UVA DAMAGE BIOMARKERS EXPRESSION ON FT-SKIN

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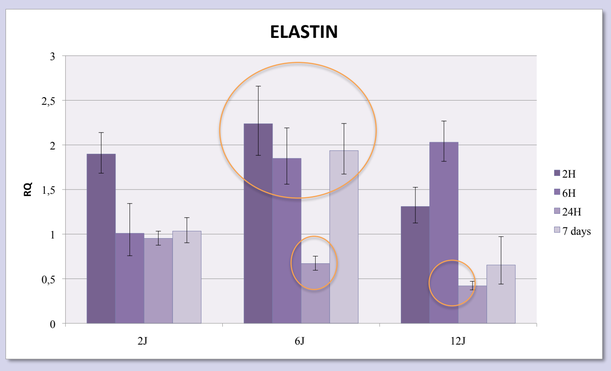
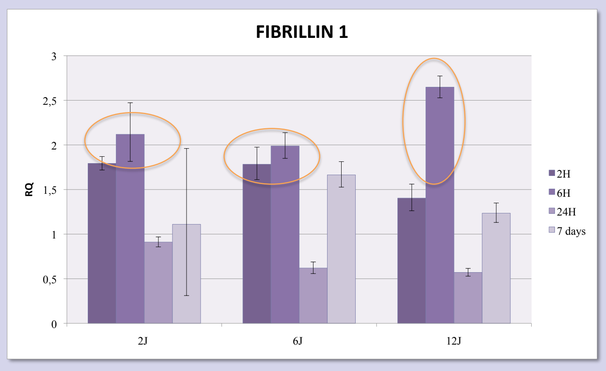
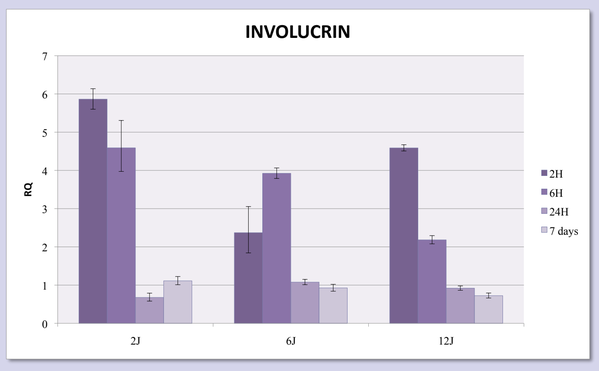
BACKGROUND

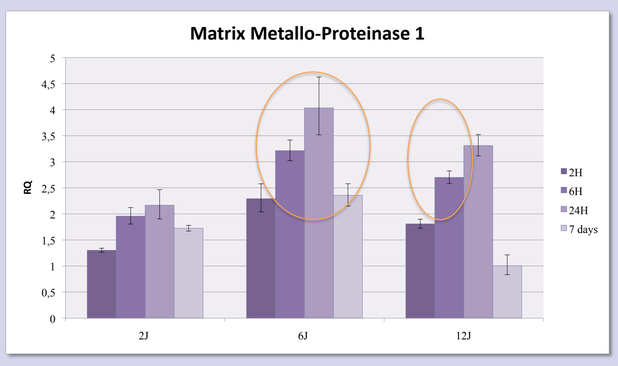
Ultraviolet A (UVA) radiation adversely affects skin health and appearance via multiple molecular pathways. Biologically relevant **UVA damages** are classified as **short-term effects** (e.g. formation of reactive oxygen species [ROS], inflammation, photo-oxidation, DNA damage, immunosuppression, photoallergy and cell-mediated contact hypersensitivity) and **long-term effects** (elastosis, photoageing and photocarcinogenesis). Single and chronic experimental exposure to UVA are limited in humans by ethical concerns and furthermore it is impossible to quantify long-term endpoints such as photoageing over the life-span of a human volunteer. In this study the molecular response to acute (6 and 12 J/cm^2) and repeated UVA exposures were investigated by monitoring the kinetics of gene expression during the post-irradiation period using the Phenion® FT skin model. Potential biomarkers of UVA damage were identified according to their roles in epidermal and dermal compartment.

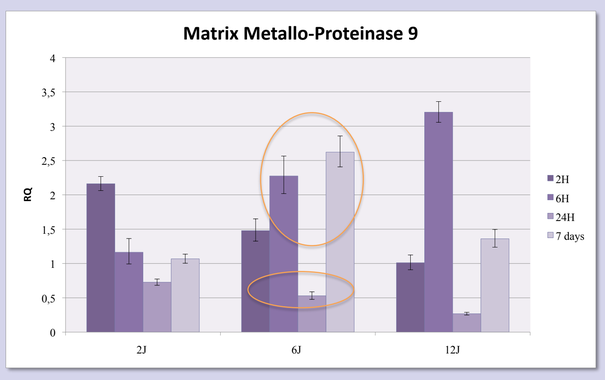
RESULTS

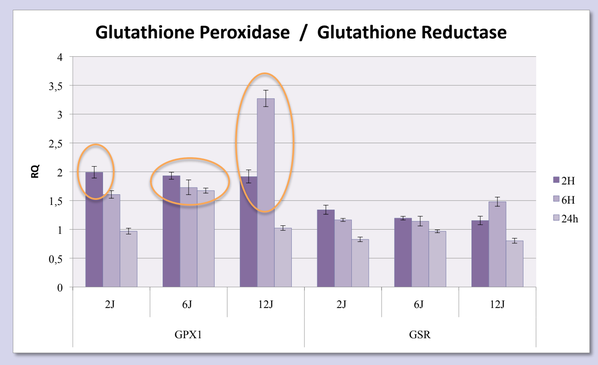












	24H recovery		
UVA J / cm²	2	6	12
	RQ Values		
COL1A1	0,915	0,624	0,739
COL7A1	0,961	2,128	0,845
CCND1	0,842	1,507	1,179
DCN	1,172	1,844	0,923
ELN	0,952	0,67	0,42
FBN1	0,911	0,62	0,571
IVL	0,678	1,078	0,92
ITGβ1	0,825	1,415	0,95
MMP-9	0,727	0,53	0,268
MMP-1	2,167	4,034	3,311
IL1-α	0,974	1,128	1,399

Quantitative analysis of mRNA expression by qRT-PCR (Taqman® assays) in irradiated FT tissue samples (duplicate cultures) recovered in incubator at 37°C with 5% CO_2 for 2h, 6h, 24h and 7 days following irradiation of 2, 6 or 12 J/cm^2 UVA.

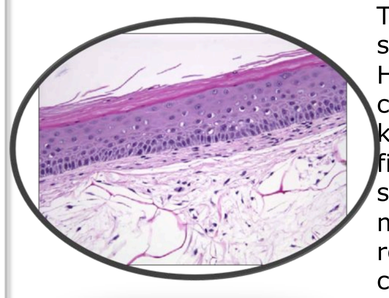
Significant gene up-regulation (RQ ≥2) results are highlighted in dark grey boxes (white text); light grey boxes (black text) indicate significant down-regulation (RQ ≤ 0.5) of the gene. GAPDH was used as a housekeeping gene. Non-irradiated tissue was used as calibrator control (CN = RQ =1).

Down-regulation of a gene at 24h post-irradiation confirmed by the repeated exposures procedure is suggestive of a long-term damage: in our study, elastin, Collagen I, Fibrillin and MMP-1 genes dynamic pathway described this response and are interesting candidates to assess the photo-protective and anti-photo-ageing efficacy of topically applied products at dermis level.

The early biological response (2h-6h) to acute UVA exposures has shown the early involvement of biomarkers related to the oxidative stress confirming an oxidative stress pathway of the UVA damage.

EXPERIMENTAL APPROACH

IN VITRO TISSUE CULTURE (FT-SKIN)



The Phenion® full thickness (FT) skin model (thickness, 1.3 cm), Henkel (Düsseldorf, Germany) is constuited by epidermal keratinocytes and dermal fibroblasts grown in a specialized stable matrix to form a multilayered skin equivalent that resembles human skin under culture conditions.

UV-A SOURCE

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

EXPERIMENTAL DESIGN		
UVA EXPOSURES	SINGLE	CUMULATIVE (on 3 days)
UVA doses	2 -6 -12 J/cm²	6 J/cm² (2+2+2)
READOUTS AFTER UVA EXPOSURE	after 2h, 6h, 24h and 7 days	after 2h and 7 days

UVA EXPOSURE and CULTURING

The FT skin model was cultivated using the ALI® culture medium. Tissues were placed in a 6 -well plate with 1 mL of PBS. Following irradiation, duplicate FT skin tissues were transferred into Petri dishes containing fresh ALI medium and recovered in incubator (37°C, 5% CO_2, 90% RH) for different times. Non-irradiated tissue was used as control.

TRANSCRIPTIONAL STUDY OF mRNA BY qRT-PCR

Molecular modification of the selected biomarkers was monitored by quantification of mRNA using qRT-PCR (Taqman® assay) in a thermal cycler (Applied Biosystems ABI PRISM 7500 Real Time PCR System)

CONCLUSION

The transcriptomic approach applied to 3D human tissues appears to be an encouraging method for gaining a deeper understanding of the UVA effects on skin and for studying the dermal response with non-invasive, ethical and quantitative techniques.

A robust and predictive gene signature has been assigned to the **UVA adaptive, defensive and damage pathways** : the quantification of the protection offered by UV filters and antioxidant molecules is currently in progress.

REFERENCE

Marisa Meloni, Anne Farina, Barbara De Servi.
Molecular modifications of dermal and epidermal biomarkers following UVA exposures on reconstructed full thickness human skin. Photochem Photobiol Sci. 2010 Apr;9(4):439-47.

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