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Sensitivity of FRDA Lymphoblasts to Salts of Transition Metal Ions

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ABSTRACT

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease resulting from decreased expression of the nuclear-encoded mitochondrial protein, frataxin. FRDA patients have characteristic iron deposits and dysfunction of mitochondrial enzymes in the heart. Inactivation of the frataxin homologue in yeast causes dysregulation of both mitochondrial iron levels and iron export. Previously, we have observed sensitivity of FRDA fibroblasts to $FeCl_3$ and hydrogen peroxide, results consistent with the hypothesis that FRDA cells may experience increased Fenton chemistry. To determine whether the sensitivity of FRDA cells to transition metal ions is a general or specific property, we have compared the sensitivity of lymphoblasts from FRDA patients and healthy controls to the transition metal salts $CoCl_2$, $CuSO_4$ $FeCl_3$ $FeSO_4$, $MnCl_2$, and $ZnCl_2$. FRDA lymphoblasts were significantly more sensitive to $FeCl_3$ and $MnCl_2$ than control cells. However, there were no significant differences observed in sensitivity to $CoCl_2$, $CuSO_4$, $FeSO_4$ and $ZnCl_2$ in the concentration ranges studied. Thus, the sensitivity of FRDA lymphoblasts exposed to transition metals appears to be specific, and could be relevant to the pathophysiological mechanism, which is discussed. Antiox. Redox Signal. 2, 461–465.

INTRODUCTION

FRIEDREICH'S ATAXIA (FRDA) is the most common hereditary ataxia, characterized by unsteady gait, neuropathy, and cardiomyopathy (Harding, 1993). FRDA is an autosomal recessive disease resulting from decreased expression of frataxin, a nuclear-encoded mitochondrial protein. The most common genetic defect is an intronic triplet expansion of GAA repeats in the frataxin gene (Campuzano *et al.,* 1996).

Results from multiple studies have suggested an iron-dependent mitochondrial oxidative pathophysiological mechanism for FRDA. Frataxin expression is localized to mitochondria in humans and yeast (Campuzano *et al.,* 1997; Wilson and Roof, 1997; Priller *et al.,* 1997; Koutnikova *et al.,* 1997). In yeast, deletion of the frataxin homologue (mYfh1p) causes mitochondrial iron accumulation, sensitivity to oxidative stress, respiratory deficiency, and rearrangements of the mitochondrial genome (Babcock *et al.,* 1997; Foury and Cazzalini, 1997; Wilson and Roof, 1997). Induced expression of frataxin in yeast increases mitochondrial iron efflux (Radisky *et al.,* 1999).

Other support for an iron-dependent mitochondrial oxidative pathophysiology is de-

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rived from heart biopsies of FRDA patients, which exhibit myodegeneration, contain iron deposits (Sanchez-Casis *et al.*, 1976), and exhibit deficiency of the mitochondrial enzymes aconitase and complexes I-III of the electron transport chain (Rotig *et al.*, 1997). These enzymes have previously been demonstrated to contain iron–sulfur clusters, which are known to be oxidant-sensitive (Fridovich, 1995; Keyer and Imlay, 1997). In addition, FRDA fibroblasts are sensitive to oxidative stress by prooxidants $FeCl_3$ and hydrogen peroxide (H_2O_2) which is rescuable by the chelators deferoxamine and BAPTA-AM (Wong *et al.*, 1999). In contrast to fibroblast cultures from FRDA patients, there is a better supply of lymphoblasts, which can be produced by Epstein-Barr virus (EBV) transformation. Thus, to determine whether earlier results regarding $FeCl_3$ sensitivity in fibroblasts were generalizable, and to determine whether the sensitivity of FRDA cells to transition metal ions was general or specific, we have tested the sensitivity of FRDA lymphoblasts to $CoCl_2$, $CuSO_4$, $FeCl_3$, $FeSO_4$, $MnCl_2$, and $ZnCl_2$.

MATERIALS AND METHODS

Cell culture, viability, frataxin expression, and statistical methods

Lymphoblasts were grown in RPMI-1640 supplemented with 15% fetal bovine serum (FBS). Previously, we have observed that expression of frataxin is about 50% the level in fibroblasts of FRDA cases versus controls by reverse transcriptase polymease chain reaction (RT-PCR) analysis (Wong *et al.*, 1999), and results of a Western blot of extracts from FRDA lymphoblasts were similar (Fig. 1). Cell viability was determined by the trypan blue exclusion assay (Wong *et al.*, 1999). Briefly, approximately 1×10^6 cells were seeded in multiwell plates and given increasing concentrations of $CoCl_2$, $CuSO_4$, $FeSO_4$, $FeCl_3$, $MnCl_2$, or $ZnCl_2$. After 6 hr, cells were harvested and resuspended in phosphate buffered saline (PBS). Equal volumes of trypan blue and cell sample were mixed and counted. Student's *t*-tests were carried out to determine the significance values for both control and FRDA lymphoblasts at individual doses and time points.

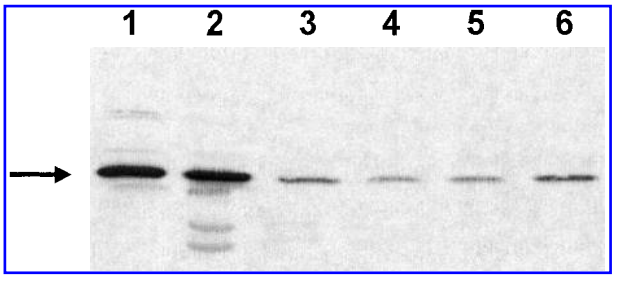

FIG. 1. Western blot analysis of frataxin expression from FRDA patients and controls. Total protein was extracted from FRDA lymphoblasts and assayed, and 100 μg/lane of protein was electrophoresed, blotted, and probed with an anti-frataxin antibody as in Taroni *et al* (1993). Lanes 1 and 2, normal controls; lanes 3–6, cells from FRDA patients.

RESULTS AND DISCUSSION

FRDA lymphoblasts are more sensitive to $FeCl_3$ and $MnCl_2$ than control cells

The viability of lymphoblasts given $CoCl_2$, $CuSO_4$, $FeCl_3$, $FeSO_4$, $MnCl_2$, and $ZnCl_2$ was examined (Fig. 2) after a 6-hr incubation period. FRDA lymphoblasts were statistically more sensitive to $FeCl_3$ than control cells, with 40% and 60% viability at 5 m*M*, respectively (Fig. 2c). On the other hand, both FRDA and control lymphoblasts were not significantly different in sensitivity to $FeSO_4$, having 25% viability at 5 m*M* $FeSO_4$ (Fig. 2d). In addition to $FeCl_3$, FRDA lymphoblasts were statistically more sensitive to $MnCl_2$ than control cells (Fig. 2e). At 5 m*M* $MnCl_2$, FRDA lymphoblasts and controls were 50 and 60% viable, respectively.

Incubation of lymphoblasts in $CuSO_4$, another transition metal salt, yielded no significant differences between FRDA and control lymphoblasts (Fig. 2b). Incubation of cells in $CoCl_2$ or $ZnCl_2$ yielded similar results; however, the cells remained >85% viable at 10 m*M* (Fig. 2a,f).

In conclusion, we observed preferential sensitivity of FRDA lymphoblasts to the salts of transition metals $FeCl_3$ and $MnCl_2$, but not to $CoCl_2$, $CuSO_4$, $FeSO_4$, and $MnCl_2$. There are at

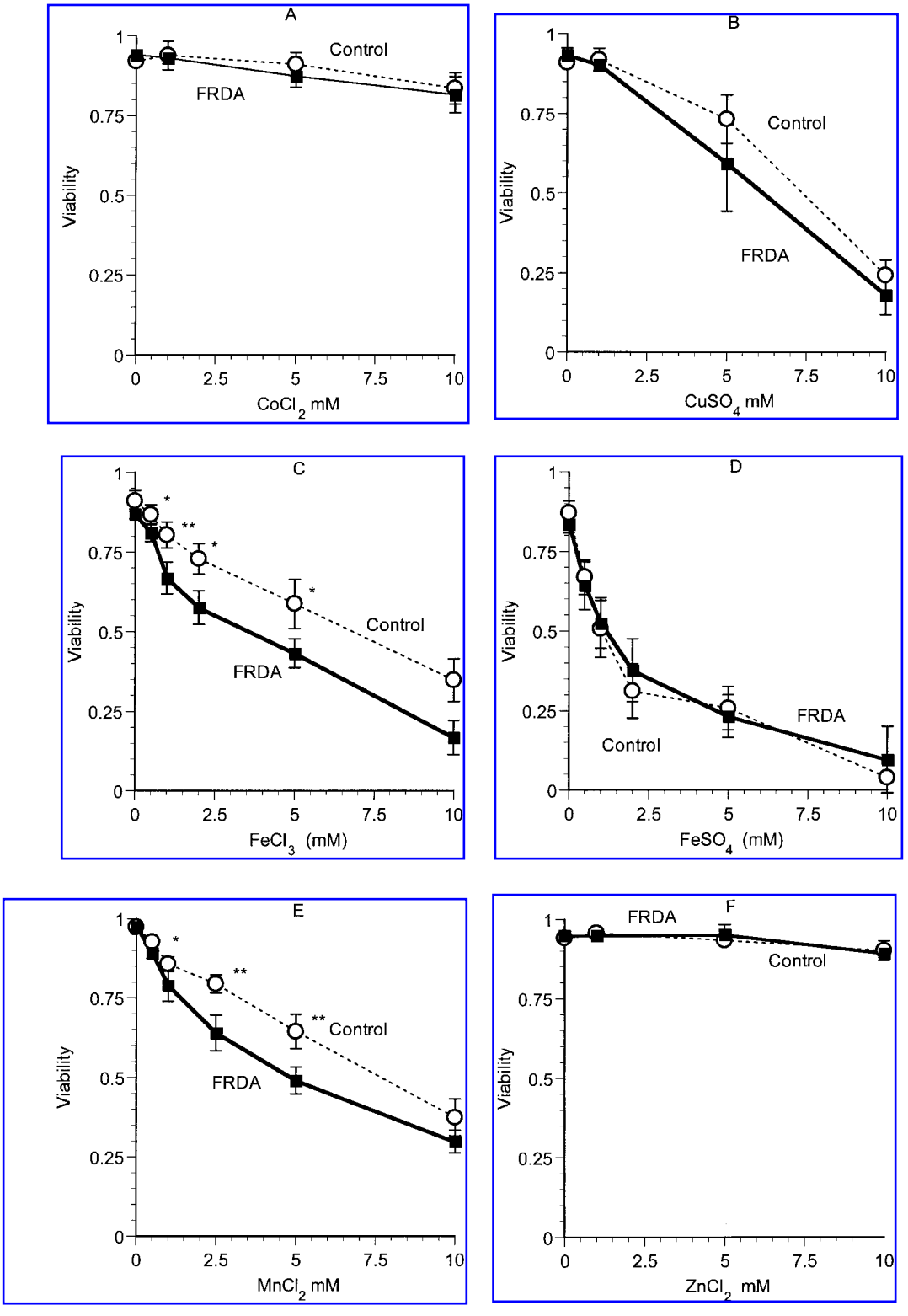

FIG. 2. Sensitivity of FRDA and control cells to increasing concentrations of heavy metal ions. FRDA (filled squares and solid lines) and control (open circles and dashed lines) cells were treated for 6 hr with increasing concentrations of $CoCl_2$ (**A**), $CuSO_4$ (**B**), $FeCl_3$ (**C**), $FeSO_4$ (**D**), $MnCl_2$ (**E**), and $ZnCl_2$ (**F**). Viability was determined by the trypan blue exclusion assay and averages from three to five independent experiments are shown. Error bars represent two standard errors of the mean, and Student's *t*-test was performed to determine *p* values ($*p < 0.05$ and $**p < 0.005$).

least three potential explanations for this observation. First, if the frataxin protein is involved in transport of transition metals or is itself an iron efflux transporter, then the specificity of that transporter could include Fe and Mn, which would not be unreasonable given their similar atomic size and properties. Second, if frataxin were a metal-binding protein, as suggested by recent data (Isaya *et al.,* 1999), it is possible that its specificity could include both Fe and Mn, and decreases in frataxin concentration would be expected to increase the concentration of free Fe and/or Mn, which could initiate Fenton chemistry and be toxic. A third possibility is that frataxin is involved in the transport and/or binding of multiple transition metal ions in mitochondria, but that Fe and Mn are preferentially involved in Fenton chemistry to produce toxic oxygen radicals. There is some evidence that Mn can produce reactive oxygen species by participating in Fenton-type reactions (Berlett *et al.,* 1990), and at high intracellular concentrations, Mn is known to be a neurotoxin (Donaldson, 1987; Aschner and Aschner, 1991).

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ABBREVIATIONS

EBV, Epstein-Barr virus; FBS, fetal bovine serum; FRDA, Friedreich's ataxia; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase polymerase chain reaction.

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