Development of an in vitro immunosuppression model

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INTRODUCTION

Ultraviolet Radiation (UVR), particularly UVB (280–320 nm), initiates inflammation, local and systemic immunosuppression and skin cancer. Substantial evidence, primarily from animal models and susceptibility of human volunteers (Youn et al., 2016), suggests that UV-induced cytokines are key mediators of these interrelated effects. Immunosuppressive effects of excessive ultraviolet radiation in humans include exacerbation of infectious diseases, skin cancer and skin aging. Relative low doses of UV exposure and early local immunosuppression may be mediated by a functional inhibition of melanocytes and a delayed systemic immunosuppression in which photoinflammatory valid factors on cytokines play a crucial role (Schwarz T et al, 2000). TNFα and IL-12 are recognized as responsible for the onset of the inflammation and the induction of the chemokines and neutrophils and macrophages into the skin after UVR and in particular TNFα is an important mediator in local UV-induced immunomodulation on human skin. In a kinetic study on human skin (suction blister) Bar et al. have demonstrated that the TNFα and IL-12 are produced immediately after exposure to UVR, and that IL-12 is secreted during UV irradiation reaching the blood stream. It is proposed that IL-12 could act as a protective molecule (Schwarz, A. et al, 2000).

METHODS

The Episkin® Model (Episkin®-L50™) is manufactured by SkinTec® (Zurzach, Switzerland). It is a reconstituted, acanthogenic culture of human keratinocytes reproducing a cross section of normal human epidermis. Cells are grown on a collagen matrix for 12 days using the air-lift technique. The Episkin Phototoxicity Assay, EPA has demonstrated the relevance of the Episkin® model in photobiology investigation.

It has been able to discriminate between phototoxic and non-phototoxic compounds in comparison with the 3T3 NMuMG bioassay (OECD TG 420) (Barclay et al., 2013) and to identify photo-protective formulations in a modified protocol. MTT assay was used to study the cytokine production (Meloni et al., 2014).

UV DOSIS AND POST IRRADIATION TIMES SELECTION

Literature reports (Young, A.R. 1996) that 2–3 MED as humans are sufficient to determine an immunosuppressive response and that a specific kinetics corresponds to the post irradiation periods: between 4–16h the maximum of cytokines was released, describing an immunosuppressive response and after 24h a prevalent DNA damage was established.

Therefore tissues were irradiated with UVR doses correspondent to 0,5, 1, 2 and 4 MED (0,125 to 1,0 Ucm²). After irradiation, skin tissues were let in the incubator for 4, 6, 16 and 24h post irradiation periods. Cell viability (MTT test) and adenosine kinase release (Toxilight®-Roche Diagnostics) have been monitored in parallel.

Based on these results the doses of 1MED and 2MED have been applied in a second trial (Fig. 6-7-8) in order to assess the kinetics of non toxic and biologically relevant UV dose inducing the expression of the relevant biomarkers of the immunosuppressive mechanism, in order to take into account the dynamics of the immunosuppressive imbalance mediated by both cytokines the post irradiation times of 4h, 16h (overnight) and 24h were selected to establish an experimental window with a prevalent, TNFα response and a minor or null IL-12 expression.

KINETICS OF THE IMMUNOSUPPRESSIVE RESPONSE

The already observed kinetics of IL-10 gene at 4h has been confirmed: a null contribution to the immunosuppressive pathway at this time (Fig. 5). IL-10 gene was significantly over-expressed at the end of 16h post irradiation period which have been proposed that IL-12 could act as a protective molecule (Schwarz, A. et al, 2000).

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