In vitro and *ex vivo* skin models to study the lipid mediators inflammatory response to stress and its modulation by active ingredients

J. Franchi[1], C. Marteau[1], V. Jeronimo[1], O. Jeanneton[1], M. Dumas[1], N. Amalric[2] and S. Schnebert[1]

1- LVMH Recherche, 185 avenue de Verdun, 45804 Saint Jean de Braye, France

2- Synelvia SAS, Prologue Biotech, 516 rue Pierre et Marie Curie, 31670 Labège, France

INTRODUCTION

Human skin is considered as the body's largest organ and constitutes the primary barrier from the outside protecting from injury, infections, water loss, solar irradiations, as well as being an important player of the immune system. Inflammation partakes in physiological mechanism mediation as healing and response to injury including stress resulting from exposure to ultraviolet irradiation (UVI) in sunlight. The cutaneous response is regulated by mediators such as cytokines and bioactive lipids that can initiate rapid reactions with a controlled inflammation, followed by an efficient resolution. UVI results in oxidative stress with an increase in malondialdehyde (MDA) reflecting lipids oxidation. Furthermore, UVI increases the release of polyunsaturated fatty acids (PUFAs) in the skin by up regulating the synthesis and activity of phospholipase A2 (PLA2) through increased oxidative stress-mediated reactions. Proteins expression such as cycloxygenases (COXs) and lipoxygenases (LOXs) are also up regulated by UVB, along with increased levels of bioactive lipids. The aim of the study was to characterize cellular and tissular models regarding the modulation of active ingredients mainly on the bioactive lipids profile after inflammatory stress.

METHODS

Cell culture and treatment

Normal human keratinocytes (NHK)

NHK were isolated from an abdominoplasty performed on a female donor. Confluent NHK were plated at passage 5 in Epilife Medium (Gibco), at a density of 125,000 cells per well (12-well plate). After a 24 hour period treatment with active ingredient, the cells were collected and total RNA extracted.

Coculture of immune cells

The Ambiotis model consists of a coculture of lymphocytes and monocytes (24-well plate) which are preincubated or not with active ingredient for 24h hours, then stimulated with PMA/A23187 (phorbol myristate) in presence or not of EPA (eicosapentanoic acid) and DHA (docosahexaenoic acid), n=3 by condition.

Organotypic skin culture and treatment

Human skin explants (abdominoplasty performed on a female donor) from BIO EC model (1.5 x 1.5 cm) or from Genoskin model (Nativeskin 0.6 cm²) were maintained in culture at the air-liquid interface in a survival medium during 4 days. The UV A+B source used is a Vilbert-Lourmat lamp. The skin explants were irradiated at 3 MED (Minimal Erythema Dose).
In case of topical treatment on skin explants (Nativeskin model), the formulated ingredient is applied during 3 days (2 days before and one day after UVI).

Evaluation of the KHN gene expression: TLDA (TaqMan Low Density Array) analysis

A TLDA microfluidic card was designed to include a list of selected genes known to code for proteins involved in keratinocyte metabolism including gene involved in inflammatory status . Genes are used for the normalization of the results.
RNA extraction, RNA quantification/normalization of the samples and the reverse transcription steps were processed through an automated robotic workstation (MicrolabStar, Hamilton Robotics). An Applied Biosystems ABI Prism 7900HT was used for amplification and fluorescence detection. Data analysis for PCR step was carried out according to the RQ analysis method using RQ Manager software program. Student's t test was used to evaluate statistically significant variations in transcriptional activity.

Evaluation of the inflammatory lipid mediators

After extraction, the measured biochemical markers of interest were: lipid peroxidation marker MDA by GC/MS method and lipid mediators involved in UV-induced inflammation, PUFAs and eicosanoids (prostaglandins, HETEs and lipoxins) by LC/MS method. Lipidomic analyses were performed at 6, 24, 48 and 72 hours after UV radiation for skin explants, or 2 hours in case of coculture of immune cell model.

CONCLUSION

Keratinocytes 2D culture, coculture of immune cells (lymphocytes and monocytes) and a developed "UV-inflammed" skin explant model were complementary models to explore UVI effects on cutaneous lipids to better define active ingredients contributing to preventive or curative efficacy of dermo-cosmetic formulations.

Bibliography:
Nicolaou A. *et al* Ultraviolet-radiation induced skin inflammation: dissecting the role of bioactive lipids. *Chemistry and Physics of Lipids*, (2011), 164, 535-543. Rhodes L. *et al* The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. *FASEB J.* (2009), 23, 3947-3956.

RESULTS

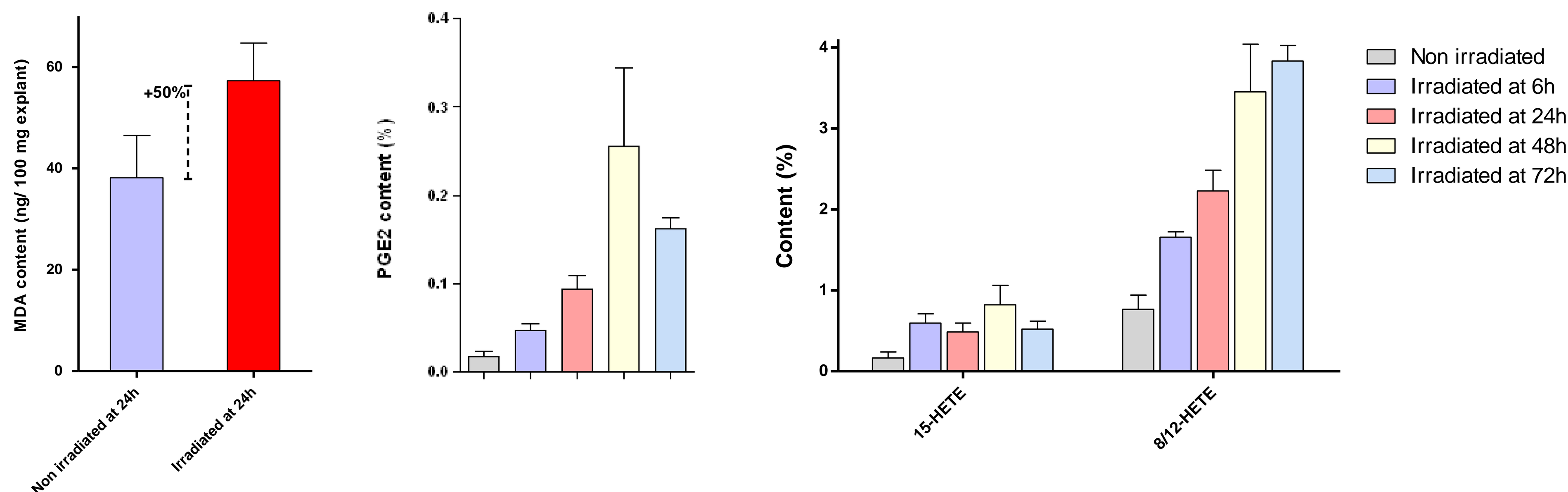
Keratinocytes 2D culture model – Inflammatory Gene profiling

Example of an active ingredient response (n=3)

The TLDA technology is a valuable tool for rapid, sensitive and quantitative gene expression profiling predictive of ingredient potential, in this case in the anti-inflammatory domain.

Skin explant models : UVI response on bioactive lipids

The skin explant model allows us to explore the effects of UVI on cutaneous bioactive lipids by measuring lipid peroxidation (MDA) and PUFAs metabolism (n=3)



As expected, 24h after a 3 MED irradiation of the skin explants, a significant increase of the lipid peroxidation marker, MDA is observed. PUFAs release was followed by an increase of prostaglandins especially PGE2 with a pic at 48 h after UV irradiation. The increase could be explained by the up regulation of COX-2. A significant increase of HETEs levels (5, 8, 11, 12 and 15 HETEs) was registered until 48 and/or 72 h, depending on the markers. This increase could be explained by the up-regulation of LOXs and CYP450 mono-oxygenase. Lipoxins A4 and B4, lipid mediators involved in the resolution of cutaneous inflammation, were detected 72 h after irradiation.
->The UV-response profile of these bioactive lipids is close to the one observed *in vivo*.

Example of an active ingredient evaluation (on Nativeskin model, n=2)

Area ratio/100 mg	Control	UV 3 DEM	UV 3DEM + active ingredient	Evolution
PGE2	5.9	25.4	20.6	- 26.5%
8+12 HETE	3.4	5.7	5.2	- 9%
PGE1	0.5	3	2.7	- 10%
PGD2	0.26	0.31	0.18	- 42%

Topically applied formulated active ingredients could be selected for their ability to modulate inflammatory status induced by UVI in skin explants maintained in culture.

Coculture of immune cells

The cellular cooperation between monocytes and lymphocytes (Ambiotis model) is particularly adapted to the evaluation of biolipids metabolism, including those implied in the resolution of the inflammation. The PMA mimics the early stage of the inflammation and DHA and EPA are naturally present in our tissues.

ng/well	Control	PMA	Active ingredient	DHA/EPA	DHA/EPA+ active ingredient
PGE2	0.73	1.42 (+94.5%)	0.58 (-20,5%)	1.03	0.77 (-25%)

ng/well	Control	PMA	PMA + active ingredient	PMA + DHA/EPA	PMA + DHA/EPA+ active ingredient
LTB4	0	21	17.4 (-17%)	11	0

In those conditions, it is possible to complete our observations on the active ingredient modulation on the resolution of the inflammation markers.

LVMH RECHERCHE
PARFUMS & COSMETIQUES

Synelvia
Your partner in life sciences

5th European Workshop on lipid mediators 23-24 October 2014 Istanbul