Protective effects of Polypodium leucotomos extract against UVB-induced damage in a model of reconstructed human epidermis

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Background

Ultraviolet radiation (UVR) is a well-known key factor in the development of skin cancer and photo-aging. UVR (UVB and UVA) causes strong cell damage at keratinocytes level inducing DNA alterations with formation of cyclobutane pyrimidine dimers (CPD), oxidation and increase expression of several proteins (p53, Ki-67 and p21) involved in the apoptosis process and abnormal skin cell proliferation. Several studies have shown that Polypodium leucotomos (PL) extract exerts potent antioxidant, photo-protective and immune-modulatory activities. A reconstructed human epidermis (RHE) is a suitable model for the evaluation of UV-induced damage and cell alteration at the keratinocytes level. So far, no data regarding the photo-protective action of PL in this model are available.

Study aim

To evaluate the effects of PL extract on the prevention of UVB-induced cell damage assessing sunburn cells, CPD formation and p53, Ki-67, p21 and Epidermal growth factor (EGF) expression.

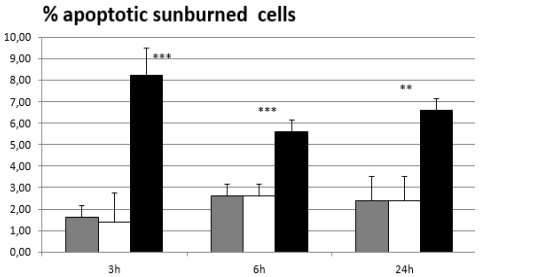
Material and Methods

RHE was incubated in standard conditions according with manufacturer's instructions. PL extract was topically applied at the concentration of 2 mg/cm^2, immediately before UVB exposition. UVB exposition (300mJ/cm2) was performed using a dedicated UVB lamp. Irradiated samples without PL and non-irradiated samples of RHE were used as positive and negative controls, respectively. After UVB exposition all cultures were maintained at 37° C, for 24 hours. Test evaluations were performed at 3, 6 and 24 hours. EGF was evaluated performing an ELISA test on supernatant. Expression of p53, p21 and Ki-67 was evaluated with immune-histochemical methods in RHE formalin-fixed samples for microscopic evaluation. CPD were measured by in situ hybridization methods using a horseradish-peroxidase monoclonal antibody.

Results

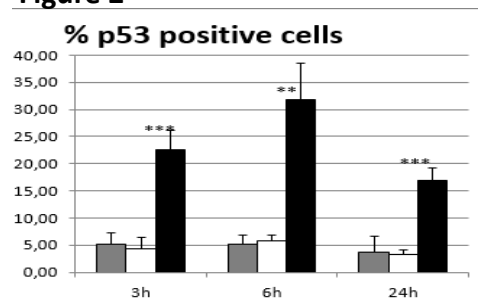
PL significantly reduced in comparison with positive control sunburned cells by 80% at 3 hours post UVB radiation. This reduction was observed also at 6 and 24 hours (Figure 1). PL prevented the increased of EGF expression (5 pg/mL vs. 8.2 pg/mL; $p<0.05$) at each tested times. PL also reduced the p53(-80%) (Figure 2), p21 (-84%) and Ki-67 (-48%) positive cells at all tested times. PL completely prevented the formation of CPD in comparison with the positive control (0% vs. 20% positive cells). Detection of sunburn cells in (E&E) and evaluation of stratum corneum (Van Gieson) in histological sections is shown in Figure 3.

Figure 1



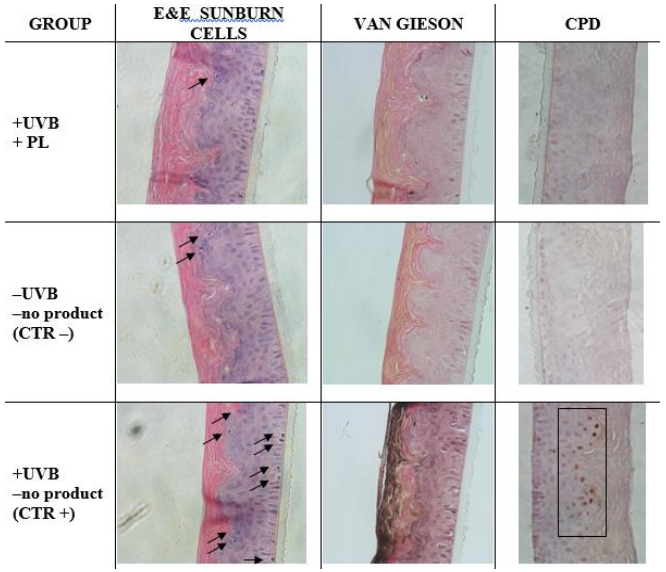
Gray Bars: PL; White Bars: Negative (no UV) CTR; Black Bars: Positive (+UV) CTR

Figure 2



Gray Bars: PL; White Bars: Negative (no UV) CTR; Black Bars: Positive (+UV) CTR

Figure 3



Conclusion

In this model of reconstructed human epidermis PL has shown to prevent UVB cell damage, the up-regulation of proliferating proteins and fully preventing the formation of CPD.