Effects of cigarette smoke on the skin in comparison to UV radiation: parallels and differences

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Abstract

The human skin is the outermost barrier of the human body and therefore an important defense system. Over the years, there has been a continuous increase in air pollutions that affects the human skin. Cigarette smoke (CS) is a highly complex aerosol composed of various chemical substances, reactive oxygen species as well as carcinogens such as several polycyclic aromatic hydrocarbons. There is a relationship between CS and the development of dermal diseases. Ultraviolet radiation (UVR) is a physical pollutant that causes extrinsic skin aging triggered by different pathways which are intersected with those that are triggered by CS exposure such as the aryl hydrocarbon receptor activation. In this study the effects of CS on the skin in comparison to UVR were studied applying the Suction Blister Method on healthy Caucasian volunteers after pollutant exposure. CS exposure and UVR resulted in an increase of matrix metalloproteinase-1 and Interleukin-8 expression levels in the interstitial fluid and an epidermal Langerhans' cell depletion. Furthermore, the influence of CS on the epidermal barrier lipid lamellae in the intracellular space of the stratum corneum (SC) was investigated using the Lipbarvis® technology. The total length of the lipid lamellae between the corneocytes was significantly reduced after CS and UVR exposure. Thus, likewise UVR, CS causes cutaneous immunosuppression which is associated with the pathogenesis of skin cancer as well as dermal collagen degradation, which is associated with extrinsic skin aging. Both pollutants verifiably damage the epidermal skin barrier.

Introduction

The skin is one of the main targets of pollution and in particular of CS as a very common environmental factor. Among other effects CS causes the generation of oxidative stress, increases transepidermal water loss (TEWL) and the expression level of matrix metalloproteinases (MMPs) with concurrent reduction in collagen formation. Further, a relationship between CS and the incidence of dermal diseases development such as psoriasis as well as skin aging was shown [1–4]. CS inhibits antioxidant enzymes such as the superoxide dismutase and leads to elevated levels of reactive oxygen species (ROS) which leads to oxidative stress and fibroblast senescence.

Additionally, the fibroblast growth and proliferation are impaired by CS [5, 6, 3]. It is already evident that CS leads to an in-

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creased MMP-1 expression level in lung tissue [7–9], aortic endothelial cells [10] and human skin explants [11].

Another environmental key factor in the development of various dermal diseases is UVR [3].

In general, the skin contains a defense line against pathogens and environmental, harmful substances – the Langerhans cells (LCs) [12]. The skin's immune system protects and eliminates mutated skin cells in the prevention of skin cancer. A high UV exposure weakens the immune system of the skin by damaging epidermal LCs. Depending on dose it leads to a depletion of LCs in the epidermis, a loss of dendritic extensions as well as a rounding of cells. In addition, there is a loss of function regarding antigen presentation to T cells [13–16]. Besides these immunosuppressive effects, UV radiation induces DNA damage which results in the generation of skin cancer [13, 17, 15, 3]. An immunosuppressive effect has also been shown in lung tissue of CS-exposed mice [18].

Interleukin (IL)-8 is a chemokine that is also known as *neutrophil chemotactic* factor which already describes its primary function [19]. An increase in IL-8 was observed after CS treatment in cell culture studies on human fibroblasts, keratinocytes and reconstructed epidermis [20, 21]. ROS are the initiating element in the increased release of various pro-inflammatory mediators from skin cells by activating transcription factors. Different signaling pathways are activated by those transcription factors which are involved in cell growth, differentiation as well as degradation of the dermal connective tissue [2].

The elimination of harmful pollutants in the environment is a tedious and difficult process, if not impossible to achieve and the air pollution is increasing rather than decreasing. As a result, there is an increasing demand for remedies that counteract the effects of the pollutants daily surrounding the human body damaging the skin and other organs. There is a need for new topical preparations against pollution effects to be tested before treatment.

For the investigation of pollution effects on the skin and the anti-pollution claim support we used the smoke chamber model. Here, the effects of smoke and UV on skin were studied for the first time *in vivo* in a clinical trial applying the Suction Blister method. After an *in vivo* treatment with smoke and UV, blisters were artificially induced. Afterwards, the complete viable epidermis with intact basal layer and the interstitial fluid within the blisters were used for analyzing the migration of Langerhans cells as well as the release of MMP-1, a collagenase of the extracellular matrix, and the release of IL-8. In addition, the lipid barrier visualization (Lipbarvis®) technique and SC lipid composition analysis (HPTLC) has been used to investigate the epidermal skin barrier.

Material and Methods

Human volunteers and clinical study design

A clinical study was conducted to obtain epidermis and skin fluids from UV-exposed as well as CSexposed and unexposed control skin of 12 healthy male and female Caucasian volunteers (age 42.3 ± 8.6 years; non-smokers) with type I – III skin phototypes. Their skin was exposed on the forearms to 1 minimal erythema dose (MED) using the sun simulator SOL 500 (Dr. Hönle AG, Gräfelfing, Germany) and/or CS from three cigarettes over 30 minutes using a self-designed smoking machine. The CS- and UV-exposition were performed after baseline measurements. Suction blisters were collected from unexposed as well as CS- and UV-exposed skin 24 hours and 48 hours after exposition. The complete viable epidermis with intact basal layer and the interstitial fluid within the blisters were used for analyzing the migration of Langerhans cells as well as the expression level of MMP-1 and IL-8. One subject was excluded from the data analysis of Langerhans cell staining, since not enough material could be obtained from the blister roofs after suction blister formation.

Suction blister chambers

SC lipid lamellae structure and lipid content were analyzed using the lipid barrier visualization (Lipbarvis®) technique.

The clinical study was conducted in the SGS INSTITUT FRESENIUS GmbH laboratories in accordance with the Declaration of Helsinki Principles and its amendment and written informed consent was obtained from all study volunteers.

Determination of the MED

The determination of the minimal erythema dose (MED), i.e. the minimal UV dose required to cause a definite erythema rated as 1.0, was carried out with the sun simulator SOL 500 (Dr. Hönle AG, Gräfelfing, Germany) prior to the irradiation of the test sites for induction of an UV erythema.

First, the light sensitivity of the subject was determined with respect to the definition of photo types according to Fitzpatrick [22]. Then, each subject was irradiated on the left or right volar forearm using six single doses with a 25 % incremental progression. The visual evaluation of the erythema and the resulting individual determination of the MED were carried out 24 hours after irradiation.

The investigator assessed the grade of erythema on each test site under daylight conditions using an erythema score according to Frosch, P.J. and Klingman, A.M. [23].

UV Irradiation

The UV irradiation was performed with the sun simulator SOL 500 (Dr. Hönle AG, Gräfelfing, Germany) equipped with a H2 filter in a distance of approx. 23 cm to the test areas. First, a light-impermeable template with one hole corresponding to the specified test sites was attached to the inner forearm. All other parts of the forearms were covered to protect these areas from UV radiation. Then test area was irradiated with 1 MED.

Cigarette smoke exposure

CS was generated by a self-designed smoking machine. For each voluntary participant, 3 cigarettes (Mohawk Classic Red, Grand River Enterprises GmbH, Rietz / Kloster Lehnin, Germany) were used. Thereby, the smoke of each cigarette was passed through custom-made plexiglass chambers, which were fixed to the respective area, in a time of approx. 5 minutes by means of a Rotilabo®-membrane vacuum pump CR-MV100 (Carl Roth GmbH + Co, Karlsruhe, Germany), operating a constant airflow to draw the smoke filtered cigarettes through the plexiglass chambers on the respective skin area. After each single cigarette, the pump was switched off and the smoke of each cigarette was left on the respective area for 5 further minutes. There were 4 areas that were exposed with CS for overall 30 minutes.

Suction blister generation

Suction blisters were generated on the skin from each test area (unexposed control area, CS-exposed area and UV-exposed area) by using custom-made plexiglass suction chambers having three circular openings of 7 mm diameter. A low negative pressure (550 – 850 mbar) was applied, and the blisters were induced during a time period of 1.5 – 2.5 hours. The blisters were formed in the diameter of the suction openings of the plexiglass chamber, whereat after approx. 2 hours the epidermis was slowly detached from the underlying dermis. The blister fluid (interstitial fluid) was extracted with a sterile syringe and the roofs of the generated suction blisters were removed afterwards under sterile conditions. The small wounds caused by this procedure were treated with Hansaplast® Active Gel Strips (Beiersdorf AG, Hamburg, Germany). All wounds had healed completely, scar-free, within 6 – 10 days.

24 (± 2) hours and 48 (± 2) hours after UV- and CS-exposition the generation of suction blisters on the exposed areas as well as on the unexposed area was performed to analyze the expression level of IL-8 and MMP-1 in the blister fluid (after 24 (± 2) hours) and the number of Langerhans cells in the epidermal cells from the blister roof (after 48 (± 2) hours).

Blister roofs preparation for Langerhans cell staining

The entire staining procedure of the blister roofs was carried out in 24-well cell culture plates with a volume of 1.0 ml per well. The preparation (blister top) was first rinsed briefly in PBS solution. It was then incubated for 20 minutes at 4°C in the fixing solution. This was followed by three rinsing steps in 0.9% NaCl solution at 4°C. It was then incubated for 30 minutes at 37°C in the staining solution. This was followed by another two 5-minute rinses with 0.9% NaCl solution. Subsequent incubation in the precipitation solution led to the formation of the dark-colored PbS precipitate. Finally, two 5-minute rinses in PBS were carried out at 4°C. The preparation was then fixed to the slide with a drop of Aquatex and covered.

Evaluation of the number of maintained Langerhans cells

The number of stained Langerhans cells in the preparation was determined microscopically (Olympus BX41, Hamburg, Germany) and this number calculated as cells per mm^2. For each stained blister roof the number of Langerhans cells of four areas was evaluated. The results were allocated to test areas without the knowledge of the evaluator.

Determination of IL-8 and MMP-1 in the interstitial fluid of suction blisters

Suction blister fluids (interstitial fluid) from each test area were collected using a sterile syringe and were stored at -80 C until further analysis. The IL-8 and MMP-1 level in the interstitial fluid of each approach was measured by using ELISA Kits (RayBio Human MMP-1 ELISA Kit, RayBiotech, Georgia, USA and Human IL-8 UltraSensitive, Invitrogen, Camarillo, USA).

Lipbarvis® technology and determination of skin lipids

A noninvasive skin sampling technique (Lipbarvis®, Microscopy Services Dähnhardt GmbH, Flintbek, Germany) was employed to determine SC lipid lamellae structure and lipid content, as previously described. [24 – 27] Briefly, corneocytes were removed from the skin surface using the adhesive Lipbarvis® and a special carrier. Samples were then prepared for analysis of intercellular lipid lamellae (ICLL) organization in the SC by transmission electron microscopy (TEM CM 10, FEI, Eindhoven, The Netherlands) [28]. Lipid content was determined using a high-performance thin layer chromatographic (HPTLC) method with densitometry [29, 30]. All Lipbarvis® samples were obtained from a region of the test areas not used for instrumental measurements. Lipbarvis® sampling was performed on the irradiated test area, the test area treated with cigarette smoke and a control area.

Statistical analysis

The analysis of the study objective was performed by SGS INSTITUT FRESENIUS GmbH using the computer software Microsoft EXCEL (Microsoft Office 2010) and STATISTICA (Version 13.3). Microsoft EXCEL was used for the calculation of the relative data, the sample size, means, standard deviations as well as the minimum and maximum values. STATISTICA was used for analyzing the distribution of the data (Kolmogorov-Smirnov-Test) and for analyzing the significance of differences (ANOVA for repeated measures with post hoc pair wise comparisons by Fisher LSD Test for normally distributed data; Friedman ANOVA with post hoc pair wise comparisons by Wilcoxon Test for paired samples for not normally distributed data). The hypothesis of a normal distribution was accepted when there was a p-value > 0.05. Concerning the differences between the treatment situations and the measurement times, in the case of a p-value ≤ 0.05 a difference was accepted as statistically significant.

Regarding the analysis of transepidermal water loss values, the original data and the data relative to the point in time after 24 hours were analyzed. The relative data reflect the changes in the

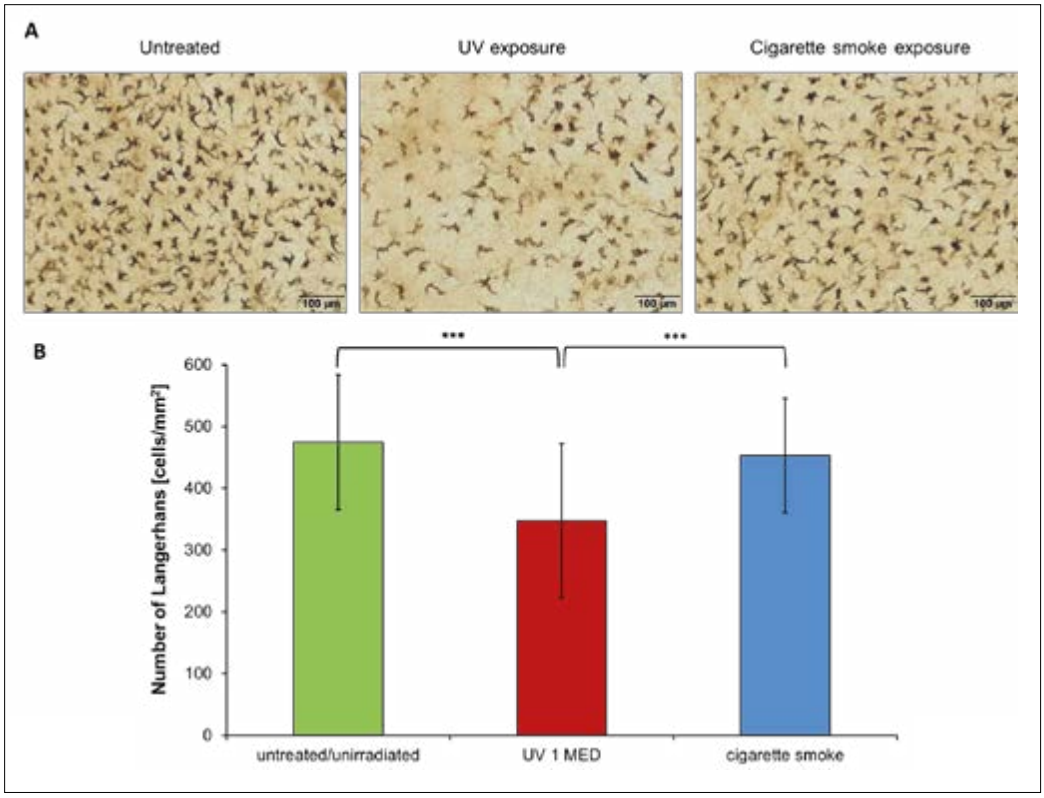

Fig. 1: Langerhans cell staining (A) and total number of Langerhans cells (B) 48 hours after exposure to pollutants; ultraviolet radiation of human skin and exposure of human skin to cigarette smoke, untreated/unirradiated skin was used as a control (A, 200 × magnification); harvest of blister roofs after 48 hours; analysis of Langerhans cell migration; ANOVA for repeated measures with post hoc pairwise comparisons by Fisher LSD Test, ***p < 0.001, n = 11.

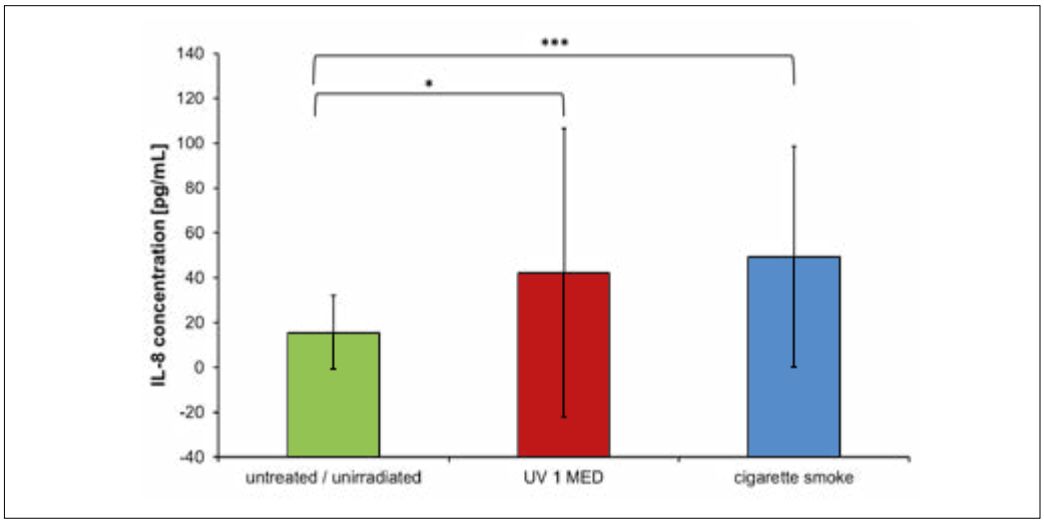

Fig. 2: Interstitial fluid analysis of suction blisters 24 hours after exposure to pollutants; cytokine concentration of Interleukin-8 (pg/ml); Friedman ANOVA with pairwise comparisons by Wilcoxon Test for paired samples for not normally distributed data,
**p < 0.01, *p < 0.05, n = 12.

parameter taking account of differences between the test sites at the point in time after 24 hours, i.e. after the UV irradiation and before exposition with UV and/or CS.

For the determination of Langerhans cell numbers, original data and difference data were analyzed 48 hours after UV- and/or CS-exposition.

Results

CS and UVR induce depletion of Langerhans cells

48 hours after exposure to pollutants, UVR induced a highly significant ($p < 0.0001$) LC depletion with an average 27 % decrease from 475 ± 109.27 cells/mm² to 347 ± 124.95 cells/mm² in the epidermal LC count. CS induced only a slight depletion with an average 4 % decrease in epidermal LC without statistical significance ($p = 0.3995$, shown in Fig. 1). In addition, a statistically significant difference in the number of LC could be observed between cigarette smoke exposure (454 ± 92.56 cells/mm²) and UVR ($p = 0.0003$), with UVR showing a higher depletion (347 ± 124.95 cells/mm²).

CS and UVR lead to increased release of pro-inflammatory mediators and matrix metalloproteinases

Both, CS ($p = 0.0022$) and UVR ($p = 0.0281$), lead to a significantly increased release of the pro-inflammatory cytokine IL-8 (Fig. 2) 24 hours after exposure to these pollutants. The starting level was on average 15.75 ± 16.39 pg/ml. No differences in IL-8 concentration between CS (49.52 ± 49.03 pg/mL) and UVR (42.28 ± 64.22 pg/ml) exposure could be found.

Furthermore, CS ($p = 0.0037$) as well as UVR ($p = 0.0022$), lead to a significantly increased release of the matrix metallo-

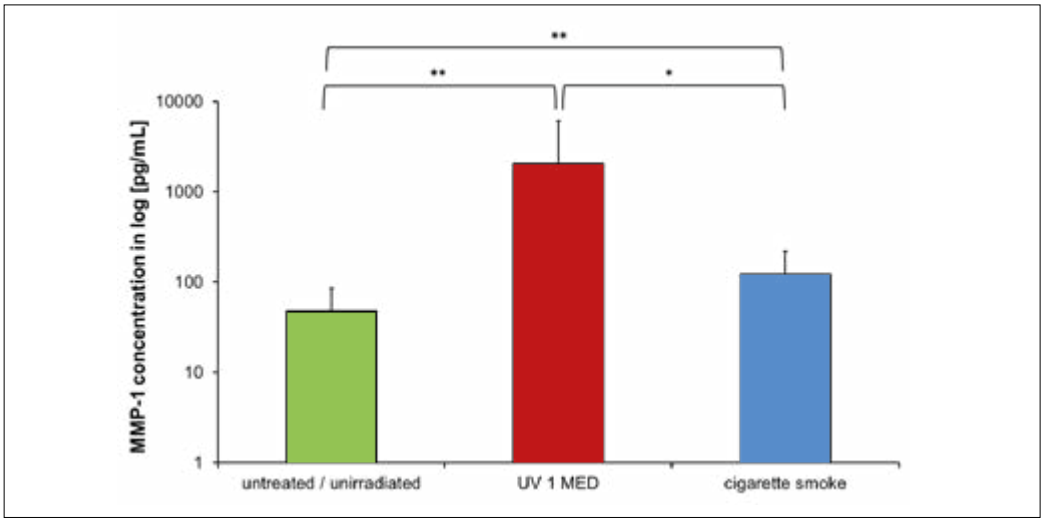

Fig. 3: Interstitial fluid analysis of suction blisters 24 hours after exposure to pollutants; concentration of matrix metalloproteinase-1 log[pg/mL]; Friedman ANOVA with pairwise comparisons by Wilcoxon Test for paired samples for not normally distributed data, **p < 0.01, *p < 0.05, n = 12.

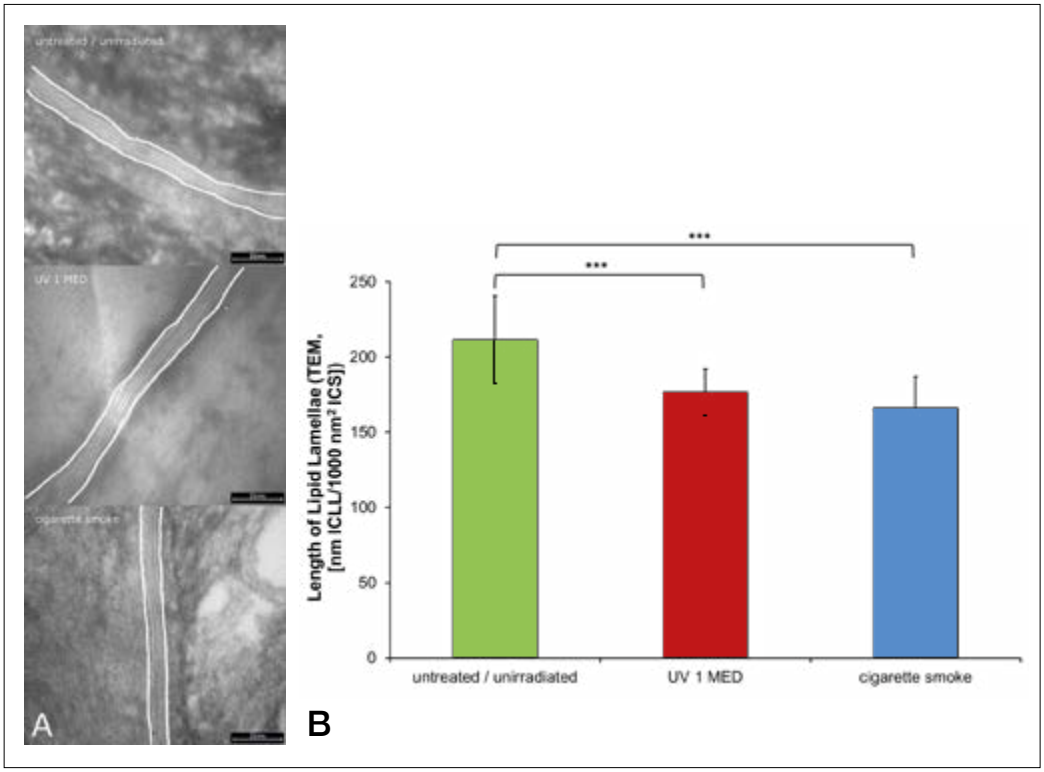

Fig.4: Investigation of the epidermal skin barrier 24 hours after exposure to pollutants; (A) Transmission Electron Microscopy (TEM) of the lipid lamellae in the intercellular space after 24 h exposure to different pollutants. The white lines marks the transition from the corneocytes to the intercellular space filled with the lipid lamellae. (B) Length of lipid lamellae per 1000 nm² intercellular space in the stratum corneum in untreated, UV irradiated and cigarette smoke treated skin samples. Skin samples in the range of 3 – 5 cell layers were generated by using a particularly gentle adhesive / carrier system and examined by transmission electron microscopy; length of lipid lamellae was determined and related to the intracellular space; ANOVA for repeated measures with post hoc pairwise comparisons by Fisher LSD Test, ***p < 0.001, n = 12.

proteinase 1 (MMP-1) 24 hours after exposure to these pollutants (Fig. 3) in comparison to the untreated/ unirradiated test site (48.24 ± 37.02 pg/ml). A statistically significant difference could be observed between the two treatments (p = 0.0229), with UVR (2055.98 ± 4025.60 pg/ml) showing a higher MMP release in comparison to CS exposure (123.13 ± 98.54 pg/ml).

CS and UVR damage the epidermal skin barrier

Electron microscopic evaluation shows that with both treatments, UVR and CS, a significant reduction of intercellular lipids in the intercellular space between the corneocytes was detectable, compared to the untreated skin (Fig. 4 A). The length of lipid lamellae was statistically significantly reduced 24 hours after UR by 15% (176.7 ± 15.2 nm | CCL/1000 nm² | CS, p = 0.0002) and CS by 21% (166.0 ± 21.0 nm | CCL/1000 nm² | CS, p < 0.0001) compared to the untreated test site (211.5 ± 29.1 nm | CCL/1000 nm² | CS). Although, the reduction after CS exposure was slightly pronounced, there was no statistically significant difference between both treatment situations (Fig.4 B).

Along with the reduction in lipid lamellae length, UVR and CS induced a reduction of free fatty acids (FFA), ceramides EOS and NP in the lipid layer of the SC (shown in Fig. 5). The untreated starting values were in a range from 2.3 ± 1.2 µg/carrier for FFA, 3.0 ± 1.4 µg/carrier for Ceramide EOS and 7.6 ± 2.9 µg/carrier for Ceramide NP. The reduction induced by UVR was slightly pronounced (FFA by 28% (1.3 ± 0.3 µg/carrier), Ceramide EOS by 20% (2.3 ± 1.0 µg/carrier) and Ceramide NP by 7% (6.2 ± 2.1 µg/carrier)) in comparison to CS exposure (FFA by 12% (1.7 ± 0.6 µg/carrier), Ceramide EOS by 17% (2.4 ± 1.2 µg/carrier) and Ceramide NP by 6% (6.6 ± 2.2 µg/carrier)). Statistical significance was reached for FFA (UV: p = 0.0008, CS: p = 0.0387) and ceramide EOS (UV: p = 0.0079, CS: p = 0.0328). No differences between the CS and UVR could be observed for any of the other analyzed lipids.

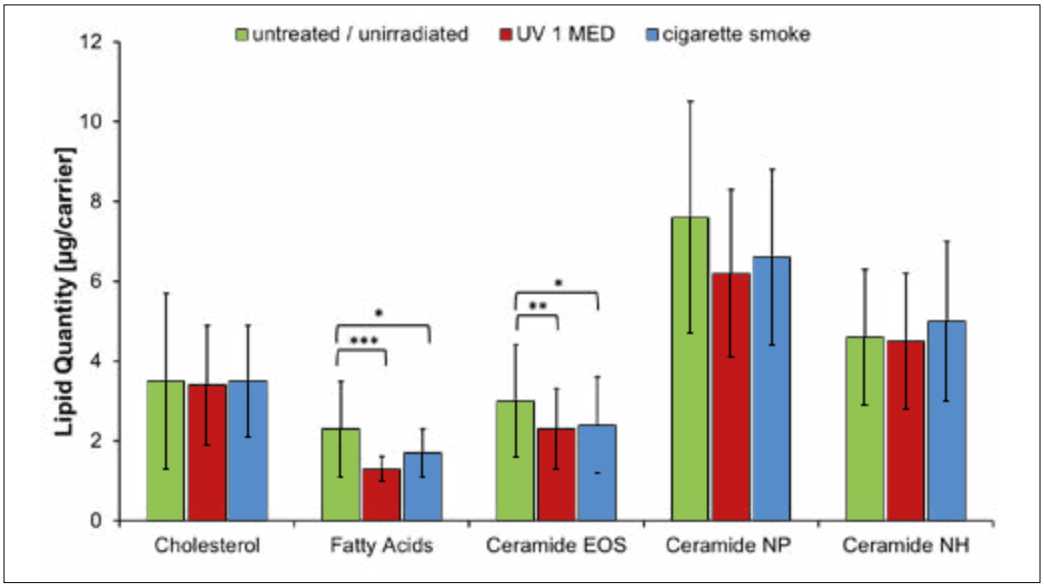

Fig. 5. Determination of skin lipids 24 hours after exposure with pollutants; Skin samples in the range of 3 – 5 cell layers were generated by using a particularly gentle adhesive / carrier system and analyzed using the High Performance Thin Layer Chromatography (HPTLC); quantitative determination of skin lipids; ANOVA for repeated measures with post hoc pairwise comparisons by Fisher LSD Test for normally distributed data or Friedman ANOVA with pairwise comparisons by Wilcoxon Test for paired samples for not normally distributed data, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $n = 12$.

Discussion

The skin is frequently exposed to pollution since it is the biggest organ of the human body and a barrier between the environment and human internal organs. The role of different pollutants in dermal diseases, immunosuppression and premature aging was studied in various epidemiological studies as well as *in vivo* animal studies and *in vitro* studies using human skin cells [17, 15, 2, 3].

The human skin consists of the stratum corneum which is the first important defense line against pollutants and also a physical barrier to prevent trans-epidermal water loss. Besides the physical barrier, the skin contains a chemical barrier composed of ceramides, fatty acids and cholesterol forming a hydrophobic matrix bound to the corneocytes inside the stratum corneum. An additional immunological barrier with Langerhans cells as the most important component forms a defense line against pathogens and maintains the immune homeostasis in the skin [31, 32, 12, 2]. Under CS and UV exposition all three, the physical, the chemical and the immunological barrier function is impaired. The total length of the lipid lamellae in the stratum corneum intercellular space of corneocytes was significantly reduced after CS and UV exposure in comparison to the untreated area on the volunteers' forearms. This is not surprising, since ceramide EOS is an important component for the stratum corneum due to its role in molecular organization in this layer of the skin [32]. The lamellar phases with periodicities of approx. 6 and 13 nm could not form here because there was a reduction in ceramide EOS and NP as well as fatty acids after pollutant exposure. Therefore, the length of the lipid lamellae was reduced as shown in former studies [33, 32]. This indicates an impairment of the skin's chemical barrier function. The immunological barrier is, similar to former studies [34, 35], suppressed due to an epidermal depletion of Langerhans cells. We firstly could show this effect on the skin after CS exposure as well. The depletion of Langerhans cells is maybe due to them, recognizing PAHs in the smoke (which is also on particulate matter in the environment) as antigenic, migrating to the draining lymph nodes and elicits T cell activation.

The dermis localized below the epidermis, is also affected by the impairment of the epidermis. Exposure to pollutants results in a reduction of collagen formation and also in an increased dermal collagen degradation due to pathways triggered by oxidative stresses and the increased expression of pro-inflammatory cytokines such as IL-8 and collagenases such as MMP-1 [2, 3]. After exposure to CS and UVR, the release of IL-8 and MMP-1 was significantly increased. That indicates an additional damage of the skin barrier triggered by the generation of ROS.

The similar effects of both pollutants might be due to related signaling pathways triggered by CS as well as by UV. One of those pathways is the activation of the aryl hydrocarbon receptor (AHR). This receptor is activated by ROS and CS as well as UV exposure cause the generation of ROS [2, 3].

We have shown that alike UVR, CS impairs the overall barrier function of the skin. Such a disturbance of the skin's functions results long-term in the development of skin diseases as well as premature skin aging [2]. The association between CS and the incidence of psoriasis, for example, could be explained by the reduction of free fatty acids and ceramides EOS and NP as well as the resulting reduction in lipid lamellae length and therefore, a reduction of epidermal barrier function.

Even through human-made sources of pollution can be controlled, the reduction of air pollution is a tedious process. Therefore, the development of strategies to protect the skin against pollution becomes very important. Besides using sunscreen, it is now also important to use topical preparations including antioxidants to prevent respectively neutralize the generation of ROS.

Conclusions

In conclusion, UV and CS exposition result in a depletion of epidermal Langerhans cells and a significantly increased expression level of IL-8 and MMP-1, shown by using the Suction Blister method. The epidermal skin barrier was verifiably damaged by CS and UVR. This was demonstrated by a significantly reduced number of intracellular lipid lamellae and the peroxidation of lipids in the SC (Lipbarvis® technology).

We protect ourselves against UV radiation by using sunscreens, but against pollution, there is not that much protection available yet. Therefore, it is important to develop protecting agents to protect our skin against the high increasing air pollution. Together with the smoke chamber model, the Suction Blister method with the analysis of IL-8 and MMP-1 and the Lipbarvis® technology are suitable to detect pollution effects and are thus suitable for anti-pollution claim support.

Conflicts of interest

There are no conflicts of interest.

References

1 *Thomsen SF, Sorensen LT. Smoking and skin disease. Skin Therapy Lett. 2010 Jun;15(6):4-7.*

2 *Valacchi G, Sticozzi C, Pecorelli A, Cervellati F, Cervellati C, Maioli E. Cutaneous responses to environmental stressors. Ann N Y Acad Sci. 2012 Oct;1271:75-81.*

3 *Puri P, Nandar SK, Kathuria S, Ramesh V. Effects of air pollution on the skin: A review. Indian J Dermatol Venereol Leprol. 2017 Jul-Aug;83(4):415-23.*

4 *Hendricks AJ, Eichenfield LF, Shi VY. The impact of airborne pollution on atopic dermatitis: a literature review. Br J Dermatol. 2020 Jul;183(1):16-23.*

5 *Yang GY, Zhang CL, Liu XC, Qian G, Deng DQ. Effects of cigarette smoke extracts on the growth and senescence of skin fibroblasts in vitro. Int J Biol Sci. 2013;9(6):613-23.*

6 *Kim KE, Cho D, Park HJ. Air pollution and skin diseases: Adverse effects of airborne particulate matter on various skin diseases. Life Sci. 2016 May 1;152:126-34.*

7 *Kim H, Liu X, Kobyama T, Kobayashi T, Conner H, Abe S, et al. Cigarette smoke stimulates MMP-1 production by human lung fibroblasts through the ERK1/2 pathway. COPD. 2004 Apr;1(1):13-23.*

8 *Mercer B, Brinckerhoff C, D'Armiento J. Activation of the MMP-1 promoter bycigarette smoke in human small airway epithelial cells requires ERK MAP kinase signaling:differential response of the 1G and 2G promoter sequences. Proc Am Thorac Soc. 2006Aug;3(6):477.*

9 *Mercer BA, Wallace AM, Brinckerhoff CE, D'Armiento JM. Identification of acigarette smoke-responsive region in the distal MMP-1 promoter. Am J Respir Cell Mol Biol.2009 Jan;40(1):4-12.*

10 *Lemaitre V, Dabo AJ, D'Armiento J. Cigarette smoke components induce matrixmetalloproteinase-1 in aortic endothelial cells through inhibition of mTOR signaling. ToxicolSci. 2011 Oct;123(2):542-9.*

11 *Curpen S, Francois-Newton V, Moga A, Hosenally M, Petkar G, Soobramaney V, etal. A novel method for evaluating the effect of pollution on the human skin under controlledconditions. Skin Res Technol. 2020 Jan;26(1):50-60.*

12 *Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. Immunity. 2012 May 25;36(5):873-84.*

13 *Berneburg M, Krutmann J. Photoimmunology, DNA repair and photocarcinogenesis. J Photochem Photobiol B. 2000 Feb;54(2-3):87-93.*

14 *Schmidt-Ullrich RK, Dent P, Grant S, Mikkelsen RB, Valerie K. Signal transduction and cellular radiation responses. Radiat Res. 2000 Mar;153(3):245-57.*

15 *Aubin F. Mechanisms involved in ultraviolet light-induced immunosuppression. Eur J Dermatol. 2003 Nov-Dec;13(6):515-23.*

16 *Norval M, McLoone P, Lesiak A, Narbutt J. The effect of chronic ultraviolet radiation on the human immune system. Photochem Photobiol. 2008 Jan-Feb;84(1):19-28.*

17 *Baudouin C, Charveron M, Tarroux R, Gall Y. Environmental pollutants and skin cancer. Cell Biol Toxicol. 2002;18(5):341-8.*

18 *Robbins CS, Dawe DE, Goncharova SI, Pouladi MA, Drannik AG, Swirski FK, et al. Cigarette smoke decreases pulmonary dendritic cells and impacts antiviral immune responsiveness. Am J Respir Cell Mol Biol. 2004 Feb;30(2):202-11.*

19 *Hoffmann E, Dittrich-Breiholz O, Holtmann H, Kracht M. Multiple control of interleukin-8 gene expression. J Leukoc Biol. 2002 Nov;72(5):847-55.*

20 *Lecas S, Boursier E, Fitoussi R, Vie K, Momas I, Seta N, et al. In vitro model adapted to the study of skin ageing induced by air pollution. Toxicol Lett. 2016 Sep 30;259:60-68.*

21 *Costa A, Facchini G, Pinheiro A, da Silva MS, Bonner MY, Arbiser J, et al. Honokiolprotects skin cells against inflammation, collagenolysis, apoptosis, and senescence causedby cigarette smoke damage. Int J Dermatol. 2017 Jul;56(7):754-61.*

22 *Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988 Jun;124(6):869-71.*

23 *Frosch PJ, Kligman AM. The soap chamber test. A new method for assessing the irritancy of soaps. J Am Acad Dermatol. 1979 Jul;1(1):35-41.*

24 *Daehnhardt-Pfeiffer S, Surber C, Wilhelm KP, Daehnhardt D, Springmann G,Boettcher M, et al. Noninvasive stratum corneum sampling and electron microscopicalexamination of skin barrier integrity: pilot study with a topical glycerin formulation for atopicdermatitis. Skin Pharmacol Physiol. 2012;25(3):155-61.*

25 *Blaak J, Dahnhardt D, Dahnhardt-Pfeiffer S, Bielfeldt S, Wilhelm KP, Wohlfart R, etal. A plant oil-containing pH 4 emulsion improves epidermal barrier structure and enhancesceramide levels in aged skin. Int J Cosmet Sci. 2017 Jun;39(3):284-91.*

26 *Stettler H, Kurka P, Lunau N, Manger C, Bohling A, Bielfeldt S, et al. A new topicalpanthenol-containing emollient: Results from two randomized controlled studies assessingits skin moisturization and barrier restoration potential, and the effect on skin microflora. JDermatolog Treat. 2017 Mar;28(2):173-80.*

27 *Dahnhardt D, Surber C, Dahnhardt-Pfeiffer S. Influence of Topical Formulations: Lipid Lamella Organization and Lipid Composition of Stratum Corneum as a Surrogate Marker for Barrier Integrity. Curr Probl Dermatol. 2018;54:166-72.*

28 *Pfeiffer S, Vielhaber G, Vietzke JP, Wittern KP, Hintze U, Wepf R. High-pressurefreezing provides new information on human epidermis: simultaneous protein antigen andlamellar lipid structure preservation. Study on human epidermis by cryoimmobilization. JInvest Dermatol. 2000 May;114(5):1030-8.*

29 *Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased level ofceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? JInvest Dermatol. 1991 Apr;96(4):523-6.*

30 *Dahnhardt D, Bastian M, Dahnhardt-Pfeiffer S, Buchner M, Folster-Holst R.Comparing the effects of proactive treatment with tacrolimus ointment and mometasonefuroate on the epidermal barrier structure and ceramide levels of patients with atopicdermatitis. J Dermatolog Treat. 2020 Jan 3:1-9.*

31 *Romani N, Holzmann S, Tripp CH, Koch F, Stoitzner P. Langerhans cells - dendritic cells of the epidermis. APMIS. 2003 Jul-Aug;111(7-8):725-40.*

32 *Bouwstra JA, Ponec M. The skin barrier in healthy and diseased state. Biochim Biophys Acta. 2006 Dec;1758(12):2080-95.*

33 *Bouwstra JA, Gooris GS, Dubbelaar FE, Weerheim AM, Ijzerman AP, Ponec M.Role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res.1998 Jan;39(1):186-96.*

34 *Kolgen W, Both H, van Weelden H, Guikers KL, Bruijnzeel-Koomen CA, Knol EF, et al. Epidermal langerhans cell depletion after artificial ultraviolet B irradiation of human skin in vivo: apoptosis versus migration. J Invest Dermatol. 2002 May;118(5):812-7.*

35 *Achachi A, Vocanson M, Bastien P, Peguet-Navarro J, Grande S, Goujon C, et al. UV Radiation Induces the Epidermal Recruitment of Dendritic Cells that Compensate for the Depletion of Langerhans Cells in Human Skin. J Invest Dermatol. 2015 Aug;135(8):2058-67.*