ATP downregulation in mononuclear cells from children with graft-versus-host disease following extracorporeal photochemistry

Graft-versus-host disease (GvHD) is a frequent and major complication of allogeneic bone marrow transplantation (BMT). Acute GvHD occurs in 40% to 50% of allogeneic BMT recipients; chronic GvHD can be observed in 30% to 60% of long-term survivors.1

Extracorporeal photochemotherapy (ECP), which is currently used for the treatment of cutaneous T-cell lymphoma, has also produced encouraging results in the treatment of rejection after organ transplantation, selected autoimmune diseases, and drug-resistant graft-versus-host disease (GvHD) even in pediatric age.1,2,3 ECP is a multistep procedure including collection of peripheral blood mononuclear cells (MNC) from the patient by leukapheresis and their treatment with 8-methoxypsoralen (8-MOP) in combination with UVA light (PUVA) in an extracorporeal system.4

There is evidence that PUVA-treated MNC stimulate an immunomodulatory response against pathologically altered T-cells;5,6 however, the exact mechanism of action of ECP is not fully understood.

We investigated ATP content in MNC from 7 pediatric patients who underwent ECP for the treatment of drug-resistant chronic GvHD after allogeneic bone marrow transplantation (BM-T), as an indicator of the importance of PUVA-induced cell damage. ATP was evaluated in the apheresic products, before and after PUVA, using a sensitive chemiluminescent assay. The clinical characteristics of patients are summarized in Table 1; GvHD was classified according to previously published criteria.6,7

Eligible children were planned to undergo ECP twice weekly for the first month, then twice monthly for two months, and monthly for the following four months. Sample collections for ATP assay were scheduled at procedure 1, 3, 5, 8, 16, and 24 (end of therapy); so far 29 samples have been analyzed as shown in Table 1. MNC were obtained by leukapheresis; mean recovery was always higher than 95%.

Mean ATP content after ECP was lower than the initial in all patients but one, who showed a slight increase before the ATP assay, by trypan blue exclusion, and it was assessed on both untreated and PUVA-treated cells immediately before and immediately after PUVA irradiation. DNA damage causes a series of biochemical events, including increased activity of poly(ADP-ribose) synthetase, a chromatin-bound enzyme which promotes the DNA excision-repair process by the successive transfer of ADP-ribose units from NAD to nuclear proteins. When DNA strand breaks are repaired, the activated enzyme can exhaust intracellular NAD and lower the cellular ATP pool, resulting in rapid cell death.9

In the present study we demonstrate that ECP induces down-regulation of intracellular ATP in viable lymphocytes, likely as a consequence of poly (ADP-ribose) synthetase activation.

Table 1. Characteristics of the patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>BM</th>
<th>PD</th>
<th>MA</th>
<th>A1</th>
<th>BI</th>
<th>BA</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/age (yrs)</td>
<td>M/8</td>
<td>M/7</td>
<td>M/6</td>
<td>M/8</td>
<td>F/16</td>
<td>M/10</td>
<td>M/12</td>
</tr>
<tr>
<td>Disease</td>
<td>ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes; SAA, severe aplastic anemia; CS, corticosteroid; CSA, cyclosporin A, IMP, improved; RES, resolved.</td>
<td>IMP</td>
<td>RES</td>
<td>RES</td>
<td>IMP</td>
<td>IMP</td>
<td>IMP</td>
</tr>
<tr>
<td>Organs involved</td>
<td>skin, liver, gut, skin, brain</td>
<td>skin, skin, liver</td>
<td>skin, liver</td>
<td>skin, liver, gut</td>
<td>skin, gut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous GvHD treatment</td>
<td>CS, CsA, CsA, ECP, CS, CS, CS, CS, CS,</td>
<td>CSA, ECP, CsA, CSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GvHD outcome</td>
<td>IMP</td>
<td>RES</td>
<td>RES</td>
<td>RES</td>
<td>IMP</td>
<td>IMP</td>
<td>IMP</td>
</tr>
<tr>
<td>Evaluated samples</td>
<td>1*</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>3*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ATP post-PUVA (%)</td>
<td>95.3</td>
<td>74.6</td>
<td>81.8</td>
<td>91.1</td>
<td>84.8</td>
<td>80.9</td>
<td>101.2</td>
</tr>
</tbody>
</table>

*two ECP cycles; +deceased; §mean value, expressed as percent of the initial content.

ATP content change was negatively correlated to the amount of lymphocytes in the apheresic products (r = 0.44, p<0.05). ATP decrease was an immediate effect of PUVA treatment: overnight storage at room temperature did not significantly affect the ATP content of cell suspensions. Evaluation of signal stability over a period of 24 h showed a signal half-life of about 4 h; the kinetics of decay was statistically indistinguishable in pre- and post-PUVA samples.

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It was shown that ECP directly induces significant levels of apoptosis in patients with cutaneous T-cell lymphoma, systemic sclerosis, and GvHD. However, based on our results, the cytotherapeutic effect of PUVA seems to be only one of the components of the mechanism of action of ECP; other mechanisms seem to contribute to the positive effect of ECP in the treatment of GvHD and other autoimmune diseases.

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References