Antioxidant enzymes in red blood cells and lymphocytes of ataxia-telangiectasia patients

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Toxic oxygen metabolites may contribute to the development of tissue damage, and play a role in the pathogenesis of malignancies, some acute and chronic pulmonary diseases, and in cell damage by radiomimetic agents, which can be seen in patients with ataxia-telangiectasia (A-T). Oxidative stress resulting from increased free radical production and/or defects in antioxidant defences is also involved in neurodegenerative disorders. Thus, oxidative stress could account for several aspects of the pleiotropic phenotype of A-T patients. The aim of this study was to determine the activities of the enzymes involved in cellular antioxidant metabolism in A-T patients to see if there is any defect which may result in constant oxidative stress. Superoxide dismutase (SOD) and catalase activities of erythrocytes, in contrast to lymphocytes, were found to be significantly higher in patients than in healthy controls. Our results may be another indication for the presence of constant oxidative stress in A-T patients as suggested previously.

Key words: ataxia-telangiectasia, antioxidant enzymes, lymphocyte, erythrocyte.

Excessive production of reactive oxygen species (ROS), which might occur in the course of recurrent infections (oxidative stress), not only damage the cellular macromolecules, including single and double-strand DNA, proteins and lipids, but also cause cell death. Under normal conditions, protective enzymes (catalase, peroxidases and superoxide dismutase) and cellular enzymatic and non-enzymatic antioxidants prevent DNA damage by reactive oxygen intermediates (ROI). Individuals may be more susceptible to damage by ROI because of overproduction, defective detoxification and/or defective DNA repair mechanisms.

Ataxia-telangiectasia (A-T) is an autosomal recessive disease with multisystem defects, due to the functional inactivation of ATM (AT-mutated), which is an essential component of the signal transduction pathway that operates under stress as well as normal physiological conditions. DNA damage is sensed by the ATM protein, without which cells fail to induce p53, cell cycle checkpoint controls, DNA repair and programmed cell death. Since patients with A-T suffer from recurrent infections, they are exposed to oxidative stress to a higher degree. Hallmarks of A-T are immunodeficiency, progressive neurodegeneration, premature aging, genomic instability, radiosensitivity, cell damage by radiomimetic agents and predisposition to chronic pulmonary diseases and malignancies, which may be at least partly caused-by cellular oxidative stress, resulting from overproduction or defective detoxification of ROI. We determined the activities of some of the key antioxidant enzymes, superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) in a group of A-T patients to investigate whether there is a defect in antioxidant mechanism resulting in constant oxidative stress in children with A-T.

Material and Methods

Patients: Twenty-seven classical A-T patients (13 male and 14 female) with ages ranging from 7-18 years (11.72±5.12 years) who admitted to...
the Immunology Unit in İhsan Doğramaci Children's Hospital, Ankara, were enrolled in the study. The majority of the patients had histories of recurrent sinopulmonary infections in varying degrees. The toxic oxygen radical scavenging enzyme system was investigated in patients and 24 healthy control children of a similar age group. The study was performed without any exposure to chemicals or antioxidant medication or serious infections that can cause oxidative stress. Due to technical reasons, all of the enzyme panel could not be studied in all patients and controls.

Superoxide dismutase (SOD), catalase, glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) activities were measured spectrophotometrically in erythrocytes and lymphocytes of A-T patients and healthy controls.

**Measurement of biochemical parameters**

Glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate and its reduced form (NADP), NADPH), reduced and oxidized glutathione (GSH, GSSG). GR xanthine oxidase, ethylene-diaminetetra-acetic acid (EDTA) and other chemicals were of analytical grade and obtained from Sigma. GR, G6PD and GSH-Px activities were determined by the methods of Beutler. Milton Roy Spectronic 3000 diode array spectrophotometer was used for protein determination and kinetic measurements. In addition, GST, catalase and SOD activities were measured. Protein concentration in all samples was determined by the method of Lowry.

**Statistical Analysis**

Results are presented as means±SD. Mann-Whitney U test analysis was used to determine the significance level of differences among sample groups, with a significance criterion of p≤0.05.

**Results**

Values for various biochemical parameters of both A-T patients and the control group are given in Table I. Statistically significant differences were found only in SOD erythrocyte and catalase erythrocyte levels and erythrocyte GSH-Px activity in A-T patients when compared to the control group. Mean values and ranges of SOD erythrocyte were 5.82±0.65 (4.71-6.56) and 3.08±0.17 (2.80-3.30) U/mg protein for A-T and control groups, respectively, showing about a two-fold increase in patients (p<0.05) than controls. All of the patients studied showed

<table>
<thead>
<tr>
<th>Enzyme Group</th>
<th>Lymphocyte Mean±SD (range)</th>
<th>n</th>
<th>Erythrocyte Mean±SD (range)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.058±0.020 (0.044-0.086)</td>
<td>9</td>
<td>0.004±0.001 (0.003-0.007)</td>
<td>10</td>
</tr>
<tr>
<td>A-T</td>
<td>0.076±0.050 (0.011-0.209)</td>
<td>14</td>
<td>0.05±0.002 (0.003-0.010)</td>
<td>12</td>
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<tr>
<td>GSSG-R</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.24±0.08 (0.16-0.39)</td>
<td>6</td>
<td>5.47±0.73 (x10^-3)</td>
<td>6</td>
</tr>
<tr>
<td>A-T</td>
<td>0.19±0.06 (0.10-0.24)</td>
<td>5</td>
<td>5.78±0.20 (x10^-3)</td>
<td>5</td>
</tr>
<tr>
<td>GSH-Px</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.27±0.13 (0.08-0.50)</td>
<td>6</td>
<td>8.46±1.10 (x10^-3)</td>
<td>6</td>
</tr>
<tr>
<td>A-T</td>
<td>0.20±0.07 (0.08-0.27)</td>
<td>5</td>
<td>10.83±1.53 (x10^-3)</td>
<td>5</td>
</tr>
<tr>
<td>GST</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.41±0.16 (0.21-0.69)</td>
<td>6</td>
<td>6.25±0.88 (x10^-3)</td>
<td>6</td>
</tr>
<tr>
<td>A-T</td>
<td>0.32±0.08 (0.19-0.40)</td>
<td>5</td>
<td>6.58±0.62 (x10^-3)</td>
<td>5</td>
</tr>
<tr>
<td>Catalase</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0.10±0.04 (0.05-0.14)</td>
<td>8</td>
<td>0.045±0.011 (0.03-0.06)</td>
<td>10</td>
</tr>
<tr>
<td>A-T</td>
<td>0.16±0.12 (0.07-0.14)</td>
<td>14</td>
<td>0.065±0.011 (0.052-0.08)*</td>
<td>9</td>
</tr>
<tr>
<td>SOD</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.12±0.03 (0.07-0.21)</td>
<td>14</td>
<td>3.08±0.17 (2.80-3.30)</td>
<td>6</td>
</tr>
<tr>
<td>A-T</td>
<td>0.12±0.02 (0.05-0.42)</td>
<td>15</td>
<td>5.82±0.65 (4.71-6.56)*</td>
<td>6</td>
</tr>
</tbody>
</table>

* p<0.05 (Statistically significant difference between A-T and controls; Mann-Whitney U test). Results of the enzyme activities are given as U/mg protein except catalase (C/mg protein).

A-T : ataxia-telangiectasia.
G6PD : glucose-6 phosphate dehydrogenase.
GSSG-R : glutathione reductase.
GSH-Px : glutathione peroxidase.
GST : glutathione-S-transferase.
SOD : superoxide dismutase.
higher values than the control mean level. The same pattern was also seen in the catalase erythrocyte levels of patient and control groups: 0.065±0.011 (0.052-0.08) and 0.045±0.011 (0.03-0.06) U/mg protein, respectively (p<0.005). Again, erythrocyte GSH-Px enzyme activity was 10.83±1.53 (x10⁻³) U/mg protein (range: 8.73-12.4) in the patient group versus 8.46±1.10 U (x10⁻³)/mg protein (range: 6.79-9.35) in the controls (p=0.05). The other enzymes in glutathione detoxification pathway (G6PD, GSSG-R, GST) failed to reveal any significant difference in patients versus controls in both erythrocytes and lymphocytes.

Discussion

Excessive production of ROS, which might occur in recurrent infection, is commonly referred to as oxidative stress. Since the majority of patients with A-T suffer from recurrent infections, they are exposed to oxidative stress to a higher degree. We studied various enzymes that prevent oxidative damage under normal conditions and found significantly higher SOD and catalase activities of erythrocytes in patients than in healthy control children. In contrast, however, the values in SOD and catalase activities in lymphocytes and enzymes in the glutathione detoxification pathway, except for GSH-Px in erythrocytes, did not show any significant difference in patients versus controls. Previous reports have suggested a reduced catalase activity12 or a defect in glutathione metabolism13 in A-T cells, although these results have not been uniformly observed by all investigators14-17. However, Reichenbach et al.18 recently found significantly reduced plasma levels of TEAC (Trolox-equivalent antioxidant capacity), which reflects the ROS scavenger activity of a variety of proteins, such as ubiquinol, uric acid and antioxidant vitamins19. Increased SOD, catalase and GSH-Px activities of erythrocytes in our patients may be an indication of the presence of constant oxidative stress and suggest that A-T patients may be able to increase the levels of these enzymes at least in erythrocytes, although it is unclear whether the levels are sufficient. The lack of significant difference between A-T patients and controls in enzymes of the glutathione detoxification pathway both in erythrocytes and lymphocytes and the lack of increase in SOD and catalase in lymphocytes in contrast to erythrocytes may be due to 1) the difference in enzyme isotypes or regulating mechanisms in different cells, 2) the fact that the SOD and catalase activities are independent of the glutathione pathway, 3) deficient re-synthesis of glutathione, 4) the lack of the ATM protein, which might act as a sensor of ROS and result in non-activation of thiol-related antioxidant mechanism, or 5) exhaustion of enzyme activities or substances (i.e. GSH) due to constant oxidative stress in these patients. Our results as well as those of previous studies suggest that A-T patients may have decreased ability to counteract oxidative stress. Reichenbach et al.18 reported that patients with A-T displayed lower values of antioxidant capacity than patients with common variable immunodeficiency and hypothesized that the ATM gene product might be an upstream sensor that is activated by oxidative damage, initiating pathways responsible for protecting cells from such damages. The absence of functional ATM in A-T cells would then result in a state of increased sensitivity to oxidative stress.

All these data are in agreement with probable involvement of increased ROS production and diminished ROS scavenger capacity in the disease process of patients with A-T and with the probable benefit of vitamin A and vitamin E in the therapy of these patients.

Acknowledgements

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REFERENCES