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Epidermal Regeneration and Occlusion

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Disruption of the stratum corneum, by tape stripping or chemical injury, results in epidermal recovery of the skin barrier. In human skin, 40–48 h after surface trauma, epidermal proliferation is maximal. In a previous study in mice, occlusion with plastic or the hydrocolloid Duoderm proved to inhibit the regenerative response.

The aim of the present investigation was to find out whether occlusion modulates epidermal proliferation following removal of the stratum corneum in normal healthy volunteers by sellotape stripping. Epidermal proliferation was assessed, using a multiparameter approach, by measuring ornithine decarboxylase activity, keratin 16 expression and DNA synthesis.

Following tape stripping without subsequent occlusion, ornithine decarboxylase activity, keratin 16 expression and DNA synthesis were induced to the same extent as observed in previous studies. However, in contrast to the experiments in mice, no indication of a modulation of these responses was observed by the application of the hydrocolloids Duoderm and Comfeel.

In human skin, a direct effect of the artificial restoration of the skin barrier on epidermal regeneration remains unsubstantiated. ***Key words: keratinization; proliferation.***

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Epidermal regeneration following disruption of the stratum corneum and damage of the "skin barrier" has been studied using various approaches. Transepidermal water vapour loss is increased dramatically following stripping of the stratum corneum (1–3). During the first week after tape stripping a fast initial recovery is seen, and complete normalization is realized after 4 weeks (3).

A chronic impairment of the skin barrier with respect to water vapour loss persists in the hyperproliferative skin disorder psoriasis (4). In psoriasis, hyperproliferation of the epidermis is regarded as a therapeutical target. In psoriasis it has been shown that artificial restoration of the skin barrier will reduce epidermal proliferation directly or indirectly (5). In this complex pathological process it is difficult to deduct the direct effect of occlusion on epidermal proliferation. In order to answer the question to what extent occlusion modulates epidermal proliferation, the response to "sellotape stripping" is an appropriate model. Experimental removal of the stratum corneum by repeated applications of sellotape until the skin surface is glistening proved to be a standardized procedure, resulting in DNA synthesis reaching maximum activity after 2 days (2, 6). It is of importance in this respect that the regenerative response following tape stripping and the chronic psoriatic plaque are both characterized by an increased recruitment of cycling epidermal cells from the resting G_0 population (7). In mice it was demonstrated that artificial replacement of the barrier with water-impermeable membranes inhibited the expected increase in DNA synthesis induced by tape stripping and chemical injuries (8).

The aim of the present study was to find out whether in human volunteers occlusion by a hydrocolloid dressing reduces epidermal proliferation following sellotape stripping. Epidermal proliferation was assessed by quantification of the percentage of epidermal cells in S and G_2 M phase, percentage of cells expressing the hyperproliferation-associated keratin 16 and by measuring the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis.

MATERIALS AND METHODS

Subjects and experimental approach

In 7 healthy volunteers, 4 females and 3 males aged between 19 and 32 years, without signs or history of skin diseases, DNA synthesis and keratin 16 expression following tape stripping were assessed. At 6 sites localized at the upper back tape stripping was carried out. Immediately following tape stripping 2 sites were left uncovered with the hydrocolloid Duoderm (Convatec, Rijswijk, The Netherlands), and 2 sites were covered with the hydrocolloid Comfeel transparent dressing (Coloplast, Amersfoort, The Netherlands). A razor-blade biopsy was taken from 3 of these stripped sites after 24 h and from the remaining 3 sites 48 h after stripping.

In 6 healthy volunteers, 2 females and 4 males aged between 18 and 30 years, without signs or history of skin diseases, ornithine decarboxylase (ODC) activity following tape stripping was assessed. A 3 sites localized at the upper back tape stripping was carried out. Immediately following tape stripping, one site was left "uncovered", one site was covered with the "hydrocolloid dressing" Duoderm and the third site was covered with the "hydrocolloid" Comfeel transparent dressing. A keratotome biopsy was taken from 3 of these stripped sites 8 h after stripping, when the induction of ODC is maximal (9). Table I summarizes the experimental protocol.

Tape stripping and biopsy technique

Tape stripping was carried out according to the methodology described by Pinkus (10). Repeated applications of sellotape were carried out at the test-sites (2 cm^2). Removal of the stratum corneum was considered to be complete if the skin was glistening. Tape-strips applied after the appearance of a glistening surface proved to contain no epidermal cells. Between 15–25 applications were required for "complete stripping".

Razor-blade biopsies were taken free-handed, using a razor-blade in conjunction with a metal guard. The average thickness and diameter of the biopsies were 0.3 mm and 5 mm, respectively. The keratotome biopsies were taken with the Castroviejo keratotome (Storz Instr. Co., St. Louis, MO, USA). Razor-blade biopsies were processed immediately for measurement of DNA content and keratin 16 expression. Keratotome biopsies were snap frozen in liquid nitrogen and stored at –80°C prior to biochemical analysis.

Analytical procedures

Cell suspensions were prepared for flow cytometrical analysis of DNA content and keratin 16 expression by trypsinization as described by Bauer & Boezeman (11). In brief, following washing in phosphate-buffered saline (PBS), the biopsies were incubated for 20 min at 37°C floating on a solution containing 1% trypsin (DIFCO, Detroit, Michi-

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Table I. *Experimental approach*

ODC = ornithine decarboxylase.

Number of volunteers	Stripped sites per volunteer	Biopsies after tape stripping (h)	Parameters
	1 site covered with Duoderm E	24	% K_s 8.12-positive cells
		48	% cells, SG_2M
7	1 site covered with Comfeel	24	% K_s 8.12-positive cells
		48	% cells SG_2M
	1 site left without occlusive	24	% K_s 8.12-positive cells
		48	% cells SG_2M
	1 site covered with Duoderm E	8	ODC activity
6	1 site covered with Comfeel	8	ODC activity
	1 site left without occlusive	8	ODC activity

gan, USA) and 0.3% dithioerythrital (Sigma, St Louis, Mo, USA) in PBS. The biopsies were fixed in 500 µl cold ethanol (70% v/v) and dissociated by sonification (55 at 70 W).

An aliquot of 200 µl of these cell suspensions was centrifuged and rehydrated in PBS containing 1% normal goat serum (NGS, Sera Lab, Sussex, U.K. Cells were incubated with the monoclonal antibody K_s 8.12 (Bio-Yeda, Rekovat, Israel; diluted 1:30 in PBS/NGS) for 30 min at room temperature. After two washes with PBS/INGS and centrifugation at 1000 g for 5 min the second antibody goat-anti-mouse IgG conjugated with fluorescein isothiocyanate (GAM-FITC; Tago, Burlingauce, California, USA) diluted 1:25 in P8S/NGS was applied to the cells for 30 min. Following washing twice in PBS, the sediment was resuspended in 200 Gµl of 40 mg/l propidium iodide in PBS (Calbiochem, San Diego, California, USA) a 50 µl 1% ribonuclease A (Sigma, St Louis, Mo, USA) in order to measure DNA content per cell (12) simultaneously with the green fluorescence of K_s 8.12-positive cells.

From each sample 5,000 cells were analyzed using an ortho 504 flow cytometer equipped with a 5 W argon laser tuned at 488 nm, as described by van Erp et al. (13). K_s 8.12-positive cells were determined as green fluorescence and DNA was quantified as red fluorescence of propidium iodide. The right angle scatter signal was measured to correct for damaged cells. The data were stored in list made and analyzed with a PDP 11/34 computer (11, 13). The percentages of cells in G_1, S and G_2M phases were calculated with the standard program described by Baisch & Linden (14).

ODC activity was measured according to the methodology of Hayaski & Kameji, with slight modifications (15). Using an all glass ice-cooled Potter type grinder, biopsies were homogenized in 0.5 ml of a freshly prepared buffer (50 mM Tris, 50 µM pyridoxal phosphate and 2 mM dithiothreital at pH 7.3). The reaction was initiated by adding 80 µl of homogenate to 20 µl of a solution containing 2 mM L-ornithine and 0.1 µl L-[1-^{14}C] ornithine (0.1 m Ci/ml, specific activity 52.3 m Ci/mmol (New England Nuclear, Boston, MA, USA) in plastic tubes. After fitting each tube with a plastic cap carrying 5 cm^2 filter paper moistened with 20 µl 10% KOH, the mixture was incubated at 37°C for 45 min. The reaction was stopped by adding 100 µl 1 M HCl and incubated at 37°C for another 30 min to ensure complete absorption of $^{14}CO_2$ onto the paper. The paper was placed directly into a scintillation vial containing 4.0 ml scintillation fluid (Aqua Luma Plus) without prior dissolution, and radioactivity was measured by scintillation counting using a Pharmacia Wallac 1410. All samples were screened in duplicate and a complete reagent blank was included with each batch.

An aliquot of the homogenate was diluted 1:55 with homogenizing buffer and centrifuged (10 min, 12.000 rpm). Protein was determined in the clear supernatant by direct fluorescence ($\lambda_{EX} = 278$, $\lambda_{EM} = 340$ nm) using bovine serum albumin as standard.

RESULTS

In all subjects the tape stripping and the applications of the occlusives were well tolerated, without inducing irritation of the skin.

Tables I–III summarize the percentages of cells in SG_2M phase, the percentages of cells expressing keratin 16 and the ODC activities following tape stripping at the uncovered and occluded test sites.

No statistically significant difference was observed with respect to each of the 3 parameters between occluded and non-occluded test sites with respect to any of the parameters analyzed (Wilcoxon ranking test for paired data).

DISCUSSION

The present study does not indicate that epidermal hyperproliferation of human skin following sellotape stripping is inhibited by occlusion with the hydrocolloids Duoderm and Com-

Table II. *Percentages of cells in SG_2M phase at 48 h after tape stripping*

Subject	Uncovered	Duoderm	Comfeel
1	21.6	16.3	19.4
2	14.6	15.5	11.6
3	10.7	11.1	11.0
4	21.7	11.6	27.0
5	17.7	16.9	18.1
6	19.6	–	18.1
7	18.4	13.1	16.8
Mean ± SEM	17.8 ± 1.5	14.1 ± 1.0	17.4 ± 2.0

Table III. *Percentages of cells expressing keratin 16 at 24 h following tape stripping*

Subject	Uncovered	Duoderm	Comfeel
1	19.2	18.6	15.5
2	6.9	6.7	8.0
3	8.8	5.9	1.9
4	31.3	23.2	38.3
5	11.8	3.5	12.4
6	10.9	13.9	–
7	15.8	15.8	25.9
Mean ± SEM	15.0 ± 3.2	12.5 ± 2.7	17.0 ± 5.5

Table IV. *Ornithine decarboxylase activity (Pmol/min/mg protein) at 8 h following tape stripping*

Subject	Uncovered	Duoderm	Comfeel
1	108	89	87
2	49	98	83
3	57	45	51
4	63	55	34
5	189	128	100
6	43	80	89
Mean ± SEM	85 ± 23	83 ± 12	74 ± 10

feel. In normal skin the percentage of K_s 8. 12^+ cells has been reported to be 1.8 ± 1.0 (13), whereas the percentage of cells in S and G_2M phase in normal skin is 4.0 ± 0.7 (20). In normal skin ODC activity is below the level where it can be measured (9). Therefore, the percentage of K_s 8.12^+ cells, cells in SG_2M phase and the levels of the ODC activities following tape stripping are substantially increased. The results of the present study are not in accordance with the observations in mice skin. Following tape stripping in mice, epidermal hyperproliferation was observed after 18 h, which appeared to be inhibited by occlusion with hydrocolloid (4, 8). The hydrocolloid Duoderm caused a pronounced inhibition of epidermal proliferation following tape stripping in mice (8).

The interpretation of the discrepancy as to the effect of occlusion in human and animal skin could well be the consequence of a more general difference between humans and mice. An intriguing discrepancy is evident as to the dynamics of epidermal regeneration in human and mice. As early as 16–24 h after skin injury DNA synthesis in mice skin is maximal, whereas in humans the maximum DNA synthesis is seen as late as after 40–48 h (2, 7, 8, 16, 17). In human skin DNA synthesis remains within the normal range until 32 h after tape stripping. This difference with respect to dynamics suggests that studies on epidermal regeneration in mice skin might not be applicable to the situation in humans.

From the observation that occlusion does not impair trauma-induced epidermal regeneration in humans, we may conclude that there is no rationale to suspect that the application of an occlusive will directly inhibit epidermal growth in skin conditions characterized by increased recruitment of cycling epidermal cells, such as psoriasis.

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