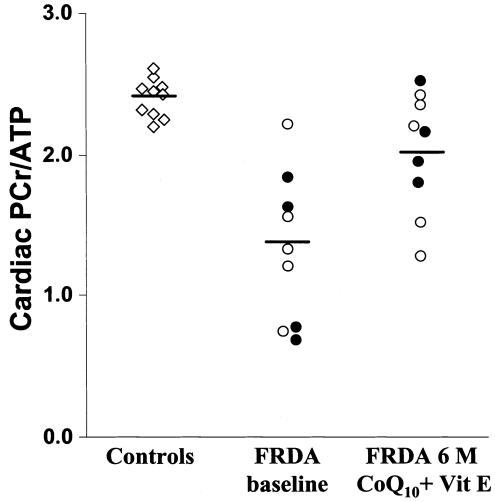

FIGURE 1 Cardiac phosphocreatine (PCr) to ATP ratios in controls and Friedreich's Ataxia patients (FA) before and after six months (6 M) of coenzyme Q_{10} (CoQ_{10}) plus Vitamin E (Vit E) administration.[28,41,42] Horizontal bars indicate group's mean value. Closed circles represent FRDA patients without left ventricle hypertrophy.

content in our FRDA patients would be associated with an increase in [ADP] which is the major driving force of mitochondrial ATP production.[30,31] However, low total cardiac creatine content has been reported in various forms of LVH and its reduction could contribute to low cardiac PCr to ATP ratios in our FRDA patients with LVH. Low total creatine would result in either no, or a proportionally smaller increase in calculated free ADP concentration and reduced fall in the free energy of ATP hydrolysis, respectively. Although we do not know the concentration of total creatine in the heart of our FRDA patients, a reduction in cardiac PCr/ATP is most likely to report on increased [ADP], reflecting impaired oxidative phosphorylation. This hypothesis is supported by the low PCr/ATP ratio also found in the heart of FRDA patients with no LVH or failing heart and by the marked increased in cardiac PCr/ATP after antioxidant therapy effecting mitochondrial function in FRDA patients with and without LVH (see below). Low [PCr] and high [ADP] is a typical finding in skeletal muscle[32] and brain[33] of patients with mitochondrial encephalomyopathies due to a deficit of oxidative phosphorylation caused by mutations within mitochondrial DNA. In mitochondrial encephalomyopathies, high cellular [ADP] represents an increased stimulus for ATP production in the presence of malfunctioning oxidative phosphorylation. We have recently found a similar reduction in cardiac PCr/ATP in patients with the MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes) syndrome[34] which is caused by the A3243G mutation of mitochondrial DNA and is associated with a profound deficit of respiratory chain complex I activity.

Our *in vivo* findings in the heart of FRDA patients show that cardiac PCr/ATP can be reduced in the absence of either left ventricular hypertrophy or failing contractile function, as detected by echocardiography. It can be speculated that the LVH process may be compensatory and caused or contributed to by the bioenergetic deficit, which is also known to stimulate myocyte hypertrophy.[35,36] This hypothesis is supported by the frequent finding of hypertrophic cardiomyopathy in patients with a deficit of oxidative phosphorylation due to mutations of mitochondrial DNA[37,38] where, as in FRDA patients,[2,7] dilated cardiomyopathy often appears when compensation eventually fails.

Skeletal Muscle

Skeletal muscle is an ideal tissue in which to assess *in vivo* mitochondrial ATP production rate by ^{31}P-MRS as it can be studied conveniently at rest, during exercise and in the subsequent recovery phase.[39] During incremental exercise there is a progressive reduction of PCr, hydrolyzed via the creatine kinase reaction in order to buffer ATP concentration. As soon as the exercise is stopped the PCr concentration begins to return to its pre-exercise values as PCr is re-synthesised from ATP. ATP production during recovery from exercise is entirely due to oxidative phosphorylation;[39] thus PCr re-synthesis rate reflects the mitochondrial rate of ATP production. Skeletal muscle ^{31}P-MRS spectra were obtained from the right calf muscle at rest, during an aerobic incremental exercise of plantar flexion and the following recovery period as previously described.[40] Relative concentrations of inorganic phosphate (Pi), phosphocreatine (PCr) and ATP were obtained. The maximum rate of mitochondrial ATP synthesis (V_{max}) was calculated from the initial rate of PCr post-exercise re-synthesis ($V = k \cdot \Delta[PCr]$) and the end-exercise [ADP] ([ADP]end): $V_{max} = V\{1 + (K_m/[ADP]end)\}$.[31]

Muscle V_{max} in FRDA patients (20 ± 6 mM/min) was below the normal range in all cases and the mean V_{max} was reduced to 34% of the normal mean (58 ± 19, $p < 0.001$) (Fig. 2).[41,42] This is also a typical finding in patients with mitochondrial myopathy due to mtDNA mutations.[40] We found that mitochondrial V_{max} was strongly dependent on the size of the GAA repeats in the smaller allele: the higher the number of GAA repeats the lower the mitochondrial V_{max} ($r = -0.74$, $p < 0.01$).[41] This is clear evidence that the GAA expansion is the cause of the mitochondrial deficit and suggests a link between degree of mitochondrial respiration deficit and clinical expression of the disease in other tissues. As reported above, the length of the GAA expansion has been shown to determine the amount of frataxin expressed.[11] Therefore, the residual

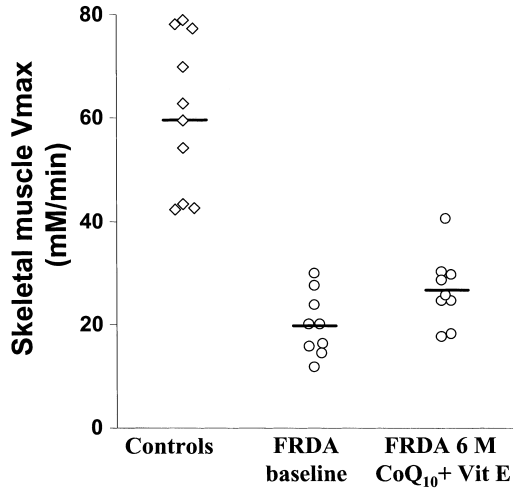

FIGURE 2 Maximum rate of mitochondrial ATP production (V_{max}) in skeletal muscle of Controls and Friedreich's Ataxia patients (FRDA) before and after six months (6 M) of coenzyme Q_{10} (CoQ_{10}) plus Vitamin E (Vit E) administration.[28,41,42] Horizontal bars indicate group's mean value.

expression of frataxin probably determines the reduced skeletal muscle mitochondrial ATP production rate we detected *in vivo*.

THERAPEUTIC PROSPECTIVE

The patho-physiological model of FRDA based on yeast and human studies indicates two possible therapeutic approaches: iron-chelation and antioxidant therapy. The possible role of iron-chelation therapy was recently evaluated.[43] Serum iron, representing available tissue iron supply, and serum ferritin, representing total iron storage, were assessed in FRDA patients. The measurements were within the normal range in all FRDA patients indicating that iron-chelation therapy may not be beneficial to FRDA patients. These results are not entirely unexpected because in FRDA, as mentioned in "Clinical, genetic and pathophysiological features of Friedreich's ataxia" section, there is a iron re-distribution rather than increase in total iron storage. Studies of yeast model of FRDA have shown a small increase in total cellular iron levels in association with a 10–12 times increase in mitochondrial iron content.[13,19] Similarly, selective accumulation of intramitochondrial iron has been found in fibroblasts[23] and cardiac samples of FRDA patients.[17] Taken together these findings suggest that iron-chelation therapy may not be beneficial to FRDA patients.

The excessive free radical production and deficit of oxidative phosphorylation shown in FRDA suggests that the mitochondrial respiration deficit may be amenable to treatment with antioxidants.[44] Three FRDA patients were treated for 4–9 months with idebenone (4 mg/kg daily), a short chain quinone analogue which acts as a free-radical scavenger.[45] Idebenone administration resulted in a reduction in septal thickness ranging from 31 to 36% and of the left ventricle posterior wall from 8 to 20%.

We evaluated the effect of antioxidant treatment (Coenzyme Q_{10}, 400 mg/day plus Vitamin E, 2100 IU/day) on *in vivo* cardiac and calf muscle energy metabolism, LVH and ataxia in a larger group of FRDA patients.[42] After 6 months of therapy cardiac PCr to ATP ratio increased by more than 50% in the patients as a group (2.02 ± 0.43, $p = 0.03$) (Fig. 1). Cardiac PCr/ATP did not increase in two patients, both with LVH, but one was the only patient with normal cardiac PCr/ATP before therapy. There was a greater degree of PCr/ATP recovery in the 4 FRDA patients without LVH (+70%) than in the five patients with LVH (+37%) (Fig. 1).[42] Skeletal muscle mitochondrial V_{max} for ATP production, after six months of CoQ_{10} and Vit E treatment, increased by 34% in the patients' group (Fig. 2), being unchanged in only two patients. Echocardiography showed unchanged interventricular septum and posterior wall thickness in patients with and without LVH.[42] FRDA patients, assessed neurologically using the semi-quantitative International Cooperative Ataxia Rating Scale (ICARS),[46] showed lack of progression of their neurological deficits after six months of therapy.[42] The follow up of the same patients after 24 months of CoQ_{10} and Vit E demonstrated a sustained improvement in cardiac and skeletal muscle energy metabolism associated with lack of progression of both neurological and echocardiographic signs[47] (manuscript in preparation).

CONCLUSIONS

Since the discovery of the genetic basis of FRDA only five years ago, the progress made in our understanding of the pathogenic mechanisms underlying FRDA has been remarkable. Although the precise function of frataxin still remains to be defined, FRDA has clearly been identified as a nuclear encoded mitochondiral disorder. Our and others' pilot studies have indicated the potential effect of antioxidant therapy in this condition. We have now a robust background for designing larger randomized trials which will confirm whether an early diagnosis of FRDA can be exploited to initiate antioxidant treatment and prevent or alleviate the progression of this disorder.

Acknowledgements

Work in the authors' laboratories was supported by the Medical Research Council, the British Heart

Foundation, the Friedreich's Ataxia Group (UK), the National Lottery (JLB), Wellcome Trust short term travel grant (RL), CNR grant #97.01029.PF49 (RL), Fondazione Cassa di Risparmio in Bologna (RL) and Progetto Giovani Ricercatori E.F.ISSS (RL) and Wellcome Clinical Research Training Fellowship (PEH).

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