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Extracorporeal photochemotherapy: an Italian panel perspective on indications, methodologies and clinical results

G. Andreola[1], A. Babic[1], P. Perseghin[2], G. Crovetti[3], P. Marson[4], C. Savignano[5], F. Ipsevich[6], A. Lanti[7], D. Laszlo[1]

[1]Stem Cell Mobilization and Collection Unit, Division of Hematoncology, European Institute of Oncology, Milano;
[2]U.O.S. Apheresis and New Transfusion technology-Laboratory of Cryobiology, San Gerardo Hospital, Monza;
[3]SIMT, Busto Arsizio Hospital, Varese;
[4]Apheresis Unit, Blood Transfusion Service, University Hospital of Padova, Padova;
[5]Transfusion Medicine Department, University Hospital of Udine, Udine;
[6]SIMT, San Camillo Forlanini Hospital, Roma;
[7]Immunohematology Section, Tor Vergata University, Roma, Italy

SUMMARY

Extracorporeal photochemotherapy (ECP) is an emerging treatment for a variety of pathological conditions. Initially described by Edelson for treatment of patients with cutaneous T cell lymphoma (CTLC), it is becoming an integrated part of the treatment of acute and chronic graft-versus-host disease, a frequent complication of allogeneic stem cell transplantation, solid organs transplant rejection, several autoimmune diseases such as scleroderma or Crohn's disease.

On June 22nd 2012, a panel of Italian experts on ECP came together in Milan, Italy, to discuss the most controversial issues on this topic. Here we report the relevant issues discussed in the meeting.

INTRODUCTION

Extracorporeal photochemotherapy (ECP) is an emerging treatment for a variety of pathological conditions and it was initially described by Edelson for treatment of patients with cutaneous T cell lymphoma (CTLC) (1). ECP is becoming an integrated part of the treatment of acute and chronic graft-versus-host disease (GvHD), a frequent complication of allogeneic stem cell transplantation, solid organs transplant rejection, several autoimmune diseases, such as scleroderma or Crohn's disease (2). A panel of Italian experts on ECP discussed the most controversial issues on this topic in a meeting held in Milan, Italy, on June 22nd 2012.

Key words: extracorporeal photopheresis.

Correspondence:
Giovanna Andreola
Stem Cell Mobilization and Collection Unit
European Institute of Oncology
Via Ripamonti, 435 - 20141 Milano, Italy
E-mail: giovanna.andreola@ieo.it

Extracorporeal photochemotherapy: state of the art

Dr. G. Crovetti (Busto Arsizio) reviewed clinical indications and results in each of the pathological conditions: CTLC including mycosis fungoides (MF) and Sezary's syndrome (Ss), GvHD both acute and chronic, solid organ transplant rejection (heart, lung, liver, kidney, face), scleroderma and the newest indications such as Crohn's disease. European Organization for Research and Treatment of Cancer (EORTC) lists ECP for stage III MF and Ss in monotherapy, in combination with interferon, with total skin irradiation and with other therapy. MF and Ss are characterized by a monoclonal proliferation of CD4+CD45+RO+ helper T lymphocytes and the loss of mature T-cell antigen CD7 or CD26 in the skin and other involved organs; the prognosis is highly dependent on disease stage and is usually poor among aggressive subtypes with a median survival of 50 months and a 5-year survival rate of 30% (3). A review and a meta-analysis published in 2003 based on 19 studies for a total of 438 patients reported an overall response rate (ORR) of 55.5% with a complete response (CR) rate of 14.8% in patients with CTLC. In 5 studies a total of 28 patients with stage III MF were evaluated; the overall response rate was 35.7%, with a CR rate of 17.9%. Eight studies looked at responses in 108 patients affected by Ss and reported ORR of 42% with a CR rate near 10% (4). A long-term follow-up on 29 patients affected by MF, showed a median survival of 60 months (5).

Studies in acute GvHD (aGvHD) are small and mostly retrospective: responses in cutaneous disease have been observed from 50 to 93%, in liver up to 65% and in gastrointestinal (GI) from 40 to 100%. A meta-analysis of 11 studies in 76 patients showed skin response up to 83% with a complete response rate of 67%, liver complete response of 38%, GI complete response of 54%; maximum responses were obtained after 6-8 weeks with an overall survival (OS) of 53%. Immunosuppressive therapy was stopped in 28% of the cases and reduced in 46% (6).

The only prospective study was published in 2006 by Greinix et al. (7) in 59 consecutive patients suffering from steroid refractory or steroid-dependent aGvHD, treated with ECP. Complete responses were obtained in 82% of patients with skin disease, in 61% with liver disease and 61% with GI involvement. Remissions were rapid, after a median of 4 cycles of treatment and a median time of 1.3 months, and durable; no flares were observed after stopping the therapy. ECP treatment also allowed a rapid withdrawal of steroid therapy and patients treated with ECP achieved a significant better 4-year OS (59% *vs* 11%) and better 4-year transplant related mortality (TRM) (36% *vs* 14%).

ECP has also been tested for the prevention of aGvHD; ECP treatment performed on 2 consecutive days within 4 days of the conditioning regimen was tested in 66 patients enrolled in a prospective, multicenter trial and compared versus historical controls. Patients were treated with a myeloablative conditioning regimen including cyclosporine and methotrexate as immunosuppressive therapy and ECP/PP was performed with the on-line system (UVAR/UVADEX, Therakos). The authors were able to show a lower rate of grade II-IV aGvHD probably due to a

delay in the onset, a trend to less TRM with higher OS and disease-free survival and more importantly a significant less incidence of infections (8).
Even in chronic GvHD (cGvHD), studies are usually small and retrospective. A meta-analysis of 20 studies in 224 patients reported skin response in 76% with a CR rate of 35%, responses in lung disease were 48% and in liver 39%, with an OS of 79%. Treatments were often heterogeneous, *personalized* on the basis of clinical situation (6). In 2006 Flowers et al. published a randomized, multicenter, phase II study for the treatment of cGVHD. When ECP was added to a standard treatment of prednisone plus calcineurin inhibitors a significant reduction of steroid dose could be achieved. Total skin score did not differ significantly but it showed a favorable trend with longer treatment (9).
As for solid organ transplant rejection, Barr et al. in 1998 proved for the first time that the addition of ECP to triple-drug immunosuppressive therapy significantly decreases the risk of cardiac rejection without increasing the incidence of infection in a multicenter, international randomized double blind trial performed in 60 patients (10), results later confirmed by other authors who showed that the use of ECP is associated with improved control of recurrent rejections and allows tapering of immunosuppressive drugs (11). In a prospective randomized study of 23 primary cardiac transplant recipients ECP was added as a prophylactic tool to standard immunosuppression which resulted in a significantly reduction of panel reactive antibodies and significantly decreased coronary artery intimal thickness, sign of chronic rejection (12). Finally in a retrospective study performed on 36 recipients of heart transplant, the risk of rejection with hemodynamic compromise or rejection death decreased by 71% vs control group (13).
ECP has been used for treatment of lung transplant rejection; ECP in lung transplant recipients has only been studied retrospectively. Furthermore, all patients treated with ECP already had evidence of rejection, and the treatment was used to halt or slow down progression of bronchiolitis obliterans syndrome.
Different studies documented that ECP induced significantly clinical and/or histological improvement in patients with acute or chronic rejection, including stabilization of lung function, measured by FEV_1 (14-17).
As for liver transplant, different studies have been published showing efficacy in acute and chronic liver rejection (18-21).
In one of the latest published by Urbani et al. (22), clinical records of 302 hepatitis-C liver transplant recipients are reported: in 133 no ECP was added; in 91 patient ECP was added as 3rd line rescue therapy for biopsy proven rejection; in 78 patients antivirals and ECP were added for maintenance of drug regimen, and was intensified if rejection occurred. In the first group the 18-month mortality was reduced by 10%, in the second group by 22%, and in the third by 28%.

In-line and off-line systems

ECP can be performed with two different systems, the on-line and the off-line system. Dr. F. Ipsevich (Rome) and Dr. D. Laszlo (Milan) described the two systems illustrating advantages and disadvantages of both systems.

On-line systems are from Therakos: Uvar XTS and the more recent CellEx were discussed. The Uvar XTS performs a discontinuous centrifugation cell separation with a single needle venous access, it is usually contraindicated for pediatric patients, weighing less than 40 kg, and it allows a collection of up to $3x10^9$ of white blood cells (WBC). The CellEx allows single or dual-needle access with a continuous and discontinuous flow and requires a shorter treatment, processes up to 2000 mL of blood with a medium buffy-coat volume of 200-230 mL, allowing a numerically greater collection up to $5x10^9$. It is also indicated for pediatric patients. They both use standardized materials and are CE and FDA certified for photoactivation procedures and as pharmacologic treatments, but costs are pretty high.

Dr. Laszlo illustrated the off-line system, UVA PIT System by Med Tech Solutions and THERAFLEX - ECP System by Machopharma which is based on two different and subsequent phases: mononuclear cell collection, which can be performed with different machines, and a second one in which 8-MOP is added on the basis of cell number and then the product is exposed to the UVA.

With the off-line system it is possible to treat low-weight pediatric patients, patients with high bilirubin levels (such as in patients with aGvHD), low-volume buffy coat, with competitive costs. There are issues correlated to the different process, since the photoactivation can actually be defined as a minimal manipulation process and safety and sterility controls are required in addition to a validation process to show the expected biologic effect. He reported his personal experience on hemocolture performed on patients before the procedure and on the reinfusion product for aerobic, anaerobic and fungi, which resulted, all negative.

Validation studies

The issue of validating the system has been reviewed by Dr. C. Savignano (Udine). As required by the *International standards for cellular therapy product collection, processing, and administration*, accreditation manual, it is essential to validate and verify processing techniques as ECP. It is essential to characterize subsets of WBC in terms of lymphocytes, monocytes, and granulocytes; there have been indeed reports on the possible cell-dose-related effects by two different publications (23, 24), *i.e.* a higher total leucocyte dose is associated with superior treatment outcomes in cGVHD. As for the biologic effects test on mitogen-induced proliferation and on apoptosis have been published (25, 26). She pointed out that even though the biologic effect verification is essential to validate the entire process, this has to be done with a safe and standardized test (Annexin VI/PI, 7AAD) (27). There is no generalized standard test and the number of procedure on which it is necessary to validate; costs may be high.

Extracorporeal photochemotherapy in pediatric patients

Dr. P. Marson (Padova) reviewed the use of ECP in pediatric patients. A study from Calore et al. showed a 73% of CR for a GvHD, 2-y OS and progression-free survival of 85 and 87% in 15 patients with steroid-refractory-de-

pendent aGvHD (28). In these patients the extracorporeal circulating volume is a key issue for the risk of hypovolemic shock. Therefore it is important to maintain a level of hemoglobin >10 g/dL and an extracorporeal volume not greater than 15%.

On the other side, the liquid overload is the other issue to take into account and therefore it is important not to reinfuse the content of the belt at the end of the apheresis, to limit the volume of isotonic solution to dilute pre-irradiation buffy-coat (40-50 mL), to reinfuse the buffy-coat in slowly and to use diuretics if necessary, in patients with a compromised cardio-circulatory function.

The ECP procedure has an excellent safety profile: side effects might be mild (asymptomatic hypocalcaemia, circuit coagulation, hematoma at the side of access, low pressure), moderate (fever, allergic reaction, nausea/emesis), severe (vagal reaction, hypovolemic shock, intravascular hemolysis, arrhythmia) and in pediatric patients hypothermia.

Nevertheless, in 2010 Perotti et al. published on a series of 2360 procedures where he observed the occurrence of only mild side effects, including chills, mild abdominal pain, fever headache, with no hypotensive or metabolic complications recorded even among low-body weight patients (29). ECP remains an important therapeutic option for children affected by GvHD. Risks and complication of immunosuppressive therapy in pediatric patients are well known and include infections, increase incidence of disease relapse, growth delay, multiorgan damage, secondary neoplasms, poor quality of life.

The on-line systems, UVAR XTS can be used only with patients >40 kg (30) while the CellEx allows to treat patients of any weight as the off-line system (31). Indications are pretty much similar to the ones for the adult population. Low-body weight is not a limitation for ECP, but necessitates a specific approach, in children weighing less than 40 kg the technique of choice is the *off-line technique* (apheresis plus standalone irradiation); in bigger children, the choice of device should be made on a case-by-case depending on the pros and cons of each technique (32).

Extracorporeal photochemotherapy for graft-versus-host disease

Indications to ECP for the treatment of GvHD were discussed by many of the experts (33).

The all agreed that they are still controversial: generally, in case of aGVHD the absence of clinical and biologic improvement after 1 week of steroid therapy (up to 2-5 mg/kg/day), with the tendency to start earlier in the event of severe aGVHD (even as 48 h after the initiation of steroid therapy) (29). The indication to continue ECP therapy until maximum improvement - maximum immunosuppressive therapy (IST) tapering and clinical response- in responders, on an individual basis, after a multidisciplinary discussion of the case, discounting after 4 weeks, in the absence of response.

For cGVHD from the onset in association to IST, in both extensive and limited disease, with any organ involvement, ECP treatment should go on until maximal response is obtained (trying to reduce, at the same time, IST), according to a multidisciplinary case discussion. If no response is obtained,

ECP should be stopped after 8 weeks. These indications suggested by Perotti et al., are wider than those suggested by Kanold et al. in 2007 (32) for cGVHD who would start the treatment in the following cases:

1. stable disease after 1 month of treatment;
2. no more than a partial response after 2 months of treatment; or
3. progressive disease 2 weeks after initiation of steroid treatment.

Schedule of treatment are different too. For aGVHD different schedules have been described:

1. 2 procedures on 2 consecutive days every 1-2 weeks until improvement; then every 2-4 weeks until maximum results (7);
2. 3 weekly procedures until maximum result, then reduction on individual basis (32); 2-3 weekly procedures until clinical improvement, then 2 weekly procedures for two weeks, 2 weekly procedures every other week for three times, and finally two procedures per month (29).

For cGvHD the different schedules are as follow:

1. 3 weekly procedures, then 2 procedures in consecutive days from week 2-12; if positive results, 2 procedures every 4 weeks until week 24 (9);
2. 3 weekly procedures, then twice weekly, then once weekly and then one procedure every 15 days, depending on clinical result (34);
3. from 2 to 4 weekly procedures, then once weekly until partial response until maintaining to 2 procedures every 2 weeks (35);
4. 2 weekly procedures twice, then two weekly procedures every 14 days for three times, and finally two procedures every month until clinical improvement and/or immunosuppressive therapy tapering (29).

Extracorporeal photochemotherapy in dermatological disease

Dr. A. Lanti (Rome) described experience with ECP in dermatological diseases. Indications for dermatological diseases are: atopic dermatitis (AD), CTCL, systemic sclerosis (SyS), erosive lichen planus, psoriasis, pityriasis rubra pilaris and pemphigus.

In the management of CTCL, ECP is mainly used for the treatment of erythrodermic patients, including those with Ss with a high number of circulating atypical mononuclear cells (Ss cells). A number of studies indicated the efficacy of ECP in the disease, and according to a meta-analysis, for all stages of CTCL the combined overall response rate was 55.7% with 17.6% complete remission. ECP is recommended as first-line treatment for patients with MF stage III and Ss by treatment guidelines of the European Organization for the Research and Treatment of Cancer.

Predominant theories today explain the therapeutic benefit of photopheresis in CTCL by apoptotic malignant T cells that are phagocytized by stimulated monocytes. Then they are processed and presented by antigen presenting cells, which evokes an anticlonotypic response by CD8+ effector cells against the CD4+ neoplastic T cells. SyS is an autoimmune disorder, characterized by abnormal deposits of collagen in the skin and within visceral organs.

Many reports in the literature show a beneficial effect of ECP on SyS; specifically improvement of the cutaneous

component may override systemic response.
The mechanism of ECP in the successful treatment of SyS patients, again is unclear, but points at an abnormal immune activation by means of activated T cells within the peripheral blood and the sites of abnormal collagen production.
AD is a troublesome inflammatory skin disease characterized by severe pruritus, typical eczematous morphology and a chronic relapsing course often associated with increased immunoglobulin (Ig) E levels. Immunomodulatory changes of the inflammatory mediator pattern might be beneficial in the treatment of severe AD. ECP induced clinical improvement and reduction of elevated serum levels of eosinophil cationic protein and total IgE. Dr Lanti showed a particular case of AD in pediatric patient favorably solved with ECP.
A great number of studies indicate that ECP may be used as monotherapy o in combination with conventional therapies for the treatment of severe skin diseases that has become intractable to standard therapeutic modalities. ECP is a safe and viable treatment option with few adverse effects. Additional clinical studies and long-term follow-up are required to assess the benefits of ECP in dermatological diseases.

Mechanisms of action of extracorporeal photopheresis: biologic studies

Dr. P. Perseghin (Monza) reviewed biological mechanisms of ECP. Multiple mechanisms have been postulated and investigated. In a rat relapsing experimental allergic encephalomyelitis [model for multiple sclerosis MS)], incidence of disease relapses in rats treated with ECP were significantly lower than in the control group (36).
Based on pre-clinical results, ECP was used in a safety and tolerability pilot study in refractory relapsing-remitting MS. Administered as one procedure every 2 weeks for 4 months and one procedure/month for 8 months, the authors showed a reduction of the total dose of steroids administered during the study period, and stabilization/reduction of encephalic lesions at the nuclear magnetic resonance (37).
The main mechanism involved in ECP is lymphocytes apoptosis, involving both the Fas/FasL system, and the Bcl-2 protein family, but since only the 1-2% of lymphocytes is treated in a single procedure that cannot be the only mechanism involved in the clinical activity. Down-regulation of pro-inflammatory cytokines, production of anti-inflammatory cytokines, clearance of apoptotic cells by antigen-presenting cells resulting into a more tolerogenic phenotype leading to decreased stimulation of effector T cells or their deletion, finally generation of T regulatory cells have all been described as possible mechanisms involved in ECP clinical effects. In particular Biagi et al. evaluated the changes in frequency and immunophenotype of circulating regulatory T cells in 10 patients undergoing allogeneic hematopoietic stem cell transplantation, receiving ECP/PP for aGvHD or cGvHD. ECP was accompanied by a significant increase of CD4+CD25+ T regs after 6 procedure, with a simultaneous increase of its associated marker GIRT (38). Sorted CD4+CD25+ T-regs were potently inhibitory toward the CD4+CD25- cell fraction when matched with an allogeneic target, through a cell-to-cell contact.

ECP was accompanied by progressive improvement of GvHD and drugs tapering, concomitantly with the detection of increasing levels of circulating T-regs (38).

GcHD associated autoimmunity and, by extension, cGVHD is attributable to the progressive loss of regulatory T cells during the course of aGvHD. This leads to the expansion of donor-derived CD4+ T cells with Th1 and Th17 cytokine phenotypes that release proinflammatory cytokines and cause autoimmune-mediated pathological damage. These T cells are present early after transplantation, indicating that the pathophysiological events that lead to cGVHD are set in motion during the acute phase of GVHD, therefore the absence of CD4+CD25+ regulatory T cells coupled with unregulated Th1 and Th17 cells leads to the development of autoimmunity and that donor-derived Th1 and Th17 cells serve as the nexus between aGvHD and cGvHD (39, 40). Di Blasio et al. documented a much higher secretion of IL-17 in 27 patients with acute and cGvHD treated with a combination of immunosuppressive drugs, which correlates with significantly lower percentages of CD24+CD25+FOXP3+ T-reg cells.

In addition, Dander et al. (41) analyzed presence of Th17 cells in 51 hematopoietic stem-cell transplantation patients and 15 healthy donors. An increased Th17 population was observed in patients with aGvHD and in patients with active cGvHD along with an inflammatory process. In contrast, the percentage of Th17 cells drastically decreased in patients with inactive cGVHD. Th-17 cells consisted of both interleukin (IL)-17+/interferon (IFN)gamma- and IL-17+/IFNgamma+ subsets and expressed IL-23 receptor. Interestingly, IFNgamma+ Th17 cells were able to infiltrate GvHD lesions as observed in liver and skin sections. Moreover, the proportion of Th-17 was inversely correlated with the proportion of regulatory T cells observed in the peripheral blood and tissues affected by GvHD.

Immune effects of ECP are still under investigation and multiple mechanisms are likely to be involved. T-regs represent an important target because they have an inhibitory function on allo-reactive T cells mediated by cell-to-cell contact and do not secrete inhibitory-cytokines, while Th17 subsets deserve further investigation.

Clinical management of the patients undergoing extracorporeal photochemotherapy

Finally A. Babic (Milan) gave an updated on the best vascular access for performing ECP procedures. The presence of good vascular access is obviously necessary; the procedure needs a constant blood flow pressure of 40-90 mL/min during the whole time (3 h approximately) with minor complication risks.

Peripheral veins (cephalic or basilical) are usually preferred, but patients' venous access is often insufficient or severely compromised by multiple chemotherapy cycles.

Therefore a central venous access is usually necessary. Patients may have a Port-a-Cath catheter, which can be used as a return way during the procedure, but in case of complete absence of peripheral access there is need of another central venous catheter in femoral or jugular vein. In case of ECP with off line system we need

two good venous accesses available during every procedures. There are a variety of schedules depending on disease, but usually the treatment takes more than six months to be completed. The choice of the central venous catheter to be used also depends on the psychophysical conditions of each patients and it needs to be evaluated carefully and on a one-to-one basis. Depending on the situation, the choice of the catheters to be used for procedures is personalized. Venous access, risks of infections and thrombosis are evaluated by the apheresis team while the nurse is dedicated to perform the procedure and to schedule catheter's medications. Choosing the right venous access we can guarantee the optimum blood flow able to collect in less time the selected cell types with low contamination of unwanted cells, minor risks due to the platelet loose, minor stress to the patient and major security to patient and to operator.

Current recommendations from the American Society of Clinical Apheresis

Current recommendations for the use of ECP from the American Society of Clinical Apheresis are the following: scleroderma (recommendation grade 1A); cutaneous acute and chronic GvHD (recommendation grade 2C and 1B respectively), non-cutaneous acute and chronic GvHD (2C), cutaneous T-cell lymphoma (mycosis fungoides and Ss) in erythrodermic phase (1B) and non-erythrodermic phase (2C), lung allograft rejection (1C), cardiac allograft rejection in prophylaxis (1A) and treatment of rejection (1B), *Pemphigus vulgaris* (2C), nephrogenic systemic fibrosis (42).

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