Kinetic Profile of Inflammation Markers in Human Skin *In vivo* Following Exposure to Ultraviolet B Indicates Synchronic Release of Cytokines and Prostanoids

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Ultraviolet B (UVB) irradiation affects epidermal cells, which respond via a cascade of inflammation markers. After initial *in vitro* and *ex vivo* experiments, this study used cutaneous microdialysis to generate a kinetic profile for 16 cytokines and 4 prostanoids in human skin *in vivo*. Skin areas 9 cm² were irradiated with UVB (2× minimal erythematous dose) 16 h after catheter placement in the dermis of the volar forearms of healthy volunteers. Dialysates were collected at 4-h intervals up to 64 h and analysed for 5- and 8-iso-PGF₂α, 9α,11α-PGF₂α and PGE₂, by gas chromatography–mass spectrometry (GC/MS). Dialysates were also analysed for interleukin (IL)-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, tumour necrosis factor (TNF)-α, Fas ligand (Fasl.), interferon-γ–inducible protein-10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), RANTES, eotaxin, and granulocyte-macrophage colony-stimulating factor (GM-CSF) using a multiplex-based cytometric-bead-array. In conclusion, 3 peaks with synchronic release of T helper (TH) 1-directed inflammatory cytokines and prostanoids could be detected post-UVB: an early phase (4–12 h), an intermediate phase (16–24 h) and a late phase (32–40 h). A TH2-directed cytokine response was detectable at intermediate and late phases. Key words: cytokines; prostanoids; cutaneous microdialysis; UVB; skin inflammation.

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Ultraviolet (UV) radiation, especially UVB (280–320 nm) radiation, is known as a major skin carcinogen that mainly affects epidermal cells, which mostly consist of keratinocytes. A complex cascade of acute inflammation of the skin, characterized by erythema, oedema, and immunosuppression, is induced by UVB irradiation, which, in cases of chronic exposure, can subsequently be linked to the initiation and progression of skin cancer. The role of inflammatory mediators, which include cytokines, nitric oxide, and oxidized arachidonic acid derivatives, in orchestrating cell-cell communication in the intercellular space in response to UVB irradiation is of critical importance. In addition to direct photochemical damage mediated by the generation of specific pyrimidine dimers, UVB generates high levels of reactive oxygen species (ROS) in the skin, presenting a risk of photo-oxidative damage to cells and the extracellular matrix. ROS can regulate not only the expression of inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), through nuclear factor kappaB (NF-kB) activation and tyrosine kinase-dependent pathways, but also cell death, as a mediator of apoptosis (1). Therefore, we analysed the kinetic profile of both cytokines and prostanoids, and especially 9α,11α-PGF₂α, PGE₂, and 5- and 8-iso-PGF₂α (2–4). Based on our initial *in vitro* studies presented here we knew that irradiation of keratinocytes results in alteration of their expression of surface molecules and secretion of cytokines, such as interleukin (IL)-1β, IL-6, IL-8, and TNF-α, in addition to prostanoids, such as PGE₂.

For a long time, microdialysis has been used to study tissue chemistry within the interstitium, pharmacokinetic drug profiles in various tissue compartments *in vivo* (5–8) and the inflammatory response by collecting inflammatory mediators from e.g. the skin (4, 9–11). The microdialysis technique seems to be superior to other techniques, such as suction blisters or biopsies, which destroy the tissue and lack the ability to elaborate a complete kinetic profile for the compounds of interest (9, 12). The principle of microdialysis is simple (7). The main problems in studying *in vivo* inflammatory responses in the skin are the low levels of mediators that can be found in the microdialysate, which need a highly sensitive analytical work-up, and the catheter placement in the skin (dermis or subcutis), which itself induces a relatively strong inflammatory response for several hours, making it difficult to separate the response to the tissue damage from the response of interest. There is also a need to establish in an animal model, using so called retrodialysis, the recovery of cytokines in *ex vivo* microdialysis experiments, before starting the experiments in humans.

In the clinical trial presented herein, we used microdialysis to establish a complete kinetic profile for inflammation markers released into the dermal inter-
The following text is a segment of a scientific report discussing the recovery of prostanoids and cytokines in vivo after UVB irradiation.

METHODS

In vivo cell culture experiments (see Appendix S1)

Animal model: ex vivo recovery experiments (see Appendix S1)

Clinical microdialysis experiments

Six healthy volunteers aged 26–45 years and without UVB exposure or any signs of skin inflammation within the test region within the last 6 weeks were included in this clinical trial after their informed consent and approval by the local review board of the Medical Faculty, Otto-von-Guericke-University Magdeburg, Germany (Approval 81/07). The principles of the Declaration of Helsinki were followed. The MED for each volunteer was first determined by the application of 5 increasing doses of UVB (Waldmann UV 3003K, Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany) to the contralateral forearm, ranging from 50 to 250 mJ/cm². Six microdialysis catheters (20 kDa cut-off membranes for the detection of prostanoids: CMA70 60/20 membranes and 100 kDa cut-off membranes: CMA71 60/20 membranes for the detection of cytokines, both from CMA Microdialysis, Sweden) were placed in the dermis on the volunteer’s volar forearm at a depth of 0.7–1.2 mm with the aid of 22 MHz ultrasound. As it is known that placement of a microdialysis catheter induces an inflammatory response that is clearly detectable for up to 10–16 h post-placement (19), UVB irradiation was performed 16 h after catheter placement. The membranes were first flushed with equilibration and basal microdialysis buffers at 5 µl/min for 2 h to eliminate the initial mediators induced by catheter insertion. The membranes were then perfused with 0.9% NaCl at a flow rate of 0.5 µl/min for the collection of prostanoids and with Dextran 60 (Deltadex 60®, Deltaselect, Germany) at a flow rate of 0.2 µl/min for the collection of cytokines using a mobile CMA107 microdialysis pump (CMA Microdialysis, Sweden), which allowed free mobility throughout the whole experiment. A lower flow rate of 0.2 µl/min was used to collect the cytokines, based on the results of the recovery experiments and the high molecular weight of cytokines relative to low-molecular-weight prostanoids (20, 21). The set-up is illustrated in Fig. S1. Microdialysate samples were collected after 2 h and then at 4 h intervals up to 62 h and immediately stored in liquid nitrogen. In case of prostanoid collection, 0.5 µl of the antioxidant BHT (end concentration 0.01%) was added. A total of 16 h after catheter placement, skin areas of 3×3 cm² above the dermal catheters were irradiated with 2×MED (350–450 mJ/cm²). For negative controls, the same set-up was used, but without irradiation, and microdialysates were also collected for 62 h in the same way. The prostanoid and cytokine content in the microdialysates was quantified altogether using sensitive gas chromatography-mass spectrometry/negative-ion chemical ionization for lipid mediators, markers of oxidative stress (the F2-isoprostanes 5- and 8-iso-PGF₂α) and markers of inflammation (the prostaglandins 9α,11α-PGF₂α and PGE₂) as well as of cytokines and chemokines. In particular, we examined Fas ligand (FasL), RANTES, eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5, and IL-13, which have not previously been studied in vivo in this context, and IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, and interferon-γ-inducible protein-10 (IP-10), which we studied more precisely and for a much longer period after irradiation than in past studies.

RESULTS

In vivo cell culture experiments

Cell culture experiments showed strong release of 9α,11α-PGF₂α, PGE₂, IL-6 and TNF-α into the cell-free supernatant from the same HaCaT keratinocytes within the first 24 h after irradiation, with a maximum at 8–12 h, whereas IL-8 was elevated up to 40 h after UVB irradiation. 5- and 8-iso-PGF₂α isoprostanes, were mainly detectable intracellular (Fig. 1A, C and D), which is consistent with previous reports (4, 9, 23). However, when we compared the intracellular vs. extracellular generation of prostanoids into the surrounding medium, we observed a second peak of 9α,11α-PGF₂α, released at 28 h and 36 h (Fig. 1A, C and D). Furthermore, the levels of prostanoids did not seem to increase continuously, which may result from certain instability of prostanoid mediators within the supernatant when cells were cultured up to 48 h and then collected.

Animal model: ex vivo recovery experiments

To switch from the in vitro situation to the in vivo situation using microdialysis, recovery of these mediators, meaning the amounts of specific mediators that can be found in a microdialysate, from the concentration in real tissue must be determined first. For this purpose, we used the method of retrodialysis in vivo to determine the recovery of cytokines in the skin, as in vitro approaches to determine recovery do not reflect the in vivo situation (20, 21). The recovery of prostanoids in vivo using porcine skin has already been determined and reported by us previously (18). As expected, high-molecular-weight cytokines are much more difficult to collect due to ultrafiltration of the dialysate, protein interactions or binding to the extracellular matrix of the dermis and to the outer microdialysis membrane (Appendix S1; SFig. 1C). To avoid ultrafiltration and to ensure the highest concentration of cytokines in the

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1http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2406
dialysate in the shortest period, we used a solution of the colloid Dextran 60 as the perfusate. The recovery of cytokines correlated with the flow rate and the molecular weight of the individual cytokines, with very low recovery for a flow rate of 1 µl/min and for higher molecular-weight chemokines and cytokines such as IL-6 and higher recovery for flow rates of 0.5 and 0.2 µl/min and for lower molecular-weight chemokines and cytokines (IL-8, eotaxin, and RANTES) (Appendix S1; SFig. 1C).

**Clinical microdialysis experiments**

In our approach, we compared the release of prostanoids and cytokines and analysed the release of these mediators in human skin in vivo until 48 h post-irradiation. Furthermore, we investigated different cytokines, such as FasL; the chemokines RANTES, eotaxin, and GM-CSF; and IL-13, which are mediators of the TH2 response. For cytokine detection in microdialysates, we used a different method, a cytometric-based cytokine array, which allows the detection of up to 36 cytokines (which can be individually selected) in a single 50 µl sample, with a lower limit of detection at 2.5–5 pg/ml. This method allows collection intervals of 4 h and, therefore, a more precise kinetic profile for cytokines until 48 h after UVB irradiation. Unfortunately, IL-5 remained undetectable throughout the study, as its concentration was below 2.5 pg/ml. Placement of the microdialysis catheters strongly induced a TH1-related immune response, which is consistent with previous reports (10, 24), as levels of IL-6, IL-8, and IL-1 beta were strongly increased, with much higher levels than after UVB irradiation. However, increased levels of prostanoids, and especially 8-iso-PGF2α; elevated levels of TNF-α, FasL, IL-10, IL-13, IP-10, and GM-CSF; and interestingly relatively high levels of RANTES were detected (Figs 2 and 3) after catheter placement and tissue trauma. After UVB irradiation, we could identify 3 time periods in which cytokines and prostanoids were increasingly detectable: an early phase, at 4–12 h; an intermediate phase, at 16–24 h; and a late phase, at 32–40 h. The prostanoids PGE2 and 8-iso-PGF2α showed increasing levels within these time periods, whereas the levels of 9α,11α-PGF2α were strongly increased at 8–12 h and 32–36 h (Fig. 2A–C). The early-phase increase in cytokine levels was more TH1 dominated, and the late phase was more TH2 dominated, whereas, in the intermediate phase, a mixed TH1/TH2 response was detectable (Figs 2 and 3). These findings indicate a slow TH1-to-TH2 shift within 40 h after UVB irradiation (Figs 2 and 3). Because not all cytokines reached baseline at UVB challenge, we compared AUC values after UVB irradiation with the AUC values for negative control dialysates without UVB irradiation. The mean AUC was clearly superior for all mediators except IL-1 beta and IL-13 (Table I). However, the negative controls showed a completely different profile, as they were nearly at baseline until 44 h, but at 44–48 h, an increase of some mediators could be recognized: IL-6, IL-8, IP-10, MCP-1 and 9α,11α-PGF2α, indicating that this release may be induced by beginning tissue-catheter irritations triggered by the immune system 58–62 h after placement (data not shown). This phenomenon is also the reason not

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**Fig. 1. Release of prostanoids and cytokines in vitro after ultraviolet B (UVB) irradiation of HaCaT cells (2.2 × 10⁷). Supernatants were repeatedly collected every 4 h in supernatants after UVB irradiation of HaCaT cells at 50 mJ/cm². Continuous release of prostanoids ((A) isoprostanes 5- and 8-iso-PGF2α and prostaglandins 9α,11α-PGF2α and PGE2) cytokines ((B) IL-6, IL-8 and TNF-α) into the surrounding medium. (C) Comparison of extracellular release vs. (D) intracellular generation of prostanoids after UVB irradiation (100 mJ/cm²) of HaCaT keratinocytes in parallel experiments. Cells and supernatants were harvested simultaneously every 4 h up to 48 h after irradiation.
all differences between UVB treatment and the control reached statistical significance, as shown for TNF-α, FasL, IL-3, IL-4, IL-10, GM-CSF, RANTES and all prostanoids (9α,11α-PGF_2α, PGE2, and 5- and 8-iso-PGF_2α) (Table I). Interestingly, FasL and 9α,11α-PGF_2α showed relatively synchronic release (Fig. 2A and D).

**DISCUSSION**

Using clinical microdialysis, we elaborated, for the first time, a complete kinetic profile for prostanoids, cytokines, and chemokines released into the dermal intercellular space in vivo 14 h before and up to 48 h after UVB irradiation (2×MED) at 4 h intervals. We identified 3 time-periods in which cytokines and prostanoids were increased: an early phase, at 4–12 h after UVB; an intermediate phase, at 16–24 h after UVB; and a late phase, at 32–40 h after UVB. The prostanoids PGE2 and 8-iso-PGF_2α showed increasing levels within these time periods, whereas the levels of 9α,11α-PGF_2α were strongly increased at 8–12 h and 32–36 h. Furthermore, we could identify an early-phase increase in cytokine levels that was more TH1 directed, and a late phase that was more TH2 directed, whereas, in the intermediate phase, we observed a mixed TH1/TH2, indicating a slow TH1-to-TH2 shift within 40 h post-irradiation.

Prostanoid mediators are formed by 2 different pathways: that is, enzymatically and non-enzymatically. Arachidonic acid is a key player whose liberation from membrane phospholipids and the formation of
prostaglandins is induced by inflammatory stimuli including UVB irradiation. Prostaglandins, such as 9α,11α-PGF₂α and PGE₂, are bioactive products that act as vasoregulators. However, the non-enzymatic pathway of arachidonic acid oxidation includes free radical-catalysed peroxidation of arachidonic acid and synthesis of F₂-isoprostanes, which are mostly bound to the backbone of phospholipids and which have vascular, proinflammatory and nociceptive functions. These products can be used as an indicator of photo-oxidative cell damage, sunburn-associated pain, and inflammation; in a dose-response study, the generation of 8-isoprostane was detected by specific antibody staining in the epidermis after UVB exposure (3, 25). Microdialysis has only been used once to detect PGE2 in UVB-irradiated skin (4×MED) at 24 h after UVB irradiation. Microdialysis was performed for only 30 min and PGE2 levels were increased in UVB-irradiated skin erythema compared with non-irradiated skin (310 pg/ml vs. 237 pg/ml) (25). In the current study, we were able to elaborate a compete kinetic profile, following catheter placement and UVB irradiation up to 48 h post-irradiation and the PGE2 value average approximately 400 pg/ml 24 h after UVB irradiation.

In another single previous report (10), cytokines were collected and a time-dependent profile generated for 24 h after UVB irradiation by combining a multiplex cytokine array with cutaneous microdialysis. In this interesting approach, the time intervals were 8 h, and microdialysis was performed 24 h before and 24 h after UVB challenge. The authors concluded that comparing cytokine responses between traumatic tissue injuries due to either catheter placement or UVB irradiation revealed significant differences; catheter placement mainly induced an unspecific proinflammatory response driven by IL-6, IL-8, IL-10, IP-10, RANTES, monocyte chemoattractant protein 1 (MCP-1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) before and after ultraviolet B (UVB) irradiation in healthy volunteers (mean with standard deviation).

Figure 2. Kinetic profile of interleukin (IL)-1β, IL-6, IL-8, IL-10, IP-10, RANTES, monocyte chemoattractant protein 1 (MCP-1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) before and after ultraviolet B (UVB) irradiation in healthy volunteers.
The cytokines, chemokines, and prostanoids were still being released (13). In previous reports, it was demonstrated that exposure to UVB irradiation resulted in increased homogeneous expression of Fas on epidermal cells from suction blisters, with the greatest expression at 24 and 72 h after irradiation, as detected by flow cytometry. The authors, however, could not detect increased levels of soluble FasL in the suction blisters by ELISA, which seems unsurprising because the levels that we have detected were relatively low (up to 25 pg/ml) and probably would not have been detectable with a commercial ELISA at that time (26, 27). Furthermore, we observed a strong increase in MCP-1, which is known to be strongly released after UVB exposure of keratinocytes in vitro, together with IL-8, and which is also mediated by ROS (1).

In conclusion, cutaneous microdialysis, together with a cytometric bead array and GC/MS, allows us to elaborate a kinetic profile for inflammation markers after in vivo immune challenge, such as UVB irradiation, in the dermal skin. Furthermore, we have evidence that release of cytokines and prostanoids is synchronized and that a slow TH1-to-TH2 shift occurs up to 40 h after UVB irradiation. Cutaneous microdialysis is possibly limited to a 58 h duration because an increase in the levels of some mediators, the cytokines, chemokine and prostanoid IL-6, IL-8, IP-10, MCP-1 and 9α,11α-PGF₂α were detected in non-UVB-irradiated skin at 58–62 h of the experiment. As volunteers were allowed free mobility throughout the experiment, methodological improvements for longer investigations might include a closer observation, possible hospitalization, and the use of smaller and shorter catheters (CMA66 catheters; 10 mm instead of 30 mm), which might reduce possible irritation induced by the catheter itself.

Long-term microdialysis could be utilized further, not only in studying skin physiology, such as response to heat and pain stimuli, but also in investigating other inflammatory skin diseases, such as psoriasis, atop dermatitis, or chronic urticaria. It could also be used to study the pharmacodynamic effects of pharmacological intervention using new drugs, such as chemicals or biologicals, delivered via the microdialysis catheters, on the in vivo release of cytokines or prostanoids within the dermal skin in real time (18, 24, 28–32).

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