LETTER TO THE EDITOR

Systemic immunomodulatory effects of *Polypodium leucotomos* as an adjuvant to PUVA therapy in generalized vitiligo: A pilot study

KEYWORDS
Adjuvant; Immunomodulation; *Polypodium leucotomos*; PUVA; Vitiligo

Although extensive research has been done to improve the treatment of vitiligo, an universal effective treatment for vitiligo is not yet available [1]. PUVA therapy has demonstrated to have immunomodulatory effects, mainly in the T lymphocyte compartment but has several adverse effects, such as skin cancer risk [2,3]. The optimization of PUVA therapy is a relevant clinical objective in the management of vitiligo patients.

*Polypodium leucotomos* (PL) extract has demonstrated to have relevant photoprotective effects in humans [4,5] and animal models [6]. These biological effects may be involved in the action mechanism of PL on vitiligo patients, and could explain the enhancement of the pigmentary response of conventional PUVA by PL when this was used as adjuvant treatment in vitiligo patients [4].

In order to optimize PUVA treatment for management of vitiligo patients, we therefore investigated potential immunomodulatory effects of PUVA associated with PL. For this purpose, a pilot randomized, double-blind, placebo-controlled, clinical trial designed to study effects of PUVA + PL on T lymphocytes from 19 generalized vitiligo patients was conducted (Table 1). At baseline (before first PUVA session) and after 12 weeks of treatment (before the last PUVA session), peripheral blood both from patients and from each corresponding control was withdrawn. Repigmentation response was evaluated by three independent dermatologists at 12 weeks and graded as none or minimal (<25%), mild (25–50%) and moderate to excellent (>50%). Peripheral blood mononuclear cells (PBMC) isolation, immunofluorescence and proliferative response (PR) assays have been previously described [7].

At baseline we observed an abnormal in vivo activation of T lymphocytes in patients (increased CD25 and HLA-DR expression and decreased percentage of CD8+CD45RO+ cells) (Fig. 1, Panel A). The percentages and absolute counts of CD56+CD3−, CD16+, CD2+, CD3+, CD3+CD4+, CD4+CD45RA+, CD4+CD45RO+, CD3+CD8+, CD8+CD45RA+, CD11a+, CD11b+, CD19+ and CD45+ cells were similar in patients and healthy controls (data not shown). Subjects treated with PUVA + placebo did not significantly change any of the parameters analyzed, however, in patients receiving PUVA + PL, we observed a significant increase in the percentage of CD8+CD45RA+ subset (24.5 ± 1.5 to 33.7 ± 2.6) with a significant decrease in the percentage of CD25+, HLA-DR+ and CD8+CD45RO+ lymphocytes when compared to baseline. As shown in Panel B, there was no significant difference in the PR of PBMC between patients and healthy controls. Whereas no significant change in the PR was seen after treatment with PUVA + placebo, the treatment with PUVA + PL provoked a significant decrease in the PR as compared to baseline and controls. The in vitro addition of ionomycin or IL-2 significantly increased PR from all subjects when compared to those found in the presence of anti-CD3+TPA alone. The addition of IL-4 did not induce significant modifications in the PR of patients treated with PUVA + placebo, but significantly reduced the PR of the group treated with PUVA + PL with respect to that found in presence of anti-CD3+TPA alone.

We found that the percentage of subjects with a skin repigmentation >50% was significantly higher in arm of PUVA + PL than in the arm with PUVA + placebo. The PUVA treatment significantly decreased the percentages of CD3+CD25+ and CD8+CD45RO+ cells in the patient group with a mild repigmentation response (Panel C). We also found that the PUVA...
treatment significantly decreased the percentage of CD3+CD25+, CD3+HLADR+ and CD8+CD45RO+ cells in the patient group with a moderate to excellent response. The treatment with PUVA in patients with a mild/moderate to excellent repigmentation provoked a significant decrease in the PR to T cell mitogens as compared to baseline and healthy controls (Panel D). Furthermore, this significant reduction was also observed in the presence of ionomycin, IL-2 or IL-4 in the culture medium. For all the patients as a whole, we observed a significant correlation between the clinical response and the percentage of CD3+CD25+ cells (correlation coefficient = −0.493).

The normal PR of PBMC from generalized vitiligo patients differs from those seen in other autoimmune diseases with similar activation of T lymphocytes [7]. PUVA therapy induces a cell cycle arrest and subsequent apoptosis in T lymphocytes from vitiligo patients [8]. Our data indicate that PUVA therapy is not able to suppress the abnormal in vivo activation of the T lymphocyte nor has it functional effects on the PR of PBMC. In contrast, PUVA + PL therapy normalized the expression of activation markers by T cells and suppressed the proliferation of PBMC to mitogens. These effects of the adjuvant use of PL agree with its known biological effects, since PL has demonstrated to have immunomodulatory activity upon T cell activation [9].

The relevance of the immunosuppression in the treatment of vitiligo has been described but the toxicity of the analyzed immunosuppressive drugs, such as cyclosporine or steroids, as well as the transitory effects of these treatments have limited their clinical use. The increased long-term skin cancer risk observed in PUVA treated patients is also a limiting factor in this treatment modality in vitiligo patients. In this study, neither significant...
phototoxic side effects nor carcinogenesis were found in both groups of patients. The immunosuppressive effects observed with PUVA + PL treatment, might explain the higher rate of patients with moderate to excellent repigmentation. These clinical results agree with previous reports of acceleration and increase in the pigmentary response of conventional PUVA therapy in vitiligo patients (extensive instead of extended) treated with oral PL [4]. The immunomodulatory effects seen with the adjuvant use of PL in PUVA treatment of vitiligo patients, and the clinical results observed, justify further clinical trials with large patient populations.

Acknowledgments

The reagents used in our study, placebo and Polypodium leucotomos, were graciously provided by its manufacturer IFC, Madrid, Spain. All authors declare any commercial or financial interest including support from drug companies that might be involved in the treatment of vitiligo. Melchor Alvarez-Mon and Salvador Gonzalez are consultants for IFC. This work was partially supported by grants from the CICYT (C001999-AX131), Feder (2FD1997-1950) and FIS (00/0806), Spain.

References

[4] Gonzalez S, Pathak MA. Inhibition of ultraviolet-induced formation of reactive oxygen species, lipid peroxidation,


Eduardo Reyes*
Pedro Jaén
Elena de las Heras
Flavio Carrión
Melchor Álvarez-Mon
Laboratory of Immune System Disease and Oncology, National Biotechnology Associated Unit (CNB-CSIC), Department of Medicine, University of Alcalá, Madrid, Spain

Pedro Jaén
Ester de Eusebio
Dermatology Service, Hospital of Guadalajara, Guadalajara, Spain

Jesús Cuevas
Pathology Service, Hospital of Guadalajara, Guadalajara, Spain

Salvador González
Wellman Laboratories of Photomedicine, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Vicente G. Villarrubia
Department of Immunology, I.F. Cantabria, Madrid, Spain

Melchor Álvarez-Mon
Medicine and Immune System Diseases and Oncology Service, University Hospital Principe de Asturias, University of Alcalá, Madrid, Spain

*Corresponding author. Tel.: +34 91 8854888; fax: +34 91 8854526
E-mail address: eduardo.reyes@uah.es (E. Reyes)

14 June 2005