doi:10.36849/JDD.S589D

April 2021 s23 Volume 20 • Issue 4 (supplement)

ORIGINAL ARTICLE

Journal of Drugs in Dermatology

Alteration to the Skin Barrier Integrity Following Broad-Spectrum UV Exposure in an Ex Vivo Tissue Model

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ABSTRACT

Dynamic changes to the skin barrier's molecular structure and ceramide profile are well-documented in skin conditions such as atopic dermatitis and psoriasis. Pathological and environmental factors have been shown to impair barrier integrity and demonstrate shifts in ceramide composition in the skin. However, the relationship between acute and prolonged sun exposure and its effects on skin barrier homeostasis is insufficiently investigated. This study aims to uncover new scientific evidence to elucidate the relationship of UV irradiation with the skin barrier using an ex vivo tissue model following simulated UVA/UVB exposure.

Fresh ex vivo human skin pretreated either with or without a broad-spectrum sunscreen was exposed to either a physiological or elevated UV condition. Following eight days in culture, structural and molecular changes were evaluated. UV irradiated skin displayed epidermal cell death and altered expression of key barrier proteins. TEM analysis demonstrated disruption to adherens junctions and dissociation between tissue layers following both physiological and extensive UV exposures. An effective broad-spectrum sunscreen containing essential skin ceramides completely protected the skin from such changes. This is one of the first works demonstrating a clear correlation of altered skin barrier integrity using a physiologically relevant dose in an ex vivo tissue model. Our findings also further support the additional importance and benefits of sun protection among the consumers.

J Drugs Dermatol. 2021;20(4 Suppl):s23-28. doi:10.36849/JDD.S589D

INTRODUCTION

The human skin barrier is a complex structure comprising of physical, chemical, immunological, and microbial components that maintain skin homeostasis whilst protecting the body from external irritants.[1] The outermost layer of the skin, or the stratum corneum (SC), is the skin's first line of defence against external irritants and comprises of corneocytes embedded in a lipid matrix composed of an equimolar ratio of cholesterol (CHOL), free fatty acids (FFAs), ceramides (CERs), and sterol/wax esters.[2] Pathological conditions, such as atopic dermatitis (AD) or psoriasis, are known to trigger barrier disturbance through disruption of the natural ceramide profile, adherens junctions, and key barrier proteins.[3–6] Furthermore, a direct correlation was observed in AD patients, where decreased FFA and CER chain lengths in AD skin caused less dense lipid organization increased transepidermal water loss (TEWL).[7]

The health concerns of prolonged UV exposure are well-documented; it is shown to induce premature photoaging, altered pigmentation, inflammation, and carcinoma.[8–10] In addition, there are several articles investigating the effects of ultraviolet (UV) radiation on skin barrier function.[11–13] UV exposure was reported to induce epidermal barrier damage by altering the tight junction protein expression, disruption to the basement membrane junctions, decrease in the level of covalently bound ceramides, increase stratum corneum (SC) thickness & increase stratum corneum (SC) thickness and TEWL, and induce degradation to the structural and mechanical integrity of the skin.[11,13–22] While each of the studies has furthered the understanding on UV on barrier function, the connection between a physiologically relevant UV dose with the molecular changes to the barrier of the skin remains unclear. This study is the first of two parts that utilized a physiologically relevant, fresh ex vivo skin model to understand how broad-spectrum UV affects the composition of epidermal barrier and illustrate how the application of sunscreen can provide barrier protection. This study also investigated the effects of a physiologically relevant dose, which mimics a chronic exposure to the maximum level of daily UV condition, and an elevated non-physiological exposure dose to serve as a contrast group.

Experimental Design

Ex Vivo Tissue Culture

Fresh ex vivo human skin was acquired from BioIVT LLC (Westbury, NY) one day post-abdominoplasty. A total of seven lots of fresh ex vivo skin was utilized in this study, (Caucasian (n=6), Hispanic (n=1), Male (n=1), Female (n=6), 29–51 years old). Tissue was defatted, cleaned of blood residue, and 1.2mm skin biopsy punches were created. Broad-spectrum CeraVe Hydrating Sunscreen SPF 50 Face Lotion, which contains Ceramides 1, 3, 6, in addition to other essential skin lipids, was applied (4.42 µL/cm^2) to the respective biopsy punches fifteen minutes prior to irradiation. At this time, the study solely studied ceramide-incorporated formulas. These biopsy punches were then exposed to one-time exposure of 20J/cm^2, a five-time exposure of 20J/cm^2 over 1 week or one-time exposure of 100J/

doi:10.36849/JDD.S589D

cm² of 96% UVA/4% UVB using a solar simulator (Sol3A Class AAA Solar Simulator, Newport Corporation, CA). The 20J/cm² and 100J/cm² UV doses falls into an average range of a 3MED and 13MED respectively based on skin phototypes II-III.[23] The 20J/cm² condition simulated the effects of a 1-week exposure to the maximal level of daily UV condition, while the 100J/cm² demonstrated an elevated and severe non-physiological level of UV exposure and serves as a contrast group.[24] Following irradiation, skin explants were cultured in a 12-well transwell at an air-liquid interface in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin at 37°C and 5% CO2. The group receiving 20J/cm²/5x over 1 week were subjected to daily UV-exposure conditions and returned to the incubator. Following the 8-day culture period, all biopsies were processed for histological and transmission electron microscopy analysis.

H&E and Immunofluorescence Staining

Skin explants were processed for hematoxylin and eosin staining (Tejas Pathology, Trumbull, TX) and frozen sectioning. Frozen section samples were fixed in methanol/acetone and blocked with 10% normal goat serum for 1 hour at room temperature. Tissue sections were then incubated with TUNEL (Reveal Biosciences, San Diego, CA), Rabbit Polyclonal to Anti-Transglutaminase 1 (Novus Biologicals, Centennial, CO), Rabbit Polyclonal to Anti-Involucrin (Abcam, Cambridge, MA), Mouse Monoclonal to Anti-Desmoglein 1 (Abcam, Cambridge, MA), Rabbit Polyclonal to Anti-Claudin 4 (Abcam, Cambridge, MA), or Anti-Laminin 5 γ2 chain (Millipore Sigma, Temecula, CA) primary antibody. Following the primary staining, tissue sections were then incubated with secondary antibody Goat Anti-Rabbit IgG H&L Alexa Fluor 594 or Goat Anti-Mouse IgG H&L Alexa Fluor 594 (Invitrogen, Carlsbad, CA) and counterstained with DAPI. Frozen sections were stained with Rabbit Polyclonal to Anti-Filaggrin (Abcam, Cambridge, MA) according to the Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (Abcam, Cambridge, MA). All sections were then imaged with a fluorescent microscope (Leica DM500, Wetzlar, Germany).

Transmission Electron Microscopy (TEM)

Tissue explants were fixed in 5% glutaraldehyde solution and prepared for TEM as previously described by Van den Bergh et al.[25] Sections were imaged on Jeol 1200 EX Transmission Electron Microscope (Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ) at 80kV.

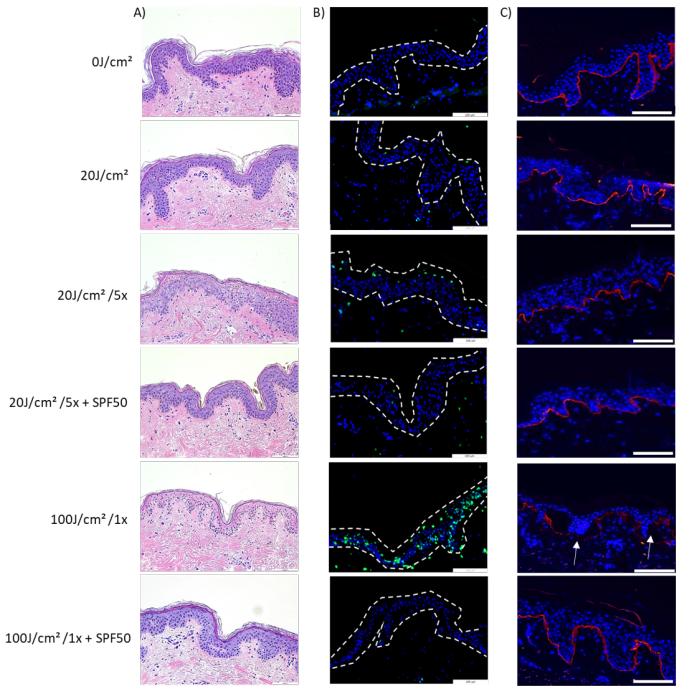
RESULTS/DISCUSSION

The health risks associated with prolonged UV exposure on unprotected skin are well-documented. While the shorter wavelength of UVB light is less able to penetrate the skin, excessive exposure to solar UVB irradiation induces DNA damage and inflammation.[26] The longer wavelength of UVA light penetrates deeper into the skin and is one of the key exogenous factors promoting premature skin aging and inducing oxidative stress. To date, there are limited studies that investigated the relationship of prolonged sun exposure with skin barrier function.[11,14,22] The objective of this study was to elucidate the effects of exposure to high doses of UV irradiations on structural and molecular properties of skin barrier using ex vivo human skin, a model that is able to closely represent physiologically relevant changes. To accomplish this, a solar simulator was utilized with a filter that allowed a balanced ratio of 4% UVB/96% UVA in order to provide physiologically relevant UV energies. The doses elected for this study are 1) 5x exposure to 20J/cm² for 1 week and 2) 1x exposure to 100J/cm². The 20J/cm² group is designed to simulate the effects of 1-week exposure to the maximal level of daily UV condition, while the 100J/cm² group serves as a contrast group when an extreme, above-physiological level of UV irradiation is applied. Multiple skin lots from different donors have provided consistent results.

Structural Changes in UV-Irradiated Tissue

The relationship of UV-exposure with tissue structure was evaluated by hematoxylin and eosin (H&E), Laminin 5 staining, and TUNEL staining to assess epidermal apoptosis. One-time exposure of 20J/cm² UV dose displayed no structural changes while a daily exposure of 20J/cm²/5x demonstrated localized apoptosis in the stratum granulosum (Figure 1A). A one-time

FIGURE 1. Representative images of H&E (A), TUNEL (B), and Laminin 5 (C) staining of ex vivo tissue following various UV energy exposure with/without the application of sunscreen. White arrows denote disruption of Laminin 5 expression following elevated UV exposure (100J/cm²).



Blue-DAPI, Green-TUNEL, Red-Laminin 5

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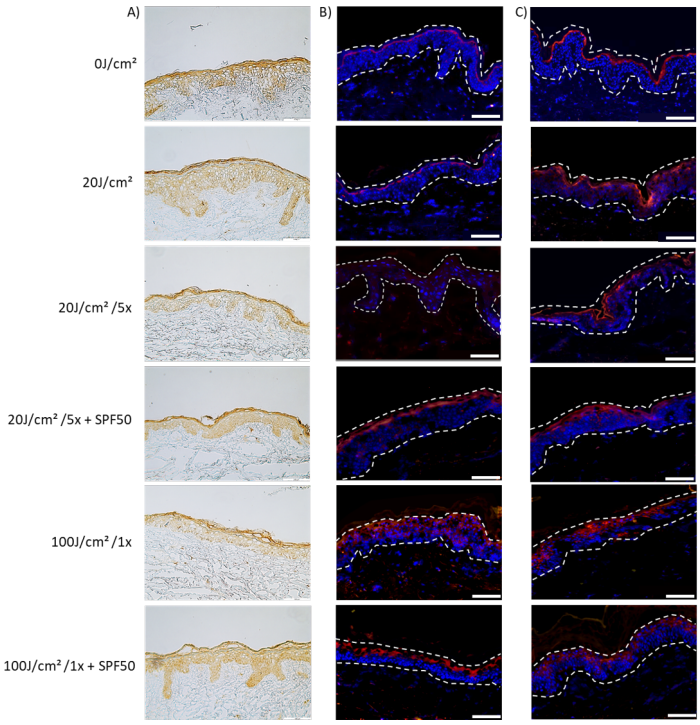
exposure to an elevated UV dose (100J/cm²) demonstrated epidermal cell death and separation of the dermis-epidermis junction (DEJ). Through evaluation of TUNEL expression, there was no significant apoptosis in a one-time 20J/cm² exposure, but there was an observed accumulated expression in the 20J/cm²/5x and 100J/cm² exposure conditions (Figure 1B). One-time and cumulative exposure of 20J/cm² maintains identical Laminin-5 expression to untreated control tissue, while a one-time elevated UV-exposure (100J/cm²) induced interrupted Laminin-5 expression (Figure 1C). The cumulative effect of UV in regard to epidermal cell death and disruption to the DEJ aligned with changes to tissue structure in histology. The application of sunscreen prior to UV irradiation demonstrated a clear protective benefit in the 20J/cm²/5x and 100J/cm² conditions as illustrated by the preservation of tissue morphology in H&E staining, reduction in the number of apoptotic cells and minimized DEJ disruption (Figure 1).

Molecular Changes in UV-Irradiated Tissue

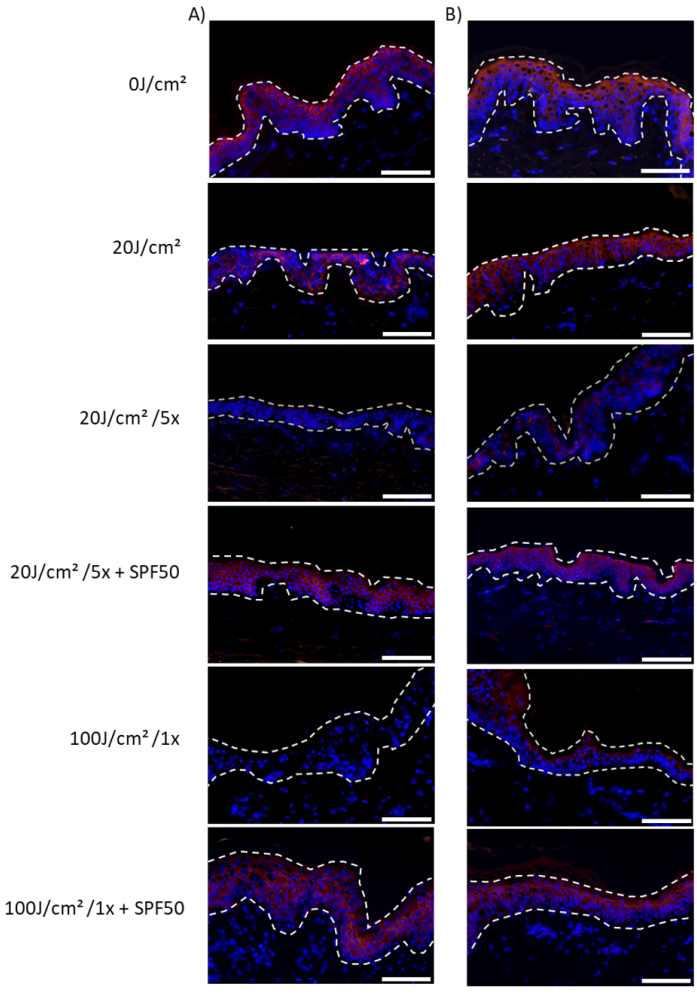
To understand how different doses of UV irradiation can compromise the skin barrier, this study evaluates some of key biomarkers related to barrier function. Immunostaining against Filaggrin (Figure 2A) demonstrated that UV exposure did not negatively influence the expression at all of the doses evaluated in this study, suggesting non-obvious impact on the external skin barrier. Additionally, staining against DSG1 (Figure 3A) and Claudin 4 (Figure 3B) evaluated the impact on the adherens junctions within the stratum granulosum layer. Figure 2B illustrates a significant decrease in expression for 20J/cm²/5x and 100J/cm²/1x treatment groups but not for 20J/cm²/1x, suggesting a cumulative and a dose-dependent effect on disrupting the adherens junctions. The tissue receiving sunscreen prior to UV irradiation showed normal level of DSG1 and Claudin 4 staining, suggesting the role of sunscreen in preventing disruption of the adherens junctions (Figure 3A and 3B). Immunostaining against TGM1 (Figure 2B) increased expression in the extreme UV condition (100J/cm²/1x), which does not hold true for the daily UV exposure condition (20J/cm²/1x and 20J/cm²/5x). This elevated expression in the higher UV dose suggests that the skin was entering a reparative state to address the altered barrier. The increase in TGM1 induced

FIGURE 2. Representative images of Filaggrin (A), Transglutaminase 1 (B) and Involucrin (C) staining of ex vivo tissue following various UV energy exposure with/without the application of sunscreen.



Scale bar = 100µm. Blue-DAPI, Red-TGM1(B)/INV(C)

FIGURE 3. Representative images of Desmoglein 1 (A) and Claudin 4 (B) staining of ex vivo tissue following UV exposure with/without the application of sunscreen.



Scale bar = 100µm. Blue-DAPI, Red-DSG1(A)/CLD4(B)

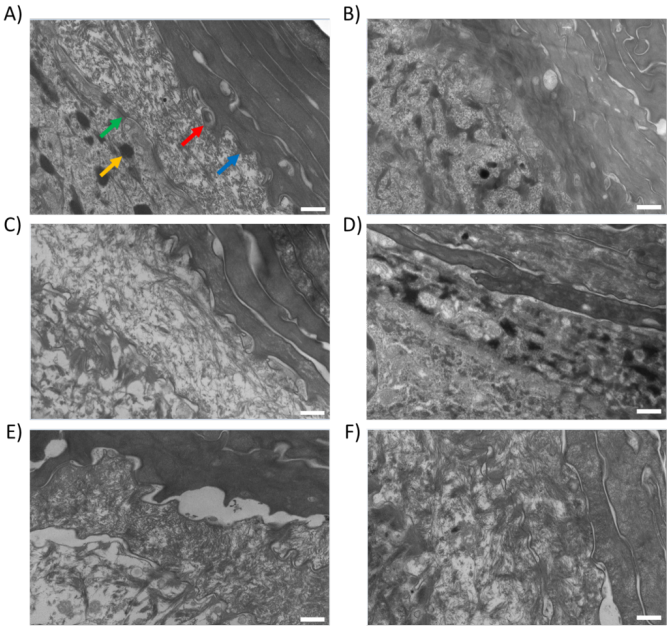
doi:10.36849/JDD.S589D

by 100J/cm² is also significantly reduced by the application of sunscreen (Figure 2B). Immunostaining against Involucrin (Figure 2C) demonstrates a modest increase in the expression level only for the 100J/cm² conditions and is ameliorated by the application of sunscreens. Altogether, these stains displayed a cohesive understanding that the skin barrier is compromised not only in the elevated UV dose (100J/cm²) but in some physiological UV conditions (20J/cm²) as well.

TEM Analysis of Ex Vivo Skin Following UV-Exposure

Figure 4 demonstrates TEM images captured to evaluate changes in tissue ultrastructure following UV irradiation. Figure 4A is able to illustrate the presence of intact corneodesmosomes (blue arrow), desmosomes (green arrow), lamellar bodies (red arrow), and keratohyalin granules (yellow arrow) in the stratum corneum and transition layer of untreated control tissue. Untreated tissue also has abundance of keratohyalin granules, an essential component of the keratinocyte cornification process, in the stratum granulosum layer. In agreement with histological and immunostaining analysis, TEM analysis illustrated limited disruption to tissue structure as a result of one-time irradiation at 20J/cm² level (Figure 4B). When the tissue was exposed to a daily irradiation of 20J/cm² over the course of 5 days, alterations to the keratin fibers and disruption to the adherens junctions can be observed. Keratinocytes in the stratum granulosum also became more apoptotic (Figure 4C). The UV irradiation effects on barrier disruption were amplified in the 100J/cm² conditions, where the tissue displayed severe disruption to the adherens junctions, disassociation between cellular layers, and marked reduction of keratohyalin granules and lamellar bodies (Figure 4E). The benefits of photoprotection were observed through TEM images, where the application of sunscreen in the daily irradiation conditions showed better preservation of the adherens junctions and the keratohyalin granules (Figure 4D). At the high UV dose (100J/cm²), photoprotection applied prior to irradiation helped maintain tissue structure, although some regions still demonstrated disrupted corneodesmosomes and reduction of the keratohyalin granules (Figure 4E).

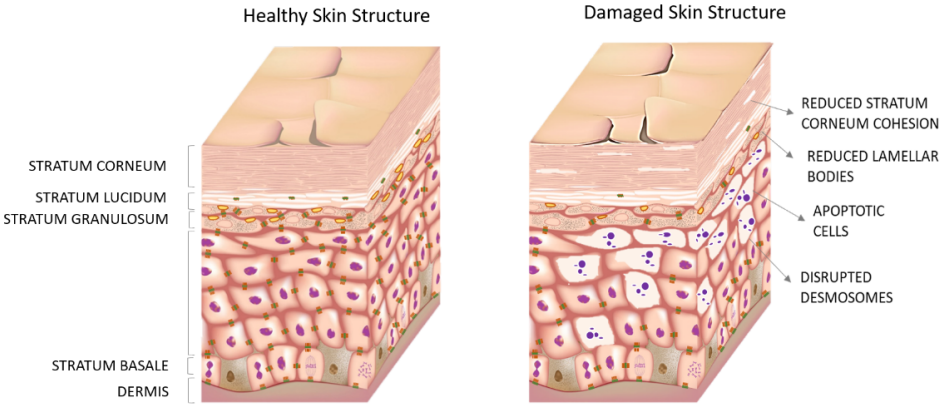
FIGURE 4. Representative TEM images of Untreated Control Tissue (A), 20J/cm² 1x (B), 20J/cm² 5x (C), 20J/cm² 5x with SPF50 Sunscreen (D), 100J/cm² 1x (E), and 100J/cm² 1x with SPF50 Sunscreen (F) treatment groups.



Scale bar = 500nm.

In this study, fresh ex vivo skin was utilized as a physiologically relevant model to understand the dynamic changes to skin barrier as a result of high-level UV irradiation. Our findings can be summarized by Figure 5, which illustrates that prolonged sun exposure significantly impacted the inside-out skin barrier, referring to cell junctions that prevent loss of water, electrolytes, and proteins, while being less potent in altering the outside-in barrier.[27] Our study demonstrated a reduction in proteins that contribute to adherens junctions (Claudin 4 and

FIGURE 5. The relationship of prolonged UV exposure on the skin barrier.



doi:10.36849/JDD.S589D

Desmoglein 1) with no effect on Filaggrin. This observation suggests a potential mechanism in which UV penetrates through the stratum corneum to induce apoptosis at the stratum granulosum layer, which in turn reduces the integrity of the adherens junctions. Since adherens junctions provide the mechanical cohesion between the cells in the epidermal layers and the key signaling cues to cytoskeletal dynamics and polarity, the disruption to the adherens junction have significant function implications.[28] In skin diseases such as psoriasis vulgaris, Occludin and ZO-1 are up-regulated and Claudins are down-regulated, suggesting that the compromised skin is undergoing active repair.[28] The irradiated skin at elevated levels also had increased levels of transglutaminase, which is a hallmark of compromised skin barrier as previously demonstrated in a SLS-challenged skin model.[29] The functional implications to the changes to transglutaminase has also been illustrated in patients suffering from atopic dermatitis (AD) and psoriasis, where lesional tissues marked increase in both TGM1 and TGM3.[5,6] The observed similarities in skin barrier changes following UV exposure with patients suffering from skin disorders further illustrates the importance of adequate photoprotection to prevent the alteration of barrier structure after receiving extended sunlight exposure. This is also one of the first works that clearly demonstrates how sunscreen can have direct benefits to the protection from UV-induced barrier damage, as previous studies have focused on the fundamental science of barrier alterations.[14,30]

Although this study has revealed very interesting structural and molecular changes the skin goes through as a result of UV irradiation, there are still many unanswered questions in the topic of barrier protection against UV irradiation. This study investigated the effect of balanced physiologically relevant UVA/UVB doses but did not explore whether the contribution of barrier disruption is driven by UVB or UVA wavelengths. One study had demonstrated that while suberythemal doses of UVB can be used as a therapeutic treatment for atopic dermatitis, elevated levels can induce barrier disruption.[31] This enables our model to be used to evaluate the therapeutic use of UV by monitoring structural and molecular changes within the tissue at varying UVB doses. While we demonstrated the benefit of sunscreen protection, further work is recommended to compare varying sunscreens with and without ceramides. Furthermore, the relationship between the structural disruption with alterations of ceramides and lipids has yet to be clarified and will be addressed in our future work. One of the key unanswered questions is the clinical and consumer relevance of the observed structural disruption and lipid ratio modification as a result of UV irradiation. The following article in this supplement will unpack the potential changes to skin barrier in a clinical study and also illustrate the benefits of a sunscreen and moisturizer routine following sun exposure. We believe through these efforts, the relationship between UV exposure and the alteration of the skin barrier can be explained, therefore illustrating the importance of barrier restoring photoprotection products.

CONCLUSION

In this study, we have introduced the use of ex vivo skin to identify additional parameters in studying barrier damage following UV exposure. Our results demonstrated that prolonged UV exposure induces epidermal cell death in addition to disruption of key basement membrane proteins. This exposure also altered expression of key differentiation proteins (TGM1, INV) and adherens junction proteins (DSG1, CLD4). Such effects can be ameliorated by the application of a ceramide containing sunscreen.

UV exposure not only induces detrimental effects on skin health and photoaging but has been demonstrated to have acute and prolonged impacts on both the structural and molecular components responsible for maintaining barrier integrity. Further work, which will be an upcoming second part to accompany this study, will be aimed to explore the relationship of the observed results with alterations to the skin ceramide composition following UV exposure. This future study permits for the understanding as to possible alteration of skin ceramide composition following UV exposure and would highlight the importance of introducing proper ceramide blends in sun care and skin care routines to help combat the UV-induced barrier damage and restore skin health following daily sun exposure. Replenishment of essential skin ceramides can not only help to recover the ideal lipid ratio within the skin but to also accelerate recovery of barrier impaired skin. Continued research in the field is needed to not only identify proper lipid combinations that lead to clinical and consumer-perceived benefits following UV exposure, but to also recognize any further benefits of photoprotection associated with UV-induced barrier disruption.

DISCLOSURE

The authors declare no conflict of interest. This research is sponsored by L'Oreal Research & Innovation.

ACKNOWLEDGMENTS

A special thank you to Francoise Bernerd and Dominique Bernard for their scientific advisements, Rajesh Patel for TEM support, and to Yung-Hao Tsou for technical support.

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