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ORIGINAL RESEARCH

TRANSFUSION

Apoptosis induction by extracorporeal photopheresis is enhanced by increasing the 8-methoxypsoralen concentration and by replacing plasma with saline

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Abstract

Background: Extracorporeal photopheresis (ECP), an apheresis-based therapy for various immunological diseases, works mainly by inducing apoptosis in lymphocytes. Several factors influence the efficacy of ECP with the photosensitizer 8-methoxypsoralen (8-MOP) and ultraviolet light A (UVA). This study aimed to optimize treatment by varying the 8-MOP starting concentration and the cell suspension medium.

Materials and Methods: All patients ($n = 13$) included in this study received photopheresis as medically indicated. Cells collected with a Spectra Optia apheresis system were suspended in plasma or physiological saline (NaCl) and incubated with 200 ng/ml versus 340 ng/ml photosensitizer before UVA irradiation (Macogenic G2 or UVA PIT system). Lymphocyte apoptosis and caspase activity were analyzed by flow cytometry and fluorimetry, and residual 8-methoxypsoralen concentrations by liquid chromatography–mass spectrometry.

Results: Raising the 8-MOP starting concentration significantly increased lymphocyte apoptosis, with values of 22% versus 35% (plasma) and 28%–46% (NaCl) at 24 h post-ECP and 37% versus 86% (plasma) and 74% versus 97% (NaCl) at 48 h for 200 ng/ml versus 340 ng/ml. Pre-transfusion residual 8-MOP levels were 168 ng/ml (plasma) and 162 ng/ml (NaCl) versus 290 ng/ml (plasma) and 266 ng/ml (NaCl) for the lower versus higher dose, respectively.

Discussion: Hence, 8-MOP concentration influences the efficacy of photopheresis as lymphocyte apoptosis rates were significantly higher with the higher starting concentration and with NaCl versus plasma. This indicates that

Abbreviations: 8-MOP, 8-methoxypsoralen; ATMP, Advanced Therapy Medicinal Product; APC, antigen-presenting cell; BC, Beckman Coulter; CMNC, Continuous Mononuclear Cell Collection; DC, dendritic cell; ECP, Extracorporeal photopheresis; GvHD, graft-versus-host-disease; HCT, hematocrit; LC-MS, liquid chromatography/mass spectrometry; MNC, mononuclear cell; Treg, regulatory T-cell; UVA, ultraviolet light A.

increased 8-MOP starting doses and saline as additional suspension medium could help in improving ECP's efficacy.

KEYWORDS
8-methoxypsoralen, apoptosis, ECP, extracorporeal photopheresis

1 | INTRODUCTION

Extracorporeal photopheresis (ECP) is an immunomodulatory therapy for T-cell mediated diseases such as cutaneous T-cell lymphoma, graft-versus-host-disease (GvHD), and solid organ transplant rejection.[1–4] In ECP, autologous leukapheresis is performed with subsequent ultraviolet A (UVA) irradiation in the presence of the photoactive substance 8-methoxypsoralen (8-MOP). This leads to transcription and replication inhibition followed by cell inactivation and apoptosis.[5–8]

The immunological mechanisms, although not completely understood, are mainly lymphocyte apoptosis,[9, 10] the initiation of dendritic cells (DC), and a rapid clearance of apoptotic cells.[11] Following ECP, the cytokine profile is modified toward upregulation of immunosuppressive factors and down regulation of co-stimulatory molecules.[12] Furthermore, antigen-presenting cells (APC) suppress T-cell effector activity and support regulatory T-cell (Treg) function.[13, 14]

Apoptosis induction is a central mechanism of ECP,[4, 15–19] and several factors influence the efficacy of 8-MOP/UVA treatment (see Figure 1). In general, the two standard starting concentrations of 8-MOP used for photopheresis are 200 ng/ml for offline ECP, and 340 ng/ml for inline ECP (calculated as product volume × 0.017 according to the instruction for use of Uvadex, Therakos).

The cell suspension matrix (plasma vs. saline) also influences the effect of 8-MOP in solution.[20] Plasma has two contradictory consequences for 8-MOP/UVA: it compromises 8-MOP availability for cellular uptake due to plasma protein binding on the one hand,[6, 21] but minimizes 8-MOP adhesion and absorbance to plastic materials on the other. 8-MOP is known for its affinity to various plastic materials, especially the polyvinylchloride used to make medical tubing and blood bags. UVA irradiation bags usually consist of ethylene-vinyl acetate, to which 8-MOP adheres to a lesser degree. Saline solution (NaCl), on the other hand, facilitates 8-MOP adhesion to bag surfaces.[22]

The efficacy of 8-MOP/UVA may possibly be influenced by the incubation time between addition of the photosensitizer and UVA irradiation. This substance diffuses within minutes into the cells.[23] Equilibrium within the cell suspension, however, may take up to 30 min.[24]

Treatment efficacy is further influenced by the UVA dose, which directly influences T-cell apoptosis.[20] A dose of 1–2 J/cm^2 is widely used at a range of wavelength of 320–400 nm.[6, 25] The efficacy of irradiation also depends on the hematocrit (HCT) of the product; it is compromised at HCT levels of more than 4% because RBCs intercept UV light.[20, 26]

The aim of this study was to optimize 8-MOP/UVA by modulating the 8-MOP concentration and the cell suspension matrix.

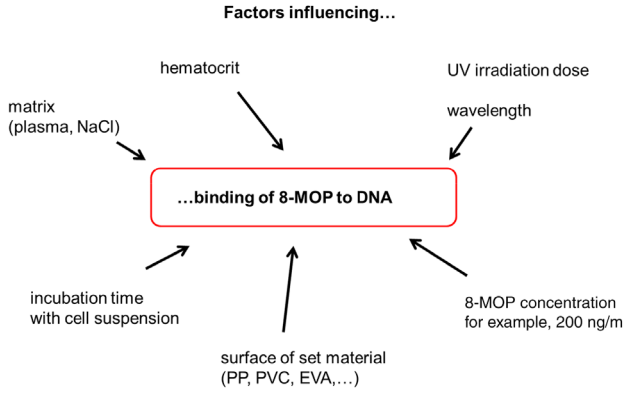

FIGURE 1 Critical parameters influencing the efficacy of photopheresis. Shown are several factors that influence binding of the photosensitizer 8-methoxypsoralen to DNA [Color figure can be viewed at wileyonlinelibrary.com]

2 | MATERIAL AND METHODS

2.1 | Patients

Data were obtained from 2018 to 2020 according to the EU Guidelines for Good Manufacturing Practice.[27] All participants gave their informed consent and received ECP as medically indicated.[28] The study included a total of 13 patients (11 male, 2 female), 5 with GvHD, 3 with cutaneous T-cell lymphoma, and 5 with cellular solid organ transplant rejection. The study protocol was approved by the local ethics committee of the University Hospital Regensburg (16-101-0046).

2.2 | Photopheresis

All ECP procedures ($n = 42$) were carried out with apheresis and UVA irradiation on separate devices (offline ECP). UV irradiation was performed either with the Macogenic G2 (Macopharma, France; $n = 14$) or the UVA PIT system (PIT Medical Systems GmbH, Germany; $n = 28$). Mononuclear cell (MNC) suspensions were obtained with a Spectra Optia (Terumo BCT, Lakewood, CO) apheresis system in the continuous mononuclear cell collection (CMNC) mode. The ECP procedures were set up to yield leukocyte and plasma volumes of 90 and 110 ml (UVA PIT) or 90 and 210 ml (Macogenic G2), respectively, after cell harvesting. Each resulting cell suspension was sterilely transferred to the irradiation system and 8-methoxypsoralen (8-MOP, Uvadex, Therakos, West Chester, PA) was injected into the bag at a concentration of 20 mg/l to yield a starting concentration of 200 ng/ml (low-dose) or 340 ng/ml (high-dose), respectively, of the drug. Patients were assigned to a high or low-dose 8-MOP starting concentration with MNCs suspended in plasma or saline, respectively, whereby high-dose 8-MOP was always delivered with UVA PIT system. After incubation with the photosensitizer, a UVA dose of 2 J/cm^2 was delivered with UVA PIT or Macogenic G2, as indicated, and the resultant cell suspension (product after sampling) was immediately infused to the patient (see Figure 2).

2.3 | Sample preparation

ECP product samples were taken before the addition of 8-methoxypsoralen (pre) and after UVA irradiation (post). Apoptosis and caspase-3/7 testing was carried out from pre- and post-samples immediately after sampling (0 h), and after 24 and 48 h incubation in TexMACS GMP medium (Miltenyi, Bergisch-Gladbach, Germany) with 1% Glutamax (Gibco) at 37°C and 5% CO_2.

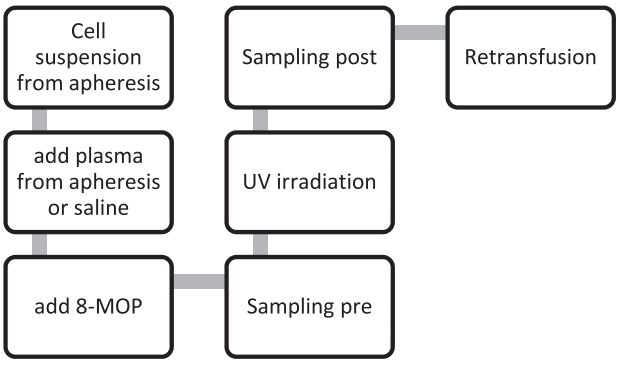

FIGURE 2 Flow chart of extracorporeal photopheresis procedure and sampling. "Pre" and "post" samples were analyzed for residual 8-MOP and incubated up to 48 h for caspase and apoptosis analysis

2.4 | Analytics

2.4.1 | Flow cytometry

Briefly, 1×10^6 MNCs/tube were stained after treatment with FcR Blocking Reagent (Miltenyi). Immunophenotyping of leukocytes was performed with commercially available antibodies from Beckman Coulter (BC): CD45-KrOrange, CD3-ECD, CD56-APC-AF750, CD20-APC-AF750, CD19-APC-AF750, and CD66b-APC-AF750. The BC Annexin V/7-AAD kit was used for apoptosis detection. All antibodies were titrated to obtain an optimal concentration.

Flow cytometric analyses were performed with the Navios Ex and Navios flow cytometers, Cytometry List Mode Data Acquisition Software, version 2.0 and 1.3, respectively, and Kaluza Analysis Software from Beckman Coulter, version 2.1. T-cells (CD3) were analyzed by excluding granulocytes (CD66b), natural killer (NK) cells (CD56), and B cells (CD19, CD20). Annexin V^+ and 7-$AAD^{+/-}$ cells were sub-gated from T-cells. Apoptosis induction levels were calculated as the percentage ratio of post/pre photopheresis.[29]

2.4.2 | Caspase 3/7 activity assay

Caspase activity was measured in ECP samples obtained with Optia and UVA PIT using the Cell Meter Fluorimetric Caspase 3/7 activity kit (AAT Bioquest, Sunnyvale, CA) according to manufacturer's instructions. In brief, 40,000 mononuclear cells per group were seeded in duplicates in 96-well plates (seeding volume: 100 µl). Exactly 24 and 48 h after ECP with Optia and UVA PIT, fluorogenic caspase-3/-7 substrate $(DEVD)_2$-R110 was added (volume: 70 µl), and the plates were incubated for 90 min at room temperature in the dark. Subsequently, light emission was quantified using a Victor3 Multilabel Reader (Perkin Elmer, MA). ECP-related caspase-3 and 7 activity was calculated and expressed as relative fluorescence units (RFU) in the post-ECP sample minus that in the pre-ECP sample.

2.4.3 | 8-MOP HPLC–MS/MS

The photopheresis supernatant (pre and post) was analyzed by liquid chromatography/mass spectrometry (LC–MS/MS) using the Agilent Technologies 1200 Series (Santa Clara, CA) and Varian 320-MS systems, if

indicated, or the Agilent Technologies 1290 Infinity and 6495 TQ Mass Spectrometer systems as already published.[30]

2.4.4 | Cell count

Cell concentrations and hematocrit were measured undiluted on an XN-550 Automated Hematology Analyzer (Sysmex, Kobe, Japan) per manufacturer's instructions.

2.5 | Statistical analysis

Data were analyzed and figures generated using Microsoft Excel 2010 and IBM SPSS Statistics 25 software. Descriptive statistics included absolute numbers, frequencies, ratios, means with standard deviations, and medians with interquartile ranges. Correlations were determined with Pearson correlation coefficient. p values below 0.05 were considered statistically significant. Normal distribution was analyzed by the Anderson–Darling test from R's package nortest.

TABLE 1 Patient and ECP product characteristics

Patient characteristics	
Age	57 years (34–65)
Sex	11 male, 2 female
Diagnosis	Graft versus host disease ($n = 5$), T-cell lymphoma ($n = 3$), solid organ transplant rejection ($n = 5$)
Leukocytes, peripheral	3.8×10^3/μl ($1.7–11.5 \times 10^3$/μl)
Lymphocytes, peripheral	11% (2.1%–55%)
Platelets, peripheral	196×10^3/μl ($16–387 \times 10^3$/μl)
ECP product characteristics	
Product volume	234 ml (178–282 ml)
Leukocytes	16.5×10^3/μl ($4.7–67.1 \times 10^3$/μl) 34×10^8 ($13–133 \times 10^8$)
Lymphocytes	32% (11%–73%)
Platelets	1.5×10^{11} ($0.2–3.5 \times 10^{11}$)
Hematocrit	1.2% (0.5%–1.9%)

Note: Characteristics of the study population and extracorporeal photopheresis (ECP) products. Data are presented as median and range.

3 | RESULTS

ECP was performed with cell separation and UVA irradiation on separate devices. The study included 13 patients with different diagnoses requiring ECP therapy. The cell suspension volumes varied depending on the irradiation system between 200 ml (UVA PIT) and 300 ml (Macogenic G2). The cellular composition of the ECP products was also variable and depended on the composition of cells in the peripheral blood of the individual patient (see Table 1). The products had a hematocrit of 0.5%–1.9%, which was in line with previously published values by Spectra Optia.[31] In general, a UVA dose of 2 J/cm^2 was delivered with both systems. The UVA PIT adjusted the exposure time according to the individual product volume and hematocrit to yield the defined UVA dose. Thus, the irradiance of UVA, in Joules, correlated with the hematocrit of the product ($R^2 = 0.9924$).

Apoptosis of CD3$^+$ T-cells, a well-known mechanism induced by ECP, was detected as soon as after 24 h incubation, as determined by discriminating between living, apoptotic (Annexin V$^+$), and necrotic (7-AAD$^+$) T-cells. Cells were suspended in plasma and irradiated with UVA PIT or Macogenic G2. Samples were taken before addition of 200 ng/ml 8-MOP and after irradiation to measure apoptosis without (pre) and with ECP treatment (post) and to calculate apoptosis induction (percentage ratio of post/pre) up to 48 h after ECP ($n = 24$; see Figure 3). After in vitro cultivation of the cells, 22% (−3% to 114%) of CD3$^+$ T-cells were apoptotic at 24 h compared with 37% (3%–86%) at 48 h. Tests for the association between cell content and apoptosis rates revealed no correlation between the proportions of leukocytes ($p = 0.497$), lymphocytes ($p = 0.260$), monocytes ($p = 0.169$), or neutrophils ($p = 0.168$) in the products and T-cell apoptosis rates 48 h after treatment.

Apoptosis is a regulated cell death process leading to the activation of proteolytic enzymes (caspases 3, 6, 7), DNA fragmentation, and chromatin condensation.[32] Therefore, caspase 3 and 7 activity was detected at ECP with the Spectra Optia and UVA PIT system with the standard conditions of 200 ng/ml 8-MOP and plasma, to verify apoptosis due to the ECP procedure ($n = 12$). Caspase activity increased from −29 RFU (median; range: 299–522 RFU) at 0 h to 8923 RFU (median; range: 133–20,869 RFU) at 24 h and 13,092 RFU (median; range: 1589–75,118 RFU) at 48 h; these levels assured advanced initiation of apoptosis already after 24 h (see Figure 4).

Comparison of the two UVA irradiation systems with 200 ng/ml 8-MOP and plasma revealed T-cell apoptosis induction rates by flow cytometry of 0%–35% (median 21%, $n = 12$) with UVA PIT versus 14%–114% (median 28.5%, $n = 12$) with Macogenic G2 24 h after treatment. At 48 h, the rates were 11%–86% (median 37.5%) and 3%–60% (median

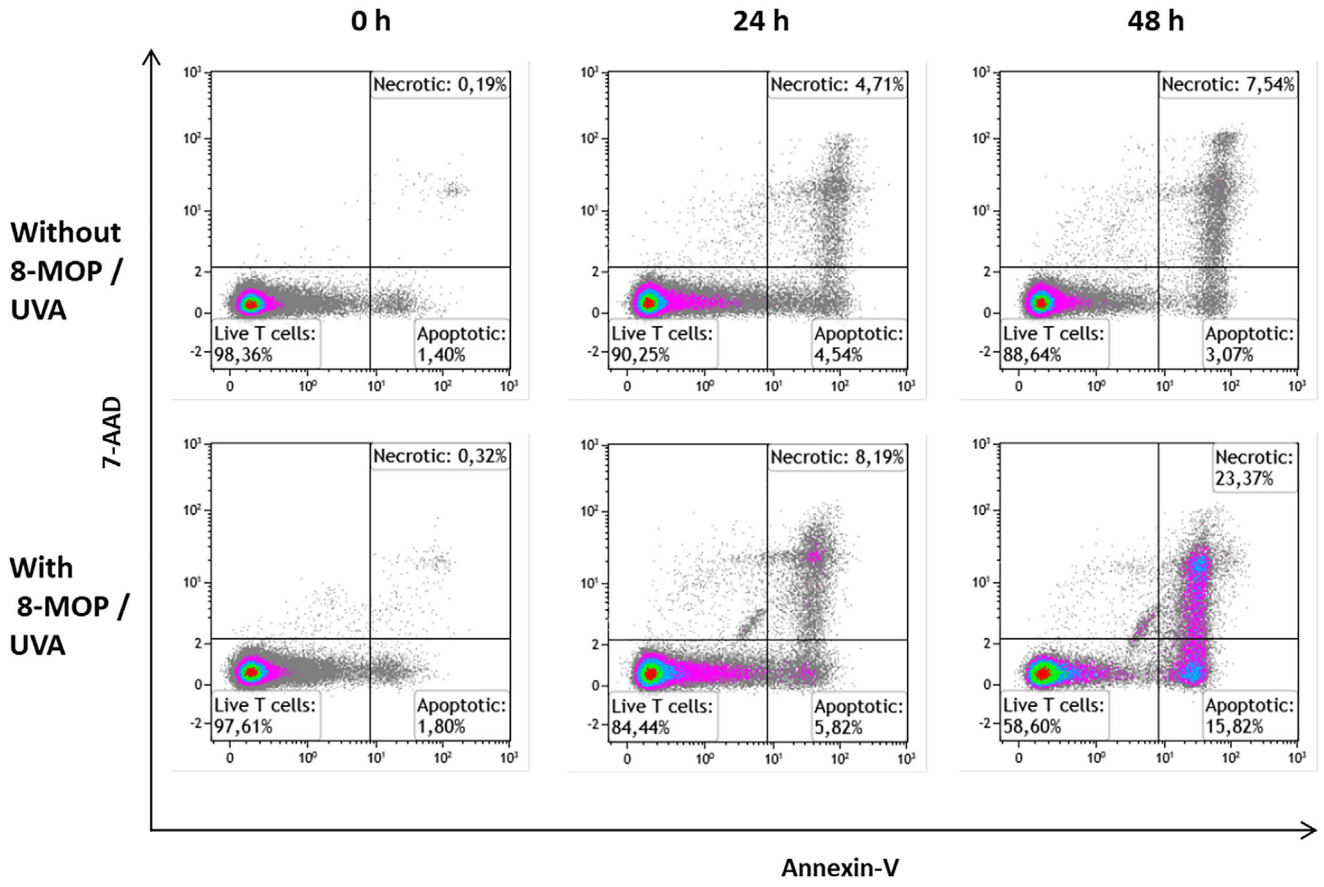

FIGURE 3 T-cell apoptosis induced by photopheresis. Extracorporeal photopheresis product samples were analyzed before versus 24 and 48 h after treatment with 8-MOP and ultraviolet light A irradiation. Necrotic and apoptotic T-cells were discriminated from live T-cells by Annexin V and 7-AAD staining [Color figure can be viewed at wileyonlinelibrary.com]

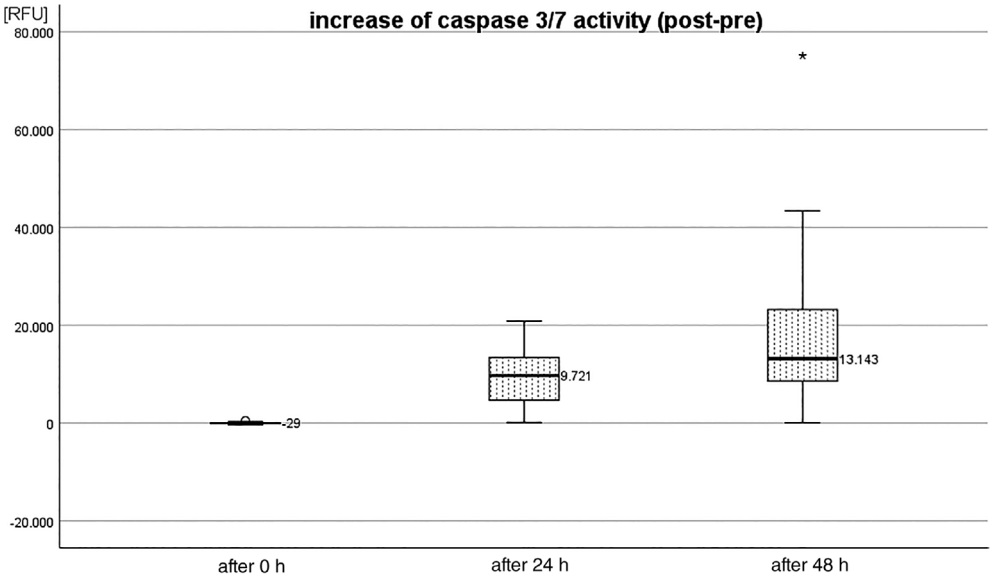

FIGURE 4 Increases in caspase 3 and 7 activity 24 and 48 h after extracorporeal photopheresis (ECP). Post–pre caspase-3/-7 activity levels were calculated by subtracting the levels measured by adding fluorogenic caspase-3/7 substrate $(DEVD)_2$-R110 to plasma-based ECP samples 24 and 48 h after irradiation (post) with the Optia and ultraviolet light A PIT system, respectively, from those detected before treatment (pre) and expressed in relative fluorescence units (RFU). Outliers are marked as asterisks

24.5%), respectively. There was no significant difference in apoptosis induction between the two UVA systems ($p = 0.164$).

Our standard ECP procedure specifies a UVA irradiation dose of 2 J/cm^2 with an 8-MOP starting dose of 200 ng/ml. With the aim of optimizing the ECP

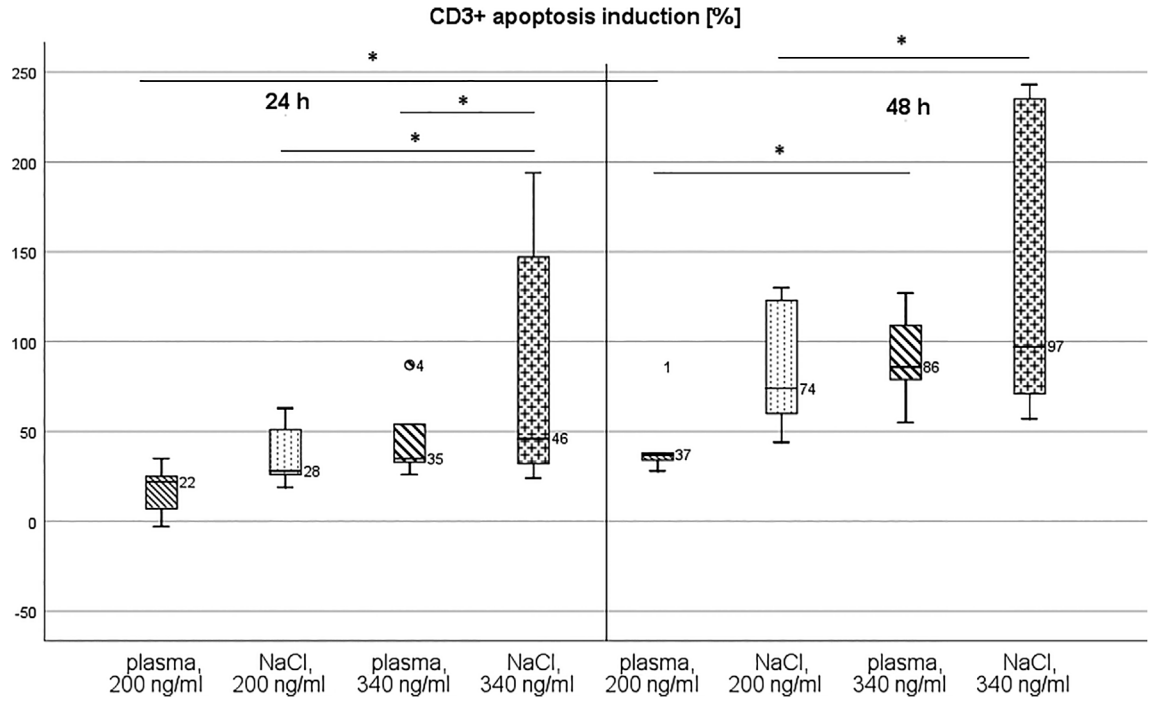

FIGURE 5 T-cell apoptosis induction rates by cell suspension matrix and 8-MOP starting concentration. Dead (Annexin $V^+7\text{-}AAD^+$) and apoptotic (Annexin V^+ $7\text{-}AAD^-$) T-cell ratio (post/pre as a percentage) detected 24 and 48 h following extracorporeal photopheresis with 8-MOP concentrations of 200 ng/ml ($n = 30$; UVA PIT and Macogenic G2) and 340 ng/ml ($n = 12$; UVA PIT) in plasma or in saline (0.9% NaCl). Asterisks (*) denote significance ($p < 0.05$). UVA, ultraviolet light A

procedure, we analyzed different combinations of 8-MOP starting doses (200 or 340 ng/ml) and cell suspension matrices (plasma or saline) (see Figure 5). With the lower 8-MOP starting concentration (200 ng/ml), apoptosis induction rates in plasma and saline as determined by flow cytometry were 22% and 28% after 24 h and 37% and 74% after 48 h, respectively. The use of an 8-MOP concentration of 340 ng/ml according to inline ECP standards (volume product × 0.017) increased apoptosis induction levels after 24 h (35% in plasma and 46% in NaCl) compared with the standard dose, and the increase observed in the saline group at 24 h was statistically significant ($p = 0.044$). After 48 h, apoptosis induction by 340 ng/ml 8-MOP was even higher: 86% (plasma) and 97% (NaCl). For NaCl, the difference between the two doses at 48 h was significant ($p = 0.02$). The level of apoptosis induction with high-dose 8-MOP (340 ng/ml) was generally higher, especially after 48 h, but the difference in apoptosis between the two cell suspension matrices on day 2 was not significant.

The availability of 8-MOP influences binding to DNA and, thus, the induction of T-cell apoptosis. Therefore, residual 8-MOP concentrations were analyzed by 8-MOP starting concentration and type of cell suspension matrix (see Table 2). At the lower starting concentration (200 ng/ml), residual 8-MOP was 119–215 ng/ml (median 168 ng/ml) in plasma and 149–203 ng/ml (median 162 ng/ml) in NaCl. The higher starting concentration led to a residual 8-MOP of 269–325 ng/ml in plasma (median 290 ng/ml) and 197–276 ng/ml in NaCl (median 266 ng/ml). With high-dose 8-MOP, there was no significant difference in residual 8-MOP between plasma and NaCl. The patients were treated on two consecutive days per treatment cycle. No remaining 8-MOP was detectable in the pre-sample on the second treatment day, confirming that 8-MOP is excreted within less than 24 h. With regards to the two UVA irradiation devices, less

TABLE 2 Residual 8-methoxypsoralen after ultraviolet light A (UVA) irradiation

	200 ng/ml 8-MOP		340 ng/ml 8-MOP	
	Plasma	**NaCl**	**Plasma**	**NaCl**
Min	119	149	269	197
Max	215	203	325	276
Median	168	162	290	266

Note: Residual 8-MOP concentrations in extracorporeal photopheresis products were determined after UVA irradiation for samples pre-incubated with an 8-MOP starting concentration of 200 ng/ml versus 340 ng/ml and with plasma versus physiological saline solution (0.9% NaCl) as the cell suspension matrix.

8-MOP remained in solution after UVA treatment with UVA PIT compared with Macogenic G2, but the difference was not statistically significant.

Considering the total amount of photoactive 8-MOP transfused with the photopheresis product, there was 24–60 μg (median 34 μg) in plasma and 29–57 μg (median 45 μg) in NaCl after an initial dose of 200 ng/ml, and 53–64 μg (median 57 μg) in plasma and 39–55 μg (median 53 μg) in NaCl after an initial dose of 340 ng/ml.

Some research groups have defined 8-MOP dosage recommendations to achieve inactivation of lymphocytes, but not of monocytes by multiplying the concentration of photosensitizer with applied UV dose.[33, 34] The resulting effective dose is approximately 50–400 ng 8-MOP·J/ml·cm^2. In the present study, ECP with UVA PIT resulted in values of 268–422 ng 8-MOP·J/ml·cm^2 (median: 315 ng 8-MOP·J/ml·cm^2), whereas ECP with Macogenic G2 yielded values of 318–430 ng 8-MOP·J/ml·cm^2 (median: 363 ng 8-MOP·J/ml·cm^2), in both cases, with plasma as the suspension matrix and with 200 ng/ml as the initial 8-MOP concentration.

4 | DISCUSSION

ECP patients benefit from its immune suppressive and immune regulatory effects. One of the main mechanisms of ECP is lymphocyte apoptosis.[9, 10] The present study elucidated critical parameters that affect the efficacy of this procedure. Increasing the starting concentration of 8-MOP from 200 to 340 ng/ml in combination with replacing plasma with saline as the cell suspension matrix had a major influence on the availability of the drug in solution, as reflected by a significant increase of T-cell apoptosis.

Besides determining cellular composition, hematocrit, and residual 8-MOP, we analyzed T-cell apoptosis as a functional measure. Whereas these tests revealed no direct relationship between cell composition and lymphocyte apoptosis, the hematocrit should be kept lower than 4% to avoid interference with the UVA irradiation.[35] For treatment optimization purposes, we varied the cell suspension matrix as well as the starting concentration of the photosensitizer 8-methoxypsoralen. UVA irradiation was done on one of two different devices, which act by agitation (Macogenic G2) or circulation of the cell suspensions (UVA PIT system). In Europe, offline photopheresis products are mainly regulated as Advanced Therapy Medicinal Products (ATMP) and are subject to pharmaceutical authority control. Photopheresis with UVA PIT can be performed as inline ECP, that is, with a continuous connection between the ECP system and the patient. Therefore, all procedures with the higher 8-MOP starting concentration were performed with the UVA PIT system. Our analyses revealed no significant difference in apoptosis induction rates between the two devices. Both systems use a different composition of container sets and plastic materials, for which the photoactive substance 8-MOP is already known to have an affinity.[22] Although 8-MOP adhesion and absorption to plastic is more prominent with saline (about 60%) than plasma (about 10%), our data revealed no significant difference in residual 8-MOP between the two cell suspension matrices. However, these data may not reflect a potential difference in availability of 8-MOP for the uptake into the cells. Although plasma is associated with lower absorption of 8-MOP to plastic containers, it facilitates plasma protein binding of the photosensitizer (80%–90%), resulting in reduced availability of 8-MOP for DNA intercalation.[21, 23, 36] In the present study, this was reflected by significantly lower lymphocyte apoptosis induction rates in plasma than in saline.

Increasing the concentration of 8-MOP to 340 ng/ml, the dose typically used for inline ECP, resulted in a clear increase of apoptosis induction of cells suspended in plasma as well as in saline after 24 h, and a further increase after 48 h. Apoptosis induction at 48 h was not significantly higher with saline compared with plasma ($p > .05$). This is in accordance with Laulhé et al., who stated that plasma reduced apoptosis induced by photopheresis.[20] Concerning the potential toxicity of 8-MOP, the total maximum dose in the ECP product was 64 μg 8-MOP, which is not harmful for the patient. 8-MOP is used orally for the treatment of psoriasis at a dose of 70–160 mg per day,[37] which is 1000-fold higher than the dose in ECP.[33]

In addition, our analysis of lymphocyte apoptosis and caspase activity 24 and 48 h after ECP indicated that cells suspended in plasma and treated with a starting dose of 200 ng/ml 8-MOP have suitable characteristics for successful treatment. However, increasing the starting concentration of 8-MOP to 340 ng/ml, the level used for inline ECP, resulted in a further increase of lymphocyte apoptosis. Suspending the cells in physiological saline instead of plasma also resulted in a significant increase of T-cell apoptosis. The latter finding suggests that the total amount of free 8-MOP in saline solution is higher despite increased adhesion to plastic.

The therapeutic action of ECP relies on apoptosis.[4, 15–19] The concept is that more apoptosis would translate to higher efficacies. However, ECP relies also on cell interactions. This involves the activation of monocytes by platelets, which adhere to fibrinogen that is deposited on plastic surfaces of the device sets.[11] Replacing plasma with saline is supposed to not affect these cell interactions because of two reasons: (1) shear is a factor in the activation of platelets, and (2) mononuclear cells were collected in a defined volume of plasma followed by supplementing the suspension with saline.

Thus, all components for the effect of monocyte and platelet interaction were present in all groups. With regard to lymphocyte apoptosis as an important effect of ECP, improving the process conditions would be beneficial for binding of the photosensitizer to DNA and thus for apoptosis induction. However, the effect of such an increase in lymphocyte apoptosis on the clinical effect and patient recovery needs to be clarified in further studies.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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REFERENCES

1. Marques MB, Adamski J. Extracorporeal photopheresis: technique, established and novel indications. J Clin Apher. 2014; 29:228–34. https://doi.org/10.1002/jca.21333
2. Adamski J, Kinard T, Ipe T, Cooling L. Extracorporeal photopheresis for the treatment of autoimmune diseases. Transfus Apher Sci. 2015;52:171–82. https://doi.org/10.1016/j.transci.2015.02.005
3. Schwartz J, Padmanabhan A, Aqui N, Balogun RA, Connelly-Smith L, Delaney M, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the writing Committee of the American Society for apheresis: the seventh special issue. J Clin Apher. 2016;31:149–62. https://doi.org/10.1002/jca.21470
4. Cho A, Jantschitsch C, Knobler R. Extracorporeal photopheresis-an overview. Front Med. 2018;5:236. https://doi.org/10.3389/fmed.2018.00236
5. Song PS, Tapley KJ Jr. Photochemistry and photobiology of psoralens. Photochem Photobiol. 1979;29:1177–97.
6. Gasparro FP, Dall'Amico R, Goldminz D, Simmons E, Weingold D. Molecular aspects of extracorporeal photochemotherapy. Yale J Biol Med. 1989;62:579–93.
7. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. Semin Hematol. 2001;38:4–11.
8. Cao H, Hearst JE, Corash L, Wang Y. LC-MS/MS for the detection of DNA interstrand cross-links formed by 8-methoxypsoralen and UVA irradiation in human cells. Anal Chem. 2008;80:2932–8. https://doi.org/10.1021/ac7023969
9. Schmid D, Grabmer C, Streif D, Lener T, Schallmoser K, Rohde E. T-cell death, phosphatidylserine exposure and reduced proliferation rate to validate extracorporeal photochemotherapy. Vox Sang. 2015;108:82–8. https://doi.org/10.1111/vox.12200
10. Holien T, Gederaas OA, Darvekar SR, Christensen E, Peng Q. Comparison between 8-methoxypsoralen and 5-aminolevulinic acid in killing T cells of photopheresis patients ex vivo. Lasers Surg Med. 2018;50:469–75. https://doi.org/10.1002/lsm.22806
11. Edelson RL. Mechanistic insights into extracorporeal photochemotherapy: efficient induction of monocyte-to-dendritic cell maturation. Transfus Apher Sci. 2014;50:322–9. https://doi.org/10.1016/j.transci.2013.07.031
12. Di Renzo M, Sbano P, De Aloe G, Pasqui AL, Rubegni P, Ghezzi A, et al. Extracorporeal photopheresis affects co-stimulatory molecule expression and interleukin-10 production by dendritic cells in graft-versus-host disease patients. Clin Exp Immunol. 2008;151:407–13. https://doi.org/10.1111/j.1365-2249.2007.03577.x
13. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997;390:350–1. https://doi.org/10.1038/37022
14. Wang Z, Larregina AT, Shufesky WJ, Perone MJ, Montecalvo A, Zahorchak AF, et al. Use of the inhibitory effect of apoptotic cells on dendritic cells for graft survival via T-cell deletion and regulatory T cells. Am J Transplant. 2006;6:1297–311. https://doi.org/10.1111/j.1600-6143.2006.01308.x
15. Lamioni A, Parisi F, Isacchi G, Giorda E, Di Cesare S, Landolfo A, et al. The immunological effects of extracorporeal photopheresis unraveled: induction of tolerogenic dendritic cells in vitro and regulatory T cells in vivo. Transplantation. 2005;79:846–50. https://doi.org/10.1097/01.Tp.0000157278.02848.C7
16. Becherucci V, Allegro E, Brugnolo F, Piccini L, Gori V, Bisin S, et al. Extracorporeal photopheresis as an immunomodulatory agent: Haematocrit-dependent effects on natural killer cells. J Clin Apher. 2017;32:257–65. https://doi.org/10.1002/jca.21490
17. Cervio M, Scudeller L, Viarengo G, Del Fante C, Perotti C. Quality control on mononuclear cells collected for extracorporeal photochemotherapy: comparison between two UV-A irradiation devices. Vox Sang. 2015;109:403–5. https://doi.org/10.1111/vox.12293
18. Florek M, Sega EI, Leveson-Gower DB, Baker J, Müller AMS, Schneidawind D, et al. Autologous apoptotic cells preceding transplantation enhance survival in lethal murine graft-versus-host models. Blood. 2014;124:1832–42. https://doi.org/10.1182/blood-2014-02-555128
19. Morelli AE, Larregina AT. Apoptotic cell-based therapies against transplant rejection: role of recipient's dendritic cells.

Apoptosis. 2010;15:1083–97. https://doi.org/10.1007/s10495-010-0469-9
20. Laulhé M, Lefebvre S, Le Broc-Ryckewaert D, Pierre M, Ferry A, Delorme B. A standardized methodical approach to characterize the influence of key parameters on the in vitro efficacy of extracorporeal photopheresis. PLoS One. 2019;14:e0212835. https://doi.org/10.1371/journal.pone.0212835
21. Schmitt IM, Chimenti S, Gasparro FP. Psoralen-protein photochemistry–a forgotten field. J Photochem Photobiol B. 1995;27:101–7. https://doi.org/10.1016/1011-1344(94)07101-s
22. Hahnel V, Weber I, Tuemmler S, Graf B, Gruber M, Burkhardt R, et al. Matrix-dependent absorption of 8-methoxypsoralen in extracorporeal photopheresis. Photochem Photobiol Sci. 2020;19:1099–103. https://doi.org/10.1039/c9pp00378a
23. Karolak L, Tod M, Leon A, Heudes AM, Petitjean O, Laroche L. In vitro kinetics of 8-methoxypsoralen penetration into human lymphoid cells. Photodermatol Photoimmunol Photomed. 1992;9:58–60.
24. Budde H, Berntsch U, Riggert J, Legler TJ. In vitro effects of different 8-methoxypsoralen treatment protocols for extracorporeal photopheresis on mononuclear cells. Cent Eur J Immunol. 2017;42:1–9. https://doi.org/10.5114/ceji.2017.67312
25. Kruger J, Christophers E, Schlaak M. Dose-effects of 8-methoxypsoralen and UVA in cultured human lymphocytes. Br J Dermatol. 1978;98:141–4.
26. Perseghin P, Dassi M, Balduzzi A, Rovelli A, Bonanomi S, Uderzo C. Mononuclear cell collection in patients undergoing extra-corporeal photo-chemotherapy for acute and chronic graft-vs.-host-disease (GvHD): comparison between COBE spectra version 4.7 and 6.0 (AutoPBSC). J Clin Apher. 2002;17:65–71. https://doi.org/10.1002/jca.10021
27. Commission E. The Rules Governing Medicinal Products in the European Union. Good manufacturing practice (GMP) Guidelines [Internet]. 2016;EudraLex, Volume 4. Available from: http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm
28. Padmanabhan A, Connelly-Smith L, Aqui N, Balogun RA, Klingel R, Meyer E, et al. Guidelines on the use of therapeutic apheresis in clinical practice - evidence-based approach from the writing Committee of the American Society for apheresis: the eighth special issue. J Clin Apher. 2019;34:171–354. https://doi.org/10.1002/jca.21705
29. Hahnel V, Dormann F, Kronenberg K, Hutchinson JA, Burkhardt R, Ahrens N. Validation of an apoptosis assay for extracorporeal photopheresis. Transfus Med. 2021;31:113–20. https://doi.org/10.1111/tme.12767
30. Hahnel V, Dormann F, Nitsopoulos A, Friedle A, Ahrens N. A method for the quantification of 8-methoxypsoralen by mass spectrometry for offline extracorporeal photopheresis. Photochem Photobiol Sci. 2017;16:193–200. https://doi.org/10.1039/c6pp00327c
31. Brosig A, Hahnel V, Orso E, Wolff D, Holler E, Ahrens N. Technical comparison of four different extracorporeal photopheresis systems. Transfusion. 2016;56:2510–9. https://doi.org/10.1111/trf.13728
32. Huerta S, Goulet EJ, Huerta-Yepez S, Livingston EH. Screening and detection of apoptosis. J Surg Res. 2007;139:143–56. https://doi.org/10.1016/j.jss.2006.07.034
33. Andreu G, Leon A, Heshmati F, Tod M, Menkes CJ, Baudelot J, et al. Extracorporeal photochemotherapy: evaluation of two techniques and use in connective tissue disorders. Transfus Sci. 1994;15:443–54. https://doi.org/10.1016/0955-3886(94)90178-3
34. McKenna KE, Whittaker S, Rhodes LE, et al. Evidence-based practice of photopheresis 1987-2001: a report of a workshop of the British Photodermatology group and the U.K. skin lymphoma group. Br J Dermatol. 2006;154:7–20. https://doi.org/10.1111/j.1365-2133.2005.06857.x
35. Jacob MC, Manches O, Drillat P, Richard MJ, Plumas J, Chaperot L, et al. Quality control for the validation of extracorporeal photopheresis process using the Vilbert-Lourmat UV-A irradiation's system. Transfus Apher Sci. 2003;28:63–70. https://doi.org/10.1016/S1473-0502(02)00101-5
36. Shephard SE, Nestle FO, Panizzon R. Pharmacokinetics of 8-methoxypsoralen during extracorporeal photopheresis. Photodermatol Photoimmunol Photomed. 1999;15:64–74.
37. Owsianowski M, Garbe C, Ramaker J, Orfanos CE, Gollnick H. Therapeutic experiences with extracorporeal photopheresis. Technical procedure, follow-up and clinical outcome in 31 skin diseases. Hautarzt. 1996;47:114–23. Therapeutische Erfahrungen mit der Extrakorporalen Photopherese. Technisches Vorgehen, Uberwachung und klinische Ergebnisse bei 41 Hautkranken.

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