Antioxidant enzymes in blood of patients with Friedreich’s ataxia

G Tozzi, M Nuccetelli, M Lo Bello, S Bernardini, L Bellincampi, S Ballerini, L M Gaeta, C Casali, A Pastore, G Federici, E Bertini, F Piemonte

Background and Aims: Increased generation of reactive oxygen species and mitochondrial dysfunction may underlie the pathophysiology of Friedreich’s ataxia, the most common inherited ataxia, due to GAA expansion in a gene coding for a mitochondrial protein (frataxin), implicated in the regulation of iron metabolism. Because iron overload would cause oxidative stress in Friedreich’s ataxia, we investigated the enzyme antioxidant system in the blood of 14 patients by determining superoxide dismutase, glutathione peroxidase, and glutathione transferase catalytic activities. We also studied the glutathione S-transferase genotype polymorphism in order to evaluate its possible influence on enzyme activity.

Methods: Blood samples were obtained from 14 unrelated patients with Friedreich’s ataxia and 21 age matched healthy subjects. Antioxidant enzyme determinations were spectrophotometrically assayed using specific substrates; the glutathione S-transferase genotype polymorphism was analysed by endonuclease restriction mapping of exon 5 and 6 amplification products.

Results: There was a significant elevation of the superoxide dismutase/glutathione peroxidase activity ratio (0.037 (0.01) v 0.025 (0.008) of controls) and an 83% rise of glutathione transferase specific activity (0.22 (0.1) v 0.12 (0.03) nmol/min/mg protein) in blood of patients with Friedreich’s ataxia than in the controls. The genotype polymorphism of glutathione S-transferase enzyme did not show any relevant differences when compared to that of healthy subjects.

Conclusions: Data show an impairment in vivo of antioxidant enzymes in patients with Friedreich’s ataxia and provide evidence of an increased sensitivity to oxidative stress, supporting a consistent role of free radical cytotoxicity in the pathophysiology of the disease.

Abbreviations: dNTP, deoxynucleotide triphosphate; FRDA, Friedreich’s ataxia; GPX, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; ROS, reactive oxygen species; SOD, superoxide dismutase
patients, and we therefore analysed the enzymatic activity of SOD, GPX, and GST in 14 patients and 21 healthy subjects. We also studied the GSTP1-1 genotype polymorphism in order to investigate its possible influence on GST activity.

METHODS

Chemicals

All substrates and reagents were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). “Ransod” and “Ransel” kits were obtained from Randox Laboratories Ltd, UK. Electrophoresis reagents were from BioRad (Hercules, California, USA). The Taq DNA polymerase, dNTPs (deoxynucleotide triphosphates), and the hydrophobic interaction column (XK 16/20) were from Amersham Pharmacia-Biotech, Inc. (Piscataway, New Jersey, USA).

Sample collection and preparation

Blood samples were obtained from 14 unrelated FRDA patients (nine males, five females) and 21 age matched healthy controls (13 males, eight females). Clinical diagnosis used accepted criteria.\(^3\) Table 1 summarises clinical and genetic information for patients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)</th>
<th>Sex</th>
<th>GAA repeats</th>
<th>Cardiomyopathy</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>M</td>
<td>848/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>B</td>
<td>448/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>J</td>
<td>10</td>
<td>750/750</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>L</td>
<td>10</td>
<td>699/699</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>14</td>
<td>848/1300</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>MA</td>
<td>22</td>
<td>300/810</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>SA</td>
<td>14</td>
<td>1160/833</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>SE</td>
<td>12</td>
<td>1160/1160</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>TC</td>
<td>19</td>
<td>680/350</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>BM</td>
<td>17</td>
<td>814/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>B</td>
<td>8</td>
<td>681/914</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>RF</td>
<td>18</td>
<td>888/888</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>BR</td>
<td>15</td>
<td>848/848</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

As fig 1 shows, SOD activity showed a significant increase in FRDA erythrocytes (1.56 (0.45) v 1.04 (0.28) U/mg).

GST activity was determined using 1-chloro-2,4- dinitrobenzene (CDNB) as cosubstrate, as described previously.\(^2\) In a typical experiment, 100 µl partially purified GST was added to 1 ml (final volume) of 0.1 mol/l potassium phosphate buffer (pH 6.5), containing 2 mmol/l CDNB and 1 mmol/l GSH. The reaction was monitored at 340 nm (e = 9600/M/cm), using a DU-640 spectrophotometer (Beckman Instruments, Inc., California, USA) equipped with a thermostated cuvette holder at 25°C.

Endonuclease restriction mapping GSTP1

Genomic DNA was amplified by polymerase chain reaction (PCR) performed with a DNA thermal cycler (Perkin Elmer 480). PCR for GSTP1 exons 5 and 6 was carried out after a preheating step at 94°C for five minutes through 30 cycles (denaturation at 94°C for one minute, annealing at 64°C for one minute, extension at 72°C for one minute) and a final extension at 72°C for eight minutes.

The mixture for each single reaction in a final volume of 50 µl was as follows: 100 ng genomic DNA, 10 mM Tris (pH 8.3) PCR buffer, 50 mM KCl, 2.5 mM MgCl2, 0.2 mM dNTPs, 5% (v/v) DMSO, 1 unit Taq polymerase, and 0.2 µM of the listed primers:

Exon 5 sense: 5′ CTC TAT TAT GGG AGG AAC CAG CAG G 3′
Exon 5 antisense: 5′ CAA GCC ACC TGA GGG GTA AGG 3′
Exon 6 sense: 5′ AAT CTG GGA CTC TGG TGT CTG G 3′
Exon 6 antisense: 5′ TCT TGC CCT CTC GGT TCT GGG 3′

The amplification product was a 192 bp fragment for exon 5 and a 182 bp product for exon 6. The 192 amplification product was digested overnight at 37°C with Alw26I, which in the presence of the variants B and C generates two fragments of 108 and 84 bp. The 182 bp amplification product was digested overnight at 37°C with Aci I, which in the presence of the variants A and B generates two fragments of 98 and 84 bp. The restriction fragments were evaluated by 12% polyacrylamide non-denaturing gel and electrophoresed for three hours under constant current (45 mA). After electrophoresis, the gel was stained with 0.01% (w/v) ethidium bromide for 10 minutes and DNA fragments were visualised with Fluor-S Max.

Statistical analysis

Data are expressed as mean (SD). The comparison between values obtained in patients and controls was performed by Student’s t test for unpaired data.

RESULTS

As fig 1 shows, SOD activity showed a significant increase in FRDA erythrocytes (1.56 (0.45) v 1.04 (0.28) U/mg,

### Table 1: Clinical data for FRDA patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)</th>
<th>Sex</th>
<th>GAA repeats</th>
<th>Cardiomyopathy</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>14</td>
<td>M</td>
<td>848/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>M</td>
<td>448/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>J</td>
<td>10</td>
<td>M</td>
<td>750/750</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>L</td>
<td>10</td>
<td>M</td>
<td>699/699</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>M</td>
<td>848/1300</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>MA</td>
<td>22</td>
<td>F</td>
<td>300/810</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>SA</td>
<td>14</td>
<td>M</td>
<td>1160/833</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SE</td>
<td>12</td>
<td>F</td>
<td>1160/1160</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>TC</td>
<td>19</td>
<td>M</td>
<td>680/350</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>BM</td>
<td>17</td>
<td>M</td>
<td>814/848</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>F</td>
<td>681/914</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>RF</td>
<td>18</td>
<td>M</td>
<td>888/888</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>BR</td>
<td>15</td>
<td>M</td>
<td>848/848</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
SOD plays a fundamental role in modulating ROS toxicity and its induction seems related to the extent of the redox abnormality in the cell. An increased SOD activity represents an adaptive response to a higher superoxide ion production, and a critical ratio of the enzymes metabolising ROS is needed to protect the cells from free radicals toxicity. Transgenic mice overexpressing SOD activity develop morphological and biochemical abnormalities of neuromuscular tissue similar to those seen in aged animals and exhibit an increased neuronal susceptibility to apoptotic death. Furthermore, changes in the physiological ratios of SOD to GPX have a profound effect on the cellular resistance to oxidant induced damage and on cell killing. An elevation in SOD/GPX ratio induces cellular senescence in transfected cell lines, suggesting a role for ROS in aging. Moreover, patients with Down's syndrome, that carry three copies of SOD and only two copies of GPX gene, display increased SOD activity resulting in higher concentrations of H$_2$O$_2$.

Furthermore, as human GSTP 1-1 gene expression is regulated by the redox status of the cell and H$_2$O$_2$ treatment has been shown to induce protein transcription in human cell cultures, we analysed GSTP1-1 catalytic activity and gene polymorphism in patients with FRDA. We obtained a significant increase of blood GSTP1-1 specific activity, but not relevant differences in frequencies of expression variants. Our suggestion is that the unbalanced SOD:GPX ratio in FRDA may contribute to an overload of H$_2$O$_2$ and, speculatively, lead to a modulation of the second defence line against ROS provided by an increase of GSTP1-1 specific activity. This enzyme activation, however, seems not to reside on a different pattern of protein expression, but it could be caused by a favourable conformational change following the oxidative insult.

In conclusion, our findings give evidence of an impairment in vivo of enzymatic antioxidants in FRDA disease, supporting a relevant role of free radical damage in the pathophysiology of neurodegeneration. This study may also assist in finding a biochemical marker of oxidative stress in FRDA, and in future therapeutic trials.

Figure 1 SOD, GPX, and GSTP1-1 enzyme activities in erythrocytes of 14 FRDA patients. Values are expressed as % of age matched controls (n = 21).

Table 2 GSTP1-1 genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FRDA patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>A/A</td>
<td>64.3</td>
<td>68.8</td>
</tr>
<tr>
<td>A/B</td>
<td>28.6</td>
<td>18.7</td>
</tr>
<tr>
<td>B/B</td>
<td>7.1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

p < 0.0001), whereas GPX activity was comparable to the controls (41.2 (13) v 43.6 (8) nmol/min/mg protein), thus leading to a consistent rise of SOD:GPX activity ratio (0.037 (0.01) v 0.025 (0.008), p < 0.05) in patients. Furthermore, the determination of GST specific activity in FRDA erythrocytes showed an 83% rise (0.22 (0.1) v 0.12 (0.03) nmol/min/mg protein, p = 0.0061), related to healthy subjects.

As at least four GSTP1-1 allelic variants exist which encode functionally different GSTP proteins, we also analysed GSTP1-1 genotype polymorphism in patients with FRDA. However, endonuclease restriction mapping of exon 5 and 6 amplification fragments did not find any significant differences in the relative frequencies of the allelic variants with respect to healthy subjects (table 2).

DISCUSSION

An increased oxidative stress, caused by accumulation of hydroxyl radicals produced by the iron catalysed Fenton reaction and/or a deficiency of iron–sulphur containing proteins with mitochondrial dysfunction are the proposed pathogenic mechanisms in FRDA. Our findings show evidence of an increased sensitivity to oxidative stress in the disease and are in agreement with two recent papers, showing increased concentrations of plasma malondialdehyde and urine 8-hydroxy-2-deoxyguanosine in patients. We assayed antioxidant enzymes in FRDA erythrocytes and obtained a disequilibrium between SOD and GPX activities, with a significant increase of SOD:GPX activity ratio.

REFERENCES

Antioxidant enzymes in Friedreich’s ataxia


ARCHIVIST

A touch of the ‘flu

When I sat my final M.B. written exams one of the questions was, “Discuss the uses and abuses of the word influenza”. I didn’t know then what the examiners were getting at and I still don’t now although I know considerably more about influenza than I did then.

Clinical ‘influenza’ (or influenza-like illness) may be caused by influenza viruses A and B, respiratory syncytial viruses (RSVs) A and B and probably other viruses including the newly identified human metapneumovirus (see Lurica page 386). Although paediatricians are familiar with RSV because of bronchiolitis it is, in fact, responsible for many influenza-like illnesses in children and adults.

During the winters of 1995–96, 96–97, and 97–98 a virological study (MC Zambon and colleagues. Lancet 2001;358:1410–6, see also editorial, ibid: 1382–3) was carried out in 10–15 general practices across England and Wales. Nasopharyngeal swabs were collected from people with an influenza-like illness (loosely defined) and examined for influenza virus and RSV by tissue culture and multiplex reverse transcription PCR. Seven hundred and sixty-two of the 2226 patients tested were children under the age of 14. In the three successive winters 32%, 33%, and 21% of the children under 5 years and 49%, 38%, and 41% of the children aged 5–14 years tested positive for influenza viruses. During the same winters 20%, 37%, and 41% of the under 5s and 7%, 22%, and 17% of the 5–14 year olds tested positive for RSV. In all three winters RSV was the predominant virus in infants.

Influenza and RSV outbreaks occur at about the same time in probably half of winters and they may be clinically indistinguishable. New virus-specific drug treatments and the development of an effective RSV vaccine may make it important to develop rapid tests to distinguish between them.

www.archdischild.com

Downloaded from http://adc.bmj.com/ on June 15, 2017 - Published by group.bmj.com
Antioxidant enzymes in blood of patients with Friedreich's ataxia

G Tozzi, M Nuccetelli, M Lo Bello, S Bernardini, L Bellincampi, S Ballerini, L M Gaeta, C Casali, A Pastore, G Federici, E Bertini and F Piemonte

Arch Dis Child 2002 86: 376-379
doi: 10.1136/adc.86.5.376

Updated information and services can be found at:
http://adc.bmj.com/content/86/5/376

These include:

References
This article cites 33 articles, 14 of which you can access for free at:
http://adc.bmj.com/content/86/5/376#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Metabolic disorders (761)
- Molecular genetics (111)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/