LPO and Apoptosis during Pulmonary Tuberculosis


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LPO and apoptosis in blood mononuclear cells were studied in patients with pulmonary tuberculosis before and during treatment with standard chemotherapeutics. Pulmonary tuberculosis was accompanied by LPO activation and intensification of apoptosis in lymphocytes and monocytes. These changes were observed before and after the course of intensive care. The intensity of lipid peroxidation returned to normal, while activity of apoptosis remained high after therapy.

Key Words: tuberculosis; lymphocytes; monocytes; apoptosis; lipid peroxidation

Monocytes and lymphocytes play a major role in antituberculous protection of the macroorganism. Structural and functional destabilization of membranes plays a role in the development of immune dysfunction during tuberculosis and is associated with free radical lipid oxidation. Hyperactivation of this process is an apoptogenic factor. M. tuberculosis initiate free radical reactions, which results in cell death due to activation of lipid-mediated apoptosis [3]. However, little is known about the mechanisms of oxidative damage to blood immunocompetent cells. The role of blood immunocompetent cells in apoptosis during pulmonary tuberculosis (PT) and involvement of these cells in the pathogenesis of tuberculous infection are poorly understood.

Here we studied lipid peroxidation (LPO) and apoptosis in peripheral blood lymphocytes and monocytes taken from patients with PT before and after antituberculous treatment.

MATERIALS AND METHODS

We examined 70 patients (men, 20-55 years) with drug-sensitive and drug-resistant infiltrative PT. The diagnosis of PT was made according to the results of microscopic examination of sputum and X-ray examination of the lungs. Drug-resistance of mycobacteria was estimated by the method of absolute concentrations. The patients were examined before and after the course of intensive care, as well as after completion of therapy (by the end of maintenance therapy).

The control group included 11 healthy volunteers (men, 22-55 years) and 11 patients (men, 22-55 years) with chronic nonspecific diseases of the lungs (chronic obstructive bronchitis and community-acquired pneumonia).

Peripheral blood was taken from the cubital vein after overnight fast.

Blood lymphocytes and monocytes were isolated on Ficoll-Urografin density gradient (1077 and 1083 kg/m³, respectively) [2]. The concentra-
tions of malonic dialdehyde (MDA) and conjugated dienes in leukocytes were measured spectrophoto-
metrically. The results were expressed in µmol/mg protein. The intensity of apoptosis was determined
by means of spectral photometry using Annexin V (BD Biosciences). The percentage of Annexin V-po-
positive cells was determined.

The results were analyzed by Statistica 6.0 software (StatSoft Inc.). Normal type of distribution in
the samples was assessed by calculating the coefficients of asymmetry and excess. Statistical treat-
ment included χ² and Kolmogorov—Smirnov test. Student's t test was used for testing the hypothesis
about equality of the means drawn from a normally distributed population. When the data did not have
a normal distribution, the differences were assessed by means of Mann—Whitney U test. The differen-
tes were significant at p<0.05.

RESULTS

The intensity of LPO in peripheral blood mononu-
uclear cells increased in patients with drug-sensi-
tive PT before the start of chemotherapy (compared
to healthy donors). In patients with drug-resistant
PT before therapy, the intensity of LPO in blood
lymphocytes and monocytes did not differ from
normal (except for the concentration of conjugated
dienes in monocytes, Table 1). The intensity of
apoptosis in lymphocytes and monocytes signifi-
cantly increased in patients with drug-sensitive and
drug-resistant PT (Table 1).

Respiratory burst resulting in the formation of
considerable amounts of reactive oxygen species is
an important mechanisms of antibacterial protec-
tion. A similar effect is typical of lymphocytes with
high killer activity. Reactive radicals damage myco-
bacteria by activation of LPO in membranes. How-
ever, high-reactivity compounds formed during free
radical oxidation can cause a strong destructive
effect not only on bacteria, but also on membrane
lipids in host cells. This effect is associated with
activation of LPO and lipid-induced apoptosis. The
observed changes probably contribute to activation
of these processes in blood mononuclear cells from
patients with drug-sensitive PT [1,3]. Activation of
apoptosis in lymphocytes and monocytes from pa-
tients with drugs-resistant PT was not accompanied
by an increase in the intensity of LPO. It was prob-
bly associated with the ability of mycobacteria
resistant to antituberculous drugs to adapt to the
internal environment in macrophages [4].

These data suggest that activation of apoptosis
in leukocytes from patients with this form of PT is
mediated by a non-lipid mechanism [6].

The intensity of apoptosis in lymphocytes and
monocytes from patients with both forms of PT
remained high by the end of intensive chemother-
apy (Table 1). It was probably related to the toxic
effect of standard antituberculous drugs [5,7]. Under
these conditions the intensity of LPO decreased in
patients with drug-sensitive PT (normal concentra-
tions of conjugated dienes in lymphocytes and mono-
cytes), but increased in patients with drug-resistant

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA, µmol/mg protein</th>
<th>Conjugated dienes, ×10³ µmol/mg protein</th>
<th>Apoptotic cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lymphocytes</td>
<td>monocytes</td>
<td>lymphocytes</td>
</tr>
<tr>
<td>Healthy donors (n=11)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.80±0.14</td>
<td>1.66±0.09</td>
<td>1.86±0.31</td>
</tr>
<tr>
<td>Patients with CNDL (n=11)</td>
<td>3.31±0.42**</td>
<td>2.90±0.35**</td>
<td>3.77±0.53**</td>
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<tr>
<td>Patients with IPT (n=70)</td>
<td></td>
<td></td>
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<td>before therapy</td>
<td></td>
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<tr>
<td>DSPT (n=15)</td>
<td>4.09±0.56***</td>
<td>6.43±1.18***</td>
<td>2.34±0.38*</td>
</tr>
<tr>
<td>DRPT (n=13)</td>
<td>1.53±0.19**</td>
<td>2.51±0.43</td>
<td>2.58±0.52</td>
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<tr>
<td>after intensive chemotherapy</td>
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</tr>
<tr>
<td>DSPT (n=11)</td>
<td>2.84±0.58**</td>
<td>4.36±0.34**</td>
<td>1.52±0.30</td>
</tr>
<tr>
<td>DRPT (n=11)</td>
<td>3.95±0.45*</td>
<td>3.51±0.43*</td>
<td>5.92±0.42**</td>
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<tr>
<td>after completion of therapy</td>
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<tr>
<td>DSPT (n=11)</td>
<td>2.17±0.19</td>
<td>2.22±0.34</td>
<td>2.48±0.34</td>
</tr>
<tr>
<td>DRPT (n=9)</td>
<td>4.31±0.76*</td>
<td>3.10±0.32*</td>
<td>1.33±0.33</td>
</tr>
</tbody>
</table>

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to healthy donors; *p<0.05 and **p<0.01 compared to patients with CNDL.