

# Previously Activated Psoralen: A Possible Novel Format of Psoralen Used in the Treatment of Graft-versus-host Disease

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## ABSTRACT

*Graft-versus-host disease (GVHD) is a lethal complication of allogeneic haematopoietic stem cell transplantation which limits its application. Psoralen was utilized in the treatment of GVHD as a second-line method, which is also known as extracorporeal photochemotherapy (ECP). In the process of ECP, mononuclear cells must be isolated from the body in advance. Then a photosensitizer, 8-methoxypsoralen (8-MOP, 200 µg/L for the final concentration), would be added to the cell suspension before its exposure to ultraviolet A [UVA; 365 nm, 2J/cm<sup>2</sup>]. The disposed lymphocytes re-infused into the body account for 5% to 15% of the lymphocytes in the body. The process of ECP is complicated, expensive and very labour intensive, which limits its popularity. We hypothesized that psoralen should be activated by UVA, and should be kept in activation for a relatively long time before it gets in contact with mononuclear cells. This kind of psoralen is called previously activated psoralen (PAP), which may have the same effects on GVHD as ECP, but would be much easier and economical to work with.*

**Keywords:** Extracorporeal photochemotherapy, graft-versus-host disease, psoralen, ultraviolet

# Psoraleno Activado Previamente: Un Novedoso Posible Formato de Psoraleno Utilizado en el Tratamiento de la Enfermedad de Injerto-contra-huésped

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## RESUMEN

*La enfermedad de injerto-contra-huésped (EICH, o GVHD por las siglas en inglés) es una complicación letal del trasplante alogénico de células madre hematopoyéticas que limita su aplicación. El psoraleno se utilizó en el tratamiento de EICH como método de segunda línea, también conocido como fotoquimioterapia extracorpórea (FEC). En el proceso de FEC, las células mononucleares tienen que ser aisladas del cuerpo por adelantado. Entonces un fotosensibilizador, 8-metoxypsoraleno (8-MOP, 200 µg/L para la concentración final), se añadirá a la suspensión de células antes de su exposición a radiación ultravioleta A [UVA; 365 nm, 2J/cm<sup>2</sup>]. Los linfocitos eliminados re-infundidos en el cuerpo representan del 5% al 15% de los linfocitos en el cuerpo. El proceso de FEC es complicado, costoso, y requiere mucho trabajo, lo cual limita su popularidad. Partimos de la hipótesis de que el psoraleno debe ser activado por rayos UVA y debe mantenerse en activación por un tiempo relativamente largo, antes de que se ponga en contacto con las células mononucleares. Este tipo de psoraleno se denomina psoraleno activado previamente (PAP), el cual puede tener los mismos efectos sobre la EICH que la FEC, pero de manera mucho más fácil y económica.*

**Palabras claves:** Fotoquimioterapia extracorpórea, enfermedad de injerto-contra-huésped, psoraleno, ultravioleta

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## INTRODUCTION

Graft-versus-host disease (GVHD), including the acute (aGVHD) and chronic (cGVHD) conditions, is a severe complication associated with allogeneic haematopoietic stem cell transplantations (HSCT), which causes significant mortality and morbidity. It has been demonstrated that aGVHD is an

inflammatory process leading to target organ damage, and cGVHD is just like an autoimmune disease where immune tolerance is broken (1–3). Traditionally, the first-line treatment of GVHD is steroids alone or in combination with calcineurin inhibitors. Long-term administration of these drugs leads to severe complications including life-threatening infections, reduced quality of life and psychosocial disturbances (4–6).

Recently, extracorporeal photochemotherapy (ECP) was considered as a safe, well-tolerated and effective regimen for refractory and recurrent GVHD (7–11). It has been demonstrated that a photosensitizer could induce apoptosis of activated lymphocytes. Cross-linking between photosensitizer 8-methoxypsoralen (8-MOP) and DNA would proceed after absorbing light energy then DNA synthesis would be inhibited which could lead to damaged lymphocytic function (12). Unlike the lymphocytes, circulating activated monocytes also have an important role in ECP treatment. It has been reported that activated monocytes could promote the release of anti-inflammatory cytokines (eg, interleukin (IL)-1, IL-10, tumour necrosis factor- $\alpha$ ) and stimulate anti-tumour responses by effector cells [NK cells, cytotoxic T cells] (13–15). Moreover, it has been theorized that ECP could induce apoptosis of treated lymphocytes, promote reversion of inflammation conducted by T helper cells and develop immune tolerance induced by T-reg cells (10). Besides, the photosensitizer could facilitate the proliferation of regulatory T cell (16). Schmitt *et al* (17) showed that ECP could enhance the suppressive function of T-reg cells. However, ECP was not able to restore the suppressive function of the patient-derived T-reg cells completely. It has been demonstrated that the disorder of T-reg cells could accelerate progression of GVHD after transplantation (18, 19). The transfer of cells treated with ECP could reverse the established GVHD by increasing donor T-reg cells and indirectly reducing the number of donor effector lymphocytes (20).

A study of 23 adult patients with steroid-refractory aGVHD (grade II–IV) revealed that 52% of patients who were given ECP had complete response. The incidence of complete response in the skin, liver and gut were 66, 27 and 40%, respectively (21). Data from 31 children with aGVHD showed that the two-year overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) were 85% vs 57%, 87% vs 67% and 87% vs 63%, respectively between the ECP and steroid group, all with no statistically significant difference (22).

The first report regarding the administration of ECP in the treatment of cGVHD was presented by Owsianowski *et al* (23). After that, several reports of clinical research have certified the efficacy of ECP in steroid-refractory cGVHD (24, 25). Dignan *et al* evaluated 82 patients with steroid-refractory, steroid-dependent or steroid-intolerant GVHD who received a bi-monthly regimen of ECP treatment for two consecutive days. Ninety-four per cent of patients observed an improvement in symptoms of cGVHD, and 77% of patients had a re-

duction in dosage of immunosuppressants. The three-year OS was 69% (26). A retrospective analysis reported ECP treatment in 32 patients with cutaneous cGVHD (27). The evaluated patients had been previously treated with steroid, mycophenolate mofetil. Complete response (CR) rate was 22% and partial response (PR) rate was 34%. A total of 34% of patients died after ECP treatment [due to overlapping cGVHD or cGVHD-related infections] (27).

Another retrospective study evaluated 30 aGVHD and 32 cGVHD patients who had received ECP treatment with a promising result after three months. There were nine patients (30%) in CR and six patients (20%) in PR for aGVHD, and two patients (6%) and 12 patients (38%), respectively for cGVHD. After three months of ECP treatment, there was a significant reduction of the hormone dose in 83% of patients with aGVHD and in 29% of patients with cGVHD. Overall survival significantly improved after allo-stem cell transplantation (28).

### HYPOTHESIS

Extracorporeal photochemotherapy is a traditional mode of psoralen therapy applied in the treatment of GVHD. Yet, ECP is just used as a second-line medication. Possible reasons may be as follows: i) a relatively complicated procedure; ii) requirement of precious instruments; iii) expensive consumptive materials; iv) limited effects (each harvest of lymphocytes account for about 5–15% of the whole count of circulating lymphocytes in the body); v) the mechanism is still not clear (29, 30).

It has been demonstrated that previously activated psoralen (PAP) had significant cytotoxic effects on primary leukaemic cells from patients with acute leukemia and K562 cells (31, 32). Moreover, the H proton spectrum structure analysis showed that the structure of PAP was different from the structure of psoralen before irradiation (unpublished data). So we present the hypothesis that psoralen should be activated by ultraviolet A before infused into the patient's body and PAP therapy should produce equivalent or better effects when compared with ECP. Currently used ECP has proven that the harvest of lymphocytes in ECP also included more or less plasma. Previously activated psoralen would encounter various plasma components similarly once it is injected into the human body, just with a difference in amounts of plasma. It is supposed that we should infuse more dosage of PAP than the dosage of psoralen in ECP, with an efficient blood concentration and ongoing effect on the lymphocytes in the body. It is conceivable that PAP therapy may be more efficient than ECP in the treatment of GVHD, with a simpler procedure and at lower expense. Consequently, PAP may be a possible novel format of psoralen used in the treatment of GVHD.

The above-mentioned mechanisms are summed up from the ECP basically. Whether the function of PAP is influenced by the imminent environment of the body and whether PAP therapy has the same effects as ECP is still not clear and thus,

worthy of further investigation. This research is now in progress by the Department of Hematology, Navy General Hospital, Beijing, PRC.

#### AUTHORS' NOTE

All authors declare no conflicts of interest.

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