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A Pilot Study to Assess the Effects of an Oral Photo Protector of Botanical Origin against Visible and Infrared Radiations in Human Volunteers

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Abstract

Background: Infrared (IR) and visible (VIS) radiations constitute approximately 95% of total solar radiation. Although less energetic than UV radiation, IR and VIS radiations are also able to penetrate to the deeper layers of the skin, promoting the generation of reactive oxygen species (ROS). Similar to UV-derived ROS, visible- and IR-derived ROS also cause cellular damage, promoting skin photoaging. This study aims to test whether an extract of *Polypodium leucotomos* (Fernblock®) that decreases matrix metalloprotease-1 expression induced by UV light has a comparable effect on MMP-1 expression induced by VIS- and IR radiation in a small group of volunteers.

Methods: Human volunteers took Fernblock® orally for 21 days, and then were irradiated with IR/visible light. After 24 h, a biopsy was collected, and MMP1 expression was assessed by immunohistochemistry and real time, semi-quantitative PCR.

Results: Oral intake of Fernblock® attenuated MMP1 expression induced by IR and visible light.

Conclusion: Oral intake of Fernblock® efficiently protected against the appearance of a key hallmark of photoaging induced by IR/visible irradiation.

Keywords Oral photoprotection; UV visible; IR light; Botanical

Introduction

Upon incidence on Earth's surface, sunlight contains a variety of electromagnetic radiations. Most of these appear in the UV-to-infrared range, with <5% belonging to the UV range (290-400 nm), approximately 55% to visible (390-780 nm) wavelengths and 40% to infrared (>780 nm) wavelengths, although these values depend on height and latitude **[1]**.

Due to its high energy and mutagenic capability, UV radiation has been the focus of sun care research, particularly its deleterious effects on the different cells and structures of the skin **[2]**. However, and due to the large amount of visible and infrared photons that reach the skin (95 of each 100) [**3**], these radiations may have accumulating effects on the skin. For example, individuals continuously exposed to an IR-based source of heat sometimes develop erythema ab ligne, which involves the emergence of hyper-pigmented sections of exposed skin **[4]**. In addition, IR-A decreases wound healing [**5**].

Due to the lower absorption of IR-A photons by the different photon-absorbing structures in the skin (e.g. melanin), these radiations have greater penetration power than UV radiations **[6]**. Because of this they reach deeper compared to UV photons, potentially affecting all layers of the skin. This is important, because previous research has shown that incidence of visible and IR photons on skin structures also generate reactive oxygen species [**7**], triggering photo-aging [**8**]. Also, the mobilization of heat sensors in the skin triggers the hallmarks of ageing, e.g. elastin breakdown and increased matrix metalloprotease expression [**9**].

A major discovery in the field was that most sunscreens do not protect against the effects of visible and IR light [**7**]. This is not really surprising, since most sunscreen formulations are designed to quench UV radiation [**10**]. However, the contribution of these radiations to photo- ageing should be taken into account.

In a previous study, we have described that a hydrophilic extract made of leaves of the fern *Polypodium leucotomos* efficiently prevents the onset of the hallmarks of photo-ageing in a controlled in vitro environment using dermal fibroblasts [**11**]. In this report, we have investigated the effect of a similar, yet novel hydrophilic extract (Fernblock®) in human volunteers exposed to single, high doses of IR-VIS light.

Materials and Methods

This study was approved by the ethics committee of the Ramon y Cajal University Hospital, Madrid, Spain. Seven healthy volunteers with no history of skin disease participated in the study, receiving and signing informed consents in agreement with Helsinki's declaration. They first underwent a gluteal biopsy (Control condition). Then, they received a single dose of visible (VIS, 200 J/cm^2) + IR (600 J/cm^2)

radiation) on a second portion of the gluteal skin, which was biopsied 24 h after exposure (IR+VIS condition). Then, patients received 960 mg/day of Fernblock® orally for 21 days. After completing the treatment, the patients were exposed to a single radiation dose as before on a third different part of the gluteal skin, which was biopsied 24 h after exposure (Fernblock® + IR-VIS condition). Each biopsy was divided in two parts. The first part was embedded in paraffin and stained for histological examination using hematoxylin/eosin or an antibody against MMP-1. Briefly, immunostaining was performed using 3 µm tissue sections in a Dako Link platform. After deparaffinization, heat antigen retrieval was performed in 10µM citrate-based buffered solution (Dako). Endogenous peroxidase was quenched using 0.03% hydrogen peroxide. Staining was performed with a validated mouse monoclonal antibody against MMP-1 antibody (Abcam), followed by incubation with anti-mouse Ig-dextran polymer coupled with peroxidase (EnVision, Dako). Sections were visualized with 3,3'- diaminobenzidine (DAB) and counterstained with Hematoxylin. Sections from the same specimens incubated with normal mouse IgG2 antibody (X0943, Dako) were used as negative controls. The evaluation of MMP-1 expression was assessed in a blinded fashion by two investigators (SG and IJD) on digital images obtained with a Nikon Eclipse 800 upright microscope.

The second portion of the biopsy was homogenized and mRNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany). The concentration and quality (A260:A280 ratio >1.8) of the mRNA were checked by spectrophotometry (NanoDropND1000, Nanodrop Technologies, Wilmington, DE, USA). The mRNA expression was analyzed by RT-PCR (Reverse Transcription Polymerase Chain Reaction), using a RT-PCR kit (Roche) and specific primers for MMP-1 and 18S as a reference (MMP-1 F: 5'-ATGAAAGGTGGACCAACAATTT-3'; MMP-1 R: 5'-CCAAGAGAATGGCCGAGTTC-3'; 18S F: 5'-GCCGCTAGAGGTGAAATTCTTG-3'; 18S R: 5'-

CATTCTTGGCAAATGCTTTCG-3'). Semi-quantitative analysis of RT-PCR results was performed using DDCt method (Light Cycler® 480 Software, version 1.5, Roche Molecular Systems, Indianapolis, IN, USA), which corrects the data obtained from each sample relative to rRNA 18S reference value from each cDNA. Results were transformed into RQ (relative quantity) values using the control samples for normalization (RA=1.00). Each sample was analyzed by triplicate. RT-PCR analyses were carried out at Genomics Unit, Parque Científico de Madrid, Madrid, Spain. Statistical significance was determined by using the Mann-Whitney test after sample distribution was deemed not normal (Shapiro-Wilk test). Statistical values of $p<0.05$ were considered significant. Each data point in the figures represents the mean ± SD.

Results and Discussion

Using semi-quantitative RT-PCR, we determined statistically significant differences in 5 out of 7 patients in terms of MMP-1 mRNA expression (patients 1 and 5 did not show any increase in the levels of MMP-1 mRNA). On average, IR+VIS induced a 9-fold increase in relative expression of MMP-1 compared to non-irradiated controls, which decreased to a combined 3-fold increase in the presence of Fernblock® (in the two patients with the highest increase in MMP-1 mRNA, Fernblock® reduced the increase by at least 50%). These results are illustrated in Figure 1A (Control vs. VIS-IR vs. Fernblock®+VIS-IR conditions) and in Figure 1B (VIS+IR vs. Fernblock®+VIS- IR conditions.

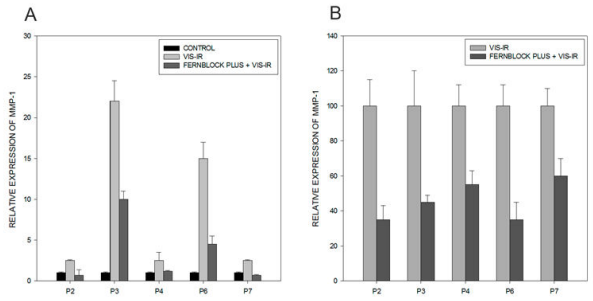

Figure 1: Expression of MMP-1 mRNA: Gluteal biopsies were extracted and subjected to mRNA extraction, cDNA production and semi- quantitative RT-PCR as indicated in Material and Methods. Data is represented as the normalized mean SD of three technical replicates of each condition (A); or normalized with respect to the VIS-IR condition set at 100% (B).

Globally, the reduction in the expression of MMP-1 was 51.6% ($p<0.05$). Interestingly, histological examination of the samples of patient 5 revealed a striking disappearance of the MMP-1 signal in the Fernblock®+VIS-IR condition compared to the VIS-IR condition alone (Figure 2).

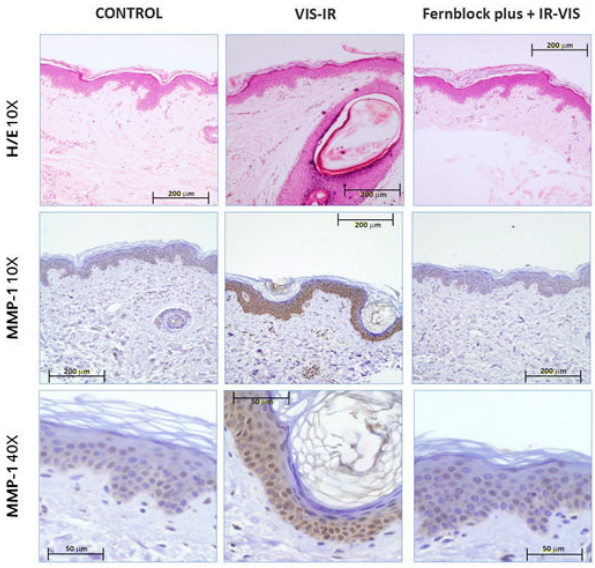

Figure 2: Expression of MMP-1 protein by immunohistochemistry: Gluteal biopsies were stained as indicated in Material and Methods. Representative images of patient 5 are shown. Scale bars are 200 µm (rows 1 and 2) and 50 µm (row 3) as indicated.

These results extrapolate to humans the conclusions of a previous study in which we showed the efficacy of a similar extract in vitro using dermal fibroblasts [**11**]. The importance of the effect of Fernblock® on MMP-1 expression is amplified by the fact that conventional sunscreens do not have this photo-protective effect whereas VIS+IR radiation (180 J/cm^2) does not induce T-T dimers [**7**], it does increase MMP-1 expression to levels comparable to those of a dose of UV

radiation (6 J/cm^2) capable of inducing T-T dimers in skin equivalents. As such, these doses of VIS+IR radiation, although not mutagenic, have a similar potential to that of UV to cause photoaging.

Conclusion

These results need to be replicated in a much larger cohort of volunteers, but early returns on the anti-photoaging potential of Fernblock® are promising beyond its ability to prevent UV-induced photo-damage.

Acknowledgments

Salvador Gonzalez and Maria Teresa Truchuelo declare that they are on the advisory board of Cantabria Labs, which produced the administered product, Fernblock®.

Conflict of Interest

The rest of the authors declare no conflict of interest. This study was partially funded by Cantabria Laboratories, Madrid, Spain.

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