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

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.[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;[2,5] 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 (BMT), as an indicator of the importance of PUVA-induced cell damage. ATP was evaluated in the apheretic 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 85.3%, observed range 44.7-99.3%. 8-MOP (Gerot Pharmazeutika, Wien, Austria) was added to the cells at a final concentration of 200 ng/mL, and the suspension was then exposed to UVA light (365 nm, 2J/cm^2, 20 min) as a 1 mm thick film, at room temperature under constant horizontal agitation (60 rpm) using the UV-A Matic irradiator (Vilber-Lourmat, Marne-la-Vallée, France).[4]

Aliquots were collected from the MNC suspension immediately before and immediately after PUVA treatment. Intracellular ATP was assayed on different cell amounts (5×10^4, 2×10^4, 1×10^4, 5×10^3) after 16 h storage at 26-28°C, using the luminescent detection assay kit, ATPLite-M (Packard BioScience, Groningen, Holland). The assay system is based on the production of light caused by the reaction of ATP with added firefly luciferase and D-luciferin; the emitted light is proportional to the ATP concentration.[8] Sample luminescence was measured using a scintillation analyzer in single photon counting mode. The ATP content of PUVA-treated cells was expressed as percent of the ATP content of untreated MNC.

Statistical analysis was performed using the Wilcoxon rank test and the Kruskal-Wallis test. Cell viability was routinely assessed on both untreated and PUVA-treated cells immediately before the ATP assay, by trypan blue exclusion, and it was always higher than 95%.

Exposure of MNC to PUVA caused a statistically significant decrease of intracellular ATP to 87.3% of the initial content (mean value of the overall procedures). Mean ATP content after ECP was lower than the initial in all patients but one, who showed a slightly increased amount after two procedures (Table 1).

Table 1. Characteristics of the patients.

Patient	BM	PD	MA	AJ	BI	BA	PS
Sex/age (yrs)	M/8	M/7	M/6	M/8	F/16	M/10	M/12
Disease	ALL	MDS	MDS	AML	CML	AML	SAA
Organs involved	skin, liver, gut	skin, brain[#]	skin	skin, liver	skin, liver, gut	gut	skin, gut
Previous GvHD treatment	CS, CsA	CS, CsA, ECP	CS, CsA	CS, CsA	CS, CsA	CS, CsA	CS, CsA
GvHD outcome	IMP	RES	RES	RES	IMP	IMP	IMP
Evaluated samples	9*	6	4	3	3+	2	2
ATP post-PUVA[§] (%)	95.3	74.6	81.8	91.1	84.8	80.9	101.2

*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. [#]See ref. #7; *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 apheretic 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.

8-MOP covalently binds to DNA pyrimidine bases after UVA 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 extensive, the stimulus for activation of poly(ADP-ribose) synthetase persists and 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.

It was shown that ECP directly induces significant levels of apoptosis in patients with cutaneous T-cell lymphoma, systemic sclerosis, and GvHD.[10] However, based on our results, the cytoreductive 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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