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). 2×10^7 HaCaT keratinocytes were seeded on 25-cm Petri dishes overnight and irradiated with 100 mJ/cm^2 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 $F_{2\alpha}$ (9α,11α-$PGF_{2\alpha}$) and 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), (21, 23).

After irradiation, HaCaT keratinocytes were exposed to 1, 10, 100 µM of either tacrolimus (Prograf® 1 mg, Fujisawa Germany, Munich, Germany), ω-3 fatty acids (Salmon Oil, Sigma Aldrich, Hamburg, Germany) or ω-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-$PGF_{2\alpha}$) and prostaglandins (9α,11α-$PGF_{2\alpha}$ and PGE_2) 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[1]). Porcine skin, due to its similarity to human skin regarding permeability, density, diameter of hair follicles, epidermal and dermal skin structure (thickness, lipids, 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-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, 9α,11α-$PGF_{2\alpha}$ and PGE_2; 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 ω-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 cm^2) with equal levels of skin darkness and erythema as assessed by a skin chromameter (Chromameter CR-300, Minolta, Osaka, Japan; (26–29)) 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% ω-fatty acid lotion (Eucerin 12% Omega lotion® (currently known as: AtopiControl® 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).

12% ω-fatty acid lotion contains 12% ω-6 fatty acids (from evening primrose oil sources containing 85% of Gammalinolenic acid and 14% of linolenic acid (LA) and grape-seed oil containing 75% LA and flavonoids, such as licochalcone A). On day 6, dermal microdialysis was performed in untreated, treated lesional and non-lesional AD skin. After flushing the 20 kDa cut-off membranes (CMA 70, CMA Microdialysis, Solna, Sweden) at a rate of 5 µl/min for 1 h (for equilibration) and performing basal microdialysis to compensate for catheter insertion, all 4 membranes were perfused at a flow rate of 0.5 µl/min with NaCl 0.9% using a CMA107 microdialysis pump (CMA Microdialysis, Solna, Sweden).

Microdialysate samples were collected at 30-min intervals for 8 h. The collected microdialysates were kept on ice during the 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-$PGF_{2\alpha}$ (F2-isoprostanes peak I; peak II co-eluating with the authentic standards of 5- and 8-iso-$PGF_{2\alpha}$); markers of inflammation; and prostaglandins (9α,11α-$PGF_{2\alpha}$ and PGE_2) were measured using gas chromatography-mass spectrometry and negative-ion chemical ionisation, as previously described (21, 24).

Skin chromamtery 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 bcba, Mariakerke, Belgium).

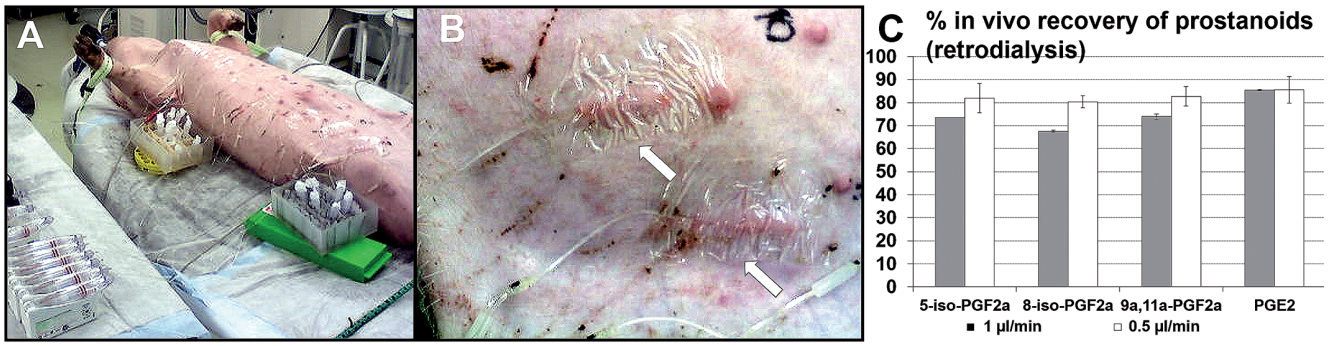

Fig. S1. In vivo recovery of eicosanoids tested in porcine skin determined by retrodialysis *in vivo* in anaesthetized domestic pigs. Microdialysis catheters (20 kDa cut-off membranes for the detection of eicosanoids and CMA70 60/20 membranes (CMA Microdialysis, Solna, Sweden) were placed within the dermal porcine skin (A) and microdialysis catheters were perfused at 0.5 and 1 µl/min with 100 ng/ml of prostanoids (5-iso-Prostaglandin $F_{2\alpha}$, 8-iso-Prostaglandin $F_{2\alpha}$, 9α,11α-Prostaglandin $F_{2\alpha}$ and Prostaglandin E_2) resulting in skin erythema surrounding the dermally placed catheters (B, *white arrows*). 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.

Table SI. *F_2-isoprostanes, 5- and 8-iso-prostaglandin $F_{2\alpha}$ and prostaglandins 9α,11α-prostaglandin $F_{2\alpha}$ and prostaglandin E2 (pg/ml*h). Area under the curve values for each patient. The means and standard deviations were obtained from dermal microdialysis in lesional, non-lesional atopic, or atopic skin treated with 0.1% tacrolimus ointment or 12% ω-6 fatty acid lotion (mean ± standard deviation (SD); *$p < 0.05$ indicates significance, as determined by the Wilcoxon rank-sum test, comparison with untreated lesional skin)*

Pat. No.	Age, years/ Sex	SCORAD	Eicosanoids	Lesional skin	Tacrolimus 0.1%	ω-6 fatty acids 12% lotion	Non-lesional skin
1	39/F	49.6	5-iso $PGF_{2\alpha}$	8,029	2,925	3,886	5,002
			8-iso $PGF_{2\alpha}$	1,035	0	2,952	2,428
			F2-isoprostanes	9,064	2,925	6,838	7,430
			9α,11α- $PGF_{2\alpha}$	6,052	4,674	225	470
			PGE_2	374	980	0	0
2	18/F	38.3	5-iso $PGF_{2\alpha}$	17,943	13,271	1,156	661
			8-iso $PGF_{2\alpha}$	1,570	659	1,163	909
			F2-isoprostanes	19,513	13,930	2,319	1,570
			9α,11α- $PGF_{2\alpha}$	2,532	547	540	320
			PGE_2	4,979	3,328	4,392	4,587
3	31/F	47.5	5-iso $PGF_{2\alpha}$	n.d.	n.d.	n.d.	n.d.
			8-iso $PGF_{2\alpha}$	5,609	2,613	6,083	2,785
			F2-isoprostanes	5,609	2,613	6,083	2,785
			9α,11α- $PGF_{2\alpha}$	3,899	1,185	2,522	5,097
			PGE_2	555	385	390	455
4	35/M	47.4	5-iso $PGF_{2\alpha}$	2,031	2,126	2,570	1,084
			8-iso $PGF_{2\alpha}$	914	825	1,245	723
			F2-isoprostanes	2,945	2,951	3,815	1,807
			9α,11α- $PGF_{2\alpha}$	0	40	102	0
			PGE_2	0	0	0	0
5	27/M	45.3	5-iso $PGF_{2\alpha}$	366	478	691	557
			8-iso $PGF_{2\alpha}$	1,358	1,421	1,971	770
			F2-isoprostanes	1,724	1,899	2,662	1,327
			9α,11α- $PGF_{2\alpha}$	1,133	823	2,402	1,285
			PGE_2	1,109	0	6,122	484
6	21/M	48.9	5-iso $PGF_{2\alpha}$	1,633	1,998	1,374	811
			8-iso $PGF_{2\alpha}$	750	727	717	529
			F2-isoprostanes	2,383	2,725	2,091	1,340
			9α,11α- $PGF_{2\alpha}$	1,336	1,173	1,003	135
			PGE_2	459	0	0	0
7	19/M	40.4	5-iso $PGF_{2\alpha}$	3,260	3,229	629	2,975
			8-iso PGF2α	1,760	2,661	710	2,086
			F2-isoprostanes	5,020	5,890	1,339	5,061
			9α,11α- $PGF_{2\alpha}$	178	293	463	0
			PGE_2	0	0	0	0
8	26/M	39.8	5-iso $PGF_{2\alpha}$	3,041	3,396	6,886	2,525
			8-iso $PGF_{2\alpha}$	2,508	2,946	2,950	1,907
			F2-isoprostanes	5,549	6,342	9,836	4,432
			9α,11α- $PGF_{2\alpha}$	1,925	3,262	3,006	1,737
			PGE_2	466	2,436	1,181	0
9	21/M	43.3	5-iso $PGF_{2\alpha}$	340	354	191	158
			8-iso $PGF_{2\alpha}$	1,854	1,182	471	408
			F2-isoprostanes	2,194	1,536	662	566
			9α,11α- $PGF_{2\alpha}$	3,216	550	137	139
			PGE_2	0	0	615	0
10	23/M	40.2	5-iso $PGF_{2\alpha}$	453	312	945	527
			8-iso $PGF_{2\alpha}$	697	421	1,876	1,237
			F2-isoprostanes	1,150	733	2,821	1,764
			9α,11α- $PGF_{2\alpha}$	1.5	447	126	452
			PGE_2	5,312	5,377	7,721	2,417
11	23/M	43	5-iso $PGF_{2\alpha}$	145	288	311	512
			8-iso $PGF_{2\alpha}$	1,383	914	552	1,166
			F2-isoprostanes	1,528	1,202	863	1,678
			9α,11α- $PGF_{2\alpha}$	8,698	1,932	2,786	3,488
			PGE_2	0	0	0	0
12	29/M	39.9	5-iso $PGF_{2\alpha}$	n.d.	n.d.	n.d.	n.d.
			8-iso $PGF_{2\alpha}$	4,607	2,625	3,618	5,476
			F2-isoprostanes	4,607	2,625	3,618	5,476
			9α,11α- $PGF_{2\alpha}$	4,261	1,723	2,842	3,649
			PGE_2	865	0	1,776	0
Mean ± SD	24.14 ± 1.89	42.09 ± 1.00	5-iso $PGF_{2\alpha}$	3,722 ± 1,513	2,838 ± 1,061 (p=0.92)	1,860 ± 574 (p=0.85)	1,481 ± 423 (p=0.06)
			8-iso $PGF_{2\alpha}$	2,004 ± 429	1,416 ± 282 (p=0.06)	2,029 ± 457 (p=1.00)	1,702 ± 392 (p=0.42)
			Sum of F2-isoprostanes	5,117 ± 1,405	3,781 ± 999 (p=0.20)	3,579 ± 756 (p=0.52)	2,936 ± 591 (p=0.03)*
			9α,11α- $PGF_{2\alpha}$	2,769 ± 732	1,387 ± 377 (p=0.05)*	1,391 ± 331 (p=0.09)	1,398 ± 481 (p=0.05)*
			PGE2	1,177 ± 522	1,042 ± 486 (p=0.55)	1,823 ± 768 (p=0.36)	662 ± 391 (p=0.01)*

SD: standard deviation; SCORAD: SCORing Atopic Dermatitis; n.d.: not determined.

Table SII. *Levels of skin darkness (d, black=0, white=100) and erythema (e>0 indicates increasing erythema) with standard deviation from lesional, non-lesional atopic, or atopic skin treated with 0.1% tacrolimus ointment or 12% ω-6 fatty acids lotion*

Patient	Levels of skin darkness (d) and erythema (e)	Lesional skin of atopic dermatitis	Tacrolimus 0.1% ointment	ω-6-fatty acids 12% Lotion	Non-lesional skin
1	d	61.62 ± 0.42	62.57 ± 0.55	58.77 ± 0.54	63.36 ± 0.83
	e	9.19 ± 0.17	8.34 ± 0.27	8.87 ± 0.41	7.76 ± 0.49
3	d	66.78 ± 0.26	67.48 ± 0.52	68.22 ± 0.11	70.78 ± 0.61
	e	13.16 ± 0.37	10.36 ± 0.96	12.70 ± 0.23	9.95 ± 0.72
4	d	69.13 ± 0.48	73.26 ± 0.69	74.16 ± 0.59	71.49 ± 0.77
	e	14.80 ± 1.19	12.30 ± 0.47	10.85 ± 0.63	12.20 ± 0.52
5	d	60.53 ± 0.83	60.21 ± 0.59	55.68 ± 0.95	64.15 ± 0.37
	e	12.71 ± 0.70	9.14 ± 0.19	15.40 ± 0.66	10.06 ± 0.45
6	d	59.03 ± 0.85	58.66 ± 0.63	58.59 ± 0.92	63.89 ± 0.34
	e	8.52 ± 0.46	7.87 ± 0.08	8.41 ± 0.39	6.51 ± 0.75
7	d	63.97 ± 0.18	63.33 ± 0.21	62.02 ± 0.25	65.39 ± 1.02
	e	12.57 ± 0.32	7.95 ± 1.33	10.42 ± 0.85	7.05 ± 0.47
8	d	60.83 ± 0.30	59.18 ± 0.27	60.27 ± 0.58	65.78 ± 0.50
	e	9.36 ± 0.32	8.97 ± 0.55	8.18 ± 0.65	6.42 ± 0.12
9	d	54.87 ± 0.57	58.33 ± 0.16	56.29 ± 0.28	63.72 ± 0.89
	e	16.61 ± 0.68	10.95 ± 0.17	15.43 ± 0.61	7.51 ± 0.56
10	d	58.69 ± 0.62	60.10 ± 1.08	56.90 ± 0.52	64.48 ± 0.33
	e	13.72 ± 0.49	11.98 ± 0.75	16.12 ± 0.86	8.83 ± 0.56
11	d	60.03 ± 0.95	63.13 ± 0.54	61.22 ± 1.05	63.19 ± 0.33
	e	10.48 ± 0.60	7.54 ± 0.72	8.33 ± 0.59	6.81 ± 0.19
Mean ± SD (*p*-value)	d	61.55 ± 1.24	62.62 ± 1.40 ($p=0.16$)	61.21 ± 1.74 ($p=0.63$)	65.62 ± 0.91 ($p=0.002$)**
Mean ± SD (*p*-value)	e	12.11 ± 0.80	9.54 ± 0.52 ($p=0.002$)**	11.47 ± 0.96 ($p=0.32$)	8.31 ± 0.57 ($p=0.002$)**

*$p<0.05$ indicates significance, **$p<0.01$ indicates high significance according to the Wilcoxon rank-sum test, comparison to untreated lesional skin.
SD: standard deviation.