PHOTO-AGING REPAIRING MECHANISMS AFTER UVA DAMAGE ON FT-SKIN

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Ultraviolet A (UVA) radiation adversely affects skin health and appearance via multiple molecular pathways. Biologically relevant UVA damages are classified as short-term effects (e.g. formation of reactive oxygen species (ROS), inflammation, photo-oxidation, DNA damage, immunosuppression, photoallergy and cell-cell and cell-matrix adhesion hyporesponsivity) and longer-term effects (elastosis, photaging and photocarcinogenesis). Single and chronic experimental exposure to UVA is limited in humans by ethical concerns and furthermore it is impossible to quantify long-term endpoints such as photo-aging over the life-span of a human volunteer. An in vitro model of photo-aging has been developed (1) on a FT-skin model in order to provide a more ethical and method based approach to investigate the activity of molecules and products claiming a protection against UVA induced damages.

In this study the molecular response induced by acute UVA (12 J/cm²) exposure has been investigated and the kinetics of selected biomarkers (COL1A1, DCN, ELN, FBN1, 11-1α, MMP1) gene expression during post-injury periods (24h and 7 days) has been monitored. The transcriptional study has been conducted on controls and treated series: the efficacy of 2 antioxidant molecules (2-oxo-1,3-thiazolidine and carnosine chloride) and of 2 O/W emulsions (including exclusively UV A+B filters) with SPF values of 10 and 50 has been assessed with the aim to capture the “pure” antioxidant and UV filters effects.

INTRODUCTION

Ultrasound A (UVA) radiation adversely affects skin health and appearance via multiple molecular pathways. Biologically relevant UVA damages are classified as short-term effects (e.g. formation of reactive oxygen species (ROS), inflammation, photo-oxidation, DNA damage, immunosuppression, photoallergy and cell-cell and cell-matrix adhesion hyporesponsivity) and longer-term effects (elastosis, photaging and photocarcinogenesis). Single and chronic experimental exposure to UVA is limited in humans by ethical concerns and furthermore it is impossible to quantify long-term endpoints such as photo-aging over the life-span of a human volunteer. An in vitro model of photo-aging has been developed (1) on a FT-skin model in order to provide a more ethical and method based approach to investigate the activity of molecules and products claiming a protection against UVA induced damages.

In this study the molecular response induced by acute UVA (12 J/cm²) exposure has been investigated and the kinetics of selected biomarkers (COL1A1, DCN, ELN, FBN1, 11-1α, MMP1) gene expression during post-injury periods (24h and 7 days) has been monitored. The transcriptional study has been conducted on controls and treated series: the efficacy of 2 antioxidant molecules (2-oxo-1,3-thiazolidine and carnosine chloride) and of 2 O/W emulsions (including exclusively UV A+B filters) with SPF values of 10 and 50 has been assessed with the aim to capture the “pure” antioxidant and UV filters effects.

IN VITRO TISSUE CULTURE (FT-SKIN)

The Phlerom® full thickness (FT) skin model (diameter, 1.3 cm), is a multilayer skin equivalent that resembles human skin and is cultivated using ALB® culture medium.

UVA EXPOSURE AND TREATMENT

FT-skin tissues were placed in a 6-well plate with 1 mL of PBS. Non-irradiated tissues were used as control. The products have been applied on the epidermal surface for 2h before UVA exposure (12 J/cm²) and let on the tissues for 24h and 7 days of recovery after irradiation.

EXPERIMENTAL DESIGN

UVA-VISIBLE SOURCE

1 kW Oriel solar simulator equipped with Spectra-Physics Lamp (Xenon 1000W), 3 Schott WG335 ± 3 mm filters emitting UVA and visible light without UVB interference.

TRANSCRIPTOMICAL STUDY: mRNA by qRT-PCR

Expression levels of the selected biomarkers (COL1A1, DCN, ELN, FBN1, 11-1α, MMP1) gene expression were monitored by mRNA quantification using qRT-PCR (TaqMan® assay) in a thermal cycler (Applied Biosystems ABI PRISM 7500 Real Time PCR System). Calibrator used: irradiated positive control = 1

Table I (updated data)

Table 1: 24h recovery

Table 10: 7 days recovery

SUN PRODUCTS

DCN core protein has a binding site for type I collagen. Recently its been shown (2) that a decreased COL-to-DCN ratio impairs collagen structure, COL2 is down-regulated by UV as a long effect term, as well as downregulated expression of DCN mRNA in the different dermal strata: a decrease in the COL-to-DCN ratio is indicative of both age and UV irradiation possibly affect collagen bundle diameter and the mechanical properties of human skin. By using the ratio between absolute expression levels (RQ) of COL1A1 to DECORIN applied to (2) a non-irradiated control and a UVA damaged, the irradiated control ratio was consistent with the damage-UV induced (ratio <1). An efficacy of sun products has been shown in term of protection of collagen network by an increased COL-to-DCN ratio. The mRNA results for decorin gene have been confirmed at protein level by 3 techniques: as shown in Fig 1 the signal was well expressed in SPF 10 and 50 treated tissues. The decorin protein signal in tissues treated with antioxidants was lower.

ACKNOWLEDGMENTS AND REFERENCES

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