Evidence of oxidative stress in erythrocyte phospholipid composition in the pathogenesis of familial Mediterranean fever (periodical disease)


Abstract

Background—Familial Mediterranean fever (FMF) is a genetically linked disorder common amongst races of the Eastern Mediterranean region. Typical symptoms include episodic pain syndrome extending throughout the chest or abdomen associated with histopathological signs of amyloidosis of the kidney.

Aim—To investigate possible connections between the aseptic inflammation that occurs during pain crises and cell membrane structural and functional integrity in patients with FMF.

Methods—Oxidative stress parameters in 42 patients in remission and during a pain crisis were compared with 21 normal subjects.

Results—The patient group had significantly greater concentrations of chemiluminescent and thiobarbituric acid-reactive substances in the blood plasma and lower concentrations of α-tocopherol than the control group while in remission; these changes were exacerbated during pain crises. Analyses of the phospholipid composition of erythrocyte membranes showed significant increases in amounts of acidic phospholipids (phosphatidic acid, monophosphatidylinositol and cardiolipin) and lysophosphatidylcholine compared with healthy subjects.

Conclusions—The pattern of differences in membrane phospholipid composition was consistent with increased oxidative stress in patients with FMF.


Keywords: oxidative stress, familial mediterranean fever, periodical disease, erythrocyte phospholipids.

Familial Mediterranean fever (FMF), sometimes referred to as periodical disease, is a genetic disorder common amongst races originating from the Eastern Mediterranean region. It may affect up to 7% of the total population in certain areas and is characterised by an atypical pain syndrome and pyrexia. During an attack, which lasts for two to three days, pain spreads throughout the chest or abdomen. Subsequently, the patient enters a period of remission. The frequency of attacks varies from one week to several months. The cause of the disorder is presently unknown, but it is associated with aseptic serositis. As there are no significant changes in blood pressure, blood cell dynamics, abnormal catecholamine metabolism, or disorders of monoamine oxidase activity, the disorder can be distinguished from others with pain of different origin. Pathological signs of FMF are typified by amyloidosis of the kidney with histological evidence indicating damage to cells at the level of the constituent membranes.

The histopathological changes observed in the kidney have led to the suggestion that the pain syndrome may be associated with loss of cell integrity by loss of plasma or subcellular membranes. This is consistent with increased plasma concentrations of histamine, serotonin and bradikinin associated with degranulation of neutrophils observed during pain episodes in addition to activation of lysosomal enzymes and abnormal arachidonic acid metabolism.

In order to investigate possible connections between the aseptic inflammation that occurs during pain crises and cell membrane structural and functional integrity, indexes of oxidative stress have been compared in blood plasma of patients during a pain attack and during a period of remission. Analysis of the membrane phospholipid composition of erythrocytes obtained from these patients was also performed to establish whether changes in oxidative stress resulted in altered membrane constituents.

Methods

PREPARATION OF SAMPLES

Blood (5 ml) from 42 patients with FMF exhibiting symptoms of a pain attack and 21 unaffected donors was collected from the ulnar vein into citrate or oxalate (9:1, by volume, 1.34% solution). Erythrocyte membranes were prepared by using the method described by Limber et al. and acetone extracted powders were prepared from the membranes.

MEASUREMENT OF OXIDATIVE STRESS

Indexes of oxidative processes in blood lipids were determined by measurement of the intensity of spontaneous chemiluminescence recorded at 39°C as described by Panich and Solomenko. Tocopherol concentration in the plasma was measured fluorimetrically using an emission maximum of 384 nm.

LIPID EXTRACTION AND ANALYSIS

Total polar lipid extracts of the acetone extracted dry membranes were prepared by the method of Folch et al. Phospholipid classes

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Table 1 Concentration of thiobarbituric acid reactive material, spontaneous chemiluminescence and α-tocopherol in blood plasma of normal subjects and patients with FMF

<table>
<thead>
<tr>
<th>Patients</th>
<th>Thiobarbituric acid reactivity (AU/mL)</th>
<th>Chemiluminescence (AU)</th>
<th>α-tocopherol (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>3.67 ± 0.16</td>
<td>70</td>
<td>3.52 ± 0.26</td>
</tr>
<tr>
<td>Patients with FMF in remission</td>
<td>4.52 ± 0.21**</td>
<td>123</td>
<td>2.34 ± 0.22**</td>
</tr>
<tr>
<td>in pain crisis</td>
<td>5.31 ± 0.18**</td>
<td>186</td>
<td>1.93 ± 0.12**</td>
</tr>
</tbody>
</table>

**p<0.01.

Table 2 Phospholipid composition of erythrocyte membranes (µg lipid phosphorus/100g wet weight) of normal subjects and patients with FMF in remission

<table>
<thead>
<tr>
<th>Phospholipid class</th>
<th>Control (µg)</th>
<th>Per cent of TPL</th>
<th>FMF (µg)</th>
<th>Per cent of TPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine</td>
<td>16.70 ± 0.8</td>
<td>35.6</td>
<td>9.30 ± 0.55**</td>
<td>21</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>8.80 ± 0.6</td>
<td>18.8</td>
<td>9.00 ± 0.7</td>
<td>20</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>5.30 ± 0.4</td>
<td>11.3</td>
<td>1.70 ± 0.2**</td>
<td>4</td>
</tr>
<tr>
<td>Phosphatidyserine</td>
<td>7.70 ± 0.7</td>
<td>16.5</td>
<td>4.00 ± 0.2**</td>
<td>9</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>3.55 ± 0.1</td>
<td>7.6</td>
<td>6.40 ± 0.3**</td>
<td>14</td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>1.20 ± 0.1</td>
<td>2.6</td>
<td>2.70 ± 0.2**</td>
<td>6</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>2.00 ± 0.2</td>
<td>4.3</td>
<td>3.80 ± 0.2**</td>
<td>8</td>
</tr>
<tr>
<td>Lysophosphatidylcholine</td>
<td>1.55 ± 0.2</td>
<td>3.3</td>
<td>8.00 ± 0.6**</td>
<td>18</td>
</tr>
<tr>
<td>Neutral phospholipid (NP)</td>
<td>32.35 ± 1.0</td>
<td>69.0</td>
<td>28.00 ± 0.7**</td>
<td>62</td>
</tr>
<tr>
<td>Acidic phospholipid (AP)</td>
<td>14.45 ± 0.5</td>
<td>31.0</td>
<td>17.20 ± 0.3**</td>
<td>38</td>
</tr>
<tr>
<td>Total phospholipid (TPL)</td>
<td>40.80 ± 1.1</td>
<td>85.2</td>
<td>45.20 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

**Values significantly different from control; p<0.01.

Results and Discussion

Under normal conditions, an equilibrium is established between oxidative processes and antioxidant activity at all levels of organisation of complex organisms. Oxidative stress occurs when antioxidant activity is unable to preserve this equilibrium and tissue damage can result. The accumulation of oxidised products in patients with FMF is evident from the data presented in table 1. This shows comparisons of thiobarbituric acid reactive material and spontaneous chemiluminescence intensities recorded in whole blood plasma of normal subjects and patients with FMF in remission and undergoing a pain crisis. Chemiluminescence arises from peroxidation of polyunsaturated fatty acids which are mainly derived from the membrane phospholipids. Products of oxidised fatty acids are also believed to be the dominant compounds that react with thiobarbituric acid. It can be seen that the patients with FMF in remission have significantly higher concentrations of thiobarbituric acid reactive material and chemiluminescent products compared with the control group and this increases dramatically when the patients experience a pain crisis. There is an inverse correlation between the two parameters indexing lipid oxidation and α-tocopherol in blood plasma of normal subjects compared with patients with FMF in remission and during a pain crisis, as might be expected if there is a shift in the balance of pro- and antioxidant activity. Clearly, patients with FMF in remission are stressed oxidatively when compared with the control group and this stress is notably increased during a pain crisis when concentrations of thiobarbituric acid reactive material and spontaneous chemiluminescent products increase further and α-tocopherol concentrations decrease proportionately. It is noteworthy that other diseases also manifest as oxidative stress in erythrocyte membranes including sickle cell disease. In this case it has been shown that agents like L-prolyl carbinol are able to protect erythrocytes from peroxidative damage of the membrane lipids.22 Products of lipid peroxidation associated with chronic ethanol ingestion such as aldehydes—for example, have been shown to form adducts with proteins and these effects are particularly prevalent in inflammatory states.23

One of the principal targets for oxidative attack are the polyunsaturated fatty acyl residues of membrane lipids. These substrates are believed to represent the products detected by spontaneous chemiluminescence and thiobarbituric acid. In order to examine possible changes in membrane lipid composition, analyses of the major lipid classes of erythrocyte membranes of patients with FMF in remission were compared with those of normal subjects. The results are summarised in table 2. It can be seen that there are significant differences in erythrocyte membrane lipid composition between the two groups. Most noteworthy was that the patients with FMF had significantly greater amounts of lysophosphatidylcholine, monophosphatidylinositol, phosphatidic acid, and cardiolipin than the controls. With the exception of lysophosphatidylcholine, these lipids represent the acidic phospholipids and their elevation may reflect increased turnover of membrane lipids as a consequence of oxidative stress. The accumulation of lysophosphatidate indicates activation of phospholipase A₂ which again indicates accelerated membrane lipid mobilisation in patients with FMF. The major substrates for endogenous phospholipase A₂ are the neutral and zwitterionic phospholipids comprising the phosphatidylethanolamine and phosphatidylcholine fractions, respectively. As can be seen from table 2, there is a decrease in total neutral phospholipids in patients with FMF relative to that observed in the control group.

It is apparent from the parameters measured in the blood of patients with FMF that, even in remission, they are subject to an oxidative stress that exceeds that in a normal control group. This can be seen both in changes in the pattern of erythrocyte membrane lipid classes, the appearance of peroxidised lipids in the
plasma and the weakening of antioxidant defenses in the form of decreased plasma α-tocopherol concentrations. There is also clear evidence that during the pain crises associated with the disorder there is further elevation of oxidative stress. Whether this stress is the cause of the symptoms associated with FMF or a consequence of the condition is not yet known, but it is possible that strengthening antioxidant defenses may serve to alleviate the symptoms. There is some evidence to support the idea that elevation of tissue antioxidant levels—for example, by dietary supplementation with α-tocopherol, prevents lipid peroxidation. Whether dietary supplementation with α-tocopherol is an effective treatment of FMF is the subject of ongoing investigations.

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