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Evaluation of cell death after treatment with extracorporeal photopheresis

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ABSTRACT

The aim of our study is to assess the mortality of leukocytes during extracorporeal photopheresis. Sixty-three photopheresis performed on 13 patients affected by chronic GvHD were evaluated. Samples were analyzed using a FACSCalibur flow cytometer. Apoptosis and necrosis of limphomononuclear cells dramatically increased after the apheretic procedure. We found a further increase of apoptotic and necrotic limphomononuclear cells after treatment with 8-MOP and UVA ($p \leqslant 0.05$). Our data suggested that the immunomodulatory effects of extracorporeal photopheresis, triggered by circulating apoptotic or necrotic cells, could play an important role in the treatment of GvHD with this procedure.

1. Introduction

Extracorporeal photopheresis (ECP) represents a link between medical physics, pharmacology and clinical immunology.

Szczepiorkowski et al., in "Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Apheresis Applications Committee of the American Society for Apheresis", suggest that ECP can be used for the treatment of several diseases, such as cardiac allograft rejection, cutaneous T-cell lymphoma (in particular mycosis fungoides and Sézary syndrome), Graft–versus–Host disease, lung allograft rejection, nephrogenic systemic fibrosis, pemphigus vulgaris and scleroderma (progressive systemic sclerosis) [1].

During ECP, leukocytes from the apheresis procedure are exposed to ultraviolet light in the presence of an extracorporeally administered photosensitizing agent, 8-methoxypsoralen (8-MOP). The cells are then re-infused to the patient [2] (Fig. 1).

8-MOP is a furocoumarin characterized by the ability to form mono- and bi-functional adducts with DNA pyrimidine bases with UV-A irradiation. The link between 8-MOP and the double helix of DNA can cause nucleotide damage [3]. During photopheresis oxidative reactions also occur within nuclear and cytosolic proteins [4].

The molecule of 8-MOP is optimally activated by exposure to UVA light (320–400 nm). The photoadducts produced, are proportional to the concentration of the psoralen (ng/mL) and to the UVA irradiation (J/cm^2), applied to the cell suspension [5].

The planar structure of 8-MOP facilitates its intercalation between base pairs of DNA. Its reactive sites can be activated by exposure to UVA [6].

The DNA damage is tightly related to the apoptotic process. Therefore, apoptosis induction by ultraviolet light A

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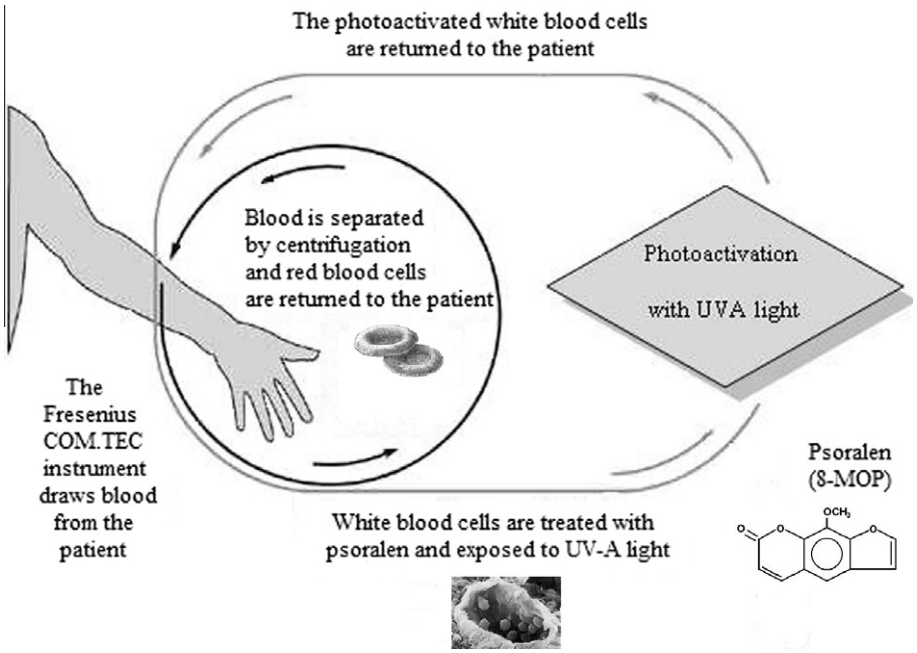

Fig. 1. By apheresis, can be obtained an optimal collection of white cells (hematocrit index ⩽2%). Red blood cells and plasma are automatically returned to the patient at the end of each cycle of harvest. Through four cycles of apheresis are obtained approximately 150 mL of apheretic collection. These 150 mL are then diluted with saline solution in a dedicated bag (Maco Pharma XUV8501Q UVA-B) in order to reach a final volume of 500 mL. After addition of 8-MOP (100 ng), the cellular suspension is exposed to irradiation with UVA light (intensity 2 J/cm^2) for 20 min and finally infused to the patient.

and 8-MOP may have an important involvement in the therapeutic action of extracorporeal photopheresis [7–9].

In this study, we have investigated the mortality of leukocytes during each step of the photopheresis procedure.

In order to reach this goal we have used a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) as this technology allowed us to detect apoptotic and necrotic cells in the total ECP-treated leukocyte population.

2. Patients and methods

2.1. Patients and control group

From March 2006 to March 2007, 13 patients affected by chronic GvHD were evaluated. The median age was 39 (range 28–50) years and the male to female ratio was 10/3.

The control group consisted of 63 healthy donors (50 males, 13 females). The median age of donors was 37 (range 27–47). Whole blood samples were collected from each donor and divided into 1 mL aliquots, in EDTA tubes.

Informed consent was obtained from the patients. The reported investigations have been performed according to the principles of the Declaration of Helsinki.

2.2. Photopheresis

In this study, a total of 63 photopheresis procedures were evaluated. Patients' 1 mL aliquots were collected at three time points: blood was drawn from peripheral blood before ECP procedure, from the apheresis unit collected at the end of the leukapheresis and 1 h after 8-MOP/UVA exposure.

Leukapheresis was performed using the COM.TEC® Continuous-Flow Blood Cell Separator (Fresenius Kabi, Sevres, France).

The photoactivation of leukocyte collections was performed through the UVA-B XUV8501Q device (MacoPharma, Tourcoing, France).

During ECP, 100 ng of 8-MOP were usually added directly in the collection bag. After exposure to UVA (135 ± 63 min), the cells were returned to the patients. The procedure was performed in approximately 4 h. It was estimated that 2.5–5% of the white blood cells were irradiated. The photopheresis was done twice a week for about 4 weeks.

2.3. Cell counts and flow cytometry

Cell counts were measured using an ethylenediaminetetraacetic acid-anticoagulated sample with an XT-1800i Hematology Analyzer (Sysmex, Meylan, France).

Apoptosis and necrosis were evaluated with the FACSCalibur flow cytometer (BD Biosciences), using Annexin V FITC (BD Pharmingen, San Diego, CA, USA) and 7-aminoactinomycin-D (7-AAD) (BD Biosciences, San Jose, CA, USA) in order to detect the expression of phosphatidylserine (PS) on the external side of the plasma membrane and to identify the non-viable cells, respectively. Cells were washed twice with PBS 1× and resuspended in Binding Buffer 1× (BD Biosciences, San Jose, CA, USA) at the concentration of 1×10^6 cells/mL. After 100 μL of the solution (1×10^5 cells) were transferred in a tube for cytometry (BD Biosciences, San Jose, CA, USA). Subsequently, 5 mL