Repetitive Exposures to UVA1 and Particulate Matter–Associated Pollutants Trigger Epidermal Barrier Dysfunction in Skin Epithelialization Model

Journal of Investigative Dermatology (2022) 142, 3331–3335; doi:10.1016/j.jid.2022.05.1091

TO THE EDITOR

Because the skin is in direct interaction with the external environment, its barrier function ensures cutaneous protection against the exosome. Most atmospheric pollutants behave as pro-oxidants, inducing damage after interacting with the skin surface (Araviskai et al., 2019). However, blood circulation may constitute another route for deep skin contamination. Indeed, particulate matter <100 nm can translocate through lung alveoli into capillaries. Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP), were detected with an estimated concentration in the nanomolar range in the plasma of smokers or inhabitants of polluted cities (Marrot, 2018). In this study, we aimed at mimicking UVA1 (340–400 nm) impact, together with photoreactive PAHs, on epidermal differentiation by developing a skin model undergoing epithelialization. Experiments were designed to ensure realistic urban exposure: low PAH concentrations such as those reported for plasmatic contamination in vivo and UVA1 irradiation corresponding to about 30 minutes in daylight. As shown in Figure 1a, 3 days (Ds) after keratinocytes had been seeded on dermis equivalents, PAHs were continuously monitored on D10 and D13. A significant impact of UVA1 on FLG transcription was observed on D10 (Figure 1b). Immunofluorescence of FLG displayed a discontinuous pattern in the stratum granulosum, and fluorescence intensity was strongly reduced. On D13, BaP delayed the recovery process leading to the normalization of epidermal status (Figure 1c). Lorincrin, a marker associated with cornification (Ishitsuka and Roop, 2020), followed the same trend: strong undertranscription after UVA1 (Figure 1d) and inhomogeneous and reduced fluorescence on D10. On D13, recovery of lorincrin was almost achieved in UVA1-irradiated samples but not in UVA1 + BaP samples (Figure 1e). Interestingly, keratin 10, a differentiation marker, was also dysregulated (Supplementary Figure S1a).

Abbreviation: BaP, benzo[a]pyrene; D, day; PAH, polycyclic aromatic hydrocarbon

Accepted manuscript published online 22 June 2022; corrected proof published online 16 September 2022

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Figure 1. Detailed experimental procedure, impact of UVA1 without or with BaP on FLG and LOR status. (a) Treatment procedure on epidermis undergoing differentiation. (b) FLG mRNA expression on D10 and D13. (c) Quantification and images of FLG immunofluorescence staining at D10 and D13 (FLG appears in green in the upper epidermis, and nuclei appear in red). (d) LOR mRNA expression on D10 and D13. (e) Quantification and images of LOR immunofluorescence on D10 and D13. Bar = 100 μm. *P < 0.05, **P < 0.01, and ***P < 0.001 (at least two independent experiments with n = 6 biological replicates per condition). DMSO control is in pink, BaP alone is in orange, UVA1 alone is in khaki, and UVA1 + BaP is in green. BaP, benzo[a]pyrene; D, day; LOR, loricrin; NHK, normal human keratinocyte.
maturation process appeared clearly impaired. In fact, some proteases involved in skin barrier maturation were impacted. Aspartic protease SASPase cleaves pro-FLG during epidermal differentiation (Matsui et al., 2011). Its staining decreased on D10 after UVA1, even more under UVA1 + BaP exposure (Figure 2b). Kallikrein-5, a serine protease, cleaves pro-FLG in the early stages of FLG maturation (Sakabe et al., 2013). Kallikrein-5 transcription (data not shown) and immunofluorescence staining in the granular layer significantly decreased on D10 under UVA1 and UVA1 + BaP (Figure 2b). Transcription of caspase 14, a cysteiny1 aspartate protease involved in pro-FLG and FLG cleavage (Hoste et al., 2011), decreased on D10 under UVA1 or UVA1 + BaP. The pro–caspase 14/caspase 14 ratio appeared slightly higher in UVA1 + BaP samples on D13 (Figure 2c). Accordingly, reduction in

Figure 2. Dysregulation of FLG processing. (a) Western blot analysis of the pro-FLG/FLG monomer ratio on D10 and D13. (b) Quantification and images of SASPase and KLK5 immunofluorescence staining on D10 (SASPase or KLK5 in green and nuclei in red). Bar = 100 μm. (c) Caspase 14 mRNA expression on D10 and D13. Western blot analysis of the pro–caspase 14/active caspase-14 ratio on D10 and D13. (d) Trans-UCA and PCA amounts on D10. (e) Parakeratosis images on D13 (arrows point to nuclei within stratum corneum). Quantification of parakeratosis granules in a defined area (mm²) on D10 and D13. Bar = 50 μm. *P < 0.05, **P < 0.01, and ***P < 0.001 (at least two independent experiments with n = 6 biological replicates per condition). BaP, benzo[a]pyrene; CASP-14, caspase 14; D, day; HES, hematoxylin, eosin, and saffron; KLK5, kallikrein-5; PCA, pyroglutamic acid; SC, stratum corneum; UCA, urocanic acid.
FLG monomer proteolysis decreased the production of the natural moisturizing factors: trans-urocanic acid and pyroglutamic acid (Figure 2d) (Hoste et al., 2011). Furthermore, parakeratosis is a marker of abnormal differentiation, characterized by the presence of nuclei and keratohyalin granules (containing pro-FLG) within the stratum corneum. On D10 and D13, quantification of parakeratosis in samples exposed to UVA1 or UVA1 + BaP confirmed pro-FLG maturation defects (Figure 2e). Interestingly, similar results on differentiation were obtained using indenopyrene, another photo-reactive PAH from particulate matter (Supplementary Figure S1b and c). Oxidative stress (Supplementary Figure S2a, b) and unexpected under-expression of aryl hydrocarbon receptor under UVA1 exposure (Supplementary Figure S2e) may explain the disruption of differentiation. Significant upregulation of several cytokines (Supplementary Figure S3a, b) and metalloproteinases also occurred (Supplementary Figure S3c).

Even if epidermal morphology was slightly and transiently disturbed in our experimental conditions (Supplementary Figure S4a) and even if keratinocyte proliferation could restart after stress (Supplementary Figure S4b), epidermal differentiation appeared strongly impaired by UVA1 and even more so with PAHs. The impaired barrier markers observed in this study (parakeratosis, FLG, and loricrin defects) have already been described in atopic dermatitis in vivo, with similar histological aspects (Naeem et al., 2015; Pellerin et al., 2013).

Aggravation of atopic dermatitis/eczema by pollution probably involves such local tissue damage in a global context of skin immune system disruption. In fact, dermatologists noticed that outdoor air quality influenced atopic dermatitis occurrence (Hendricks et al., 2020), and several reports underlined the deleterious role of particulate matter (Ngoc et al., 2017). Our results on barrier function impairment by UVA1 + PAHs are consistent with and complementary to recently published data on reconstructed epidermis or human skin explants treated topically by particulate matter (Ferrara et al., 2020; Kim and Lim, 2021). Several markers associated with in vivo and ex vivo barrier impairment were found in our dynamic organotypic model (Supplementary Figure S5). This approach should thus be helpful to define skin barrier protection strategies against pollution and more broadly against environmental (photo)toxic insults.

Compliance with ethical standards
The studies were conducted in accordance with the Declaration of Helsinki Principles. Normal human skin was obtained from surgical residues of breast reduction surgery in France, with the patients’ written informed consent in accordance with the Helsinki Declaration and with Article L. 1243–4 of the French Public Health Code. Patients’ written informed consents were collected and kept by the surgeon. The authors did not participate in sample collection. Given its special nature, surgical residue is subject to specific legislation included in the French Code of Public Health (anonymity, gravity, sanitary/safety rules, and no publicity for donation). This legislation does not require previous authorization by an ethics committee for sampling or use of surgical waste.

Data availability statement
No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST
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ACKNOWLEDGMENTS
Aude Foucher, Yohann Phallente, Therese Balde-weck, Joanna Albaud, Vanessa Perrault, Nathalie Deshayes, and Valérie Haydont (L’Oreal Advanced Research, Aulnay-sous-Bois, France) are gratefully acknowledged for excellent support.

AUTHOR CONTRIBUTIONS
Conceptualization: OZ, HZ, AD, LM; Investigation: OZ, HZ, AD; Methodology: OZ, HZ, AD; Supervision: LM; Writing - Original Draft Preparation: LM

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2022.05.1091

REFERENCES

3334 Journal of Investigative Dermatology (2022), Volume 142
EASI p-EASI: Predicting Disease Severity in Patients with Atopic Dermatitis Treated with Tralokinumab

TO THE EDITOR

Numerous targeted treatments for atopic dermatitis (AD) are currently under investigation in clinical trials. Comparability of the efficacy of new drugs is therefore becoming more and more important. However, this remains challenging given the high intra-observer and interobserver variability of primary endpoints (Flohr, 2011). The use of serological biomarkers may overcome this problem. Recent studies have shown that a combination of serum biomarkers, including TARC, IL-22, and sIL-2R (using a formula called predicted Eczema Area Severity Index [EASI] [p-EASI]), predicts disease severity in patients with AD treated with dupilumab, cyclosporine A, and topical corticosteroids (Bakker et al., 2020; Thijs et al., 2019, 2017). The advantage of the use of serum biomarkers is that they are objective and do not require subjective assessment of disease activity by clinicians (Renert-Yuval et al., 2021). This study reports on the use of p-EASI in a randomized controlled trial with tralokinumab treatment. The safety and efficacy of tralokinumab in AD have been shown in several clinical trials.

A total of 198 adult patients with moderate-to-severe AD were randomly included from the total population of the ECZema TRAlokinumab Trial No. 1 trial (N = 802) (NCT03131648). Written informed consent was obtained from all participants, and the trial was approved by the ethics committee of the Medical Faculty at the Ludwig-Maximilian University of Munich (Munich, Germany) (Wollenberg et al., 2021).

Baseline characteristics were well-balanced across treatment and placebo groups (Supplementary Table S1). A total of 149 patients were treated with subcutaneous 300 mg tralokinumab every other week, and 49 patients received a placebo for 16 weeks. If medically necessary (e.g., intolerable AD symptoms), rescue treatment for AD was provided at the discretion of the investigator. Disease severity was assessed by EASI, and serum was collected before initiation of treatment (time 0) and after 16 weeks of treatment (time 3). Serum TARC, sIL-2R, and IL-22 levels were measured using a multiplex immunoassay, as previously described (Bakker et al., 2020; Thijs et al., 2019, 2017). Differences between the two time points were tested by Wilcoxon signed-rank tests. All patients provided informed consent.

Tralokinumab treatment significantly decreased median EASI scores from baseline (30.9, interquartile range [IQR] = 22.5–42.3) through week 16 (13.5, IQR = 6.6–22.5, \( P < 0.0001 \)). At week 16, the median percentage changes from baseline in the levels of TARC measured were significantly larger in the tralokinumab-treated patients than in the placebo-treated group (Figure 1). The largest difference was observed for TARC levels in tralokinumab versus placebo (−28.33%, \( P = 0.0007 \)) and IL-22 (−23.63% vs. −24.98, \( P = 0.94 \)) showed comparable decreases in both groups (Figure 1 and Supplementary Table S2).

Serum biomarker levels were used to calculate p-EASI scores at two time points using the formula: \( b_0 + b_1 \times \log (\text{TARC}) + b_2 \times \text{IL-22} + b_3 \times \text{sIL-2R} \) (Thijs et al., 2017), a linear combination of biomarkers with coefficients that can vary over the treatment course. p-EASI is an algorithm to evaluate severity on the basis of serum markers rather than a static formula. At time point \( t \), the coefficients are estimated that can vary over the treatment course. Application of this algorithm on datasets with different ranges in EASI and biomarkers (values that are not limited to a certain maximum) will generate different coefficients. In addition, the output of p-EASI is not constrained to the range of EASI (0–72). Therefore, we proposed a translation factor with the existing data to facilitate its use in clinical practice. Consequently, p-EASI can be compared across different studies by mapping its value into the same range of EASI.

The EASI and p-EASI scores showed a moderate correlation (Spearman correlation \( r = 0.59, P < 0.0001 \)).
SUPPLEMENTARY MATERIALS AND METHODS

Chemicals
Benzo(a)pyrene (B-1760) and Indeno [1.2.3-cd] pyrene (4-8499) were provided by Sigma-Aldrich (St. Louis, MO).

Cells and reconstructed skin culture and tissue treatments
The procedure for this is shown in Figure 1a. Skin model was reconstructed as already reported (Bernerd and Asselineau, 2008). Lattices containing collagen and normal human adult fibroblasts were provided by Episkin (Lyon, France). Keratinocytes were prepared from plastic mammary surgery explants, with written and informed consent of subjects provided to the surgeon (two donors). On day (D) 0 keratinocytes were seeded on the dermal equivalent containing type 1 collagen and normal human fibroblasts. Keratinocytes proliferated and differentiated progressively; tissues were raised to the air–liquid interface on D6 just after the second UVA1 exposure. Epidermis reconstruction was performed until D13. Polycyclic aromatic hydrocarbons (15 nM benzo[a]pyrene or 7.5 nM indeno.pyrene) were provided in the medium throughout the reconstruction from D3. UVA1 exposures (7.5 J/cm²) were on D4, D6, and D7. Fresh polycyclic aromatic hydrocarbons solution prepared in PBS were provided 30 minutes before each UVA1 exposure and when the medium was changed (after each exposure and every 2 days).

Light source and spectral measurement
The light source was a solar UV simulator (Oriel, Stratford, CT) equipped with a 1,000 W xenon short-arc lamp and a dichroic mirror. UVA1 was obtained using WG360 (Monaderm, Rue des Violettres, Monaco). Incident UV spectrum (Supplementary Figure S6) and UV doses were assessed with a spectroradiometer (9910-V7, Macam, Livingston, Scotland).

Histology staining and immunolabelling
Histology was performed on D10 and D13. Reconstructed skin samples were fixed in 4% formaldehyde solution and treated for classical histology. A total of 5 mm paraffin sections were stained with hematoxylin, eosin, and saffron (Novaxia, Saint-Laurent-Nouan, France). Hematoxylin, eosin, and saffron histology images were acquired on a Nanozoomer (Hamamatsu, Massy, France). Immunostainings were performed on paraffin-embedded sections by Exicoline (Elancourt, France) using an autostainer system (Ventana Ultra, Roche, Boulogne-Billancourt, France). Briefly, after deparaffinization and rehydration steps, antigen retrieval was performed; then, slides were blocked with normal serum and incubated with primary antibodies. Specific primary mAbs were used to detect proteins involved in epidermis differentiation and proliferation: FLG (ab17808, Abcam, Cambridge, United Kingdom), Ki-67 (Ma5-14520, Tebu-Bio, Le Perray-en-Yvelines, France), loricin (BLE90101, Ozyme, Saint-Cyr-l’Ecole, France), SASpase (NB2-33981, Novus Biologicals, Littleton, CO), kallikrein-5 (AF1108, R&D Systems, Minneapolis, MN), cytokeratin 14 (NB2-33455, Novus Biologicals, Centennial, CO), and keratin 10 (ab111447, Abcam, Tel Aviv, Israel). Then specific secondary antibodies were applied: Alexa fluor 488 or Alexa fluor 568 (Molecular Probes, Invitrogen, Waltham, MA). Nuclear counterstaining was performed with DAPI.

Morphology study
Total epidermis and living epidermis thickness were quantified on hematoxylin, eosin, and saffron histology images using a specific image processing tool designed to automatically identify three limits of the tissue: surface, stratum corneum/living epidermis limit, and basal layer, as recently reported (Dimitrov et al., 2021). Different color thresholds and morphological operators are used to segment RGB color nanozoomed images (large field view). These specific algorithms were introduced into a user-friendly dedicated software developed by the company ADCIS (Saint-Contest, France). This software allows us to process images in a high-throughput way, including quantification of the morphological parameters of the tissue, especially the thickness of each layer. Representative images were extracted using the viewing software NDP View (Hamamatsu, Massy, France) for morphology observation. For fluorescence signal distribution analysis in the reconstructed skin layers, we used ImageJ (National Institutes of Health, Bethesda, MD). Fluorescent staining was quantified using an intensity profile along a line that was drawn manually. FLG, loricin, kallikrein-5, and SASpase staining was quantified using an intensity profile along a line that was drawn manually, and the mean intensity for each pixel was identified. Keratin 10 and 14 staining was quantified with average thickness area positively stained. The amount of Ki-67–positive cells in the basal layer of the epidermis was assessed using pixel intensity, and results were expressed as a number of Ki-67 per unit length of the epidermis.

Lipid peroxidation
Culture media were collected on D10 of epidermis reconstruction, and 8-isoprostane level was measured using Cayman’s 8-isoprostane Elisa kit 516351 (Interchim, Montluçon, France) as per the manufacturer’s instructions.

Quantification of excreted markers
Cytokines IL-6, IL-8, IL-1Ra, matrix metalloproteinase 1, and matrix metalloproteinase 3 concentrations were measured in the culture medium on D10 and D13 of epidermis reconstruction using the R&D Systems (Minneapolis, MN) kit (LXSAHM-10 96 tests-kit Luminex 10 plex) as per the manufacturer’s instructions with the Bio-Rad BioPlex 200 Array Reader (Marnes la Coquette, France).

Quantitative Real-Time RT-qPCR reverse transcription analysis
Briefly, RNA was extracted using RNeasy (Qiagen, Courtaboeuf, France), and purified RNAs were checked for integrity and quantified using LabChip (Perkin Elmer, Villebon-sur-Yvette, France). Reverse transcription of mRNA into cDNA was performed using O Zobiri et al. UVA1 and Pollution Impair Skin Barrier Function
a QuantiTect Reverse Transcription kit (Qiagen). Real-time PCR was performed using Sybr green I mastermix and the LightCycler 480 instrument (Roche, Boulogne-Billancourt, France) according to the manufacturer’s optimized procedure. The expression of each target gene (Supplementary Table S1) was normalized with the average expression of RPL13 as a housekeeping gene. Data analysis was completed using the Biosystems 7500 real-time PCR software, version 3.1, and relative quantification was determined by the 2-\(\Delta\Delta Ct\) method.

**Protein extraction and western blot analysis**

On D10 and D13, protein assays were performed on the epidermis using Wes automated western blot system (ProteinSimple, San Jose, CA) (Harris, 2015) by Synelvia (Labe`ge, France). Briefly, total protein was extracted using RIPA lysis buffer, and protein concentration was estimated using the BCA protein assay kit. Antibodies targeting FLG (sc-66192, Santa Cruz Biotechnology, Dallas, TX) and anti–caspase 14 (sc-48336, Santa Cruz Biotechnology) were used. Total protein measurement was used for western blot loading controls.

**Urocanic acid trans and pyroglutamic acid quantification**

On D10 and D13, the amount of pyroglutamic acid and trans-urocanic acid was analyzed on the epidermis by Synelvia by liquid chromatography-tandem mass spectrometry, and results were expressed in \(\mu\text{g/mg}\) of proteins.

**Statistical analysis**

Analysis of variance was performed with GraphPad Prism 8.0.0 software. Ordinary one-way or two-way ANOVA with Dunnett’s multiple comparison test versus DMSO UVA1 was applied to determine the \(P\)-value. Data with \(P < 0.05\) were considered statistically significant: *\(P < 0.05\), **\(P < 0.01\), or ***\(P < 0.001\).

**SUPPLEMENTARY REFERENCES**


Supplementary Figure S1. Impact of UVA1 without or with BaP on immunostaining of K10 and impact of UVA1 without or with IcdP on parakeratosis and immunofluorescence staining pattern of FLG. (a) Images of K10 immunofluorescence staining and quantification on D10 (K10 appears in green in the suprabasal epidermis layers, and nuclei appear in red). K10, generally associated with differentiated suprabasal cells, was also significantly impacted by UVA1. Impact of UVA1 without or with IcdP on differentiation. (b) Zoom on representative parakeratosis images of HES staining on D13. Quantification of parakeratosis (number of nuclei or granules) in a defined area (mm²) in SC on D13. (c) Images of FLG immunofluorescence staining and quantification (FLG appears in green in the upper epidermis, and nuclei appear in red). Bar = 100 μm. *P < 0.05, **P < 0.01, and ***P < 0.001 (at least two independent experiments with n = 6 biological replicates per condition). Control DMSO appears in pink, BaP or IcdP alone appears in orange, UVA1 alone appears in khaki, and UVA1 + BaP or UVA1 + IcdP appears in green. BaP, benzo[a]pyrene; D, day; HES, hematoxylin, eosin, and saffron; IcdP, indenopyrene; K10, keratin 10; SC, stratum corneum.
The 8-isoprostane release in culture medium, gene expression of oxidative stress and inflammation markers and of AhR, CYP1A1, and CYP1B1 after exposure to UVA1 without or with BaP.

(a) The 8-isoprostane concentration in the culture medium was measured using ELISA on D10. (b) qRT-PCR analysis of HMOX1, TXNRD1, COX2/PTGS2, and TNFα mRNA expression on D8. (c) qRT-PCR analysis of AhR mRNA expression on D8 and of CYP1A1 and CYP1B1 on D8 and D13. mRNA expression was normalized to housekeeping gene RPL13. *P < 0.05, **P < 0.01, and ***P < 0.001 (at least two independent experiments with n = 6 biological replicates per condition). DMSO control appears in pink, BaP alone appears in orange, UVA1 alone appears in khaki, and UVA1 + BaP appears in green. The 8-isoprostane, a marker of lipid oxidation, was still released in the culture medium on D10 (especially in UVA1 + BaP samples), 3 days after the last UVA1 exposure. In line with this, overtranscription of antioxidant genes (HMOX1, TXNRD1) or of inflammation genes associated with oxidative stress (COX2, TNFα) occurred immediately after the last UVA1 irradiation on D8 and was enhanced by BaP, except for TNFα.

Surprisingly, UVA1 negatively impacted the AhR pathway. On D8, the AhR gene was underexpressed after UVA1 exposure (without or with BaP). On D8, the CYP1B1 gene was significantly induced by BaP in the dark as expected, whereas UVA1 exposure decreased its expression. On D8, CYP1A1 maintained basal expression in all samples. Exposure to UVA1 significantly disturbed AhR expression and reversed its activation by PAHs occurring in the dark (as shown by modulation of CYP1B1 expression). AhR dysregulation probably had a negative impact on FLG and LOR expression, contributing to skin barrier impairment (Yurita et al., 2015). Moreover, induction of COX2 or TNFα by PAH + UVA1 may also have aggravated defects in FLG maturation/processing, as already reported for PM in the dark (Kim et al., 2021, 2011; Lee et al., 2016). AhR, aryl hydrocarbon receptor; BaP, benzo[a]pyrene; D, day; HMOX1, heme oxygenase-1; LOR, loricrin; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter; TXNRD1, thioredoxin reductase 1.
Supplementary Figure S3. Expression and/or secretion of cytokines and MMPs after exposure to UVA1 without or with BaP.

(a) qRT-PCR analysis of IL8, IL1α, and IL1β mRNA expression on D10 and D13. mRNA expression was normalized to housekeeping gene RPL13. (b) Cytokines IL-6, IL8, and IL-1ra concentrations were measured in the culture medium on D10 and D13. (c) MMP1 and MMP3 concentrations were measured in the culture medium on D10 and D13. *P < 0.05, **P < 0.01, and ***P < 0.001 (three independent experiments with n = 6 biological replicates per condition). DMSO control appears in pink, BaP alone appears in orange, UVA1 alone appears in khaki, and UVA1 + BaP appears in green. IL8 was particularly overexpressed at mRNA and peptide level in UVA1 and UVA1 + BaP. At mRNA level in the epidermis, IL1α was overexpressed on D10 and more significantly on D13 in UVA1 + BaP samples. However, IL1α was not released in the culture medium, probably in line with the low cytotoxicity of the treatments. IL1α pathway stimulation was indirectly confirmed by IL-1ra expression, an antagonist reported after IL1α modulation (Martin et al., 2021), which particularly increased after exposure to UVA1 + BaP. IL1β

D10 IL1a

D10 IL1b

D10 IL6

D10 IL8

D10 MMP1

D10 MMP3

D13 IL1a

D13 IL1b

D13 IL6

D13 IL8

D13 MMP1

D13 MMP3
overtranscription was significant in the epidermis on D10 and D13. *IL1β* overexpression occurred mainly in UVA1 + BaP samples; however it could not be detected in the medium. In our model, late overexpression of *IL1α* and *IL1β* might be related to recovery of barrier function after stress (Hanel et al., 2013). The significant induction of *IL6* by UVA1 appeared further enhanced in the presence of BaP on D10 and D13. *IL6* excess production might stimulate keratinocyte proliferation and could provide the cells required for epidermal neodifferentiation after stress. Modulation of these markers suggested that epidermal regeneration recurred after stress in our dynamic 3D model.
Supplementary Figure S5. Summary diagram of epidermal marker modulation by UVA1 and UVA1 + PAHs. Modulation by UVA1 only is in black font, and aggravation by UVA1 + PAHs is in red font. Italic font refers to mRNA assessment only. This diagram highlights the induction of oxidative stress, cytokine expression, MMP release, and epidermal differentiation impairment. Some of these defects are reported in the epidermis of patients with AD. AD, atopic dermatitis; BM, basal membrane; Casp14, caspase 14; HMOX1, heme oxygenase-1; KLK5, kallikrein-5; MMP, matrix metalloproteinase; PAH, polycyclic aromatic hydrocarbon; PCA, pyroglutamic acid; SC, stratum corneum; TXNRD1, thioredoxin reductase 1; UCA, urocanic acid.

Supplementary Figure S6. Spectral power distributions of UVA1 from solar simulator used in experiments (purple) compared to sunlight as defined by COLIPA (blue).
Supplementary Table S1. List of Primers Used for RT-qPCR

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Abbreviations: KLK5, kallikrein-5; LOR, loricrin; MMP, matrix metalloproteinase. Primer list and references are provided.