



Feasibility and safety of a new technique of extracorporeal photochemotherapy: experience of 240 procedures

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ABSTRACT

Background and Objective. Extracorporeal photochemotherapy (ECP) is a therapeutic approach based on the biological effects of ultraviolet light (UV) - A and psoralens on mononuclear cells collected by apheresis. Recently, ECP has been under investigation as an alternative treatment for various immune and autoimmune diseases. The aim of this study was to evaluate the safety and feasibility of a new three-step ECP technique, in terms of reproducibility, acceptance, tolerability, and short and long term side effects.

Design and Methods. Seventeen patients affected by acute or chronic graft-versus-host disease (GvHD), pemphigus vulgaris, or interferon-resistant chronic hepatitis C and one patient being treated for prevention of heart transplant rejection underwent 240 ECP procedures. MNC collection and processing parameters were recorded, biological effects of UV-A/8-methoxy-psoralen (8-MOP) were evaluated, and short and long term side effects were monitored.

Results. At a mean follow up of 7 months (range 2-19) 240 ECP had been completed, a mean of 7,136 mL (range 1,998-10,591) of whole blood having been processed per procedure. The mean of total nucleated cells collected per procedure was 6.5×10^9 (range 0.65-23.8), with a mean MNC percentage of 85% (41.4-98%) in a mean final volume of 115.5 mL (37-160). No severe side effects were documented and no infectious episodes occurred throughout the course of the treatment.

Interpretation and Conclusions. The new ECP technique was highly reproducible as regards the collection and each processing step. Short and long term side effects were mild. No increase in infectious episodes was recorded. All patients willingly underwent ECP, demonstrating an excellent tolerability for the procedure even after several courses.

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Key words: extracorporeal photochemotherapy, 8-methoxy-psoralen, ultraviolet-A light

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Biological effects of ultraviolet (UV) light have been documented for many years. Three types of UV light are available. UV-C light is toxic and causes cell death. UV-B light is responsible for several effects on the circulating blood cells including the cells of the immune system and also potentially plays a role in prevention of HLA-alloimmunization related to platelet transfusion.¹⁻³ UV-A light is active only in the presence of photosensitizing tricyclic compounds such as psoralens, producing photoadducts with pyrimidine bases. This mechanism abrogates cell proliferation, induces apoptosis in lymphocytes and increases the immunogenicity of cells involved in tumoral or immune processes.⁴⁻⁶

The photopheresis technique was first developed by Edelson in 1981 and consisted in oral administration of 8-MOP, collection of MNC cells with a discontinuous flow cell separator (UVAR-Therakos) and irradiation of the buffy coat with the same apparatus.⁷ For many years this was the sole available technique and suffered from many weak aspects.

The technique of photochemotherapy that we have adopted was initially created by French groups and has many potential advantages over the historical Edelson system.⁸ Extracorporeal photochemotherapy (ECP) is based on the collection of mononuclear cells (MNC) with a third generation cell separator, their irradiation with UV-A light in the presence of 8-methoxy-psoralen (8-MOP) and subsequent reinfusion of the treated cells into the patient.¹ The mechanism underlying the ECP-induced response is still under investigation. Current experimental evidence points to ECP being an amplifier of the immunogenicity of class I associated peptides that are present on the surface of the collected mononuclear cells.^{8,9}

ECP is FDA-approved for the treatment of cutaneous T-cell lymphoma and encouraging results have been reported in the management of non-malignant disorders of the immune system such as pemphigus vulgaris, scleroderma, systemic lupus erythematosus, rheumatoid arthritis, autoimmune diabetes mellitus, rejection of cardiac and renal allograft and chronic GvHD.⁹⁻¹⁷

We employed ECP in the treatment of acute and chronic GvHD, pemphigus vulgaris, drug-resistant hepatitis C and in the prevention of heart transplant rejection.

The aim of this study was to verify the feasibility and safety of the new three step ECP technique, its tolerability to and acceptance by patients in the perspective of a long term treatment.

Design and Methods

Patients

Inclusion criteria:

- confirmed diagnosis of acute or chronic GvHD, pemphigus vulgaris resistant to standard immunosuppressive therapy, hepatitis C resistant to interferon, prevention of heart transplant rejection;
- WBC count > 1000/ μ L;
- ANC >500/ μ L;
- normal or near normal value of T-suppressor and cytolytic lymphocytes (CD8⁺);
- no concomitant treatment with ATG.

Exclusion criteria:

- age < 3 years and > 75 years;
- severe cardiac, renal or liver impairment;
- bacterial or viral infection in progress with particular regard to active CMV infection.

The treatment schedules varied according to the different protocols used in our Institution. Informed consent was always obtained before starting the ECP program.

We evaluated 240 ECP procedures performed in 18 patients affected by chronic GvHD (11 patients; mean age 22.5 yrs), acute GvHD (2 patient; mean age 14 yrs), pemphigus vulgaris resistant to standard treatment (3 patients; mean age 48 yrs), interferon-resistant chronic hepatitis C (1 patient, 53 yrs), prevention of heart transplant rejection (1 patient; 46 yrs). The characteristics of the enrolled patients are summarized in Table 1.

Table 1. Patients' characteristics.

Enrolled patients	18
Gender	13 males, 5 females
Mean age	28.1 years (range 7-68)
Diseases	
<i>cGvHD</i>	11
<i>aGvHD</i>	2
<i>pemphigus vulgaris</i>	3
<i>drug-resistant hepatitis C</i>	1
<i>heart transplant</i>	1
Mean follow-up	7 months (2-18)
Total ECP procedures	240

Methods

ECP consisted of 3 distinct steps:

1. *collection of mononuclear cells*: we employed a third generation cell separator Spectra Cobe (version 4.6 and 6.0), program MNC, in all cases processing 2 blood volumes at a flow rate ranging from 25 to 65 mL/min. Collection pump was set at a flow rate of 0.8 mL/min. Anticoagulant (acid citrate dextrose, formula A)/blood flow rate ratio was 1:12. The final MNC volume to be collected was less than 150 mL, with a hematocrit value not exceeding 5%. The maximum procedure duration was set at 180 min;
2. *processing of MNC buffy-coat*: the product was adjusted to a constant volume of 300 mL by addition of normal saline and of 3 mL of 8 methoxypsoralen (8-MOP) aqueous solution (Gerot Pharmazeutica), to obtain a final concentration of 200 ng/mL of the drug. Then the buffy-coat so diluted was transferred into a special UV-A-permeable bag (Maco Pharma, Tourcoing, France) and UV-A irradiation was performed with the UV-MATIC irradiator (Vilber Lourmat, Marne-la-Vallée, France) at 2 joule/cm²;
3. *reinfusion*: the 8-MOP photo-activated MNC were reinfused to the patient within 30 min, using a blood infusion device.

Biological effects of UV-A irradiation on MNC were evaluated monthly by a) response to phytohemagglutinin (PHA); b) ability to respond to allogeneic MNC in mixed lymphocyte culture; c) ability to stimulate allogeneic MNCs in mixed lymphocyte culture.

Venous access

A 17-gauge needle was employed for the inlet line and a 19-gauge needle for the return line when peripheral venous access was practical. Alternately, a central venous catheter (sometimes previously positioned for chemotherapy) was utilized for the procedure.

Patient care and surveillance

Blood pressure was monitored every 15 minutes and heart rate continuously throughout the collection procedure. Body temperature was recorded every 30 minutes. Prophylaxis of hypocalcemia consisted in routine administration of calcium gluconate (5 mL diluted in 5 mL normal saline) every 45 minutes.

The patients' hemocytometric values, liver and renal function and coagulation parameters were determined every procedure. The hemocytometric values are reported in Table 2.

During the reinfusion and post-reinfusion phases the patients were monitored for development of fever, chills, headache, rash, erythema, urticaria, itching, vesiculation, edema.

Adverse effects secondary to MNC collection were graded according to our institution's protocol.¹⁸

Table 2. Hemocytometric values of the patients before ECP.

	Min	Max	Mean
WBC ($\times 10^6/L$)	0.95	15.9	6.7
MNC (%)	9.9	78.5	36.1
Hct (%)	21.6	48.5	34.6
PLT ($\times 10^6/L$)	12	540	225

Table 3. Characteristics of the collected cells.

	Min	Max	Mean
Volume (mL)	37	160	115.5
Hct (%)	0.1	8	1.4
MNC (%)	41.4	98	85
Total WBC ($\times 10^9$)	0.65	23.8	6.5
Total PLT ($\times 10^{11}$)	0.04	7.8	2.1

Results

Two hundred and forty ECP were completed in 18 patients over a mean follow up of 7 months (range 2-19). The mean blood volume processed per procedure was 7,136 mL (range 1,998-10,591). The mean of total nucleated cells collected per procedure was 6.5×10^9 (range 0.65-23.8), with a mean MNC percentage of 85% (range 41.4-98%) in a mean final volume of 115.5 mL (range 37-160). The main parameters of the collected cells are summarized in Table 3.

Side effects documented were sporadic and mild (see Table 4). No significant infectious episodes occurred.

The effectiveness of the irradiation was evaluated by the response of the irradiated cells to phytohemagglutinin (PHA), which was in all cases < 3% of the pre-treatment autologous control response, and the ability of the irradiated cells to respond to allogeneic mixed MNC in mixed lymphocyte culture, which was < 4% that of the autologous controls. The ability to stimulate allogeneic MNCs in mixed lymphocyte culture was maintained at 75% of control values.

No significant modifications of liver, renal or coagulative parameters related to ECP were documented.

The collection and reinfusion procedures were well tolerated and completed in all cases with only mild and sporadic side-effects (chill, fever, headache) occurring (Table 4). No hypotensive episodes were recorded even in low body weight patients.

One patient discontinued the treatment, but this was because of catheter malfunction (CVC positioned 10 years earlier).

At a mean follow-up of 7 months no significant infectious episodes had occurred throughout the course of the treatment.

Discussion

ECP has been used to treat T-cell lymphoma with interesting results. This has led to investigation of the efficacy of ECP in various non-malignant immune and autoimmune disorders.¹⁹⁻²¹

The classical method of ECP, first developed by Edelson (UVAR, Therakos), was based on the use of a discontinuous flow cell separator, oral administration of 8-MOP and irradiation of the cells with the same apparatus. Efforts to improve on the historical technique are addressed particularly at avoiding the discomfort of 8-MOP oral administration, improving the irradiation efficacy, reducing the extracorporeal volume during the collection, and shortening the time of the procedure.²²

The ECP technique we have adopted was first developed by French groups and has significant advantages over the classical technique in all of the above mentioned features.

Injecting the photosensitizing agent, 8-MOP, directly into the irradiation bag avoids oral administration with the advantage of eliminating drug-related side effects such as nausea and vomiting. It also overcomes the variability of psoralen gastrointestinal absorption.

As shown in Table 3 MNC collections resulted in a final product with a very low granulocyte and red cell

Table 4. ECP-related side effects in the 18 enrolled patients.

Case	Age (years)	Disease	ECP procedures	CVC	Follow-up (months)	Side effects (n. of episodes)
1	19	cGvHD	31	No	18	-
2	16	cGvHD	24	Yes	15	Fever (1)
3	11	cGvHD	18	No	12	Headache (3)
4	16	cGvHD	21	Yes	8	Fever (1)
5	7	cGvHD	14	Yes	5	-
6	36	cGvHD	12	No	3	-
7	24	cGvHD	10	Yes	3	Chills (3)
8	19	cGvHD	8	Yes	2	Fever (1)
9	47	cGvHD	8	No	2	-
10	41	cGvHD	6	No	1	-
11	13	cGvHD	2	No	-	-
12	16	aGvHD	6	Yes	1	-
13	12	aGvHD	1	No	-	-
14	29	pemphigus vulgaris	14	No	10	-
15	68	pemphigus vulgaris	13	No	6	-
16	47	pemphigus vulgaris	4	No	1	-
17	53	drug-resistant hepatitis C	25	No	8	-
18	47	heart transplant	7	Yes	14	-

contamination regardless of the initial leukocyte count, which indicates the high reproducibility of the harvesting technique. Red cell and granulocyte contamination of the product are critical factors influencing the efficacy of irradiation on the one hand (red cells strongly adsorb UVA energy) and lowering the effects related to cytokine release on the other (collection and manipulation can cause granulocytes disruption).

The technique allows optimal irradiation of the cells in the presence of a constant drug concentration. The efficacy of the irradiation is demonstrated by the inability of the irradiated cells to respond to PHA (decrease of response > 97%) and to allogeneic MNC in mixed lymphocyte culture.²⁵

Using this ECP technique the final volume of the collection was always less than 150 mL. It is crucial to minimize extracorporeal volume when low body weight patients are being treated. Indeed the results from our series confirm that this new ECP technique is feasible in patients with various diseases and consequently in different clinical situations. Even patients of low body weight (< 20 kg) or with a poor performance status have been demonstrated to tolerate the procedure well.²³

Moreover, the small amount of plasma collected lowers the risk of photoallergy (erythema, edema, vesiculation, itching) due to the photomodification of plasma proteins induced by UV-A irradiation.²⁴

Manipulation of MNC after collection is simple and not time consuming, taking only about 10 minutes.

Alongside the advantages of being feasible in low weight in debilitated patients with a range of clinical disorders, a low white blood cell count and thrombocytopenia are not absolute contraindications to starting ECP. Furthermore, moderate cardiac, renal or liver impairment due to previous intensive chemotherapy did not restrict the possibility of treatment.

Platelets contaminating the MNC harvested were not functionally damaged by UV-A irradiation and their reinfusion into the patients resulted in the reestablishment of the precollection platelet count.

The problem of venous access is, however, crucial: the lack of adequate peripheral veins may be bypassed by using a central catheter previously placed for chemotherapy or positioning a new one in order to guarantee a good flow rate.

The tolerability of every procedure was excellent and all patients willingly consented to the long term therapeutic program. No patient discontinued the treatment except for patient #2 because of catheter malfunction (CVC positioned 10 years before!).

All the collection procedures were completed without significant complications and no life-threatening side effects were recorded. Chills were observed in one patient and were promptly controlled by additional calcium gluconate administration. No hypotensive episodes were recorded, even in low body weight patients.

During the post-reinfusion phase mild fever (38-39°C) occurred in 3 patients within 12 hours of the MNC reinfusion, probably due to the release of cytokines by the UV-A damaged cells. In one patient headache occurred three times but this was easily treated with paracetamol.

ECP did not increase the transfusional demand of red cell concentrates and platelets. The moderate hemoglobin decrease sometimes observed after the procedure in a few patients was probably due to the dilutional effect related to ACD-A infusion and to the addition of saline to the collected cells.

In this series of patients, some of whom have now been followed up for over a year (mean follow-up 7 months), no serious infectious episodes have been reported despite concomitant immunosuppressive therapy, indwelling CVC, low WBC count, and positive serology for CMV and HCV.²⁶

In conclusion, ECP proved to be safe with particular regard to the feared infection risk. Moreover, the acceptance by the patients was excellent, especially when ECP treatment permitted immunosuppressive therapy to be tapered down or stopped. The reproducibility of the collection and processing technique reported here will permit comparison of results among different centres which is the starting point for developing multicenter controlled trials in order to clarify the role of ECP in the treatment of various immune and autoimmune diseases.

Contributions and Acknowledgments

CP and LT were the principal investigators and formulated the design of the study. LS supervised all the study and participated in writing the paper. GLV, SB, SC, and CDF performed the leukaphereses and irradiation of collected MNC. LR and RLT collected the data. FL and FB selected patients affected with cGVHD. PG, MB selected patients affected with pemphigus vulgaris. The order in which the names appear is based on the contribution given to the study and on the time spent performing the leukaphereses and collecting the data.

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Disclosures

Conflict of interest: none.

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