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Neuroprotective effects of a new skin care formulation following ultraviolet exposure

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Abstract

Background: Chronic ultraviolet (UV) exposure is a major environmental factor involved in extrinsic skin ageing (photo-ageing). Skin nerve fibres are significantly reduced in number following UV irradiation and new skincare compounds with neuroprotective effects are thus highly warranted.

Objectives: We developed a new skincare formulation from a plant extract and evaluated its neuroprotective effects of *ex vivo* UV irradiation.

Materials and methods: The new skincare emulsion was formulated from *Echinacea purpurea* extract and was enriched with antioxidants (patent no. PROV020110087075). Skin samples were obtained from 20 healthy patients enrolled for plastic surgery and were immediately treated with placebo (SPF 15) or test emulsions. Skin samples were exposed to UVA and UVB for 60 min. Nerve fibres were identified by immunofluorescence using a monoclonal antibody, anti-human CD56. Cell damage was quantified by image analysis.

Results: UVA and UVB significantly reduced (40–60%) densities of nerve endings in control samples treated with placebo ($P < 0.001$). Samples treated with test emulsion completely blocked UV-related effects on skin nerve endings. These neuroprotective effects were similarly observed regardless of age or tissue analysed (breast versus abdomen).

Conclusions: Our new skincare formulation obtained from *E. purpurea* provides important neuroprotective effects of UV irradiation and could be used together with SPFs to prevent chronic deleterious effects of solar exposure.

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Introduction

Chronic ultraviolet (UV) exposure is a major environmental factor involved in skin ageing by extrinsic means (photo-ageing). Photo-ageing overlaps chronological (intrinsic) ageing and can be compared to body areas that remain protected from solar exposure (1). This process is characterized by complex remodelling of the dermis, including morphological and biochemical changes in nervous components of the skin. There is both autonomic (sympathetic) and sensory innervation in the skin, originating from post-ganglionic sympathetic neurons, and from neurons located in dorsal root ganglia, respectively. The fibres link to mechanoreceptors (free nerve endings), thermoreceptors and nociceptors of the skin. We have recently observed reduced numbers of skin free nerve endings following *ex vivo* UV exposure (2). Peripheral neuropathy or damage to nerves of the peripheral nervous system is also associated with reduced nerve endings in several diseases, including sensorimotor neuropathy, neuritis and diabetic neuropathy (3). Development of new skin care formulations with neuroprotective properties is thus highly desirable.

Insights gained into molecular bases of photo-ageing provide new opportunities for therapeutic intervention aimed at prevention. UV irradiation induces a complex sequence of specific molecular responses that damage skin connective tissue (4) and are initiated by generation of reactive oxygen species (ROS). We speculate that ROS would damage skin nerve fibres following UV exposure, and thus antioxidants should be included in new skincare formulations, to limit these effects. Sunscreens are commonly used to reduce damage caused by solar exposure by decreasing penetration of radiation, through absorption or reflection mechanisms. Although sunscreens are highly effective at reducing erythema and sunburn, they do not

reduce chronic effects of solar radiation (5). Many research groups are thus seeking new active compounds (for example, antioxidants) to diminish acute and chronic effects of UV radiation, such as DNA damage, immunosuppression, apoptosis and nerve fibre damage (6). Polyphenols were the first plant compounds with antioxidant potential studied; these can be found in green tea and in *Echinacea purpurea*. The latter has shown greater antioxidant activity against ROS generated by UV radiation (7). In this study, we developed a new skincare formulation from a plant (*E. purpurea*) extract, and evaluated its neuroprotective effects of *ex vivo* UV irradiation.

Materials and methods

Subjects

Twenty healthy, female patients (all Caucasian), mean age 42 ± 20 years, were recruited at the Plastic Surgery Unit of the Hospital São Lucas (Porto Alegre, Brazil). All patients underwent cosmetic plastic surgery to reduce either breast (mammoplasty) (n = 9) or abdomen (abdominoplasty) (n = 11). Exclusion criteria included indoor tanning, diabetes, neoplasia, infectious or chronic inflammatory disease. A written informed consent was obtained from all subjects. This study was approved by the Pontifical Catholic University of Rio Grande do Sul (PUCRS) Ethics Committee.

Skin biopsies

Biopsies consisted of skin fragments, approximately 10 cm^2. Tissues were separated into smaller portions (1 cm^2) for all treatments, at room temperature. Biopsies were immersed in saline solution and were taken immediately to the laboratory for processing.

Skincare formulations

The skincare formulation was produced as an oil/water emulsion. Plant extracts were obtained from *E. purpurea*, which is known to be enriched with antioxidant components. Briefly, plant components were transferred to a glass container (this is the oil phase), and the mixture was placed in a water bath and heated at high temperature for a few minutes until complete fusion of the components was observed. Components of the aqueous phase were then weighed in a glass container and heated at high temperature. Following continuous shaking, the aqueous phase component was poured slowly into the oil phase component. Agitation continued for a few minutes to ensure complete emulsification then the emulsion was cooled and the preservative system was added. The test cream formulation (+*E. purpurea*) and placebo (SPF 15) were composed of cetostearyl alcohol 30/70, PEG-7 glyceryl cocoate, coco-caprylate/caprate, dicaprylyl ether, propileneglycol, glycerin, macadâmia oil, isopropyl myristate, EDTA, urea, propylparaben, methylparaben, avobenzone, octylmetoxicinnamato. Emulsions were gently, manually, applied to the skin biopsies (2 mg/cm^2) and allowed to remain there for 30 min (to complete penetration, prior to UV exposure) in Petri dishes with saline (without covering up the samples). The formulation and process are intellectually protected and a PIBR registration has been received (# PROV020110087075).

UV irradiation

UVA and UVB lamps (Philips, 15 W, Barueri, SP, Brazil) were used to irradiate the skin biopsies for 60 min, in sterile conditions, provided in a laminar flow cabinet. Control skin samples were left untouched on the bench over the same period. UVA and UVB levels of irradiation had previously been measured (2.18 ± 0.10 W/m^2) using spectro-radiometry, at the Faculty of Physics of PUCRS.

Immunofluorescence

The biopsies were treated with isopenthane (Vetec, Duque de Caxias, RJ, Brazil) and frozen briefly in liquid nitrogen. Briefly, longitudinal sections of the epidermis were cut using a cryostat (Sundown), and skin free nerve endings were identified using monoclonal antibody anti-CD56-FITC (NCAM; FK Biotec, Porto Alegre, Brazil). Samples were counterstained with DAPI and analysis was performed using a fluorescence microscope (Zeiss Axioskop 40; Carl Zeiss do Brasil Ltda., São Paulo, Brazil). Further details of this technique can be found elsewhere (2).

Image analysis

Briefly, five fields (100×) per treatment were analysed and positive cells/axons were identified using excitation in blue (488-nm) light; images were acquired with an Image Pro Plus Capture Kit digital camera (Media Cybernetics, Silver Spring, MD, USA). Further details of the image analysis have previously been described (2). Tissue densities of cells were quantified by automatic pixel selection (Adobe Photoshop CS3); all images had a spatial resolution of 4 pixels per μm^2. Evaluation of damage was performed in a double-blind fashion.

Statistical analysis

Variables were tested for normality of distribution using the Kolmogorov–Smirnov test. Data were analysed by

factorial ANOVA, including two levels for the two creams (test cream containing *E. purpurea* and placebo) and three levels of irradiation (absent, UVA and UVB). Differences between treatments were investigated by *post hoc* analysis (Tukey). Data were analysed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

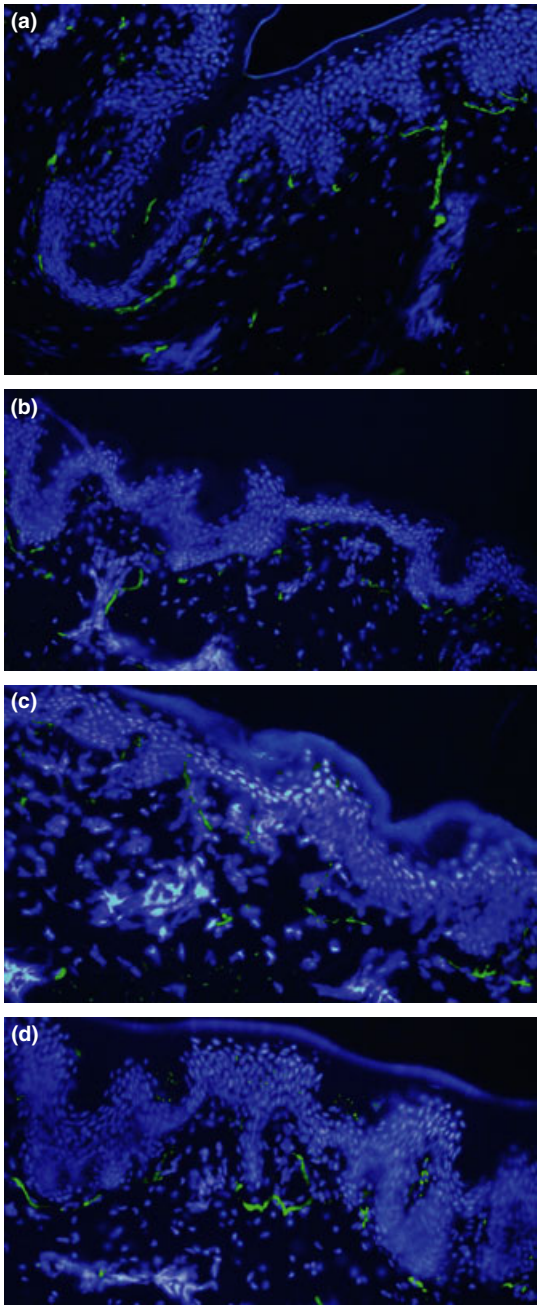

Figure 1. Effects of ultraviolet A (UVA) radiation on free nerve endings. (a) Control skin; (b) UVA-treated; (c) UVA-placebo; (d) UVA-*E. purpurea*. Magnification: 200×.

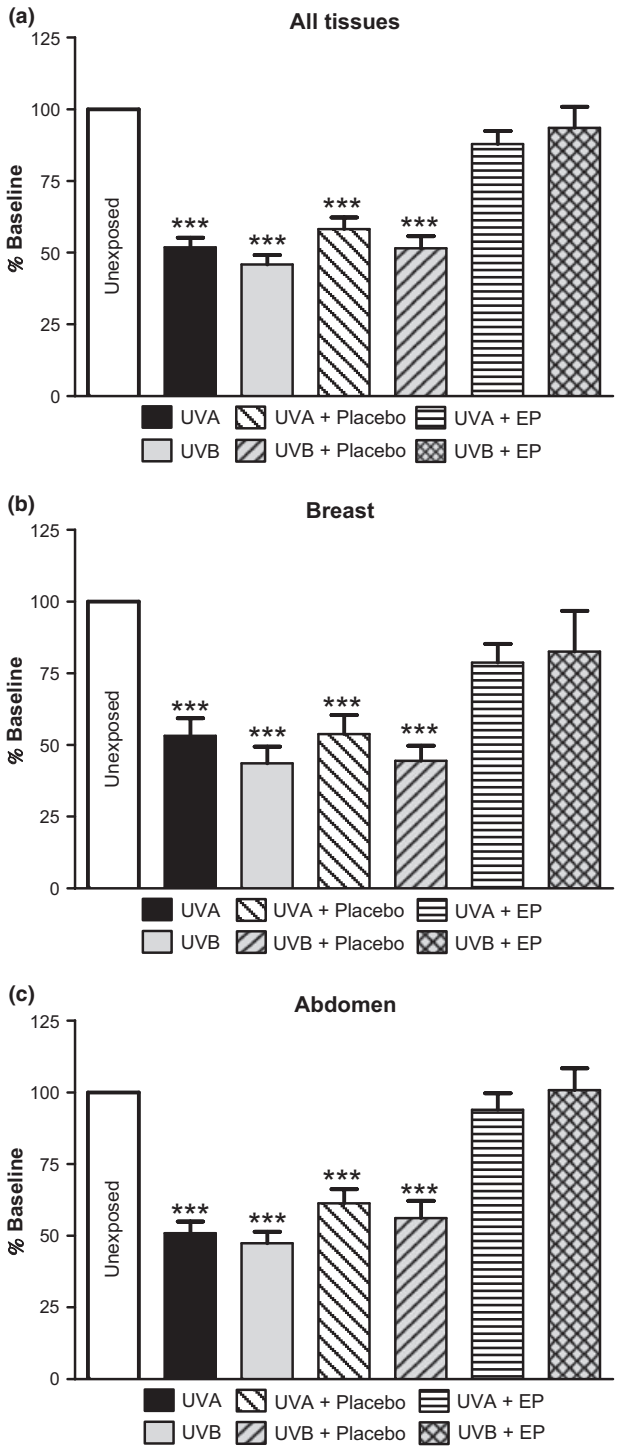

Figure 2. Analysis of nerve damage induced by ultraviolet radiation and assessment of neuroprotection. Test cream containing extract of *E. purpurea* (EP) completely blocked UV-related damaging effects of nerve endings ($P > 0.05$ versus non-exposed), regardless of tissue analysed. Data are shown as percentage of baseline (non-exposed tissue). Statistically significant differences are indicated $^{**}P < 0.001$ versus non-exposed samples.

Results

We investigated effects of UV radiation on NCAM+ densities and assessed neuroprotective effects of the new skincare formulation; diffuse nerve endings were identified in the dermis by specific NCAM+ staining (Fig. 1). *Ex vivo* ultraviolet irradiation (UVA and UVB) significantly reduced (40–60%) density of cells in untreated or placebo-treated samples ($P < 0.001$), Fig. 2a. Samples treated with test cream containing extract of *E. purpurea* completely blocked UV-related damaging effects on nerve endings ($P > 0.05$, versus non-exposed samples).

Skin biopsies had been obtained from breast and abdomen that could theoretically present differential patterns of innervation or to solar exposure. Therefore, we performed additional statistical analyses to test whether reported changes were tissue-specific. Test cream, containing *E. purpurea*, had a significant neuroprotective effect in both breast and abdomen biopsies ($P > 0.05$ versus unexposed samples), suggesting that its effect was not tissue-specific (Fig. 2b and c).

Ageing is associated with significant morphological and anatomical changes in the skin. Thus, we also sought to investigate neuroprotective efficiency of the test cream to skin samples obtained from young adults (20–40 years) and older adults (40–60 years). Test cream containing *E. purpurea* had significant neuroprotective effects regardless of age ($P > 0.05$ versus unexposed samples), Fig. 3.

Discussion

In accordance with previous studies, we confirm here that exposure to UV irradiation significantly decreases density of nerve fibres in human skin (2,8). These UV-induced effects are desirable in attenuating pruritus observed in certain skin diseases (for example, psoriasis) where phototherapy is a therapeutic option. In this study, however, we have developed a new skincare formulation based on a plant extract, potentially enriched with antioxidants, and we assessed its anti-UV neuroprotective actions. The hypothesis proved to be true and the formulation was identified as having significant neuroprotection effects.

Chronic UV exposure is a major environmental factor involved in photo-ageing. The underlying events following UV exposure include erythema, generation of ROS and associated DNA damage, increased p53 production and apoptosis (9); cumulative UV-related damage is potentially associated with development of skin cancer. Our data suggest that UV is capable of inducing apoptosis of cells with peripheral free nerve endings in the skin. However, it should be noted that our observations do not exclude the possibility that UV-induced effects are due to downregulation of NCAM molecules expressed on free nerve endings. More research is necessary to further explore this area.

Antioxidants may be important targets of prevention of photo-ageing due to their capacity to reduce production of free radicals. Pellati *et al.* (10) showed that all *Echinacea* species can have free radical scavenging activity and that *E. purpurea* was the most efficient amongst them. Antioxidant activity could be ascribed to the phenolic content of roots, and cichoric acid present in *E. purpurea*. Polyphenols are better antioxidants than monophenolics (11,12); substitution of the aromatic ring in the ortho- or para-position may enhance antioxidant efficacy, leading to increased stability of the antioxidant. Cichoric acid has two orthodihydroxies that may thus provide the plant with better free radical scavenging properties than flavonoids. Cichoric acid is only present in *Asteraceae,* and *E. purpurea* is highly enriched with this compound (10). Sunscreens are major pharmacological agents used to protect against deleterious effects of solar UV radiation. Data from our above study indicate that bioactive compounds present in *E. purpurea* may function differently from

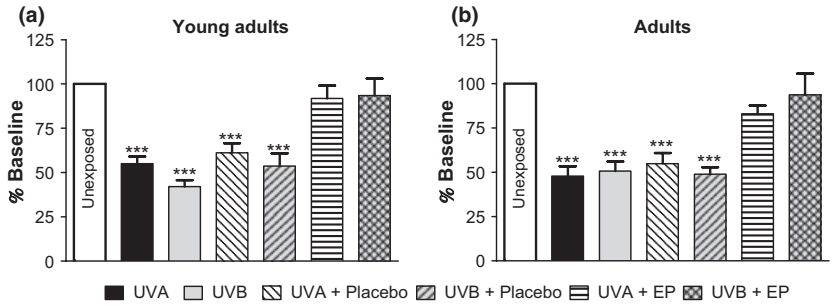

Figure 3. Neuroprotection induced by test cream is independent of age group. Assessment of UV-related damage to skin nerve endings was compared between samples obtained from young adults (20–40 years) and older adults (41–60 years). The test cream containing extract of *E. purpurea* (EP) completely blocked UV-related damaging effects to nerve endings ($P > 0.05$ versus non-exposed), regardless of age group. Data are shown as percentage of baseline (non-exposed tissue). Statistically significant differences are indicated $^{**}P < 0.001$ versus non-exposed samples.

sunscreens. Unlike sunscreens, these compounds do not appear to absorb significant amounts of light in the UV range. We demonstrated here that plant-derived substances, most likely antioxidants, combined with traditional sunscreens, had additive photoprotective effects when compared to sunscreens alone.

There are some limitations in this study to be further addressed. First, future studies should identify the exact plant bioactive products involved with skin neuroprotection following UV exposure. Although we speculated that plant antioxidants would be the likely candidates, soluble factors produced by skin also could be involved in neuroprotection. For instance, the plant extract may induce secretion of local neurotrophins (for example, NT-3, NGF, BDNF) involved with neuron survival and maintenance of skin nerve fibres. These factors need to be measured in future studies. Second, the neuroprotection should also be demonstrated following chronic UV exposure as this would better mimic long-term skin photo-ageing. Finally, future studies should also correlate UV-related changes in density of cells with absolute number of skin nerve fibres. Taking into account these limitations, the present study has two major implications: (a) confirming the impact of UV radiation on human skin nerve fibres and (b) presenting a new skincare compound with highly efficient neuroprotective properties.

Acknowledgements

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