Appendix S1.

MATERIALS AND METHODS

In vitro experiments

For in vitro experiments, we used human HaCaT cells, which are immortalized human keratinocytes that differ from the in vivo situation of the deficient epidermal skin in AD, but have been established as an in vitro model to study the generation of eicosanoids and to test potential inhibitors (6, 21, 22). 2×10^5 HaCaT keratinocytes were seeded on 25-cm Petri dishes overnight and irrigated with 100 ml/cm² ultraviolet B (UVB) (Waldmann UV 3003K, Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany), which is a sublethal dose known to induce apoptosis of HaCaT keratinocytes and release the eicosanoids 9α,11α-prostaglandin F₂α (9α,11α-PGF₂α) and 8-iso-prostaglandin F₂α (8-iso-PGF₂α) (21, 23).

After irradiation, HaCaT keratinocytes were exposed to 1, 10, 100 µM of either tacrolimus (Prograf® 1 mg, Fujisawa Germany, Munich, Germany), ø-6 fatty acids (Gamma-linolenic acid/linoleic acid, Sigma Aldrich, Hamburg, Germany) or 0-6 fatty acids (Salmon Oil, Sigma Aldrich, Munich, Germany), 0-3 fatty acids (Salmon Oil, Sigma Aldrich, Hamburg, Germany) or ø-6 fatty acids (Gamma-linolenic acid/linoleic acid, Sigma Aldrich, Hamburg, Germany) in Dulbecco’s Modified Eagle Medium 10% FCS for 24 h. The levels of F2-isoprostanes (5-iso and 8-iso-PGF₂α) and prostaglandins (9α,11α-PGF₂α and PGE₂) in the cell culture supernatants and in HaCaT keratinocytes stored intracellularly were determined by gas chromatography–mass spectrometry negative ion chemical ionization, as previously described (21, 24).

In vivo recovery of eicosanoids

The recovery of eicosanoids was determined by retrodialysis in vivo in anaesthetized domestic pigs (in vitro experiments are not well suited for the determination of in vivo recovery (Fig. S1A–B)). Porcine skin, due to its similarity to human skin regarding permeability, density, diameter of hair follicles, epidermal and dermal skin structure (thickness, lips, elastic fibres), is a good in vivo model to study recovery with microdialysis (25). However, both, retrodialysis and porcine skin are models that are approaching the human in vivo situation, but do not completely reproduce it. For retrodialysis, 4 microdialysis catheters (20 kDa cut-off membranes for the detection of eicosanoids and CMA70 60/20 membranes (CMA Microdialysis, Solna, Sweden)) were placed within the dermal porcine skin, which has the best dermal properties of all animals in relation to human skin (25). The microdialysis catheters were placed at a depth of 0.9–1.2 mm and were controlled by a 22-MHz ultrasound (taberna pro medicum, Luneburg, Germany). The microdialysis catheters were perfused at 0.5 and 1 µl/min with 100 ng/ml of eicosanoids (5-iso-PGF₂α, 8-iso-PGF₂α, 9α,11α-PGF₂α and PGE₂; Cayman Chemical, Axxora, Lörrach, Germany) in a solution containing NaCl 0.9% and 5% ethanol. The perfusates were replaced at 20-min intervals and the dialysates were pooled for analysis. Recovery was calculated as the difference between the starting concentration and the concentration found in the dialysate, which was the percentage of mediators that passed through the membrane into the surrounding dermal tissue.

Clinical microdialysis

Twelve 18–39-year-old patients with moderate AD clinically present on both arms (SCORAD>50) with a necessity for topical immunosuppressive treatment were recruited from the outpatient service of the clinic of dermatology and venereology at the Otto-von-Guericke University Magdeburg to be included in a mono-centre clinical trial.

Exclusion criteria were: systemic or local immunosuppressive treatment (including extensive UV light, corticosteroids and calcineurin-inhibitors); any pre-treatment with ø-6 fatty acids within the last 6 weeks prior to inclusion; and bacterial or mycotic skin superinfections. Informed consent was obtained from each patient, and the protocol was approved by the local ethics committee (approval 43/06) in accordance with the principles of the Declaration of Helsinki and good clinical practice.

Patients with clinically affected skin (minimum 10×10 cm²) with equal levels of skin darkness and erythema as assessed by a skin chromameter (Chromameter CR-300, Minolta, Osaka, Japan) were randomized by simple randomization (numbers assigned to the specific treatment and area) to areas with either no treatment, treatment with tacrolimus 0.1% ointment (Protopic 0.1%, Astellas Pharma, Munich, Germany) or 12% ø-6 fatty acid lotion (Eucerin 12% Omega lotion® (currently known as: AtopicControl® Lotion), Beiersdorf AG, Hamburg, Germany). The patients applied the treatment twice daily for 5 days, according to the dermatologist’s instructions (see the scheme in Fig. 1A).

The recovery of eicosanoids was determined by retrodialysis in vivo experiments. We used human HaCaT cells, which are a non-parametric Wilcoxon rank-sum test for the AUC values from each, tacrolimus 0.1% treated skin, omega-6 fatty acids treated skin and non-lesional skin, were compared with those from untreated lesional skin intra- and inter-individually. The mean values, standard deviation (SD), and Student’s -tests were calculated with Microsoft Excel (Version Office 2007, Microsoft Germany, Unterschleißheim, Germany). For statistical calculation, area under the curve (AUC) values from each experiment, and butylhydroxytolene was added to a final concentration of 0.1% after collection. The samples from each of the 4 membranes were analysed together to quantify the lipid mediators and markers of oxidative stress. 5- and 8-iso-PGF₂α (F2-isoprostanes peak I; peak II co-eluating with the authentic standards of 5- and 8-iso-PGF₂α); markers of inflammation; and prostaglandins (9α,11α-PGF₂α and PGE₂) were measured using gas chromatography-mass spectrometry and negative-ion chemical ionisation, as previously described (21, 24).

Skin chromametry had been used not only to evaluate UVB damaged skin (28), but also erythema levels in AD patients challenged with 2% sodium dodecyl sulphate (26, 27). Previously, levels of skin erythema were correlated with levels of eicosanoids found in the skin (29). Therefore, skin darkness and erythema were measured in all test areas at the end of microdialysis (Chromameter CR-300, Minolta, Osaka, Japan) in 10 of 12 patients (30).

Statistics

The mean values, standard deviation (SD), and Student’s t-tests for the in vitro experiments were calculated with Microsoft Excel (Version Office 2007, Microsoft Germany, Unterschleißheim, Germany). For statistical calculation, area under the curve (AUC) values from each, tacrolimus 0.1% treated skin, omega-6 fatty acids treated skin and non-lesional skin, were compared with those from untreated lesional skin intra- and inter-individually. A non-parametric Wilcoxon rank-sum test for the AUC values was used for the in vivo experiments and was calculated using MedCalc 10 (MedCalc beba, Mariakerke, Belgium).