Polypodium leucotomos Extract: A Natural Antioxidant and Photoprotective Tool for the Management of UV-Induced Skin Damage and Phototherapy

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The main causal agent of skin aging, immune depression, and cancer is UV light. Photoprotective agents can be divided into 2 classes: those that prevent photon-induced damage (sunscreens) and those that counter the deleterious effects of UV exposure systemically. In this review, the cellular and molecular mechanisms underlying the photoprotective effects of a *Polypodium leucotomos* (PL) fern extract are outlined. The effects of PL have been characterized in vivo and in vitro. It inhibits generation of reactive oxygen species and prevents UV-induced DNA damage, isomerization, decomposition of *trans*-urocanic acid, and also UV-induced cell death. Furthermore, oral treatment with PL combined with narrowband UVB therapy significantly promotes repigmentation of patients with vitiligo. Together, these effects postulate PL as a natural photoprotectant and potential adjuvant to phototherapy of various skin diseases.

Folkloric medicine is partly based on the use of natural extracts. Several extracts of *Polypodium leucotomos* (PL), which is a tropical fern plant of the Polypodiaceae family,[1] have been investigated during the last 30 years to evaluate its effects on the treatment of several skin maladies and has been long known to possess beneficial properties for the skin.[2] However, direct application of unrefined plant macerates in cases of psoriasis and atopic dermatitis, providing no significant improvement, perhaps due to the low concentration of its active principles. More recently, a purified hydrophilic extract of the leaves of PL (brand names Fernblock and Heliocare) has been shown to reduce skin sensitivity to damaging UV radiation and to possess a wide array of beneficial effects in the prevention of sunburn and related processes.

In this paper, an overview on the composition, pharmacologic properties, and reported effects of PL are presented, focusing on the molecular mechanisms involved in photoprotection from UV light, particularly DNA reparation. Collectively, its beneficial effects on photodamaged skin together with its photoprotective features make a strong case for its employment as an adjuvant in different photobased therapies.

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EFFECTS OF UV LIGHT ON THE SKIN

UV light includes wavelengths from 200 nm to 400 nm. It is usually divided further into 3 components,

UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). Whereas UVC does not reach the surface of earth, exposure of the skin to UVA and UVB causes numerous biological effects, as follows.

Generation of Reactive Oxygen Species

Exposure of the skin to UV induces formation of reactive oxygen species (ROS).[3,4] Reactive oxygen species are routinely removed by cellular antioxidant systems,[4] but chronic exposure to UV light causes accumulation of ROS that results in cell death due to oxidative stress, leading to premature aging (photoaging) and mutations due to direct DNA damage and expression of mutated genes, inducing tumorigenesis. In addition, ROS induce oxidative stress in cell membranes by peroxidation of fatty acids that are part of the inner structure of the membrane. They also generate lipid peroxide radicals and lipid hydroperoxides that amplify oxidative damage.[3]

UV Damage to DNA

The heterocyclic bases of DNA are the major acceptors of UVB photons in skin, which results in direct DNA damage due to the formation of pyrimidine dimers (mainly thymine-thymine) and pyrimidine-pyrimidone photoproducts.[5,6] On the other hand, cutaneous exposure to UVA photons indirectly via ROS generate 8-hydroxy-2'-deoxyguanosine, a major marker of oxidative DNA photodamage. These mutagenic products initiate tumorigenesis.[7] In addition, DNA damage has been linked to systemic immunosuppression.[8]

Inflammation

UV-induced erythema is due to increased blood flow and vasodilation,[9] in which nitric oxide and prostaglandins are key players.[10,11] Cell death caused by UV irradiation contributes to inflammation due to release of apoptotic debris[12] and subsequent inflammatory neutrophil infiltrates, which amplify ROS damage.[13] Recently, it also has been shown that photoprotection is, at least partly, mediated through reduction of acute inflammation via cyclooxygenase (COX)-2 inhibition.[14]

Immunosuppression

UV light causes tolerogenicity in epidermal Langerhans cells (eLCs), which results in clonal anergy of T_H1 cells.[15] UV light also induces photoisomerization of *trans*-urocanic acid (*t*-UCA), a photoprotective molecule[16] to *cis*-urocanic acid (*c*-UCA), which induces severe immunosuppression due, at least partially, to its effect on eLCs[17] and abnormal mast cell degranulation.[18]

In summary, UV irradiation on the skin results in immediate burns, cell death and inflammation, DNA damage and local immunosuppression, and also long-term effects from repetitive UV damage such as premature aging and tumor development.

PL: MOLECULAR COMPOSITION AND PHARMACOLOGY

Polypodium leucotomos contains multiple components, including monosaccharides (fructose and glucose); quinic, shikimic, glucuronic, and malic acids; and a high percentage of phenolics (mainly benzoates and cinnamates).[19] These phenolics are nonflavonoid catecholic compounds that are endowed with anti-inflammatory, antimutagenic, and anticarcinogenic properties,[20-23] which mainly depend on their antioxidant capability.[24] Caffeic and ferulic acid inhibit UV-mediated peroxidation by inhibiting propagation of the lipid peroxidative chain reaction, and also inhibit nitric oxide.[25] In addition, ferulic acid is a strong UV photon acceptor.[26] Both of them inhibit UVB-induced skin erythema and are employed in skin lotions and sunscreens.[27]

Coumaric, ferulic, and vanillic acid are partially conjugated to glucuronic acid and sulfates and metabolized efficiently ($t_{1/2}$ ≈4–6 hours) by cytochrome P450–dependant monooxygenases.[28,29] In addition, PL is absorbed readily through the skin.[30] Finally, PL is neither mutagenic nor toxic orally and allows for repetitive treatments with no long-term toxicity (A. Escario, MD, et al, unpublished data, November 2001).

PHOTOPROTECTIVE PROPERTIES OF PL

Early studies on extracts of PL and similar ferns have described their antitumoral properties.[31,32] Consistent with this, PL inhibited the appearance of skin tumors after irradiation with UVB light in hairless albino mice.[33] Several effects underlie the antitumoral properties of PL, and PL inhibits UV-induced damage to DNA, inhibiting the formation of thymidine dimers.[34] This seems to be related to enhanced elimination of UV-induced metabolic by-products, such as cyclobutane pyrimidine dimers.[14] In addition, PL induced activation of the tumor suppressor p53 and inhibited expression of the inducible form of COX-2,[14] which is induced by UV and its inhibition blocks carcinogenesis (Table).[35,36]

Other studies focused on their anti-inflammatory properties, particularly in the treatment of skin inflammatory diseases such as psoriasis, vitiligo, and atopic dermatitis.[44-47] In addition, other reports suggested the use of these extracts not only to treat but also to prevent skin inflammation.[48] To demonstrate this, guinea pigs were exposed to UVB light in the presence of placebo or topically applied PL; in these experiments, PL efficiently blocked UVB-induced skin damage. These experiments

Photoprotective Effects of PL

	Mechanism	Outcome	References
Generation of free radicals	Inhibits ROS generation	Relieves photoaging, prevents tumor formation	28,37
Generation of other deleterious metabolites	Inhibits *t*-UCA photoisomerization, photodecomposition	(Hypothetical) Inhibits photoaging and immunosuppresion	38
Inflammation	Inhibits cell death and apoptosis	Inhibits photoaging and carcinogenesis	30,34,39-41
DNA damage	Decreases the formation of pyrimidine dimers	Inhibits carcinogenesis	34
	Activation of p53		14
Immunosuppresion	Prevents depletion of eLCs	(Hypothetical) Inhibits chronic inflammation and carcinogenesis	34,40,42
Alterations in gene expression	Inhibition of COX-2	(Hypothetical) Inhibits carcinogenesis	14
	Inhibition of TNF-α and iNOS	Inhibits inflammation	43

Abbreviations: COX, cyclooxygenase; eLC, epidermal Langerhans cell; iNOS, inducible nitric oxide synthase; PL, *Polypodium leucotomos*; ROS, reactive oxygen species; *t*-UCA, *trans*-urocanic acid; TNF-α, tumor necrosis factor α.

were extended to human participants exposed to small amounts of UVA light; in these experiments, total photoprotection was achieved.[39] Further experiments in a larger group of human participants demonstrated that PL prevented acute sunburn and significantly reduced the phototoxic effects of exposure to sunlight after oral ingestion of psoralens,[30] postulating its beneficial effect in phototherapy used for the treatment of various skin disorders. For example, therapy combining psoralens and UVA light (PUVA) is a very successful treatment for psoriasis.[49-51]However, its deleterious side effects may include skin cancer, which limits its widespread application.[52] Use of PL as an adjuvant reduced phototoxicity during PUVA therapy.[30,40] In addition, oral administration of PL inhibited PUVA-induced sunburn and infiltration of neutrophils and mast cells and reduced loss of eLCs associated with these treatments.[41] This effect correlated with a significant reduction of skin photodamage at a histologic level, including reduced sunburn cells and inflammatory infiltrates, decreased levels of UV-induced DNA damage, and enhanced epidermal cell proliferation.[34]

In addition, PL also offers promise as an adjuvant in other types of phototherapy, such as repigmentation in cases of vitiligo vulgaris.[53,54] Oral treatment with PL concomitant to narrowband UVB therapy significantly enhanced repigmentation, particularly in light skin phototypes.[53]

At a cellular level, PL has been shown to inhibit fibroblast and keratinocyte cell death induced by UVA and restore cell proliferation. It also prevented disarray of the cellular actin cytoskeleton and loss of adhesive cell-cell and cell-matrix contacts.[55] Finally, PL inhibited protease secretion and preserved the integrity of the membranes of UV-irradiated cells.[56]

In summary, there is growing evidence that supports the photoprotective role of PL, providing a rationale for its use as an adjuvant in phototherapy treatments, such as PUVA-therapy and narrowband UVB. Its photon acceptor capability and lack of toxicity also support its inclusion in sunscreens and as an oral supplement.

MOLECULAR MECHANISMS OF PL PHOTOPROTECTION

Direct Antioxidant Activity

The nonflavonoid phenolics present in PL are endowed with antioxidant activity.[28,57] They prevent UV-mediated peroxidation by inhibiting propagation of the lipid peroxidative chain reaction.[25] In addition, PL can also scavenge ROS, including superoxide anion, hydroxyl radicals, singlet oxygen, and hydrogen peroxide.[37] Interestingly, PL

also prevents nitric oxide synthesis by inhibiting inducible nitric oxide synthase expression.[43] In the hairless rat model, PL effectively reduced glutathione oxidation in both blood and epidermis, suggesting a potent systemic antioxidant effect. In addition, PL inhibited UVR-mediated Langerhans cell depletion.[58]

Prevention of DNA Photodamage

Oral administration of PL inhibited generation of thymine dimers in humans[34] and animals.[14] In the xeroderma pigmentosum group C animal model, PL supplementation significantly decreased the number of 8-hydroxy-2'-deoxyguanosine (+) cells even before UV-irradiation, suggesting that PL reduces constitutive oxidative DNA damage.[14]

Inhibition of Photoisomerization and Photodecomposition of *t*-UCA

The main by-product of histidine metabolism is *t*-UCA. Its photoprotective capability relies on its ability to absorb UV photons that cannot induce tissue damage. However, UV photon absorption induces its isomerization to *c*-UCA, whose accumulation causes immunosupression.[16,57,59,60] PL inhibited *t*-UCA photoisomerization and the appearance of *c*-UCA in a dose-dependent fashion in the presence of hydrogen peroxide and also prevented the oxidative breakdown of *t*-UCA in the presence of ROS and a catalyst such as titanium dioxide.[38]

Immunoregulation

PL prevents UV-induced immunosuppression by inhibiting depletion of co-stimulatory cells on the skin such as eLCs. Elimination of eLCs by UV irradiation is induced by the combination of direct apoptosis, inflammation, induction of an aberrant morphology,[17] and inhibition of the expression of adhesion molecules required for the migration of eLCs to the skin.[61,62] Upon UV irradiation, PL efficiently blocked eLC depletion and prevented the appearance of abnormal morphologies.[34,41,58] Similar results were obtained using blood dendritic cells irradiated using a solar simulator, inhibiting dendritic cell apoptosis and promoting secretion of anti-inflammatory cytokines by irradiated dendritic cells.[42] In addition, PL inhibited expression of proinflammatory cytokines such as tumor necrosis factor α.[43] Finally, PL has been shown to prevent UV-induced immunosupression in contact hypersensitivity reactions.[63]

CONCLUSION

PL combines extremely low toxicity with proven beneficial effects in the prevention and treatment of UV-related skin damage, even by oral administration, postulating its employment in phototherapy to prevent side effects such as carcinogenesis. Its benefits stem from its antioxidant properties, the capability to inhibit *t*-UCA photoisomerization, inhibition of UV-induced apoptosis and DNA photodamage, and prevention of immunosupression. Oral treatment with PL provides a very effective complement for light Fitzpatrick skin types and adds extra protection in cases in which exposure to UV radiation cannot be avoided, such as those in UVB phototherapy and PUVA treatments.

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