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Clinical Monitoring in a Patient with Friedreich Ataxia and Osteogenic Sarcoma

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Abstract

Friedreich ataxia is an autosomal recessive neurodegenerative disorder caused by mutations in the *FXN* gene that result in abnormally low levels of the mitochondrial protein frataxin. We recently used a lateral flow immunoassay to measure frataxin levels in a large cohort of controls, carriers, and patients with the condition. Our findings show that frataxin levels do not appreciably change over time and correlate well with GAA₁ repeat length and age of onset; thus, frataxin is a reliable and stable marker for severity of disease. In this paper we present a patient diagnosed as having Friedreich ataxia and osteosarcoma who received combined methotrexate, doxorubicin (Adriamycin), and cisplatin (MAP) chemotherapy over 8 months. We assessed the effect of treatment on frataxin levels, blood cell counts, and clinical markers of cardiomyopathy. Results of the regimen and the use of MAP chemotherapy for treatment of neoplasms in individuals with Friedreich ataxia are discussed.

Keywords

Frataxin; Friedreich ataxia; osteosarcoma; MAP chemotherapy

Friedreich ataxia is an autosomal recessive neurodegenerative disease caused by mutations in the frataxin gene, *FXN*, that result in decreased levels of the mitochondrial protein frataxin.¹ In 98% of patients, there is a homozygous GAA repeat expansion in the first intron on the *FXN* gene, reducing gene transcription; most other patients are heterozygous for GAA expansion on one allele and have a missense mutation on the other allele.¹ The first symptoms typically begin during childhood but age of onset can vary. Progressive limb and

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gait ataxia, posterior column sensory loss, absent tendon reflexes, dysarthria, and muscle weakness are characteristic signs of Friedreich ataxia, but patients may also experience hypertrophic cardiomyopathy and scoliosis.²⁻⁴

The role of frataxin in mitochondria is becoming increasingly clear. Frataxin is involved in iron homeostasis through delivery of iron to the Nfs1/ISCU scaffolding complex and ferrochelatase in Fe-S cluster and heme biosynthesis, respectively.⁵⁻⁷ Frataxin deficiency results in increased mitochondrial iron, decreased activity of Fe-S cluster-containing enzymes, and increased sensitivity to oxidative stress.^{8,9} The current drug pipeline for Friedreich ataxia contains several agents aimed at correcting these mitochondrial deficits.¹⁰

No cure currently exists for Friedreich ataxia, but several potential therapies that aim to restore frataxin protein or mRNA levels (ie, histone deacetylase inhibitors, gene therapy, and erythropoietin mimetics) are being investigated.¹¹⁻¹⁶ Interestingly, cisplatin, a DNA-crosslinking drug used in cancer chemotherapy, can induce expression of both frataxin mRNA and protein in cisplatin-resistant ovarian carcinoma cell lines to promote defense mechanisms against reactive oxygen species damage.¹⁷ For trials of such agents, it will be helpful to measure biomarkers to assess disease severity or effectiveness of potential treatments. Clinically affected tissues are largely inaccessible, so alternatives are needed. Because frataxin protein measured from peripheral blood parallels levels in clinically affected tissue, the use of whole blood for frataxin measurements is clinically relevant as a biomarker.¹⁸

As Friedreich ataxia becomes better characterized, patients are being identified with significant concomitant medical illnesses. Some of these diseases and/or their treatments may interact pathophysiologically with Friedreich ataxia. In this paper, we describe an individual with Friedreich ataxia receiving an 8-month course of methotrexate, doxorubicin (adriamycin), and cisplatin (MAP) chemotherapy. This therapy may intersect the pathophysiology of Friedreich ataxia in 2 ways: first, in the association of increased frataxin levels in vitro with cisplatin treatment, and second, in the reactive oxygen species-based cardiac disease associated with doxorubicin, which could potentially synergize with the mitochondrial dysfunction of Friedreich ataxia to cause major adverse effects. Anthracycline antibiotics, including doxorubicin, are known to cause dose-limiting cardiotoxicity, which may exacerbate the already existent cardiac dysfunction in Friedreich ataxia patients.^{19,20} We sought to determine MAP chemotherapy had an effect on Friedreich ataxia disease state and measured serial frataxin levels to assess changes throughout the course of treatment.

Methods

Frataxin Measurements

We used a lateral flow immunoassay to measure frataxin protein levels as previously described.²¹

Statistical Analysis

Data analysis was performed using STATA SE 11 software and MS Office Excel 2007. Frataxin levels in patient samples were expressed as percentage of average control. Changes in cardiac parameters were analyzed through linear regression.

Case

A 19-year-old woman developed balance and gait difficulties at age 9 and was subsequently diagnosed with Friedreich ataxia at age 13. She carried GAA repeat lengths of 700/1000, and baseline frataxin protein levels were 20.5% of control. Hypertrophic cardiomyopathy,

scoliosis of 35 degrees, and pes cavus were present at baseline evaluation in 2008. This patient began using a walker at age 16. Her initial Friedreich Ataxia Rating Scale score was 65.5, corresponding to an individual with overt and significant symptomatology, including the need for assistance with ambulation. At that time, she was on a supplement-based regimen, including vitamin E, alpha lipoic acid, and coenzyme Q10.

At the age of 19, this individual presented with right leg pain after a fall. Evaluation with X-ray and MRI revealed a lesion of the distal femoral metaphysis on the medial side, which was confirmed to be osteosarcoma by bone biopsy. She was treated with 8 months of MAP chemotherapy, which included methotrexate (12 g/m²; weeks 4, 5, 9, 10, 15, 16, 20, 21, 24, 25, 28, 29), doxorubicin (37.5 mg/m²; weeks 1, 6, 12, 17, 22, 26) and cisplatin (60 mg/m²; weeks 1, 6, 12, 17) (Table 1). After initial therapy, her primary lesion was removed at week 11. Throughout therapy, she experienced alopecia, oral mucosal irritation, neutropenic fevers, and constipation. After therapy, no residual tumor was noted. She had no worsening cardiac symptoms, and although her gait was altered by removal of a portion of her femur, subjectively she had no increase in her neurologic symptomatology.

As cisplatin therapy may increase frataxin levels in vitro, we followed frataxin protein levels over time using both whole blood and cheek swab samples collected during the course of treatment to assess any drug-induced increases or decreases in frataxin protein levels. Because frataxin levels remained relatively stable in control and Friedreich ataxia patient samples over the course of several weeks (data not shown), any changes in frataxin from baseline levels could be attributed to the MAP therapy. Average frataxin levels measured from whole blood and buccal cells were significantly lower than control (28.57% and 12.48%, respectively, $P < .001$). Frataxin levels in buccal cells at the end of the study remained relatively unchanged (Figure 1) when compared with before chemotherapy (13.3% and 16.8%, respectively), though slight variations were seen throughout the study; these may be attributed to drug-induced oral mucosal irritation. In whole blood, frataxin levels increased from approximately 10% of control to over 40% of control after Day 50 of treatment and remained elevated through Day 170 (Figure 1). No whole blood samples were available for frataxin protein analysis after Day 170.

As frataxin levels in blood are associated with several different cell types (platelets, white blood cells, and red blood cells), changes in frataxin levels might simply reflect a change in the relative proportion of these cells in blood as caused by her treatment. Hematocrit, hemoglobin, and red blood cell count decreased during treatment, with an increased red blood cell distribution width above normal limits (Figure 2A). Although white blood cell count decreased slightly, levels remained within the normal range. Mean corpuscular volume remained unchanged, while platelet count decreased slightly toward the end of 8 months of treatment ($R^2 = 0.459$, $P = .004$), consistent with a general thrombocytopenia associated with chemotherapy (Figure 2B).

The anthracycline class of antibiotic drugs, including doxorubicin, is associated with cardiotoxicity due to reactive oxygen species production, which seems highly likely to exacerbate the cardiac deficits already associated with Friedreich ataxia. Cardiac parameters, including intraventricular septum thickness, ejection fraction, and left ventricular posterior wall thickness, were evaluated before, during, and after treatment via echocardiogram to ensure cardiac function did not worsen with MAP therapy (Table 2). Intraventricular septum thickness was not significantly altered before or during MAP therapy ($P = .773$). Ejection fraction did not deviate from the normal range during treatment, and importantly, did not significantly decrease ($P = .418$). Left ventricular posterior wall thickness increased slowly before, during the course of therapy, and after completion ($P = .012$); intraventricular septum thickness and left ventricular posterior wall thickness were both above normal

before start of treatment. Thus, during this short follow-up period, doxorubicin did not markedly worsen cardiomyopathy in this patient.

Discussion

In this study, we followed an individual with Friedreich ataxia undergoing chemotherapy for osteosarcoma and measured frataxin protein levels from whole blood and cheek swab samples during the course of 8 months of treatment. Since the 1980s, most treatment regimens for osteosarcoma have consisted of a combination of methotrexate, doxorubicin, and cisplatin, referred to as MAP chemotherapy.²² Cisplatin is a cancer chemotherapeutic agent that induces apoptosis through formation of platinum-DNA adducts that inhibit DNA replication and transcription.²³ Cisplatin may also induce frataxin expression in ovarian carcinoma cell-lines through promotion of defense mechanisms against reactive oxygen species-induced damage.¹⁷ While these agents are vital for treatment of osteosarcoma, it remained unclear as to what effects the chemotherapy would have on Friedreich ataxia disease state and frataxin protein levels, in particular. In this patient, frataxin protein levels in cheek swab cells are similar pre- and posttreatment, with any fluctuations likely associated with therapy-induced oral mucositis. Frataxin protein levels in whole blood appeared to increase after Day 50 of treatment. This is potentially consistent with the studies in cell lines, but the exact cause of elevated frataxin levels is unclear. These changes could be a result of drug-induced frataxin increase, but also could represent possible selection for blood cells containing higher levels of frataxin, either by selecting cells with shorter GAA repeats or by changing the maturational state of circulating blood cells. Still, the data clearly show that MAP chemotherapy does not suppress frataxin synthesis further than at baseline.

Doxorubicin (adriamycin), another component of MAP chemotherapy, is a member of the anthracycline class of antibiotics that exerts its cytotoxic effects through intercalation of DNA, interfering with replication and slowing proliferation of tumor cells.^{24,25} Cardiac toxicity after treatment for osteosarcoma is exclusively caused by doxorubicin, potentially through increased free radical production and oxidative stress.²² Since cardiac hypertrophy and an increased sensitivity to oxidative stress are already hallmarks of Friedreich ataxia, doxorubicin treatment could potentially exacerbate disease progression. One of the agents in trials for therapy of cardiomyopathy in Friedreich ataxia, deferiprone, is protective against doxorubicin-induced cardiotoxicity in animals, suggesting a link between the mechanisms of Friedreich ataxia and doxorubicin cardiomyopathy. However, there was no systematic loss of cardiac function or significant hypertrophy in this subject, allaying fears that Friedreich ataxia and doxorubicin may synergistically cause progressive cardiac disease. While already outside the normal range, intraventricular septum thickness did not significantly change during treatment. Left ventricular posterior wall thickness increased during treatment; however, the change during treatment was similar in magnitude to that of before and after treatment, suggesting normal progression of hypertrophy in Friedreich ataxia. Mean ejection fraction increased during treatment, but returned toward baseline after treatment; importantly, the ejection fraction remained within normal range throughout the duration of the study.

This patient's family reported that her neurological symptoms improved during the treatment course, but formal neurological assessment is confounded by effects of disease and by the surgical intervention on her femur. Still, the present report shows that an individual with Friedreich ataxia can undergo MAP chemotherapy without the risks of significantly worsened cardiac parameters, neurological deterioration, or further lowering of frataxin levels.

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Ethical Approval The Institutional Review boards of the Children's Hospital of Philadelphia and the University of California at Los Angeles approved the sample collection for frataxin protein analysis as well as the collection of demographic and neurological data. Written informed consent was obtained before any procedures were performed.

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Figure 1.

Frataxin levels measured from buccal cells and whole blood during the course of MAP chemotherapy. Average frataxin protein levels measured from buccal cells and whole blood were significantly lower in patient samples ($n = 21/n = 8$) when compared with samples from a representative control ($n = 6/n = 3$, 12.48%/28.57% of control). Frataxin levels in buccal cells after completion of the study remained relatively unchanged when compared with Day 1 of MAP therapy. In whole blood, frataxin levels slowly elevated after Day 50 of treatment and remained high through Day 170. No whole blood samples after Day 170 were available for frataxin protein analysis.

Figure 2.

Blood cell composition was analyzed during the course of MAP chemotherapy. **(A)** Hematocrit, hemoglobin, and red blood cell count all decreased below normal levels after the start of MAP treatment, with an increased red blood cell distribution width outside of normal limits. White blood cell count decreased slightly, but levels remained within normal range. **(B)** Mean corpuscular volume remained unchanged during treatment, while platelet count decreased slightly toward the end of 8 months of treatment ($R^2 = 0.459$, $P = .004$). All blood cell counts and composition were in the normal range before treatment began. Abbreviations: WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume.

Table 1**MAP Chemotherapy Schedule**

Week	Chemotherapy Agent
1, 6	Doxorubicin 37.5 mg/m ² per day over 24 hours, days 1 and 2 Cisplatin 60 mg/m ² per day over 4 hours, days 1 and 2
4, 5, 9, 10	High-dose methotrexate (12 g/m ²) over 4 hours followed by leucovorin rescue
11	Surgery
12, 17	Doxorubicin 37.5 mg/m ² per day over 24 hours, days 1 and 2 Cisplatin 60 mg/m ² per day over 4 hours, days 1 and 2
22, 26	Doxorubicin 37.5 mg/m ² per day over 24 hours, days 1 and 2
15, 16, 20, 21, 24, 25, 28, 29	High-dose methotrexate (12 g/m ²) over 4 hours followed by leucovorin rescue

Week 1 marks day that first treatment was received; drugs administered in sodium chloride 0.9% IV (doxorubicin) or IVPB (cisplatin); high-dose methotrexate followed by leucovorin rescue to minimize methotrexate toxicity. MAP, methotrexate, doxorubicin (Adriamycin), and cisplatin chemotherapy.

Table 2**Cardiac Parameters Before and During MAP Treatment**

Day	IVS (cm)	EF (%)	LVPW (cm)
-850	1.54	-	-
-681	1.54	-	-
-156	1.32	55–60	1.14
72	1.30	60–65	1.30
178	1.72	65–70	1.48
408	1.65	60–65	1.65

Day 1 marks day that first treatment was received. Measurements made at the Kaiser Permanente Medical Center.

Abbreviations: EF, ejection fraction; IVS, intraventricular septum thickness; LVPW, left ventricular posterior wall thickness; MAP, methotrexate, doxorubicin (Adriamycin), and cisplatin chemotherapy.