 doi:10.20944/preprints202012.0067.v1

Article

Effect of ultraviolet radiation on the expression of drug transporters in *in vitro* skin models

Manon Barthe[1], Jean-Paul Thénot[1] and Hanan Osman-Ponchet[1,*]

[1] PKDERM Laboratories; Grasse Biotech, 45 Boulevard Marcel Pagnol, 06130 Grasse – FRANCE. Manon.barthe@pkderm.com (M.B.) ; jpthenot@icloud.com (J.P.T.)

* Correspondence: hanan.osman.ponchet@pkderm.com ; Tel.: +33-0670-252-856

Abstract: The majority of skin cancers are caused by over exposure to ultraviolet (UV) radiation. The effects of UV radiation on the expression of drug transporters expressed in human skin has never been studied. In this the effects of UVA and UVB irradiation on the expression of ATP-binding cassette (ABC) transporters and Solute carrier (SLC) transporters was evaluated in normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF) in primary culture. First experiments were intended to validate the inflammatory reaction in response to stimulation by lipopolysaccharide (LPS) in NHEK, NHDF and 3D-reconstructed human epidermis (3D-RHE) model. LPS treatment has shown to increase the expression of IL-8 and TNF-alpha in all three *in vitro* models. Expression of the most expressed ABC and SLC transporters was then measured in NHEK and NHDF after UVA (30 J/m²) and UVB (40 mJ/m²) irradiation. The most striking result was a significant 29-fold increase of the expression of SLCO4A1 in normal human dermal fibroblasts. In summary, this study shows for the first time a significant regulation of the expression of SLCO4A1 in human dermal fibroblasts induced by UVA irradiation. This finding is of particular interest as most of skin cancers are caused by over exposure to ultraviolet radiation and need to be considered in pharmacokinetic evaluation of topical drugs.

Keywords: ABC transporters; SLC transporters; in vitro; skin; inflammation; cytokines; UVA; UVB.

1. Introduction

The human skin is organized into two primary layers, epidermis and dermis. The epidermis is the outermost layer and serves as the body's point of contact with the environment. Keratinocytes are the most abundant cells in the epidermis that forms an effective physicochemical barrier. The dermis underlies the epidermis and harbors cutaneous structures including hair follicles, nerves, sebaceous glands and sweat glands.

The human skin is among the largest organs that covers the body [1] and represents the body's first line of defense. It protects the body from various environmental stressors such as infectious pathogens, chemical agents and air pollutants [2-6].

Among air pollutants with effects on the skin include the solar ultraviolet radiation (UV), polycyclic aromatic hydrocarbons, volatile organic compounds, nitrogen oxides, particulate matter, and cigarette smoke. The solar UV radiation consists of three spectral areas: UVA (320–400 nm), UVB (280–320 nm), and UVC (180–280 nm). The atmospheric ozone layer effectively blocks UVC, so that the UV radiation reaching the skin surface is a mixture of 5% UVB and 95% of UVA [7]. The depth of penetration of UV radiation into the skin is dependent on the wavelength, and the effects of UV radiation on human skin differ depending on the wavelength (Figure 1). Longer wavelength UVA penetrates deeply into the basal layer of the epidermis and dermal fibroblasts. In contrast, UVB is largely absorbed by the epidermis, with little reaching the dermis [7-10].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

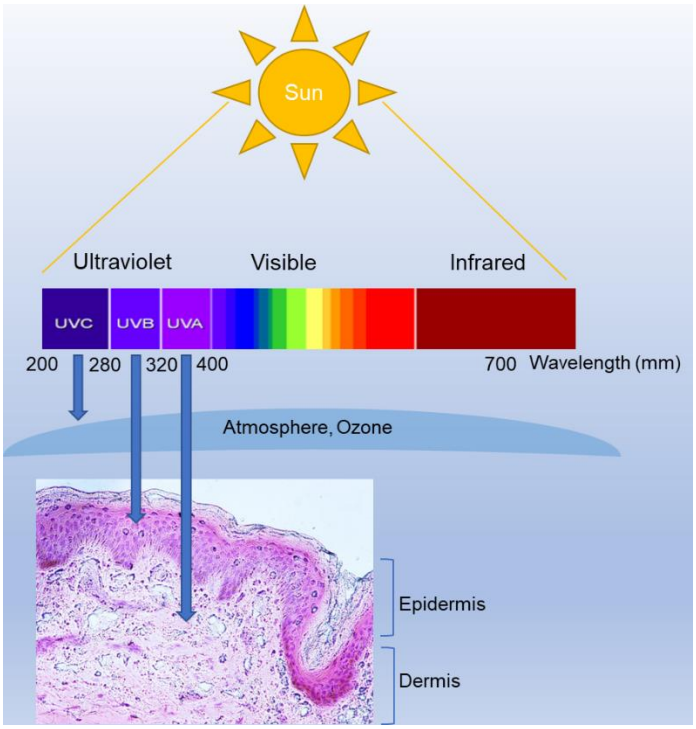


Figure 1. Spectrum of solar radiation and penetration into the skin. Solar UV radiation is subdivided into UVA, UVB and UVC components. UVC is absorbed by atmospheric ozone. UV penetrates the skin in a wavelength-dependent manner. UVA penetrates deeply into the dermis reaching well into the dermis. In contrast, UVB is almost completely absorbed by the epidermis, with comparatively little reaching the dermis. Adaptation from [11].

Excessive exposure to solar UV radiation is one of the most impactful environmental factors affecting human skin and leads to a variety of skin maladies including cancer and inflammation [11,12]. Both UVA and UVB induce DNA damage in skin cells but with different and distinct way [13,14]. UVA induces formation of reactive oxygen species that can damage DNA via indirect photosensitizing reactions [15-17]. UVB is directly absorbed by DNA which induces the formation of DNA photolesions [14,18]. These DNA modifications can lead to mutations and skin cancer initiation and progression [19–21].

Acute exposure to UV radiation induces inflammation of skin characterized by erythema and oedema. Inflammation induced by UVA and UVB irradiation results in the release of pro-inflammatory cytokines, especially interleukins (e.g., IL-1, IL-6 and IL-8), tumor necrosis factor (TNF-alpha) and interferon gamma (IFN-γ) as an acute phase response [3,4,6,22,23]. For example, UVA irradiation induces synthesis of IL-6, IL-8 and TNF-alpha in human keratinocytes and fibroblasts [24, 25]. On the other hand, UVB irradiation induces synthesis of IL-1alpha and TNF-alpha in human keratinocytes [26].

The pro-inflammatory cytokines are known to impact the expression and activity of drug transporters [27,28]. Drug transporters are membrane transporters belonging to both the ATP-binding cassette (ABC) and Solute carrier (SLC) families [29-37]. Drug transporters have broad specificity and are involved in both uptake (influx) and secretion (efflux) of their substrates, thereby affecting their cellular disposition. Published reports from different laboratories including ours have shown that human skin contains a range of influx and efflux transporters capable of drug transport, although expression of some of them is more limited than in other organs [29-39]. Using reverse transcription polymerase chain reaction (RT-PCR) we have shown expression of ABCB1, ABCC1, ABCC2 and ABCG2 in *ex vivo* human skin and in 3D-reconstructed human epidermis models, with ABCB1 and ABCG2 being barely expressed and ABCC1 being with the highest expression level. Functional analysis has shown that MDR1 and MRP1 expressed in the skin facilitate drug

 doi:10.20944/preprints202012.0067.v1

transdermal delivery the ABC transporter-mediated mechanism of absorptive transport represents a critical component of the effectiveness of the topical products [38,40]. In a subsequent RT-PCR analysis for the SLC gene family, we have shown expression of SLCO3A1, SLCO2B1, SLC47A2, SLCO4A1 and SLC47A1 in *ex vivo* human skin model, with SLC47A1 being highly expressed [36].

Drug transporters are critically important for the absorption, distribution, metabolism, and excretion of many drugs and endogenous compounds. Therefore, alteration of transporters activity can have profound effects on drug pharmacokinetics which impact drug efficacy and toxicity. Inflammation-induced changes in the expression and function of membrane transporters have been documented in various models of acute inflammation *in vitro* and *in vivo* reviewed in [28,41]. Most often, studies use IL-6, IFN, IL-1β, TNF-α, and liposaccharides (LPS) as inducers of inflammation in in vitro models. Although there has been significant progress in our understanding of the expression of inflammatory markers in response to UV radiation, little is known about the effects of inflammation induced by UV radiation on the expression of drug transporters in human skin.

The purpose of this study was to investigate if UVA and UVB irradiation modulates the expression of ABC and SLC transporters in human keratinocytes and fibroblasts.

We first validated the inflammatory effect of LPS in primary culture of normal human epidermal keratinocytes (NHEK), normal human dermal fibroblasts (NHDF) and 3D-reconstructed human epidermis (3D-RHE) model. We measured the change of expression of IL-8 and TNF-alpha induced by LPS. We then measured constitutive expression of ABC and SLC transporters in the three *in vitro* models. Finally, we measured the effects of UVA and UVB irradiation on the expression of inflammatory markers IL-8 and TNF-alpha to ensure the efficacy of UV radiation in NHEK and NHDF and the changes of expression of ABC and SLC transporters induced by UVA and UVB radiation.

In this study, we show for the first time that UVA irradiation induced a significant increase of the expression of SLCO4A1 in normal human dermal fibroblasts and that UVB irradiation induced significant downregulation of the expression of SLC47A1 in both human epidermal keratinocytes and human dermal fibroblasts.

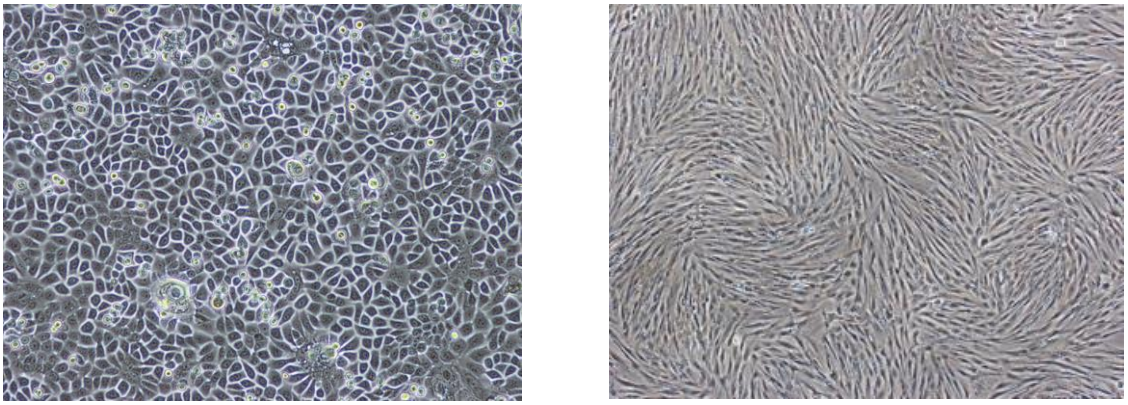
2. Materials and Methods

2.1 In vitro skin models

2.1.1. Primary culture of normal human epidermal keratinocytes and normal human dermal fibroblasts

Pre-plated (6-wells), normal human epidermal keratinocytes (NHEKs) and normal human dermal fibroblasts (NHDFs) were obtained from Sterlab (Vallauris, France). Isolated from the foreskin, epidermal keratinocytes (Figure 2a) and dermal fibroblasts (Figure 2b) were cultured in MCDB 153 culture medium in the case of NHEK or DMEM in the case of NHDF according to the supplier's recommendations. Upon arrival, cells were allowed to equilibrate in cell incubator set at 37°C in an atmosphere of 5% CO_2 for 24 hours. After the cells had equilibrated, the culture medium was removed, and 3 mL of fresh medium was placed in all the wells. The cells then remained in the 37°C/5% CO_2 incubator until they were at 80% confluent. Five different batches of keratinocytes were used through the study: PKD-KER-01; 02; 03; 04 and 05. One single batch of dermal fibroblasts was used in the study: PKD-FIB-01.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1



(a) (b)

Figure 2. Primary culture of normal human epidermal keratinocytes and human dermal fibroblasts. Representative images of normal human epidermal keratinocytes batch number PKD-KER-01 (a) and dermal human fibroblasts batch number PKD-FIB-01 (b) observed with phase contrast microscope.

2.1.2. 3D-Reconstructed human epidermis model

3D-reconstructed human epidermis (RHE) tissues were provided by Sterlab laboratories (Vallauris, France) in 24-well plate. The epidermis tissues of 0.5 cm^2 surface area were originated from a primary culture of human keratinocytes isolated from the foreskin and were cultivated for 12 days in a defined medium and reconstituted by airlifted culture on insert polycarbonate filter of 0.4 µm (Figure 3). The tissues were cultured and kept in MCDB 153 culture medium (Sigma-Aldrich - Merck KGaA, Germany) without hydrocortisone during the assays in cell incubator set at 37°C, 5% CO_2 and saturated humidity. The tissue manipulation process was carried out in sterile conditions. Seven different batches were used through the study: PKD-RHE-05; 06; 09; 10; 11; 12 and 13.

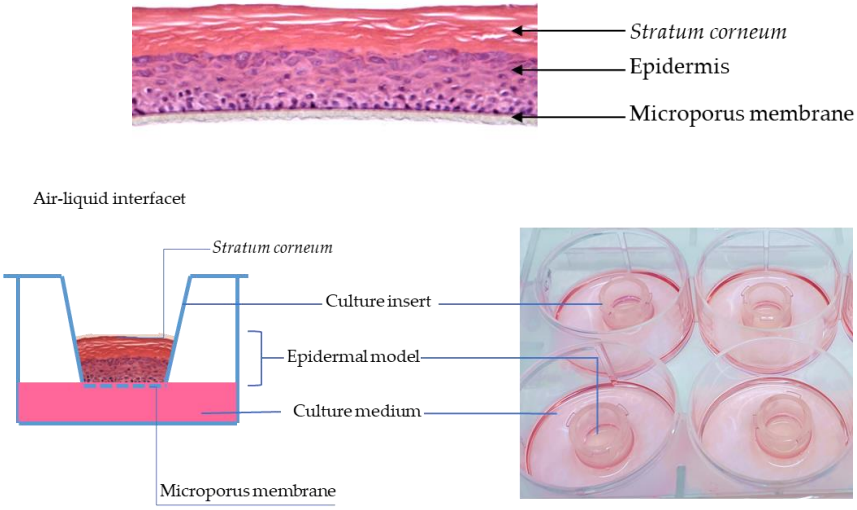


Figure 3. 3D-Reconstructed human epidermis model.

2.2 Lipopolysaccharide treatment

Lipopolysaccharides (LPS) act as endotoxins and highly immunogenic antigens which elicit strong immune responses in different models. In this study, LPS was used as a positive control of inflammation to validate our *in vitro* skin models. A preliminary experiment was performed to determine optimum concentrations of *Escherichia coli* lipopolysaccharide (LPS) for stimulating the cells. Stock solution of LPS from E. Coli 055:B5 (Sigma-Aldrich - Merck KGaA, Germany) was diluted in culture medium to achieve a final concentration of 100 µg/mL. NHEK and NHDF at 80% confluency were treated with LPS in culture medium for 24 hours. On the other hand, 3D-RHE tissues were

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

treated with LPS in culture medium for 24 hours. Incubations were done in cell incubator set at 37°C, 5% CO_2 and saturated humidity. At the end of treatment period, culture medium was collected, and cells and tissues were lysed using 500 µL lysis buffer of ReliaPrep™ RNA Tissue miniprep System (Promega France).

2.3 Exposure to UV radiation

Cell cultures of NHEK and NHDF were irradiated with 30 J/cm² UVA or 40 mJ/cm² UVB. UV exposure was performed using Bio-Link UV irradiation system (Vilber-Lourmat, France). BLX-365 was used for exposure to UVA (wavelength 365 mm), and BLX-312 for exposure to UVB (wavelength 312 nm). After exposure to UV radiation, cells were incubated for 24 hours in cell incubator set at 37°C, 5% CO_2 and saturated humidity.

2.4 Microscopic observation

At the end of treatment period, keratinocytes and fibroblasts were fixed with 4% formaldehyde in PBS, permeabilized with 0.5% Triton X-100 and then incubated with 3% BSA in PBS according to Image-iT FixPerm kit instructions (Molecular probes, USA). Polymerized actin was stained with ActinRed™ 555 in keratinocytes or ActinGreen™ 488 in fibroblasts, and nuclei were stained with NucBlue® (Molecular Probes, USA). Cells were observed using an Eclipse 80i inverted microscope (Nikon Instruments Inc., USA). Image acquisition was done using a DS-Ri1 high resolution camera coupled to NIS-Elements AR software (Nikon Instruments Inc., USA).

2.5. Measurement of lactate dehydrogenase release

Lactate dehydrogenase (LDH) is a marker of cell membrane integrity. An increase of LDH release in culture medium indicates increasing cytotoxicity. Release of LDH in culture medium was measured using LDH-Glo™ Cytotoxicity Assay according to the instructions provided by the manufacturer (Promega France). Luminescence was measured using GloMax® Explorer plate reader (Promega France). Each measurement was done in triplicate.

2.6. Isolation of total RNA

Total RNA was isolated using ReliaPrep™ RNA Tissue miniprep System (Promega France) according to the instructions provided by the manufacturer. RNA concentrations were quantified using QuantiFluor® RNA System (Promega France) according to the instructions provided by the manufacturer.

2.7. Reverse transcription and quantitative real-time PCR

Total RNA (150 – 500 ng) was converted into cDNA using High Capacity RNA to cDNA Master Mix kit according to the instructions provided by the manufacturer (Applied Biosystems).

Real-time PCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Validated PCR primers and TaqMan MGB-FAM labelled probes (TaqMan® Assay on Demand; Applied Biosystems) were used in the study. The references of the sequences used are indicated in Table 1.

PCR amplifications were performed in a total volume of 25 µL using TaqMan® Universal PCR Master Mix No Amperase® UNG according to the manufacturer's instructions (Applied Biosystems). Thermal cycling parameters were as follows: Polymerase activation (10 min, 95°C) followed by 40 cycles denaturation (15 s, 95°C) and combined annealing/extension (1 min, 60°C). Target and reference gene sequences were amplified independently in separate reactions and each PCR reaction was performed in triplicate. The PCR fluorescence data (provided by FAM probe) were analyzed with 7500 software (version 2.0.6, Applied Biosystems). The results were expressed as threshold cycle (Ct), which is inversely proportional to the amount of gene in the sample.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

Table 1. Assay-on-demand used in the gene expression experiments.

TaqMan® Assay on Demand	
Gene/Protein	**Reference**
GAPDH / GAPDH	Hs99999905_m1
CXCL8 / IL-8	Hs00174103_m1
TNF-alpha / TNF-alpha	Hs00174128_m1
ABCB1 / MDR1	Hs01067802_m1
ABCC1 / MDR1	Hs01561502_m1
ABCC2 / MRP1	Hs00166123_m1
ABCG2 / MRP2	Hs01053790_m1
SLCO2B1 / OATPB	Hs01030343_m1
SLCO3A1 / OATPD	Hs00939778_m1
SLCO4A1 / OATPE	Hs00983988_m1
SLC47A1 / MATE1	Hs00217320_m1
SLC47A2 / MATE2	Hs00945650_m1

The quantification approach used is termed the comparative Ct method [42]. This involves comparing the Ct values of the target genes in treated samples and control untreated samples. The Ct values of target genes in both control and treated samples were normalized to GAPDH used as reference gene, giving the ΔCt: Ct of target gene minus Ct of GAPDH.

The increase of the expression of a target gene was expressed as fold change and calculated as $2^{-\Delta\Delta Ct}$, where ΔΔCt = ΔCt of target gene in treated sample minus ΔCt of target gene in control untreated sample. On the other hand, the constitutive expression of a given gene was expressed as $2^{-\Delta Ct}$, where ΔCt = Ct of target gene minus Ct of reference gene.

2.8. Statistical Analysis

All experiments were performed at least three times, except where indicated. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using a two-way without replication analysis of variance (ANOVA) test using Excel's Data Analysis ToolPack. A *p* value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Cytotoxicity assay

Table 2 and Table 3 show the results of cytotoxicity in normal human epidermal keratinocytes and human dermal fibroblasts, respectively. Cytotoxicity was expressed in percentage of control unexposed cells increased significantly following the treatment of both UVA and UVB in normal human epidermal keratinocytes and decreased in human dermal fibroblasts. On the other hand, cell morphology observation (Figure 4) clearly showed morphological changes in cultured keratinocytes and fibroblasts after UV irradiation. Moreover, a marked reduction of cell number compared to control unexposed cells can be seen in both keratinocytes and fibroblasts after UV irradiation, especially after UVB irradiation.

Table 2. Effect of UVA and UVB exposure on LDH release in normal human epidermal keratinocytes. Data represent the mean and the standard deviation (SD) of 3 experiments normalized to unexposed cells set at 100%.

Normal human epidermal keratinocytes		
Treatment	**% Cytotoxicity**	***p* Values[1]**
	Mean ± SD	
Control	91.7 ± 7.4	
UVA	411.6 ± 61.3	0,0038
UVB	801.3 ± 53.2	0,000013

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

[1] *p*-values are based on the comparison to the control unexposed treatment.

Table 3. Effect of UVA and UVB exposure on LDH release in human dermal fibroblasts. Data represent the mean and the standard deviation (SD) of 3 experiments normalized to unexposed cells set at 100%.

Human dermal fibroblasts		
Treatment	**% Cytotoxicity**	***p* Values[1]**
	Mean ± SD	
Control	101.7 ± 3.8	
UVA	72.2 ± 5.0	0,011
UVB	83.0 ± 8.0	0,088

[1] *p*-values are based on the comparison to the control unexposed treatment.

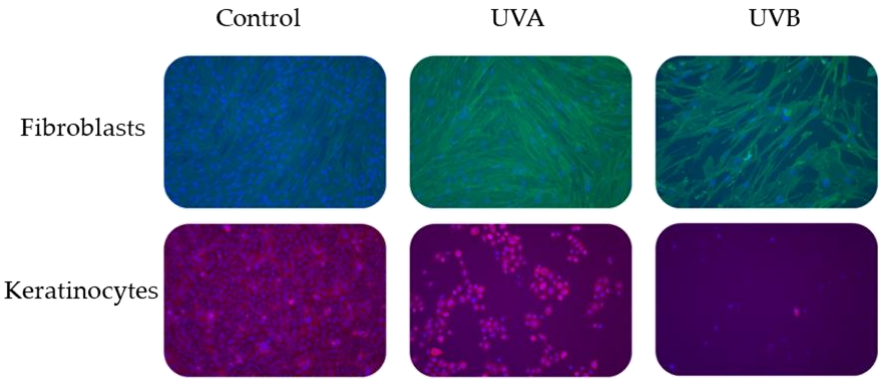


Figure 4. Cells morphology 24 hours after UV exposure. Cells cytoskeleton was stained by using ActinGreen™ 488 (fibroblasts) and ActinRed™ 555 (keratinocytes) and the cell nuclei were stained by using NucBlue™ (blue).

3.2. Constitutive expression of ABC and SLC transporters in in vitro skin models

Constitutive expression of four ABC transporters (ABCB1, ABCC1, ABCC2, ABCG2) and five SLC transporters (SLCO3A1, SLCO4A1, SLCO2B1, SLC47A1, SLC47A2) was measured in normal human epidermal keratinocytes, normal human dermal fibroblasts and 3D-reconstructed human epidermis model. Figure 5 shows that expression profile of ABC transporters was similar in the three *in vitro* skin models, with the highest expression of ABCC1 followed by ABCG2 and ABCC2. Expression of ABCB1 was very low, or even non detected in all the three *in vitro* skin models. In contrast, expression profile of SLC transporters was different depending on the model used. In normal human epidermal keratinocytes and 3D-RHE models, expression of SLCO4A1 was the highest and that of SLCO2B1 was very low or even non detected. In normal human dermal fibroblast, expression of SLC47A1 was the highest and that of SLC47A2 was very low or not detected. Overall, expression profile of ABC and SLC transporters was similar in normal human epidermal keratinocytes and in 3D-RHE model.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

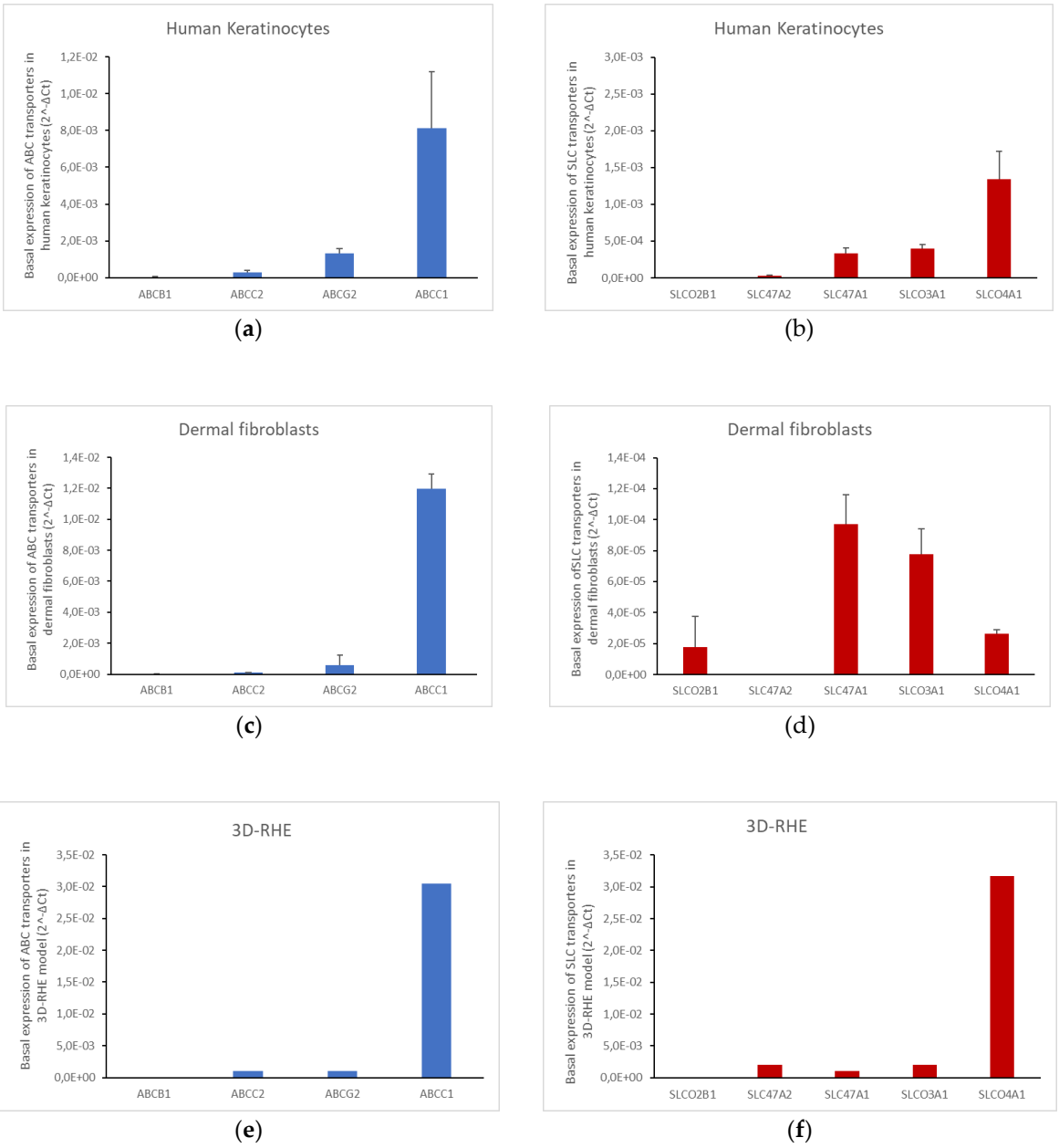


Figure 5. Constitutive expression of ABC and SLC transporters in *in vitro* skin models. mRNA expression of ABC transporters (a, c, e) and SLC transporters (b, d, f) was measured in control untreated normal human epidermal keratinocytes (a, b), normal human dermal fibroblasts (c, d), and 3D-reconstructed huma epidermis (RHE) model (e, f). mRNA expression of each transporter was measured by quantitative real-time PCR and expressed as $2^{-\Delta Ct}$. Data represent mean and standard deviation of 3 experiments for keratinocytes and fibroblasts. One single experiment was done with 3D-RHE.

3.2. Effect of Lipopolysaccharide on the expression of inflammatory markers in in vitro skin models

LPS was used as positive control of inflammation to validate the *in vitro* skin models used in the study. Figure 6 shows that treatment with LPS induced increase of the expression of the two inflammatory markers TNF-alpha and IL-8 in normal human epidermal keratinocytes, normal human dermal fibroblasts and 3D-RHE model. The extent of increase of TNF-alpha and IL-8 expression in keratinocytes was of the same order, whereas in dermal fibroblasts and in 3D-RHE model, increase of IL-8 expression was much more higher compared to increase of TNF-alpha expression. On the other hand, the extent of increase of TNF-alpha and IL-8 expression in 3D-RHE model was much higher than in epidermal keratinocytes. Overall, all three *in vitro* skin models

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

respond to LPS stimulation by increasing expression of inflammatory markers and can be used as valid model to evaluate the effect of UV irradiation in subsequent experiments.

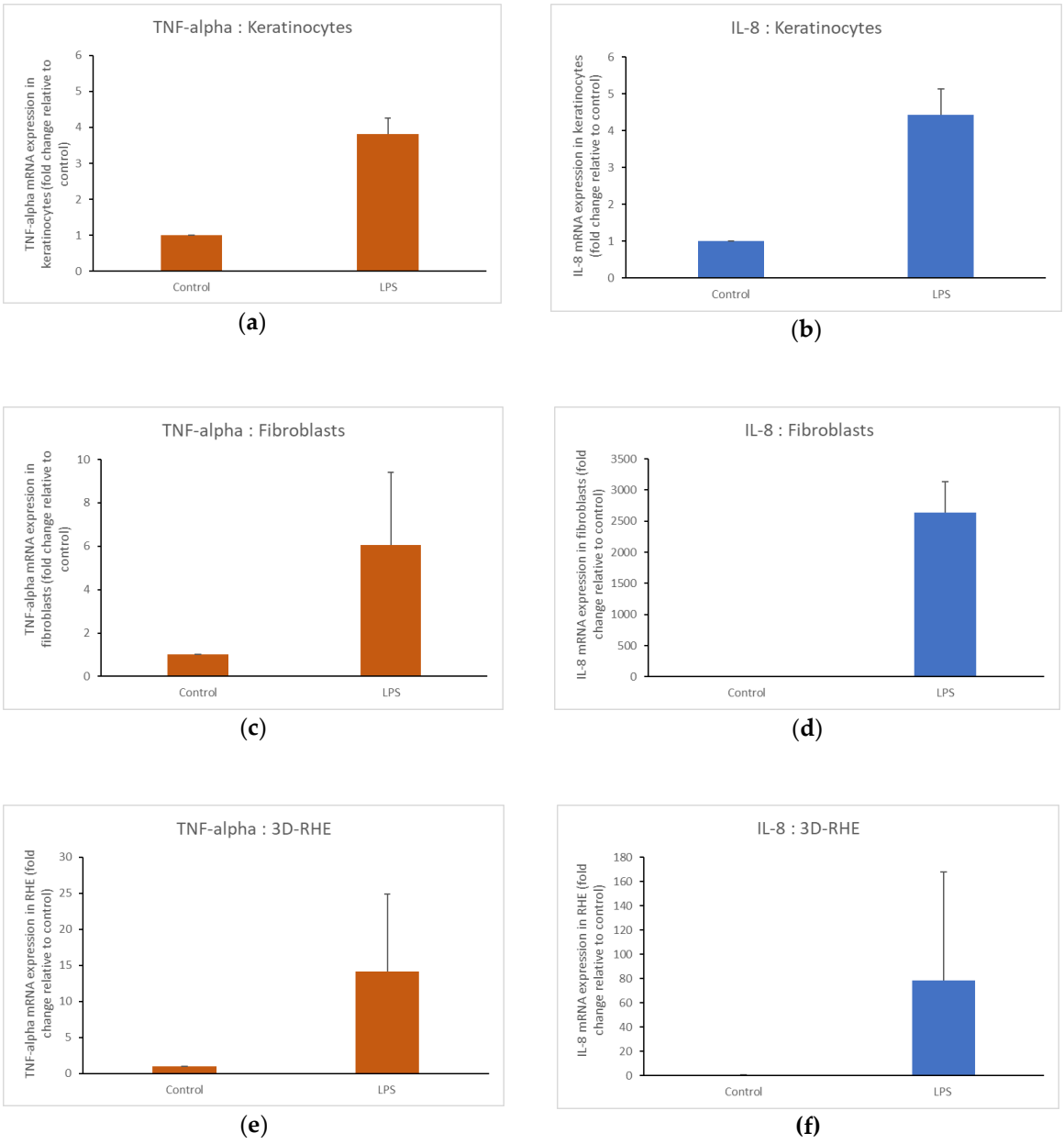


Figure 6. Effect of lipopolysaccharide treatment on the expression of inflammatory markers in *in vitro* skin models. Normal human epidermal keratinocytes (a, b), normal human dermal fibroblasts (c, d) and 3D-reconstructed human epidermis (RHE) model (e, f) were treated with 100 µg/mL lipopolysaccharide (LPS) for 24 hours. Untreated conditions were used as control. mRNA expression of TNF-alpha (a, c, e) and CXCL-8 or IL-8 (b, d, f) was measured by quantitative real-time PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ and represent mean and standard deviation of 4 different experiments for keratinocytes, 3 experiments for dermal fibroblasts and 7 different experiments for 3D-RHE model.

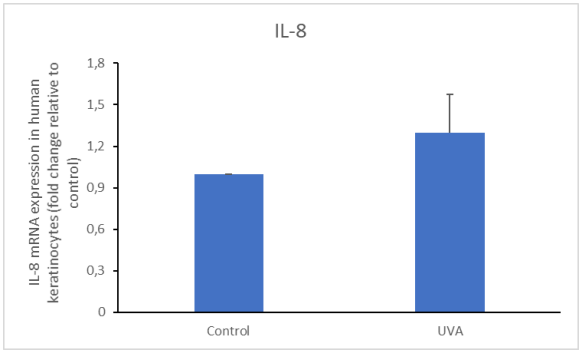
3.2. Effect of UV irradiation on the expression of inflammatory markers in in vitro skin models

Effect of UV irradiation on the expression of inflammatory markers was evaluated in normal human epidermal keratinocytes and normal human dermal fibroblasts.

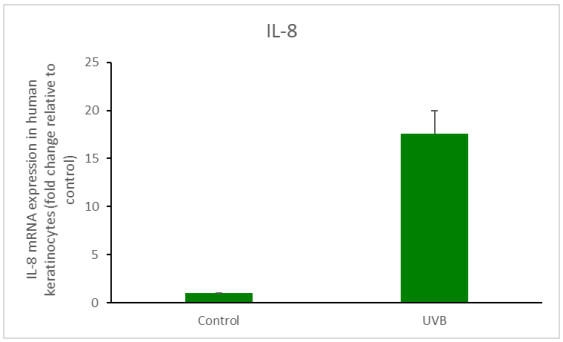
3.2.1. Normal human epidermal keratinocytes

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

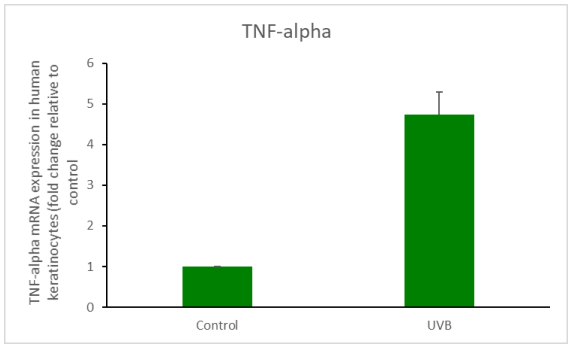
Figure 7 shows that exposure of normal human epidermal keratinocytes to 30 J/cm² UVA induced low increase of the expression of IL-8. Expression of TNF-alpha could not be measured because very little RNA could be isolated. On the other hand, exposure of normal human epidermal keratinocytes to 40 mJ/cm² UVB increased IL-8 expression 18-fold and TNF-alpha expression 5-fold.



(**a**)



(**b**)

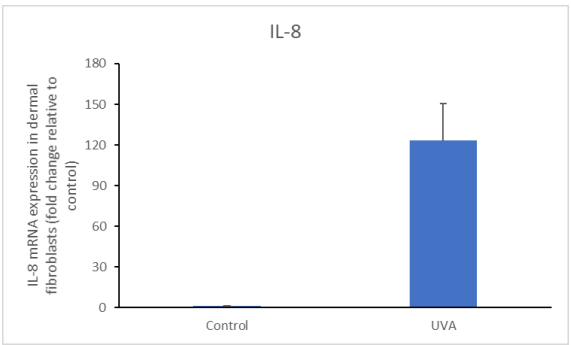


(**c**)

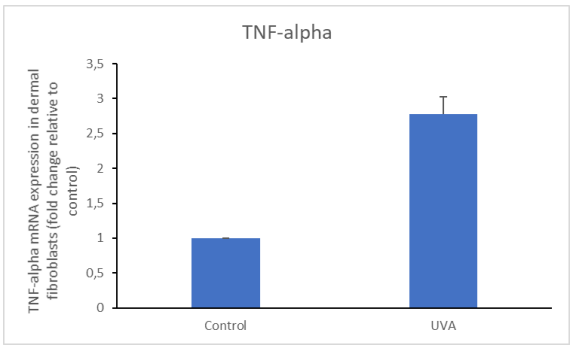
Figure 7. Effect of UVA and UVB exposure on the expression of inflammatory markers in normal human epidermal keratinocytes. Human keratinocytes were exposed to 30 J/cm² UVA (a) or 40 mJ/cm² UVB (b, c) and cultured for 24 hours. Unexposed cells were used as control. mRNA expression of CXCL-8 or IL-8 (a, b) and TNF-alpha (c) was measured by quantitative real-time PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ and represent mean and standard deviation of 3 experiments.

3.2.2. Normal human dermal fibroblasts

Figure 8 shows that exposure of normal human dermal fibroblasts to 30 J/cm² UVA increased IL-8 and TNF-alpha expression by 123-fold and 3-fold, respectively. On the other hand, exposure of normal human dermal fibroblasts to 40 mJ/cm² UVB increased IL-8 and TNF-alpha expression by 19-fold and 5-fold, respectively.

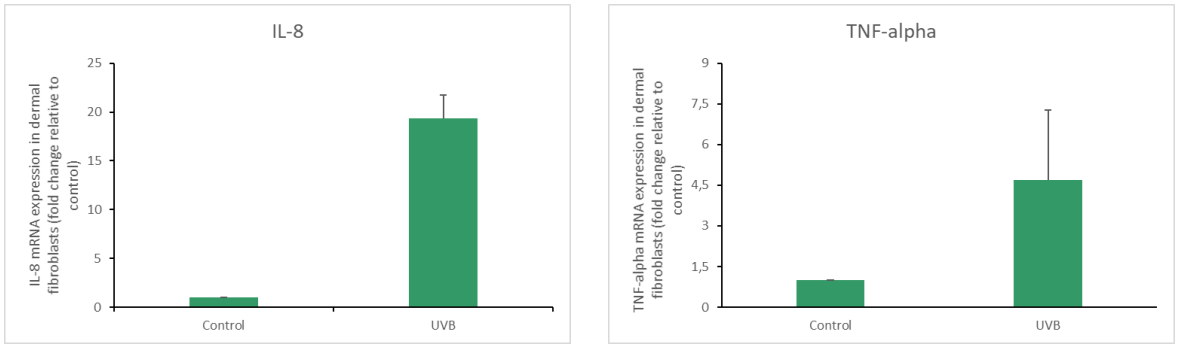


(**a**)



(**b**)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1



(c) (d)

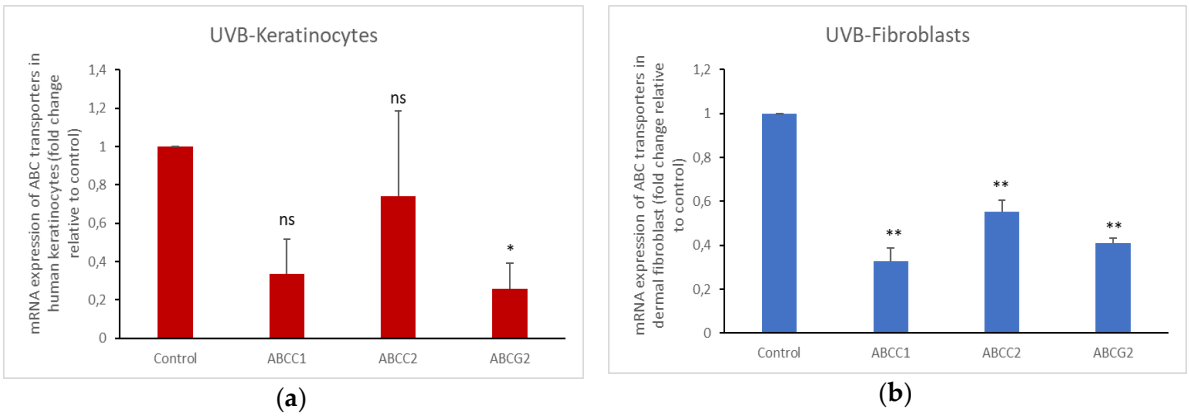
Figure 8. Effect of UVA and UVB exposure on the expression of inflammatory markers in normal human dermal fibroblasts. Normal human dermal fibroblasts were exposed to 30 J/cm² UVA (a, b) or 40 mJ/cm² UVB (c, d) and cultured for 24 hours. Unexposed cells were used as control. mRNA expression of CXCL-8 or IL-8 (a, c) and TNF-alpha (b, d) was measured by quantitative real-time PCR. Data are expressed as $2^{-\Delta\Delta Ct}$ and represent mean and standard deviation of 3 experiments.

3.3. Effect of UV irradiation on the expression of ABC and SLC transporters in in vitro skin models

Effect of UVA and UVB irradiation on the expression of ABC and SLC transporters was evaluated in normal human epidermal keratinocytes and normal human dermal fibroblasts.

3.3.1. ABC transporters

Effects of UV irradiation on the expression of ABCB1 have not been measured due to low expression of ABCB1 in in vitro skin models. UVA (30 J/cm²) irradiation had no significant effect on the expression of ABC transporters in normal human epidermal keratinocytes and normal human dermal fibroblasts (data not shown). Conversely, UVB irradiation (40 mJ/cm²) induced significant decrease ($p < 0.01$) of the expression of ABCC1, ABCC2 and ABCG2 in normal human dermal fibroblast and ABCG2 expression ($p < 0.05$) in normal human epidermal keratinocytes as shown in Figure 9.



(a) (b)

Figure 9. Effect of UVB exposure on the expression of ABC transporters in normal human epidermal keratinocytes and normal human dermal fibroblasts. Normal human epidermal keratinocytes (a) and normal human dermal fibroblasts (b) were exposed to 40 mJ/cm² UVB and cultured for 24 hours. Unexposed cells were used as control. mRNA expression of ABCC1, ABCC2 and ABCG2 was measured by quantitative real-time PCR. mRNA expression was normalized with GAPDH mRNA for each experimental condition. Data are expressed as $2^{-\Delta\Delta Ct}$ and represent mean and standard deviation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 December 2020 doi:10.20944/preprints202012.0067.v1

of 3 experiments. Statistically significant differences in mRNA expression over control were determined by ANOVA * $p < 0.05$; ** $p < 0.01$.

3.3.2. SLC transporters

We only focused on SLCO4A1 and SLC47A1 which are the most expressed transporters in keratinocytes and fibroblasts, respectively. Figure 10 shows that exposure to 30 J/cm² UVA induced significant ($p < 0.01$) 2-fold and 29-fold increase of the expression of SLCO4A1 in normal human epidermal keratinocytes and normal human dermal fibroblasts, respectively. No significant change of the expression of SLC47A1 has been observed. On the other hand, exposure of normal human epidermal keratinocytes and normal human dermal fibroblasts to 40 mJ/cm² UVB significantly decreased ($p < 0.05$) the expression of SLC47A1 by 80% and 50%, respectively. In addition, expression of SLCO4A1 decreased by 20% in epidermal keratinocytes ($p < 0.05$), while it was not modified in normal human dermal fibroblasts after exposure to UVB.

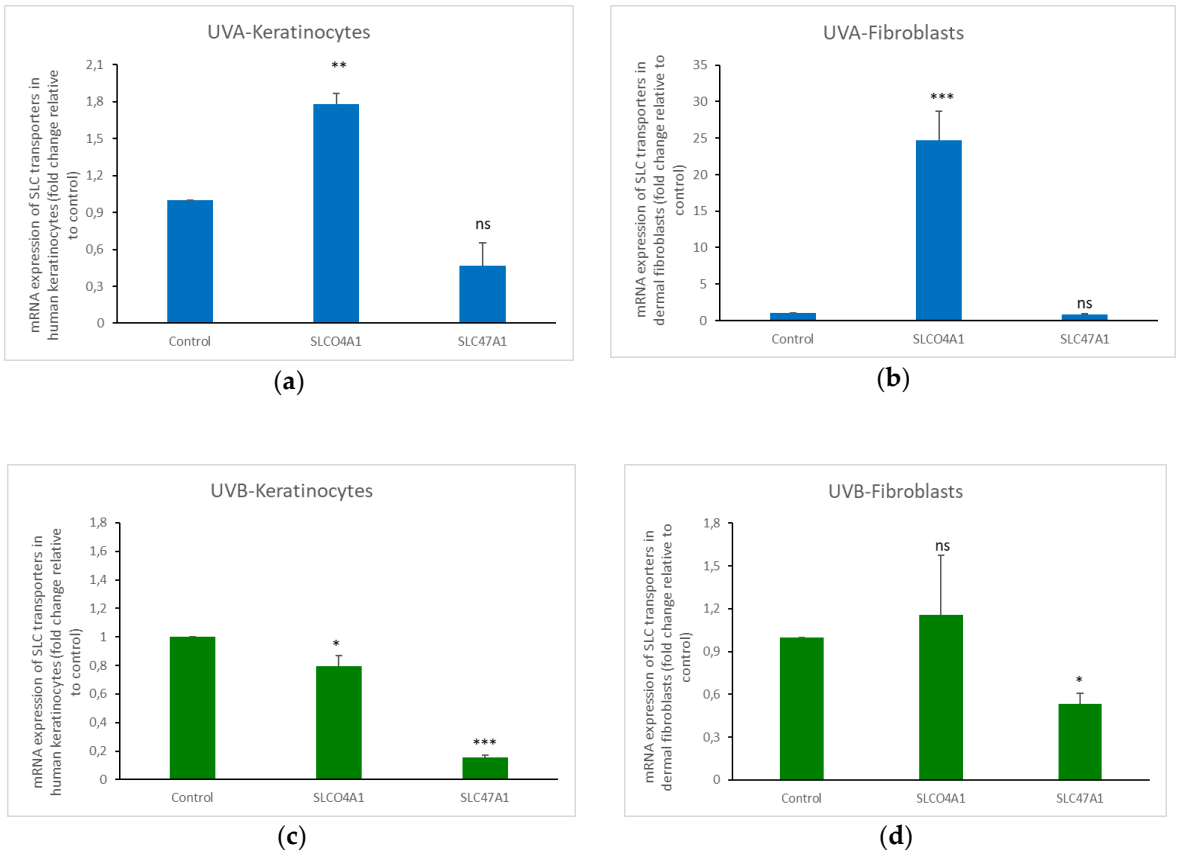


Figure 10. Effect of UVA and UVB exposure on the expression of SLC transporters in normal human keratinocytes and normal human dermal fibroblasts. Normal human epidermal keratinocytes (a, c) and normal human dermal fibroblasts (b, d) were exposed to 30 J/cm² UVA (a, b) or 40 mJ/cm² UVB (c, d) and cultured for 24 hours. Unexposed cells were used as control. mRNA expression of SLCO4A1 and SLC47A1 was measured by quantitative real-time PCR. mRNA expression was normalized with GAPDH mRNA for each experimental condition. Data are expressed as $2^{-\Delta\Delta Ct}$ and represent mean and standard deviation of 3 experiments. Statistically significant differences in mRNA expression over control were determined by ANOVA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

 doi:10.20944/preprints202012.0067.v1

4. Discussion

The main objective of the study was to evaluate the effects of UVA and UVB irradiation on the expression of drug transporters in skin. Three different in vitro skin models were evaluated: normal human epidermal keratinocytes in primary culture, normal human dermal fibroblasts in primary culture and 3D-reconstructed human epidermis, a much more complex differentiated model. We first wanted to ensure how these models responds to stimulation with LPS. LPS is an inducer of inflammatory markers. We focused on the measurement of IL-8 and TNF-alpha, as both markers are constitutively well expressed in both keratinocytes and fibroblasts. The results showed that as expected, LPS induced increase of both IL-8 and TNF-alpha mRNA expression in all three *in vitro* models, and consequently, they can be used as validate models to assess the effects of UV irradiation.

We therefore analyzed the constitutive expression of ABC and SLC transporters in the three *in vitro* skin models. We mainly focused on transporters involved in drug-drug interactions. The results showed that ABC transporters had similar expression profile in all the three models, with ABCB1 (MRP1) being with the highest expression and ABCB1 (MDR1 Pg-p) and ABCG2 (BCRP) were barely expressed, even not detected. These results are in accordance with our previous results in *ex vivo* skin in organ-culture model [32,28].

On the other hand, expression profile of SLC transporters was similar in both keratinocytes and 3D-RHE model, with SLCOA41 being with the highest expression. However, expression profile SLC transporters in fibroblasts was different with SLC47A1 having the highest expression while SLC47A2 was barely expressed. In our previous work on *ex vivo* human skin in organ-culture model, we have shown that SLC47A1 was the most expressed transporter followed by SLCO4A1 [36]. *Ex vivo* human skin model contains both keratinocytes and fibroblasts. Therefore, expression results reflected the sum in both keratinocytes and fibroblasts and can in part explain the results of the current work. We therefore focused on the two most expressed SLC transporters in the subsequent analyses, *i.e.* SLC47A1 and SLCO4A1.

UVA irradiation (30 J/m^2) induced little increase of IL-8 mRNA expression in keratinocytes, and high increase of IL-8 and TNF-alpha mRNA expression in fibroblasts. On the other hand, UVB irradiation (40 mJ/m^2) induced high increase of both IL-8 and TNF-alpha mRNA expression in both *in vitro* skin models. Theses results are in agreement with published report showing skin inflammation and increased production of cytokines following exposure to UV radiation [43,44].

We finally analyzed the effects of UV radiation on the expression of ABC and SLC transporters. We showed that UVB radiation significantly downregulated the expression of ABCC1, ABCC2 and ABCG2 in human dermal fibroblasts. As ABC transporters are involved in dermal absorption, downregulation of their expression may have an important impact on the dermal absorption of topical products applied after acute exposure to sunlight. Further functional investigations are needed to confirm this hypothesis.

On the other hand, UVB radiation significantly downregulated the expression of SLC47A1 in both keratinocytes and fibroblasts, with a more marked effect on keratinocytes. This result confirms our previous work showing significant downregulation of SLC47A1 expression in *ex vivo* human skin in organ-culture after exposure to solar simulator [36].

One of the most striking result was the marked increase of the expression of SLCOA41 induced by UVA irradiation in human dermal fibroblasts, where more than 20-fold increase was observed. The biological significance of this increase is to date not understood and further proteomic and functional analyses are needed to confirm this finding. The next question to be answered is whether a treatment with LPS may have the same effect on the expression of SLCO4A1, or whether the effect we have shown is specific to UVA irradiation. Also, this question can shed light on the potential link between cytokine production and transporter expression.

SLCOA41 encoding OATP4A1 (OATP-E) is ubiquitously expressed and OATP4A1 transports steroid hormones, prostaglandins, and bile acids. However, the exact function and role of OATP4A1

 doi:10.20944/preprints202012.0067.v1

in human skin are poorly known, and it would be very interesting to better characterize this transporter and to investigate its role in drug disposition. Moreover, the overexpression of OATP4A1 has been previously associated with tumor recurrence and progression in colorectal cancer [45]. The overexpression of SLCO4A1 shown in current study in human dermal fibroblasts in response to UVA irradiation indicates that this transporter might serve as a biomarker for skin cancers, particularly those induced by solar exposure or could play an important role in chemosensitivity of cutaneous cancer cells and thus may be the basis for the discovery of novel treatment strategies for skin cancers. This finding is of particular interest as the majority of skin cancers are caused by over exposure to ultraviolet radiation.

In summary, our findings show for the first time a significant regulation of the expression of SLCO4A1 in human dermal fibroblasts induced by UVA irradiation. Further investigations still needed to identify the potential role of this transporter in skin cancers induced by solar exposure and in pharmacokinetics of topical drugs.

Author Contributions: Conceptualization, Manon Barthe and Hanan Osman-Ponchet; Methodology, Manon Barthe; Supervision, Hanan Osman-Ponchet; Writing – original draft, Manon Barthe and Hanan Osman-Ponchet; Writing – review & editing, Jean-Paul Thénot.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Leider, M. The largest organ. *JAMA* **1947**, 134, 1565
2. Lowe, N.J. An overview of ultraviolet radiation, sunscreens, and photo-induced dermatoses. *Dermatol Clin* **2006,** 24, 9–17
3. Slominski, A.; Wortsman, J. Neuroendocrinology of the skin. *Endocr Rev* **2000,** 21, 457–487.
4. Slominski, A.; Wortsman, J.; Luger, T.; Paus, R.; Solomon, S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiol Rev* **2000,** 80, 979–1020
5. Fuchs, E. Scratching the surface of skin development. *Nature* **2007,** 445, 834–842
6. Slominski, A.T.; Zmijewski, M.A.; Skobowiat, C.; Zbytek, B.; Slominski, R.M.; Steketee, J.D. Sensing the environment: Regulation of local and global homeostasis by the skin's neuroendocrine system. *Adv Anat Embryol Cell Biol* **2012,** 2012: v-115
7. Dessinioti, C.; Antoniou, C.; Katsambas, A.; and Stratigos, A. J. Basal cell carcinoma: What's new under the sun. *Photochem Photobiol* **2010,** 86, 481–491
8. Fisher, G.J.; Wang, Z.Q.; Datta, S.C.; Varani, J.; Kang, S.; and Voorhees, J.J. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* **1997,** 337, 1419–1428
9. Dessinioti, C.; Tzannis, K. ; Sypsa, V. ; Nikolaou, V. ; Kypreou, K. ; Antoniou, C. ; et al. Epidemiologic risk factors of basal cell carcinoma development and age at onset in a Southern European population from Greece. *Exp Dermatol* **2011,** 20, 622–626
10. Valacchi, G.; Sticozzi, C.; Pecorelli, A.; Cervellati, F.; Cervellati, C.; Maioli, E. Cutaneous responses to environmental stressors. *Ann. NY Acad Sci* **2012,** 1271, 75–81
11. D'Orazio, J., Jarrett, S., Amaro-Ortiz, A.; Scott, T. UV Radiation and the Skin. *Int J Mol Sci* **2013,** 14, 12222–12248
12. Elwood, J.M.; Jopson, J. Melanoma and sun exposure: An overview of published studies. *Int J Cancer* **1997,** 73, 198–203
13. Polefka, T.G.; Meyer, T.A.; Agin, P.P.; Bianchini, R.J. Effects of solar radiation on the skin. *J Cosmet Dermatol* **2012,** 11, 134–143
14. Sklar, L.R.; Almutawa, F.; Lim, H.W.; Hamzavi, I. Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: A review. *Photochem PhotoBiol Sci* **2013,** 12, 54–64
15. Tyrrell, R.M. Ultraviolet radiation and free radical damage to skin. Biochem Soc Symp **1995,** 61, 47–53
16. Sander, C.S.; Chang, H.; Hamm, F.; Elsner, P.; Thiele, J.J. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. Int J Dermatol **2004,** 43, 326–335
17. Burke, K.E. Photoaging: The role of oxidative stress. G Ital Dermatol Venereol **2010,** 145, 445–459.
18. Pfeifer, G.P.; You, Y.H.; Besaratinia, A. Mutations induced by ultraviolet light. *Mutat Res* **2005,** 571, 19–31
19. De Gruijl, F.R. Photocarcinogenesis: UVA vs. UVB. *Methods Enzymol* **2000,** 319, 359–366
20. Ikehata, H.; Ono, T. The mechanisms of UV mutagenesis. *J Radiat Res* **2011,** 52, 115–125
21. Sage, E.; Girard, P.M.; Francesconi, S. Unravelling UVA-induced mutagenesis. *Photochem PhotoBiol Sci* **2012,** 11, 74–80
22. Clydesdale, G.J.; Dandie, G.W.; Muller, H.K. Ultraviolet light induced injury: Immunological and inflammatory effects. Immunol. *Cell Biol* **2001,** 79, 547–568.
23. Matsumura, Y.; Ananthaswamy, H.N. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol* **2004,** 195, 298–308
24. Avalos-Díaz, E.; Alvarado-Flores, E.; Herrera-Esparza, R. UV-A irradiation induces transcription of IL-6 and TNF alpha genes in human keratinocytes and dermal fibroblasts. *Rev Rhum Engl Ed*. **1999**, 66, 13-9
25. Kondo, S. The roles of keratinocyte-derived cytokines in the epidermis and their possible responses to UVA-irradiation. J Investig Dermatol Symp Proc 1999, 4, 177-183
26. Bashir, M.M.; Sharma, M.R.; Victoria P. Werth, V.P. UVB and pro-inflammatory cytokines synergistically activate TNF-α production in keratinocytes through enhanced gene transcription. *J Invest Dermatol* **2009,** 129, 994–1001
27. Le Vee, M.; Lecureur, V.; Stieger, B.; Fardel, O. Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-alpha or interleukin-6. *Drug Metab Dispos* **2009,** 37, 685–693
28. Morgan, E.T. ; Goralski, K.B. ; Piquette-Miller, M. ; Renton, K.W. ; et al. Regulation of drug-metabolizing enzymes and transporters in infection, inflammation, and cancer. *Drug Metab Dispos* **2008,** 36, 205-216

29. Li, Q.; Tsuji, H.; Kato, Y.; Sai, Y.; Kubo, Y.; Tsuji, A. Characterization of the transdermal transport of flurbiprofen and indomethacin. *J Control Release* **2006,** 110, 542-546
30. Ito, K.; Nguyen, H.T.; Kato, Y.; Wakayama, T.; Kubo, Y.; Iseki, S., Tsuji, A. P-glycoprotein (Abcb1) is involved in absorptive drug transport in skin. *J Control Release* **2008,** 131, 198-204
31. Haslam, I.S.; El-Chami, C.; Shahmalak, A.; O'Neill, C.A.; Paus, R. Differential expression and functionality of ATP-binding cassette transporters in the human hair follicle. *Br J Dermatol* **2015,** 172, 1562-1572
32. Osman-Ponchet, H.; Boulai, A.; Kouidhi, M.; Sevin, K.; Alriquet, M.; Gaborit, A.; Bertino, B.; Comby, P.; Ruty, B. Characterization of ABC transporters in human skin. *Drug Metabolism and Personalized Therapy,* **2014,** 29, 91–100
33. Takenaka, S.; Itoh, T.; Fujiwara, R. Expression pattern of human ATP-binding cassette transporters in skin. *Pharmacology Research & Perspectives,* **2013,** 1, 1-8
34. Hashimoto, N.; Nakamichi, N.; Uwafuji, S.; Yoshida, K.; Sugiura, T.; Tsuji, A.; Kato, Y. ATP binding cassette transporters in two distinct compartments of the skin contribute to transdermal absorption of a typical substrate. J Control Release 2013, 165, 54–61
35. Bleasby, K.; Castle, J.C.; Roberts, C.J.; Cheng, C.; et al. Expression profiles of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition. *Xenobiotica* **2006,** 36, 963-988
36. Alriquet, M.; Sevrin, K.; Gaborit, A.; Comby, P.; Ruty, B.; Osman-Ponchet, H. Characterization of SLC transporters in human skin. *ADMET & DMPK* **2015,** 3, 34-44
37. Fujiwara, R.; Takenada, S.; Hashimoto, M.; Narawa, T.; Itoh, T. Expression of human solute carrier family transporters in skin: possible contributor to drug-induced skin disorders. *Scientific Reports* **2014,** 4, 1-8
38. Osman-Ponchet, H.; Gaborit, A.; Linget, J.; Wilson, C.E. Expression of drug transporters in the human skin: comparison in different species and models and its implication for drug development. *ADMET & DMPK* **2017,** 5, 75-84
39. Clerbaux, L.A.; Paini, A.; Lumen, A.; Osman-Ponchet, H.; Worth, A.P.; Fardel, O. Membrane transporter data to support kinetically-informed chemical risk assessment using non-animal methods: Scientific and regulatory perspectives. *Environ Int* **2019,** 126, 659-671
40. Li, L.; Cataisson, C.; Flowers, B. ; Fraser, E. ; et al. Topical application of a dual ABC transporter substrate and NF-κB inhibitor blocks multiple sources of cutaneous inflammation in mouse skin. *J Invest Dermatol* **2019,** 139, 1506-1515
41. Fardel, O.; Le Vee, M. Regulation of human hepatic drug transporter expression by pro-inflammatory cytokines. *Expert opinion on drug metabolism & toxicology* **2009,** 5, 1469–81
42. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **2001,** 25, 402–8
43. Song, P.I.; Young-Min Park Y.M.; Abraham, T.; Harten, B.; et al. Human keratinocytes express functional CD14 and Toll-like receptor 4. The Society for Investigative Dermatology 2002, 119, 424-432
44. Arlian, L.G.; Morgan, M.S.; Neal, J.S. Modulation of cytokine expression in human keratinocytes and fibroblasts by extracts of scabies mites. *Am J Trop Med Hyg* 2003, 69, 652-656
45. Buxhofer-Ausch, V.; Német, O.; Sheikh, M.; Andrikovics, H.; et al. Two common polymorphic variants of OATP4A1 as potential risk factors for colorectal cancer. Oncology letters 2020, 20, 1-11