Topical or oral administration with an extract of *Polypodium leucotomos* prevents acute sunburn and psoralen-induced phototoxic reactions as well as depletion of Langerhans cells in human skin


Sunburn, immune suppression, photoaging, and skin cancers result from uncontrolled overexposure of human skin to solar ultraviolet radiation (UVR). Preventive measures, including photoprotection, are helpful and can be achieved by topical sunscreening agents. *Polypodium leucotomos* (PL) has been used for the treatment of inflammatory diseases and has shown some *in vitro* and *in vivo* immunomodulating properties. Its beneficial photoprotective effects in the treatment of vitiligo and its antioxidant properties encouraged us to evaluate *in vivo* the potentially useful photoprotective property of natural extract of PL after topical application or oral ingestion. Twenty-one healthy volunteers [either untreated or treated with oral psoralens (8-MOP or 5-MOP)] were enrolled in this study and exposed to solar radiation for evaluation of the following clinical parameters: immediate pigment darkening (IPD), minimal erythema dose (MED), minimal melanogenic dose (MMD), and minimal phototoxic dose (MPD) before and after topical or oral administration of PL. Immunohistochemical assessment of CD1a-expressing epidermal cells were also performed. PL was found to be photoprotective after topical application as well as oral administration. PL increased UV dose required for IPD (*P*<0.01), MED (*P*<0.001) and MPD (*P*<0.001). After oral administration of PL, MED increased 2.8±0.59 times and MPD increased 2.75±0.5 and 6.8±1.3 times depending upon the type of psoralen used. Immunohistochemical study revealed photoprotection of Langerhans cells by oral as well as topical PL. The observed photoprotective activities of oral or topical PL reveal a new avenue in examining the potentially useful field of systemic photoprotection and suggests that PL can be used as adjunct treatment and can make photochemotherapy and phototherapy possibly safe and effective when the control of cutaneous phototoxicity to PUVA or UVB is a limiting factor in such phototherapies.

Key words: *Polypodium leucotomos*; photoprotection; sunburn; phototoxicity; Langerhans cells

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Sunlight is a part of our everyday life and has become the major environmental factor that damages the structure and physiology of skin. Although a controlled exposure to ultraviolet radiation may have some beneficial effects (e.g., vitamin D synthesis), it also contributes to well-known local and systemic harmful effects, including skin cancer (1–6) caused particularly by ultra-
In the early 1970s, photomedicine entered a new era with the discovery of PUVA (psoralen plus UVA) as a source of skin targeted phototoxic reaction, by inducing a controlled cell injury at the DNA level, and with the subsequent discovery of its therapeutic effect in treating patients with psoriasis, mycosis fungoides, vitiligo, and other skin diseases (7). Although UVA radiation at that time was considered innocuous, emerging evidence demonstrated the role of UVA as well as PUVA on cell damage and their oncogenic potential (7–9).

Topical sunscreens have become an essential armament to protect normal and photosensitized human skin against the acute and chronic effects of solar radiation (1). Research efforts are attempting to develop ideal broad spectrum photoprotective agents that are effective against UVB and UVA radiation (1, 10, 11). On the other hand, it has also been established that UV radiation causes alterations in the immunologic functions of epidermal cells, particularly of Langerhans cells and keratinocytes (12–14). The abrogation by UV radiation of the Langerhans cells, the major antigen-presenting cells, is also accompanied by the irruption of infiltrating macrophages, which are able to activate "suppressor T cells" (15, 16) and hence cause induced antigenic tolerance as well as increased susceptibility of skin to UV-induced skin cancer. It is also known that UV radiation causes alterations in immunologic functions of epidermal cells, including suppressive mediator release from keratinocytes [e.g., interleukin (IL)-1, IL-3, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α] (14, 17–19) that lead to activation of suppressor T cells. This depletion of Langerhans cells and subsequent alterations of the immune surveillance of skin represents an interesting and challenging problem in elucidating the effects of sunlight at cellular and molecular levels.

Although topical sunscreens that contain UVB and UVA absorbing sun-active chemicals may reduce UV-induced Langerhans cells depletion when they are used on a regular basis (20), the development of systemic photoprotective agents against UV-induced depletion of Langerhans cells and immunosuppression is appealing and worth investigating. In this respect, the use of systemic photoprotective agents has been suggested and examined in the past (1, 11, 21); however, evidence for their effectiveness in providing photoprotection after oral administration has remained anecdotal and inferential. We evaluated the photoprotective property of a naturally occurring plant extract known as Polypodium leucotomos (PL), a tropical fern plant belonging to a natural order Polypodiaceae, has been used for the treatment of inflammatory disorders and other skin diseases, as a part of the folk medicine of Central America (22, 23). Recently, the ability of PL extract to modulate some aspects of the inflammatory/immune response have been reported (24–28). In clinical dermatology, the PL extract has been used orally for the treatment of psoriasis (29), atopic dermatitis (30), and more recently for the repigmentation of achromic macules of vitiligo vulgaris patients (31–33). Furthermore, investigators from our laboratories recently have shown an in vitro antioxidant activity of PL as well as its capacity to inhibit UVR-induced lipid peroxidation reaction triggered by photo-oxidative stress (34).

These findings led us to examine and evaluate in vivo the potentially useful photoprotective and antioxidant activity of this natural extract, either applied topically or taken orally. Clinical and experimental parameters were adopted with and without topical application or oral administration of PL to investigate and determine: a) the minimal ultraviolet dose required to induce immediate pigment darkening reaction (IPD), b) the minimal erythema dose (MED) required for eliciting sunburn reaction, c) the minimal melanogenic dose (MMD) required for delayed tanning reaction, and d) the minimal phototoxic dose (MPD) required for eliciting a phototoxic reaction of skin by oral 8-methoxypsoralen or 5-methoxypsoralen in the presence of UVA irradiation. In addition, the depletion or preservation of Langerhans cells under these conditions was also examined. The results obtained from these determinations provide supporting evidence for the use of this natural extract for the photoprotection of both normal skin and psoralen photosensitized-skin against the photo-oxidative stress of solar ultraviolet radiation.

Materials and methods

Subjects

Out of 30 subjects, a total of 21 paid volunteering subjects of skin phototype III and IV, as outlined by Pathak and Fitzpatrick (1), were enrolled for this investigation after giving their written consent. Volunteers received a complete physical examination and some were excluded because of: a) subjects manifesting an abnormal response to sunlight; b) family history of skin cancer, photosensitivity diseases; c) pre-existing intense skin tanning response, or d) use of any prescriptive or over-the-counter anti-inflammatory drugs. Subjects were randomly divided into two major groups (outlined in Table 1) and identified for this study either as
Table 1. Characteristics of PL study groups and treatments assigned to non-sensitized volunteers and psoralen-sensitized volunteers

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sensitized volunteers</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
</tr>
<tr>
<td>Standard sunscreen</td>
<td>5*</td>
</tr>
<tr>
<td>Topical PL (10, 25, 50% PL)</td>
<td>5*</td>
</tr>
<tr>
<td>Oral PL (1080 mg/subject)</td>
<td>7</td>
</tr>
<tr>
<td>Psoralen-sensitized volunteers</td>
<td></td>
</tr>
<tr>
<td>Psoralen sensitized</td>
<td>1</td>
</tr>
<tr>
<td>Psoralen+topical 10% PL</td>
<td>1</td>
</tr>
<tr>
<td>Psoralen+oral PL</td>
<td>1</td>
</tr>
</tbody>
</table>

PL: Polypodium leucotomos extract; n: number of subjects enrolled; age is given in years; *they represent the same subjects and were used for standard sunscreen and topical PL studies. Non-sensitized volunteers refer to subjects exposed to solar radiation without receiving oral 8-MOP or 5-MOP; psoralen-sensitized volunteers refer to subjects receiving oral psoralen (either 8-MOP or 5-MOP) plus solar radiation.

non-photosensitized or as photosensitized subjects receiving an oral dose of a known psoralen compound. Briefly, 13 subjects identified as non-photosensitized were distributed in two subgroups: (A) having five test subjects and (B) having eight test subjects. The five test subjects of subgroup A received a topical application of 2 µl/cm² of each test product, as recommended by FDA for sunscreen testing (35), or no treatment at six different back sites (Table 2). The eight remaining subjects of subgroup (B), identified earlier as having non-photosensitized skin, were evaluated for the photoprotection of orally administered PL extract (1080 mg per subject) in capsule form.

Eight other subjects, identified as sensitized subjects, as outlined in Table 1, were randomly assigned to receive topical application of PL lotion at 10% concentration or oral administration of PL. Four of these subjects were to receive topical application of 10% PL lotion. The remaining four subjects were to receive oral ingestion of PL. For the evaluation of psoralen-induced phototoxicity and its protection by PL, each enrolled subject receiving oral psoralen acted as his/her own control for establishing minimum phototoxic dose (MPD) values. Other data concerning age and gender are presented in Table 1.

UV light source and dosimetry: exposure conditions

This study was carried out at Málaga (Spain) (36° 45' north; 4° 25' west) during the summer season in the month of August. Sunlight, between 11:00 a.m. and 2:00 p.m. (local time), was used as a natural light source for irradiation of skin and assessment of the photoprotection of PL. The objective of the study was not to unnecessarily provoke a strong sunburn reaction in volunteers by exposing them to multiple MEDs, as is recommended to determine the sun protection factor (SPF) value of a sunscreen (35), but to assess the nature of protection provided by topically applied, or orally administered, PL after a moderate exposure of up to 120 min mid-day solar radiation (Table 2). The intensity or irradiance of sunlight was measured with a precalibrated radiometer (International Light Co., Newburyport, MA, USA) and averaged 27 W/m². With regard to the intensity value of ultraviolet components of sunlight, the flux in the UVB (290–320 nm) region was found to be 2.66×10⁻² mW/cm² and the flux of UVA radiation (320–400 nm) was estimated to be 4.45×10⁻³ W/cm². This irradiance level of flux, when shone on untreated skin for 30 min, was found to be equivalent to about 50 mJ/cm² of solar UVB radiation, a dose adequate to produce a minimally perceptible sunburn reaction in most individuals of skin type II.

Sun exposures of test volunteers were completed on two consecutive, cloudless, clear days.

Test volunteers were asked to lie down in the prone position and several adhesive templates, each with at least six precut 2×2 cm size exposure win-

Table 2. Photoprotective substances applied and exposure times used in the evaluation of topical PL

<table>
<thead>
<tr>
<th>MED site exposure (min)</th>
<th>Control site (min)</th>
<th>10%PL site (min)</th>
<th>Standard SPF site* (min)</th>
<th>Exposure doses UVB (mJ/cm²)</th>
<th>UVA (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20 min=33.3±5.3</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30 min=50±8</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40 min=66.6±10.6</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60 min=100±16</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80 min=133.3±21.3</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100 min=166.6±26.6</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120 min=200±32</td>
<td></td>
</tr>
</tbody>
</table>

MED: minimal erythematous dose; PL: 10% hydroalcoholic extract of Polypodium leucotomos; exposure times are given in minutes; 30 min of sun exposure corresponded to about 50 mJ/cm² of UVB radiation and 8 J/cm² of UVA; test area per product application was 100 cm²; amount of test product applied was of 2 µl/cm²; *standard sunscreen contained 3% Parsol 1789 (avobenzone), 5% oxybenzone, 5% octyl methoxycinnamate, and 3% padimate O). SPF15.
dows, were affixed on the back. Exposure windows were placed horizontally and symmetrically on the left and right side of the vertebra, to provide paired comparison sites; these windows were involved in each treatment and graded sun exposure dose. After delivering an appropriate dose of sun exposure, test sites were covered with UV opaque tapes.

Treatments evaluated

The Polypodium leucotomos extract (PL) used in this study was provided by Industrial Farmacéutica Cantabria, S.A. (Madrid, Spain) in two forms: a) dry powdered capsule form; b) lotion form containing 10, 25 and 50% PL extract (v/v). The capsule form of PL (DIFUR®) was used for the oral administration and evaluation of photoprotection. Each capsule contained 120 mg of dried PL extract. Each subject, prior to sun exposure had received 240 mg PL three times on one day prior to sun exposure and an additional dose of 360 mg PL 3 h before the sun exposure. This was a total oral dose of 1080 mg PL. The preparations for the topical use contained 10, 25, or 50% of an hydroalcoholic extract of PL (based on dry weight determination of PL extract) prepared in the form of water in oil lotion (milk-white) for uniform application. Additional information on composition, antioxidant activity and UV absorption properties of PL has been recently described by us (34).

Methoxsalen [8-methoxy psoralen, 8-MOP in pulverized capsular form (10 mg/capsule), sold as Oxsoralen®, Galderma S.A., Spain] was given orally to four subjects at doses ranging from 0.5 to 0.6 mg/kg body weight; 8-MOP was administered 2 h before sun exposure (7).

Bergapten [5-methoxy psoralen, 5-MOP in solubilized coated capsule form (20 mg/capsule), sold as Geralen®, Gerot Pharmazeutika, Vienna, Austria] was administered to four subjects at doses ranging from 1.0 to 1.2 mg/kg body weight, 2 h before sun exposure (7).

A brand name sunscreen SPF 15 was used as an internal standard; it contained 3% Parsol 1789 (avobenzone), 5% oxybenzone, 5% octylmethoxycinnamate, and 3% padimate O. It was determined to be effective against UVB-induced sunburn reaction and PUVA-induced phototoxic reaction when it was evaluated by us under indoor SPF testing conditions required by the FDA in the United States (35).

Treatment and irradiation regimens

Topical application of PL formulations or standard sunscreen to normal non-sensitized skin. Five women were randomly selected to receive topical applications of both test products (Table 1). Test products were delivered indoors by topical application of 2 µl/cm² of each substance on a pre-assigned body site at least 15–30 min prior to exposure. Test products were applied with the aid of a micropipette at six different back sites. Each test site (20×5 cm in size) received an adhesive template with six exposure windows (2×2 cm in size) for graded doses of sun exposure (Table 2). Site 1 received standard sunscreen lotion; sites 2, 3 and 4 received three test products containing 10, 25, and 50 percent PL extract (v/v) at three separate sites; the fifth site received topical application of the vehicle (or base); and the sixth site served as an untreated control for the determination of MED response of each enrolled test volunteer.

Evaluation of the systemic photoprotective property of PL in normal non-sensitized subjects. PL was administered orally to eight subjects for a total dose of 1080 mg, as described above.

Topical application of PL or a standard sunscreen to psoralen-sensitized skin. For assessing the photoprotective efficacy of 10% PL, topical formulations of this extract as well as standard sunscreen with SPF15 were evaluated in subjects deliberately made photosensitive by the oral administration of psoralens, as previously described (36). With this goal in mind four subjects were enrolled in this subgroup, two of them receiving oral 8-MOP (0.6 mg/kg) and the remaining two receiving oral 5-MOP (1.0–1.2 mg/kg) (Table 1). All four volunteers were exposed to solar radiation 2 h after psoralen administration to determine the MPD values for each psoralen with and without topical PL treatment.

Evaluation of the systemic photoprotective effect of PL on sensitized skin. In this experiment, the remaining four male subjects were enrolled and as in the previous experiment, they were divided into two subsets according to the type of psoralen administered (Table 1). All subjects had received PL orally at doses similar to those administered to non-sensitized subjects.

Evaluation criteria

Because the photoprotective efficacy was assessed under outdoor field conditions with natural sunlight, the evaluation of clinical parameters was carried out visually using subjective criteria without the use of a skin reflectance meter. The clinical criteria for the evaluation of photoprotection of PL included the determination of solar radiation dose required for: a) immediate pigment darkening re-
Table 3. Subjective scales adapted to evaluate immediate and delayed pigmentation reaction and erythema reaction induced by exposure of skin to solar UV radiation

<table>
<thead>
<tr>
<th>Grades</th>
<th>Pigmentation response</th>
<th>Erythema reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Trace (barely visible)</td>
<td>No Trace (barely visible)</td>
</tr>
<tr>
<td>±</td>
<td>Minimal (with four defined borders)</td>
<td>Pink (with four defined borders)</td>
</tr>
<tr>
<td>++</td>
<td>Light brown</td>
<td>Pink red without edema</td>
</tr>
<tr>
<td>+++</td>
<td>Dark brown</td>
<td>Strong red with edema</td>
</tr>
<tr>
<td>++++</td>
<td>Black or intense brown</td>
<td>Violaceous with painful edema</td>
</tr>
</tbody>
</table>

action (IPD), minimal erythema dose (MED) and delayed tanning (DT) in non-photosensitized subjects, and b) minimal phototoxic dose (MPD) of skin for psoralen-sensitization reaction. The IPD and DT responses at each site were respectively assessed 30 to 45 min and 5 days after the end of sun exposure. The MED readings were obtained 20–24 h post-exposure to solar radiation and MPD readings were obtained at 48 and 72 h after exposure. The criteria used for assessing these photobiological responses are outlined in Table 3. MPD is usually done for the determination of UVA protection factor for evaluation of UVA sunscreens (36–38).

All results were recorded as an agreed score of two clinical investigators experienced in the field of photoprotection, in the evaluation of sunscreens, and in assessing the degree of melanin pigmentation.

Skin biopsies

Skin punch biopsy specimens (size: 6 mm in diameter) were obtained from 13 subjects (9 non-sensitized, and 4 psoralen-sensitized). These skin biopsies were obtained at a specific time interval after sun exposure (at 24 h from non-sensitized subjects, and at 48 h from those who had been photosensitized with oral psoralen and UV radiation). Each biopsy specimen was fixed in 10% formaldehyde and embedded in paraffin by routine methods. Five µm thick paraffin sections were cut perpendicular to the epidermal surface for routine hematoxylin-eosin and immunohistochemical staining. Concerning immunohistochemical analysis, preheating of paraffin-embedded skin sections was achieved in order to get a better penetration of antigen prior to reaction with monoclonal antibody CD1a (Immunotech, clone O10, cat no. 1590, Marseille, France) (39).

Statistical analysis

The IPD, MED, DT, and MPD values before and after topical or oral PL-treatment and sun exposure are expressed as mean±standard deviation from the mean. The results were analyzed statistically by using unpaired or paired Student’s t-tests.

Results

Before the data of photoprotection related to 10, 25, and 50% PL extract are interpreted, it is essential to explain why only the data pertaining to 10% PL are presented. The two test products (25 and 50% PL) gave complete protection at all six exposure sites of graded exposure doses in all test subjects (5 subjects) and no threshold values for IPD, MED, MPD and DT could be assessed or interpreted as is recommended for determining sun protection factors (35).

Effect of topical application or oral administration of PL and a standard sunscreen in normal non-photosensitized skin

The degree of sunburn reaction in control skin (unprotected but exposed) was mild to moderate

Table 4. Topical application or oral administration of Polypodium leucotomos extract (PL) helps to prevent sunburn reaction in non-sensitized skin of healthy subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>IPD</th>
<th>MED</th>
<th>MMD</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.9±10.62</td>
<td>34.0±5.47</td>
<td>72.0±22.8</td>
<td>—</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunscreen</td>
<td>80.0±14.14**</td>
<td>CP</td>
<td>CP</td>
<td></td>
</tr>
<tr>
<td>n=5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% PL</td>
<td>56.0±16.73**</td>
<td>80.0±0**</td>
<td>88.0±10.95</td>
<td>2.41±0.45</td>
</tr>
<tr>
<td>n=5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral PL (1080 mg)</td>
<td>75.0±17.32**</td>
<td>98.0±15.35**</td>
<td>82.5±25.9</td>
<td>2.81±0.59</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IPD: immediate pigment darkening reaction (<1 h); MED: minimal erythema dose at 24 h; MMD: minimal melanogenic dose at 5 days; PF: estimated protection factor; IPD, MED, and MMD values are given in minutes for sunexposure; CP: complete protection at the exposure doses given in this study; 30 min of sun exposure in these studies corresponds to 50 mJ/cm² of solar UVB radiation. The amount of each applied test substance was of 2 µl/cm²; P-values were calculated by using a paired Student t-test; *P<0.01, and **P<0.001 vs control; 1P<0.05 vs sunscreen and oral PL.
The oral PL treatment provided slightly better protection against IPD reaction than the topical treatment with 10% PL. Although the DT values indicated higher sun exposure dose values with topical as well as oral PL treatments compared with the DT values of the corresponding control sites, these differences were not significant.

### Effects of topical application of PL or standard sunscreen to 8-MOP-sensitized or 5-MOP-sensitized skin

The results in Table 5 also support the photoprotective efficacy of topical 10% PL and standard sunscreen SPF 15 against photosensitization reaction induced by 8-MOP or 5-MOP. The MPD values indicate a) as anticipated, orally administered 8-MOP was more phototoxic than 5-MOP and required a lesser dose of sun exposure for skin photosensitization than that required for 5-MOP-induced skin photosensitization; b) the MPD values (presented in minutes of sun exposure) for 8-MOP and 5-MOP at 48 h were, respectively, 13.12±7.18 and 20.6±3.75, and at 72 h were 7.5 and 18.0±4.1, respectively; c) the sites treated topically with 10% PL or standard sunscreen were protected significantly better than the unprotected control sites at 48 and 72 h post-exposure, where there were phototoxic responses.

### Evaluation of the systemic photoprotective efficacy of PL in 8-MOP and 5-MOP sensitized skin

Data shown in Table 6 indicate the oral treatment with PL noticeably increased the MPD values at 72 h in all subjects taking oral psoralens, either 8-MOP or 5-MOP. The increases in MPD values were also significantly higher in the group receiving 8-MOP than the MPD values of the group receiving 5-MOP. The protection factor (PF) value in the group treated with 8-MOP+PL was 6.8±1.3, in contrast to the PF value of 2.75±0.5 in the group receiving 5-MOP+PL.

### Histological and immunohistochemical findings

The histological study of the specimens obtained from non-sensitized individuals showed a lesser degree of photodamage in those sun-exposed biopsy sites treated and photoprotected with oral or topical PL than the untreated but sun-exposed (control) skin biopsy sites of the same individuals. Sunburn cells were not quantified; however, there were no apparent differences in their numerical values before and after PL treatment. Whereas slight but distinctly noticeable differences between them (control and PL-treated sites) regarding the CD1a+ epidermal cell density were observed, the
Discussion

The data presented in this study concerning the topical and systemic photoprotection by PL extract reveal the following interesting properties of PL: 1) Epicutaneous application of 10, 25 and 50% PL lotion was found to be photoprotective against UVR-induced sunburn reaction and UVA-induced phototoxicity reaction in psoralen-sensitized skin. In non-sensitized skin, the calculated SPF value of 10% PL lotion was 2.40±0.45, whereas the extrapolated SPF value of higher strength PL lotions (at 25 and 50%) was distinctly better than that of 10% PL (SPF >3.0 or more) to the extent that none of the test volunteers showed any evidence of sunburn reaction at stated exposure doses [we did not anticipate this degree of protection and had no intention to induce uncomfortable painful sunburn reaction in test volunteers with a period of sun...
PL prevents acute sunburn and phototoxicity to psoralen

Fig. 3. Photomicrographs of skin specimens (immunohistochemical anti-CD1a stain; original magnification, ×80 and ×100, respectively). A) Skin section from 5-MOP-sensitized individual exposed to solar UV radiation (30 min, about 8 J/cm² of UVA) shows a significant disappearance of CD1a expressing epidermal cells. B) Skin section from the same 5-MOP sensitized subject who previously was treated by oral PL and exposed to solar UV radiation (45 min, about 12 J/cm² of UVA). This section shows preservation of dendritic CD1a-expressing epidermal cells (arrowhead).

exposure greater than 120 min, especially when predetermined MED was less than 30 min (50 mJ/cm²). 2) Orally administered PL at the 1080 mg dose level was found to be photoprotective not only against erythemogenic effects of solar radiation in non-photosensitized subjects but also against a phototoxic reaction in subjects receiving oral 8-MOP (0.6 mg/kg) or 5-MOP (1.0–1.2 mg/kg) and sunlight exposure, as indicated in Table 4.

Since the IPD reaction represents a photooxidation reaction of pre-existing melanin within the epidermis (40), the results shown in this study clearly support and indicate, as well, the antioxidant activity of PL extract after topical application or oral administration and are in agreement with our recently reported in vitro studies that documented the antioxidant effect of PL (34). The antioxidant effect of topical PL in IPD reaction appeared to be less than the effect of orally administered PL. This may be related either to its photolabile nature or to the barrier function of stratum corneum that limits the diffusion of topical PL extract to retard the photo-oxidation of pre-existing melanin in epidermal cell layers.

The analysis of MED data in non-sensitized subjects also demonstrated the same type of photoprotective effect of PL. In fact, all treatments (topical as well as oral PL) significantly illustrated the photoprotective action of PL (Table 4), although again revealing the fact that better results were obtained in those subjects taking oral PL than in those receiving topical application of 10% PL. These results were translated or extrapolated to sun protection factor (SPF) values and showed that oral PL and 10% PL preparations were able to minimize or prevent the sunburn reaction if the UV exposure doses were lower than four MED equivalent doses.

To account for its photoprotective properties against UVB radiation and against a phototoxic reaction induced by PUVA, we examined the ultraviolet absorption properties of concentrated and diluted PL extract. Although 50 and 100% PL extract was brown and dark brown in color and showed detectable absorption of UV radiation, this chromogenic material showed no well-defined absorption spectrum or absorption peaks in the UVB or UVA region. The diluted form (1–10%) of the PL extract, with or without treatment with activated charcoal (to remove chromogenic material), showed no ultraviolet absorption peaks in the 290–320 nm region to account for its topical photoprotective and sun-screening efficacy in the UVB region. This is further supported by the fact that orally administered PL extract containing the chromogenic material also exerted photoprotective properties against UVB radiation as well as against a phototoxic reaction induced by orally administered 8-MOP or 5-MOP in the presence of UVA radiation. The chromogenic material present in the orally administered PL extract appeared to have little or insignificant UV screening effect in exerting its photoprotective property.

Concerning MMD values, the mild increases in melanogenic dose values observed at 72 h after both topical or oral PL treatments were not appreciably high or significant, probably due to the poor UV absorption capacity of PL and its inability to filter out UVB and UVA radiation or to the high melanogenic ability of test subjects responding to less than the minimal erythema dose of sun exposure. In this regard, we wish to refer to our earlier publications (40–42) as well as those of Gange et al. (43) where it has been established that melanogenesis in subjects of skin type III or IV can be easily stimulated with less than MED doses.
of solar ultraviolet radiation. In any case, these results suggest PL extract does not appreciably inhibit or augment delayed melanogenesis in vivo.

The data in Table 5 provide additional confirmatory evidence that topical application of 10% PL extract clearly increased the minimal dose required for the development of a phototoxic reaction (MPD value) in psoralen-sensitized subjects receiving either oral 8-MOP or 5-MOP. The photoprotective activity of topical PL extract was discernible not only at 48 h after photosensitization but also at 72 h when phototoxic reaction of 8-MOP or 5-MOP is maximally manifested (7, 36). Two additional findings of interest can be recognized from the data presented in Table 5 and these include: a) the standard SPF 15 lotion contains a strong UVA absorber known as Parsol 1789 that effectively filters out UVA radiation (320–400 nm) and blocks the anticipated phototoxic reaction induced by 8-MOP or 5-MOP (36–38); b) the MPD values of subjects receiving 8-MOP and 5-MOP (Table 5) reveal 8-MOP to be more reactive and photosensitizing than 5-MOP, thus confirming the previously recognized greater photosensitizing potential of 8-MOP than 5-MOP (7).

Additionally, we found that oral PL extract is also capable of exerting photoprotective qualities against a phototoxic reaction induced by orally administered 8-MOP and 5-MOP (Table 6). This can be rationalized in three ways: a) the phototoxic action mechanism of psoralen has been shown by Pathak & Carraro (44) to involve the generation of reactive oxygen species (singlet oxygen and superoxide anion) and membrane lipid peroxidation that contribute to an inflammatory response; b) by using selective quenchers for generation of singlet oxygen, superoxide anion and lipid peroxidation, the phototoxic reaction induced by psoralens could be inhibited, both in vitro and in vivo; c) PL extract acts as an antioxidant and effectively inhibits the generation of reactive oxygen species and membrane lipid peroxidation (34). These supporting observations strengthen our hypothesis that topically applied PL or orally administered PL behaves as an antioxidant and quenches the generation of harmful reactive oxygen species and inhibits the formation of lipid peroxides. This prevents the onset and cascade of events that eventually lead to a phototoxic reaction in skin. It is also possible that some unknown ingredients of PL, with or without binding to 8-MOP or 5-MOP, inhibit the photoexcitation of psoralens to singlet and triplet states and prevent the formation of reactive oxygen species (singlet oxygen and superoxide anion) under in vitro and in vivo conditions, and block the events associated with the photo-oxidative stress caused by psoralen. However, yet another non-contradictory hypothesis may also explain the photoprotective action of PL and the absence of free radicals and reactive oxygen species as due to the immunomodulatory effects of PL (25, 28). In fact, it is well recognized that the final consequences of the immune/inflammatory response consist of the generation of reactive intermediates and the release of cytokines by cells of the inflammatory infiltrate, thus leading to tissue damage (4).

With regard to this proposed immunological hypothesis for modulation of photoprotection, the preliminary results obtained in the skin biopsies are pertinent, and of significance. In fact, only psoralen-sensitized subjects taking oral PL showed a good and significant preservation in the morphology of functional Langerhans cells (Figs. 2 and 3). It is widely known that the disappearance of Langerhans cells (LC) is one of the proposed mechanisms implied in support of local immunosuppression in PUVA-treated patients (45–47). The disappearance of LC has been pathologically related to the toxic effects of oxygen intermediates (48), and physiologically related to the action of TNF-α during the course of the antigen presentation within the skin-draining lymph nodes (49). For all these reasons, and taking into account the ability of PL to inhibit IL-1 (27) and platelet activating factor (PAF) production (28), it could reasonably be suggested that the topical as well as oral photoprotective effects of PL may be due to the inhibition of the inflammatory response provoked by the solar irradiation or by PUVA reaction, involving 8-MOP+UVA or 5-MOP+UVA.

In summary, the oral administration as well as the topical application of an extract of *Podophyllum leucotomos* appear to protect skin against the acute sunburn reaction and PUVA-induced phototoxic reactions by 8-MOP+UVA or 5-MOP+UVA. The antioxidant activity of the extract, together with its ability to modulate the immune/inflammatory response, strongly support the results presented in this communication. While the protective effects of topical PL could be due to its antioxidant property and other biophysical properties (e.g., quenching of $\cdot O_2^-$, and other free radicals), the observed activity of the oral PL, particularly in topical and oral phototoxic reactions, opens a new avenue in examining the field of systemic photoprotection. After more than 5 years of pharmacological surveillance of the oral PL treatments conducted not only in Spain (50) but also in South and Central American countries, no recognizable toxic side effects have been observed by our colleagues or reported by other investigators (32, 33), including alterations in liver function tests,
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References


