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

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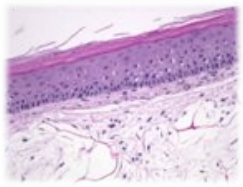
INTRODUCTION

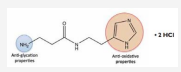
Ultraviolet A (UVA) radiation adversely affects skin health and appearance via multiple molecular pathways. Biologically relevant **UVA damages** are classified as **short-term effects** (e.g. formation of reactive oxygen species [ROS], inflammation, photo-oxidation, DNA damage, immunosuppression, photoallergy 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 photo-ageing over the life-span of a human volunteers. An in vitro model of photo-ageing has been developed (1) on a Ft-skin model in order to provide a more ethical and mechanism 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/cm^2) exposure has been investigated and the kinetics of selected biomarkers (COL1A1, DCN, ELN, FBN1, IL-1α, MMP1) gene expression during post-irradiation 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 carcinine chlorohydrate) 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 Phenion® full thickness (FT) skin model (diameter, 1.3 cm), is a multilayered skin equivalent that resembles human skin and is cultivated using ALI® 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/cm^2**) and let on the tissues for 24h and 7 days of recovery after irradiation.

EXPERIMENTAL DESIGN

UVA EXPOSURE	ACUTE 12 J/cm^2	
PRODUCTS	DECARBOXY CARNOSINE HCl (CARC-HC) 	2-OXO-1,3,THIAZOLIDINE -4-CARBOXYLIC ACID (OTZ) 
	O/W SPF 10	O/W SPF 50
RECOVERY AFTER UVA EXPOSURE	24h and 7 days recovery	

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

TRANSCRIPTIONAL STUDY: mRNA by qRT-PCR
Expression levels of the selected biomarkers (COL1A1, DCN, ELN, FBN1, IL-1α, MMP1) 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

Quantitative analysis (RQ values) of mRNA expression by qRT-PCR: published data[1] versus new data

UVA	24H 12J/cm^2	7 DAYS RECOVERY 12J/cm^2
COL1A1	0,739	1,792
DCN	0,923	1,57
ELN	0,42	0,654
FBN1	0,571	1,235
MMP1	3,3	1
IL1 α	1,3	1,5

Table I (published data[1])

UVA	24H 12J/cm^2	7 DAYS RECOVERY 12J/cm^2
COL1A1	0,932	1,424
DCN	0,642	2,604
ELN	0,684	0,464
FBN1	0,908	0,806
MMP1	1,226	2,206
IL1 α	0,987	-

Table II-present study

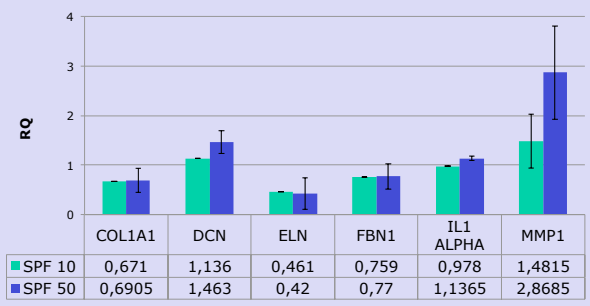
Tissue response for positive/ irradiated control was in good agreement with our previously published data. Thanks to the dynamic apporach and to the phisiological response of the FT-skin model to biologically relevant and non toxic UVA doses it has been possible to capture not only the damage /defensive response but also the adaptive one.

GENE SIGNATURES FOR EARLY (24H) AND DELAYED (7 DAYS) EFFECT OF UVA EXPOSURE:

The **24H READ-OUT** after acute UVA exposure nicely modelize a damage response as indicates the down regulation of dermal biomarkers Decorin, Fibrillin and Elastin and the upregulation of MMP-1 (published data). 24h corresponds to an early and acute damage to the dermal matrix: at this time it is possible to describe product in term of a defensive and protective efficacy against acute effect of photo damage.

The **7 DAYS** response to acute UVA exposure has shown the down regulation of dermal matrix components suggesting a damage (Elastin down regulation) but also an adaptive response (Decorin, COL.1 up-regulation): 7 days read-out seems to be adapted to assess products efficacy as protection of the main target proteins for elastosis and photoageing in vivo.

SUN PRODUCTS

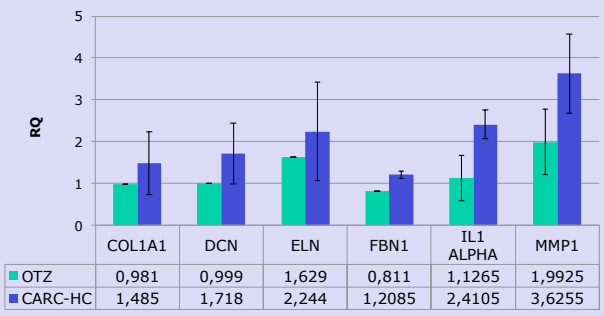


	COL1A1	DCN	ELN	FBN1	IL1 ALPHA	MMP1
SPF 10	0,671	1,136	0,461	0,759	0,978	1,4815
SPF 50	0,6905	1,463	0,42	0,77	1,1365	2,8685

24h: EARLY DAMAGE AND DEFENSE

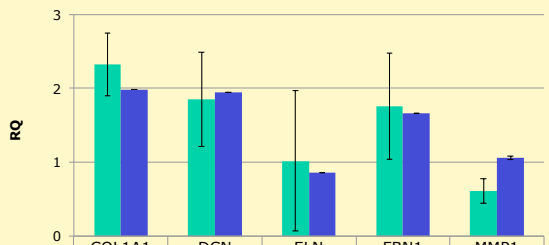
The sun products efficacy has been described based on IL-1α gene results: a control of inflammation pathways has been established. They were not efficient in counteracting the early damage to the dermal matrix biomarkers as suggested by the down regulation of ELN, FBN and Collagen I genes.
Both antioxidants on the contrary activate a direct defensive response against elastin gene damage that was upregulated and more significant for CARC-HC. Interestingly OTZ was able to limite the IL-1α gene expression to values near to 1.

ANTIOXIDANTS



	COL1A1	DCN	ELN	FBN1	IL1 ALPHA	MMP1
OTZ	0,981	0,999	1,629	0,811	1,1265	1,9925
CARC-HC	1,485	1,718	2,244	1,2085	2,4105	3,6255

SUN PRODUCTS



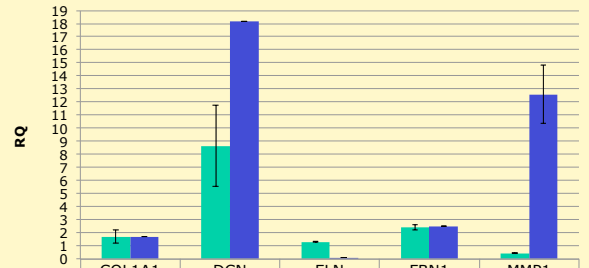
	COL1A1	DCN	ELN	FBN1	IL1 ALPHA	MMP1
SPF 10	2,323	1,8495	1,016	1,7585	0,6095	
SPF 50	1,983	1,943	0,853	1,655	1,0605	

7 DAYS: DELAYED ADAPTIVE RESPONSE

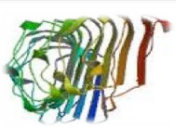
OTZ was effective in reducing the MMP-1 gene expression confirming the 24h results on the efficacy in counteracting the inflammatory response. Both antioxidants were able to activate an adaptive response to the UVA damage by inducing an overexpression of COLI, ELN, FBN1 and DCN. The overexpression of MMP1 induced by CARC-HC can be explained by the reduced penetration of the active applied here as aqueous solution.

The Sun products with no differences between SPF 10 and SPF 50 have shown a strong effect in blocking the matrix degradation as suggested by down regulation of MMP-1 confirming the down regulation of IL-1α observed at 24h.
The protection of dermal matrix was also demonstrated by the up-regulation of COLI, DCN, FBN and the recovery of ELN gene expression levels near to 1.

ANTIOXIDANTS



	COL1A1	DCN	ELN	FBN1	MMP1
OTZ	1,705	8,6595	1,322	2,4215	0,434
CARC-HC	1,724	18,163	0,123	2,521	12,594



Gualtieri A. Matrix Biology 31, (2): 141–149

COLLAGEN I / DECORIN RATIO	7 DAYS	MECHANISM
IRR	0,828	DAMAGE
SPF 10	**1,256**	PROTECTION
SPF 50	**1,02**	PROTECTION

COL I / DCN RATIO

DCN core protein has a binding site for type I collagen. Recently has been found (2) that a **decreased COL-to-DCN ratio impairs collagen structure.** COLI is down-regulated by UV as a long term effect as well as altered expression of DCN mRNA in the different dermal strata: **a decrease in the COL-to-DCN ratio inflicted by 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 7 days results** corresponding toa delayed damage, 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/DCN >1.

The mRNA results for decorin gene been confimed at protein level by IF tecnique: 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.

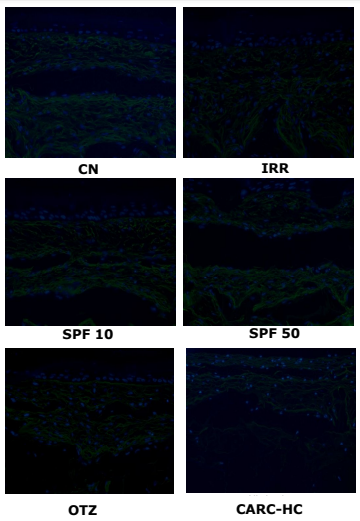


Fig. 1 Decorin immunofluorescence staining

CONCLUSION

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

The repeated cycles of the collagen degradation and imperfect remodeling during repeated exposures to UVA result in accumulation of the amorphous elastin showing an old and wrinkled appearance = photodamage.

The experimental model described is currently in progress to investigate protocol modifications and new testing parameters in order to better addres efficacy testing needs.

It seems a promising mechanism based model to reach a better understanding of skin response to UVA damage mimicking the in vivo dynamic response.

A robust and predictive gene signature has been assigned to the **UVA adaptive, defensive and damage pathways**.

AKNOWLEDGMENTS AND REFERENCES

The IF staining of decorin has been performed within SEDIFA laboratories (MONACO): their support is gratefully aknowledged

1. Meloni M., Farina A, De Servi B. 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.
2 Lochner K. et al. Expression of decorin and collagens I and III in different layers of human skin in vivo: a laser capture microdissection study. Biogerontology. 2007 Jun;8(3):269-82.

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