Anti-inflammatory Effects of Topical Formulations Containing Sea Silt and Sea Salt on Human Skin *In Vivo* During Cutaneous Microdialysis

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Silt is sediment formed in estuaries and coastal regions along the seashore. It occurs along the entire North Sea coast and it is used in skin therapy. A single mud treatment induces normalization of stratum corneum hydration, transepidermal water loss, skin surface pH and sebum content (1). Mud therapy has been used successfully in several inflammatory skin diseases, such as psoriasis vulgaris (2), atopic dermatitis (3), acne vulgaris (4) and skin ulcers (5). The aim of this study was to elucidate possible anti-inflammatory effects of sea silt and sea salt-containing topical formulations on human skin *in vivo*.

MATERIALS AND METHODS

Different topical formulations containing sea silt essences and sea salt were tested: La mer MED Sea-salt cream® (SSC, 7.5% sea silt, 10% sea salt), La mer MED sea salt lotion® (SSL, 5% sea silt, 3.5% sea salt) and La mer MED fat cream® (FC, 7.5% sea silt, 0.5% sea salt) (La mer, Cuxhaven, Germany). The silt extract in these formulations contains approximately 0.6% fatty acids (hexadecanoic acid, hexadecenoic acid, eicosapentaenoic acid, octadecatrienoic acid and eicosatetraenoic acid) and 0.3% sulphur. All formulations (except for SSL) also contain 1% hydrolysed enteromorpha compressa extract and up to 5% hydrogenated vegetable and palm kernel oil, which contains 82% saturated and 18% unsaturated fatty acids (i.e. oleic acid and linoleic acid). Twenty healthy volunteers aged 22–29 years were tested for tolerability and efficacy of sea silt formulations after approval by the local ethics committee. To test tolerability, ten healthy volunteers (age range 22–27 years) applied SSC to one-half of the body's skin surface (either left or right). In addition, five volunteers applied SSL to one side of the body and FC to the other half; the head and back were left as untreated control areas. After 2 h, skin areas on the left and right upper and lower arms, legs and back were measured for skin pH, transepidermal water loss (TEWL) (Derma Unit SSC3 and Tewameter TM300, both from Courage & Khazaka, Cologne, Germany) and skin colour (Chromameter CR-300, Minolta, Osaka, Japan) (6, 7). Measurements were repeated 0.5 h and 24 h after irradiation with a minimal erythematous dose (MED) of ultraviolet A (UVA) and ultraviolet B (UVB) (Waldmann UV 3003K, Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany). To test anti-inflammatory efficacy, well-defined areas of 9 cm^2 on the volar forearms of 10 healthy volunteers (age range 23–29 years) were exposed to UVB irradiation with twice the minimal erythematous dose (450–550 mJ/cm^2), followed by treatment with test formulations, diclofenac gel (DG, Voltaren Emulgel®, Novartis AG, Nuremberg, Germany) or base cream (BC, a cream composed primarily of water, paraffin, citric acid, sodium cetearyl sulphate and cetearyl alcohol; Laticort base cream®, Almirall Hermal, Reinbek, Germany) as a negative control. Two hours after irradiation, a thin layer of each formulation (approximately 500 mg) covering the entire test area was applied six times every 2 h and gently rubbed in for approximately 5 min until it was absorbed. Cut-off membranes of 20 kDa (CMA71 60/20 membranes, CMA microdialysis, Sweden) were placed in the dermis at 0.7–1.2 mm depth, as determined by 22 MHz ultrasound (taberna pro medicum, Luneburg, Germany) and cutaneous microdialysis was started 24 h after UVB irradiation in irradiated and treated skin as well as in non-irradiated and untreated skin as described earlier (8). After flushing the membranes at a rate of 5 µl/min for 1 h for equilibration, membranes were perfused at a flow rate of 0.5 µl/min with sodium chloride (NaCl) 0.9%, using a CMA107 microdialysis pump (CMA Microdialysis, Solna, Sweden). Microdialysate samples were collected at 30-min intervals for 8 h and analysed for 5- and 8-iso-$PGF_{2\alpha}$ F_2-isoprostanes and 9α,11α-$PGF_{2\alpha}$ and PGE_2 prostaglandins using sensitive gas chromatography-mass spectrometry and negative ion chemical ionization, as described previously (8). Since it has been demonstrated previously that the intensity of skin erythema correlates with levels of prostanoids (9), skin darkness and erythema of all test areas were measured at the end of microdialysis, 36 h after UVB irradiation in six volunteers (Chromameter CR-300, Minolta, Osaka, Japan). Mean values, standard errors (SE), significance (Wilcoxon signed-rank test) and area under the curve (AUC) were calculated with MedCalc 10 (MedCalc, Mariakerke, Belgium).

RESULTS

All test products were well-tolerated without any side-effects or increase in skin pigmentation throughout the study (data not shown). All preparations prevented a decrease in pH and an increase in transepidermal

Table I. *Changes in skin pH, transepidermal water loss (TEWL in $g/m^2 \cdot h$) and skin erythema (values > 0 indicates increasing skin erythema) at baseline (2-h treatment with topical formulations containing sea silt and sea salt or untreated), 0.5 h, and 24 h after ultraviolet A/ultraviolet B (UVA/UVB) irradiation (mean of 10 volunteers with standard error (SE))*

	Skin pH	TEWL	Erythema
Untreated skin			
2 h treatment	5.15 ± 0.57	7.74 ± 0.19	9.92 ± 2.89
0.5 h post-UV	4.96 ± 0.13	7.74 ± 0.18	10.56 ± 0.35
24 h post-UV	5.14 ± 0.14	7.81 ± 0.26	11.73 ± 0.67
Fat cream			
2 h treatment	5.00 ± 0.49	6.94 ± 0.22	10.65 ± 1.28
0.5 h post-UV	5.04 ± 0.45	6.97 ± 0.29	10.83 ± 1.22
24 h post-UV	5.30 ± 0.56	5.90 ± 0.4	11.16 ± 1.24
Sea salt cream			
2 h treatment	5.11 ± 0.42	6.72 ± 0.19	10.10 ± 1.15
0.5 h post-UV	5.17 ± 0.40	7.59 ± 0.23	10.26 ± 0.75
24 h post-UV	5.28 ± 0.47	6.05 ± 0.32	11.06 ± 1.56
Sea salt lotion			
2 h treatment	5.35 ± 0.26	6.75 ± 0.17	9.66 ± 1.16
0.5 h post-UV	5.42 ± 0.29	7.13 ± 0.15	9.78 ± 1.01
24 h post-UV	5.17 ± 0.42	5.22 ± 0.23	10.47 ± 1.49

Table II. *Prostanoid levels presented as area under the curve (AUC; mean values in pg/ml · h ± standard error (SE)) from microdialysates of 10 volunteers, untreated or treated skin areas with topical formulations containing sea silt and sea salt or diclofenac following ultraviolet B (UVB) irradiation (no significant differences compared to Base cream)*

Treatment	5-iso-$PGF_{2\alpha}$	8-iso-$PGF_{2\alpha}$	Total F2-isoprostanes	9α,11α-$PGF_{2\alpha}$	PGE_2
Base cream	251 ± 75	640 ± 252	865 ± 289	287 ± 98	403 ± 95
Fat cream	328 ± 98	512 ± 140	807 ± 151	560 ± 247	469 ± 102
Sea salt cream	290 ± 40	475 ± 98	736 ± 125	318 ± 62	661 ± 198
Sea salt lotion	278 ± 95	385 ± 70	636 ± 138	188 ± 50	406 ± 124
Diclofenac gel	162 ± 28	448 ± 116	594 ± 120	171 ± 52	380 ± 87
Untreated and non-irradiated skin	203 ± 35	452 ± 93	635 ± 110	302 ± 89	393 ± 84

water loss (TEWL) observed at 24 h post-UVA/UVB in untreated skin (Table I). Furthermore, FC and SSL strongly decreased TEWL and all sea silt preparations inhibited increase in skin erythema 24 h post-UV irradiation compared with untreated skin (Table I). Microdialysis showed lower mean values of AUC for 8-iso-$PGF_{2\alpha}$ and total F2-isoprostanes, obtained from dialysates of treated skin areas in all 10 volunteers with any treatment compared with BC (Table II). Treatment with DG resulted in lower amounts of mean AUC for all markers, whereas treatment with SSL resulted in lower amounts of mean AUC for 9α,11α-$PGF_{2\alpha}$ in all 10 volunteers tested. In six volunteers, we were able to analyse changes in skin darkness and erythema of treated skin areas at the end of microdialysis 36 h after UVB irradiation (Table III). There was a significant decrease in skin redness and darkness at 36 h for untreated non-irradiated skin and skin treated with SSC, SSL and DG, but not for FC compared with skin areas treated with BC as a negative control (Table III). When comparing these results with the mean values of AUC for 5- and 8-iso-PGF2α, total F2-isoprostanes and 9α,11α-PGF2α and PGE2 prostaglandins from the microdialysates of the same volunteers, decreases were observed in untreated non-irradiated skin and skin areas treated with the same topical formulations; that is, SSL, SSC and DG.

Table III. *Skin darkness (black = 0, white = 100) and erythema levels of treated skin areas 36 h after ultraviolet B (UVB) irradiation (at the end of microdialysis) in 6 volunteers (mean values in pg/ml · h ± standard error (SE))*

Treatment	Skin darkness	Erythema (values > 0 indicates increasing skin erythema)
Base cream	62.70 ± 0.24	10.57 ± 0.21
Fat cream	64.27 ± 0.34 (p=0.01*)	10.15 ± 0.29 (p=0.29)
Sea salt cream	65.19 ± 0.24 (p<0.01**)	9.22 ± 0.43 (p=0.04*)
Sea salt lotion	64.00 ± 0.31 (p<0.01**)	9.61 ± 0.42 (p<0.01**)
Diclofenac gel	64.65 ± 0.21 (p<0.01**)	9.43 ± 0.34 (p=0.05*)
Untreated, non-irradiated skin	67.62 ± 0.08 (p<0.01**)	6.88 ± 0.11 (p<0.01**)

*p-values <0.05 indicate significance compared with base cream,
**p-values <0.01 indicate strong significance.

DISCUSSION

We used cutaneous microdialysis to detect differences in prostanoid levels of irradiated and treated skin. However, microdialysis is an invasive method (10), leading to release of prostanoids. This, together with the small number of patients, may have prevented the detection of significant differences between treatment areas, although sufficient time was allowed for tissue recovery and equilibration as determined in previous experiments (8, 11). Sea silt extract contains various active substances from sea silt, such as unsaturated fatty acids, sulphur and algae. These ingredients could contribute to sea silt's anti-inflammatory efficacy, which are known to derive from omega-3 and omega-6 fatty acids (12). Omega fatty acids inhibit the formation of pro-inflammatory eicosanoids, but can also form potent anti-inflammatory lipid mediators, such as resolvins and protectins, suppress NFκB activity and reduce the production of pro-inflammatory enzymes and cytokines (COX-2, TNF-α, IL-1β) (13). We were able to demonstrate that all tested sea silt- and sea salt-containing topical formulations suppressed the UVB-provoked release of 8-iso $PGF_{2\alpha}$, which is a well-known marker of oxidative stress. Furthermore, skin redness and skin darkening was significantly decreased by sea silt- and sea salt-containing formulations (more for lotion than for cream). However, the effect was lower than that observed following treatment with oral diclofenac, a known inhibitor of COX-1 and -2. FC, the only formulation that did not contain sea salt, failed to exert a suppressive effect on prostanoids 9α,11α-PGF2α, PGE2 and 5-iso-PGF2α.

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