Mechanistic insights in the use of a *Polypodium leucotomos* extract as an oral and topical photoprotective agent

Salvador Gonzalez,*[a,b] Yolanda Gilaberte[c] and Neena Philips[d]

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Photoprotection is essential to prevent the deleterious effects of ultraviolet (UV) light, including skin cancer, photoaging and immunosupression. Photoprotective agents can be classified according to their main mechanism of action. Some of them absorb or deflect UV photons (sunscreens), whereas others prevent or fix the deleterious effects of UV exposure. Here, we review recent evidence on the cellular and molecular mechanisms underlying the photoprotective effect of a *Polypodium leucotomos* fern extract (PL). PL is a natural mixture of phytochemicals endowed with powerful antioxidant properties. Its short-term effects include inhibition of reactive oxygen species production induced by UV radiation, DNA damage, isomerization and decomposition of *trans*-urocanic acid, prevention of UV-mediated apoptosis and necrosis, as well as degradative matrix remodeling, which is the main cause of photoaging. These short-term effects translate into long-term prevention of photoaging and photocarcinogenesis. A striking property is that PL can exert its effect when administered orally. Together, these effects postulate PL as a natural photoprotective agent and a potential adjuvant to phototherapy for various skin diseases.

1. Introduction

Polypodium leucotomos is a tropical fern plant of the Polypodiaceae family[1] with well-known beneficial properties for the skin, and used in folkloric medicine in South America to make poultices to treat psoriasis and atopic dermatitis.[2] However, the effect of unrefined plant extracts in areas of the skin affected by psoriasis and atopic dermatitis is not very significant, probably due to a low concentration of active principles. A concentrated hydrophilic extract of the leaves of *Polypodium leucotomos* (hereafter referred to as PL) is available commercially, and has been shown to inhibit several deleterious effects of UV radiation on human skin.

In this review, we will describe our current knowledge on the composition, pharmacological properties and short- and long-term molecular, cellular and tissue effects of PL. We will also dissect the molecular mechanisms involved in its photoprotective effect, particularly its antioxidant properties and DNA-related effects. Finally, we will discuss recent studies that have highlighted its effect in preventing UV-mediated immunosupression and skin photoaging.

[a]*Dermatology Service, Memorial Sloan-Kettering Cancer Center, 160 East 53rd Street, New York, NY, 10022, USA. E-mail: gonzals6@mskcc.org; Fax: +1 212 308 0530; Tel: +1 212 610 0185*
[b]*Dermatology Service, Ramon y Cajal Hospital, Madrid, Spain*
[c]*Dermatology Service, Hospital San Jorge, Huesca, Spain*
[d]*School of Natural Sciences, University College, Fairleigh Dickinson University, Teaneck, NJ, USA*

Salvador Gonzalez

Salvador González is a faculty member in the Dermatology Service at Memorial Sloan-Kettering Cancer Center, New York. After his MD, PhD and dermatology training, he began a fellowship in the Dermatology Department in the Massachusetts General Hospital, Harvard Medical School, Boston. Later, he joined the faculty of this institution as Assistant Professor. His work pioneers oral photoprotection and in vivo *confocal microscopy. He has received several international awards and has authored over 200 peer-reviewed papers, over 20 book chapters and is editor of a book that contributes to the development of the areas of his expertise.*

2. Overview of the deleterious effects of UV radiation on human skin

Ultraviolet (UV) radiation includes wavelengths ranging from 100 nm to 400 nm. It is usually divided into three wavebands, UVA, ranging from 315–400 nm, UVB (280–315 nm) and UVC (100–280 nm), according to the Commission Internationale de L'Éclairage (CIE). UVC does not reach the surface of earth, but incidence of UVA and UVB photons on human skin causes several biological effects. These include:

2.1. Generation of reactive oxygen species (ROS)

UV photons induce local formation of ROS in the skin.[3,4] ROS are eliminated by cellular antioxidant systems,[4] but chronic exposure to UV light overwhelms these housekeeping antioxidant systems, resulting in their accumulation. Oxidative stress is the leading cause of premature aging ("photoaging"), and contributes to cell death and cancer. At a cellular level, ROS induce peroxidation of

fatty acids at the plasma membrane. The lipid peroxide radicals and lipid hydroperoxides amplify oxidative damage.[3,5] Therefore, scavenging of ROS by antioxidants is an important research field against deleterious effects of UV radiation.[6,7]

2.2. Ultraviolet damage to DNA

The heterocyclic bases of the DNA of cutaneous cells are important UVB photon acceptors. UVB causes DNA damage directly by forming cyclobutane pyrimidine dimers (CPD, mainly thymine–thymine) and pyrimidine–pyrimidone photoproducts.[8,9] These premutagenic lesions that lead to tumourigenesis[10] also cause immunosuppression.[11] Also, UVA photons cause DNA mutations directly by generating ROS. For example, UV-derived ROS induce production of 8-hydroxy-2′-deoxyguanosine (8-oxo-dG),[12] an important marker of DNA oxidative damage linked to tumorigenesis.[13]

2.3. Inflammation

UV induces skin swelling and erythema; this is due to increased blood flow, endothelial cell activation with concomitant immune cell infiltration, and vasodilation.[14–16] Cell death generates apoptotic debris, which also contributes to immune cell infiltration[17] and the amplification of oxidative stress.[18]

2.4. Immunosuppression

UV radiation induces immunological tolerance. Epidermal Langerhans cells (eLCs) are depleted, which results in clonal anergy of Th1 cells.[19] It also induces photoisomerization of *trans*-urocanic acid (*t*-UCA), which is a UVB photoacceptor with putative photoprotective properties,[20] to *cis*-urocanic acid (*c*-UCA) which induces severe immunosuppression due, at least partially, to its effect on eLCs[21] and on mast cell degranulation.[22]

In summary, the effect of UV radiation on the skin can be divided into: immediate, including erythema and sunburn, cell death and inflammation, DNA damage, and local immunological tolerance; and long-term, including premature aging and cancer.

3. Photoprotection: sunscreens and other photoprotective agents

In addition to physically blocking UV radiation (*e.g.* by limiting exposure, or wearing appropriate clothes, hats and sunglasses), there are two main types of photoprotective measures: sunscreens and other photoprotective agents.

Sunscreens can be defined as substances that protect the skin from the harmful effects of solar ultraviolet (UV) by absorbing, reflecting, scattering or otherwise deflecting UV photons. These substances physically shield the skin, preventing photons from reaching the epidermis. They are generally very useful to prevent solar erythema and sunburn caused by high-energy UV photons. However, some of the long-term deleterious effects of UV exposure, *e.g.* photoaging, are caused by repeated exposure to low-energy photons, and most sunscreens do not stop these.

The term non-sunscreen photoprotective agent, on the other hand, refers to groups of a wide array of substances that compensate, ameliorate, repair or otherwise prevent the short and long-term effects of UV radiation. These substances are usually antioxidants, and prevent, for example, the overwhelming of the natural antioxidant systems of the skin that contribute to DNA damage, immunosupression and photoaging.

A novel and exciting trend in pharmacology and dermatology is the use of oral treatments to prevent the deleterious effects of UV radiation. An important fraction of these compounds are phytochemicals or botanical extracts with photoprotective properties that are very useful due to their low toxicity, good absorption and systemic antioxidant effects. These compounds will not, and are not meant to, substitute the appropriate use of sunscreens and physical blockers, but they are conceived and designed to raise the antioxidant threshold of the whole body. Thus, the reaction of oxidative injury is diminished and recovery is achieved faster and more completely.

PL, an extract from the fern *Polypodium leucotomos*, stands out as a multifaceted photoprotector, useful both orally and topically. In the next sections, its molecular composition and existing data on its effects at the cellular and systemic levels are described.

4. Molecular composition and pharmacology of PL

PL contains a high percentage of phenolics (mainly benzoates and cinnamates, like caffeic acid and its derivative ferulic acid).[23] These are non-flavonoid catecholic compounds endowed with antioxidant properties, which provide a molecular basis for their anti-inflammatory, anti-mutagenic and anticarcinogenic properties.[24–26] Caffeic acid inhibits UV-mediated peroxidation by inhibiting propagation of the lipid peroxidative chain reaction, and also inhibits nitric oxide production.[27] On the other hand, ferulic acid is a UV photon acceptor.[28] Both of them inhibit UVB-induced skin erythema and are employed in skin lotions and sunscreens.[29] PL also contains monosaccharides (fructose, glucose and others), quinic, shikimic, glucuronic and malic acids, as well as coumaric, ferulic and vanillic acid, which are partially conjugated to glucuronic acid and sulfates, and metabolized efficiently ($t_{1/2}$ = 4–6 h) by CYP450-dependent monooxygenases.[30,31]

Pharmacological data indicate that PL is absorbed readily through the skin.[32] It is neither mutagenic nor toxic orally, thus being an ideal candidate for repetitive treatment (A. Escario, *et al.*, unpublished results).

5. Photoprotective properties of PL

Early studies have described the antitumoral effect of extracts of PL and similar ferns.[33,34] These results have been confirmed in a hairless albino mouse model, in which PL inhibited skin tumor formation after UVB irradiation.[35]

The antitumoral properties of PL likely reside in its protective effect against UV-induced DNA damage. A decrease in the formation of thymine dimers has been reported,[36] and attributed to their repair,[37] possibly achieved by preventing oxidative damage to the DNA repair enzymes, a critical component of oxidative damage of DNA.[38] In addition, PL decreased the levels of 8-oxo-dG, a marker of oxidative stress, even before UV irradiation, supporting the idea that PL raises the systemic antioxidant threshold.[37]

Also, PL blocked the effect of UV radiation on the expression of cyclooxygenase-2 (Cox-2). Cox-2 is an inducible enzyme

responsible for prostaglandin synthesis and also involved in carcinogenesis.[39–41] Finally, PL induced activation of the tumor suppressor p53.[37]

Other studies have reported the anti-inflammatory properties of PL. Different *Polypodium* extracts were used in folkloric medicine for the treatment of skin inflammatory diseases such as psoriasis, vitiligo and atopic dermatitis.[42–45] PL successfully blocks the inflammatory response either in the skin of guinea pigs irradiated with UVB light or in a small group of human volunteers irradiated with small amounts of UVA light.[46] Additionally, in the latter case, total photoprotection was achieved.[46] In a larger sample of human volunteers, PL prevented acute sunburn and reduced the phototoxic effect of exposure to sunlight after oral ingestion of psoralens.[32] This postulates its use as adjuvant in phototherapy, for example, PUVA (psoralens + UVA).[47–49] PUVA therapy is a very successful treatment for psoriasis; however, it is not widely used due to its deleterious side effects, which may include skin cancer.[50] Use of PL as an adjuvant reduced phototoxicity during PUVA therapy.[32,51,52] Moreover, oral PL inhibited PUVA-induced sunburn and infiltration of neutrophils and mast cells, and reduced loss of eLC associated to these treatments.[52] This effect is probably due to decreased apoptosis and DNA damage, and correlates well with reduced skin photodamage, including reduced sunburn cells and inflammatory infiltrates, decreased levels of UV-induced DNA damage and enhanced epidermal cell proliferation.[36]

These data support its widespread use as an adjuvant in other types of phototherapy, such as repigmentation of vitiligo vulgaris.[53,54] Oral treatment with PL concomitant to narrow band UVB therapy significantly enhanced repigmentation, especially in light skin phototypes, probably due to decreased photoinduced damage, which prompts a more robust response to therapy.[53]

In addition to its antioxidant activity, PL is a promising agent in the treatment and prevention of photoaging due to its effects on extracellular matrix remodeling. PL inhibits the activity and expression of different matrix metalloproteinases, and promotes expression of tissue inhibitor of metalloproteinase (TIMP), TGF-β, elastin and different types of collagen.[55] In summary, PL inhibits the expression and function of molecules involved in matrix degradation (MMPs) and induces the expression of protective (TIMP), proliferative (TGF-β) and extracellular matrix molecules, thus promoting regeneration and compensating for the deleterious effects of irradiation that cause photoaging.

At a cellular level, PL prevents lipid peroxidation and membrane damage induced by UV;[56] it also blocks UV-mediated disarray of the actin cytoskeleton and loss of adhesive cell–cell and cell–matrix contacts;[57] finally, PL inhibits fibroblast and keratinocyte cell death induced by UV radiation.[58]

6. Molecular effects of PL-mediated photoprotection

PL efficiently compensates and provides repair for most of the deleterious effects of UV irradiation. Most of them are related to its antioxidant capability, but some rely on specific effects in distinct cellular cohorts, such as eLCs.

6.1. Antioxidant activity

The phenolic moieties of PL endow it with antioxidant activity.[30,59] PL prevents UV-mediated peroxidation in the skin by inhibiting propagation of the lipid peroxidative chain reaction.[27] PL also scavenges ROS, including superoxide anion ($^{\bullet}O_2$), hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and H_2O_2.[60] The effect of PL is not limited to ROS; it also prevents NO synthesis by inhibiting iNOS expression.[58] In a hairless rat model, PL effectively reduced glutathione oxidation in both blood and epidermis, suggesting a potent systemic antioxidant effect, and inhibited UV-mediated eLC depletion, counteracting UV-induced immunosupression (see below).[59]

6.2. Prevention of DNA photodamage

Oral systemic administration of PL inhibits UV-mediated formation of thymine dimers in humans[36] and in a Xeroderma pigmentosum rodent model (XPC).[37] These animals exhibit an exacerbated inflammatory response to UV irradiation and a decreased DNA repair capability. As a result, they are prone to develop skin cancer. Supplementation of the diet with PL decreases the level of basal oxidative stress (quantified as number of (8-ox-dG)-positive cells), suggesting that PL reduces constitutive oxidative DNA damage.[37]

6.3. Inhibition of photoisomerization and photodecomposition of *t*-UCA

t-UCA is the main byproduct of histidine metabolism. Its photoprotective capability relies on its ability to absorb UV photons that then cannot induce tissue damage. However, UV photon absorption induces its isomerization to *c*-UCA, whose accumulation causes immunosupression.[20,61–63] PL inhibited *t*-UCA photoisomerization and the appearance of *c*-UCA in a dose-dependent fashion in the presence of H_2O_2, and also prevented the oxidative breakdown of *t*-UCA in the presence of ROS and a catalyst such as TiO_2.[64]

6.4. Immunoregulation

PL prevents UV-induced immunosuppression by inhibiting depletion of antigen-presenting 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[21] and inhibition of the expression of adhesion molecules required for the migration of eLCs to the skin.[65,66] PL efficiently blocked eLC depletion upon UV irradiation and prevented the appearance of abnormal morphologies.[36,52,59] Similar results were obtained using blood dendritic cells irradiated using a solar simulator, inhibiting DC apoptosis and promoting secretion of anti-inflammatory cytokines by irradiated DC.[67] In addition, PL inhibited expression of pro-inflammatory cytokines such as TNF-α.[58] Finally, PL prevents immunosupression caused by UVB in a rodent model of contact hypersensitivity.[68]

7. Conclusions and future perspectives

PL has very low toxicity as well as proven effects in the prevention and treatment of UV-related skin damage. These effects are based on its antioxidant properties, the capability to inhibit *t*-UCA photoisomerization, inhibition of UV-induced apoptosis and DNA photodamage and prevention of immunosupression. Of outstanding interest is its effect *via* oral administration, which

strongly suggests that it can be used as an adjuvant in situations where exposure to elevated amounts of UV radiation cannot be avoided, such as phototherapy. Its effect in the regulation of the systemic level of oxidative damage suggests that its beneficial effects may extend beyond the realm of the skin. Further research will be required to investigate this extent, and to delineate the specific contributions of the active principles contained in PL to its beneficial effects.

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