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Response of the Clinically Uninvolved Skin of Psoriatic Patients to Tape Stripping during Acitretin Treatment

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The aromatic retinoids etretinate and acitretin are widely used in the systemic treatment of severe psoriasis. The purpose of the present investigation was to further elucidate the mode of action of acitretin on abnormal keratinization and epidermal hyperproliferation in an in vivo model. Studies on the interference of acitretin with epidermal hyperproliferation and abnormal keratinization in psoriatic plaques are difficult to interpret, as acitretin-induced changes might be due to direct effects of acitretin or be the indirect effect of retinoid-induced modulation of cutaneous inflammation.

Using an immunohistochemical assessment, we examined the in vivo effect of systemic acitretin (>35 mg daily) on the expression of filaggrin, involucrin, and on the recruitment of cycling epidermal cells, in the tape-stripped uninvolved skin of psoriatic patients, a model which provides the opportunity to study epidermal regeneration in the absence of significant accumulation of T-lymphocytes. During acitretin therapy and 3 weeks after withdrawal of acitretin, we took biopsies from uninvolved skin following tape-stripping in 6 patients with psoriasis. Six patients with psoriasis who had never used acitretin served as controls. We did not observe a Koebner response in our patients after tape stripping. Filaggrin expression was decreased, while the recruitment of cycling epidermal cells and the involucrin expression were increased in the biopsies taken from patients who did not use acitretin. During acitretin treatment, however, the filaggrin expression was similar, whereas the Ki-67 positive nuclei and the involucrin expression showed a statistically significant decrease compared to those parameters in the patients who did not use acitretin. Our findings indicate that epidermal hyperproliferation and abnormal keratinization are modulated directly by acitretin. ***Key words: psoriasis; filaggrin; involucrin; recruitment of cycling epidermal cells.***

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It has been demonstrated that retinoic acid can inhibit profilaggrin synthesis in cultured human epidermis keratinocytes (1). Asselineau et al. reported that retinoic acid prevents the conversion of profilaggrin into filaggrin in cultured human epidermal keratinocytes (2). The transcription of the type I (epidermal) transglutaminase mRNA is down-regulated by retinoids (3). Transglutaminase 1 is responsible for the catalyzation of the crosslinking of involucrin during the synthesis of the cornified envelope (4).

In psoriatic plaques, epidermal hyperproliferation and abnormal keratinization are associated with a pronounced infiltration of various cell types. Therefore, in vivo studies on the interference with epidermal hyperproliferation and abnormal differentiation in psoriatic plaques are difficult to interpret, as those changes might be due to direct effects of acitretin or be the indirect effect of retinoid-induced modulations of cutaneous inflammation.

The purpose of the present investigation was to further elucidate the mode of action of acitretin on abnormal keratinization and epidermal hyperproliferation in an in vivo model. We used the tape stripping model, which was first described by Pinkus (5). After repeated application of sellotape, thereby removing almost all of the horny layer, Pinkus observed a regenerative response of the human epidermis (6). Tape stripping is a useful model of synchronized growth, allowing us to study drug influences on cell kinetics accurately, without significant infiltrate formation (7).

Using an immunohistochemical assessment, we examined the in vivo effect of acitretin on the presence of filaggrin and involucrin, and on the recruitment of cycling epidermal cells in the uninvolved skin of psoriatic patients.

MATERIAL AND METHODS

Patients

Six patients (age 41–70 years) with severe psoriasis vulgaris for 2–37 years participated in this study. Each patient was informed about the experiment and gave written consent. The mean dose of acitretin was 34.2 mg ± 9.7 (mean ± standard error of the mean, 0.4–0.8 mg/kg body weight/day), and all the patients had mucocutaneous side-effects. In 5 patients the acitretin-free dynamics of trauma-induced hyperproliferation was assessed 3 weeks following discontinuation of acitretin, whereas in one patient the acitretin-free phase was studied before treatment. Although a period of 2 years is adopted as safe with respect to teratogenicity, it was shown that a relapse in palmoplantar pustules occurred only 2 weeks after discontinuation (8). Dierlich et al. demonstrated that it takes 3 weeks of continuing medication with oral retinoids to affect the accelerated epidermal cell proliferation in psoriatic patients (9). This made us decide to take the biopsies after 3 weeks.

Tape stripping was performed at day 0 and day 23 on the uninvolved skin (back) of the psoriatic patients. After 48 h, when epidermal proliferation is most pronounced (7, 10, 11), punch biopsies (3 mm) were taken, embedded in Tissue Tek compound, snap frozen in liquid nitrogen and stored at −80°C until use. After the biopsies were taken on day 2, the acitretin therapy was discontinued.

Six patients of matched age with psoriasis vulgaris for 2–21 years and who did not use acitretin during the experiment and had not used acitretin in the past served as controls. They were also tape-stripped at the uninvolved skin (back). Again, after 48 h a punch biopsy (3 mm) was taken from the tape-stripped area. Sections of 6 µm were cut, air-dried and fixed for 10 min in acetone-ether 60/40 vol.% (Ki-67 staining) or in aceton (other stainings) and stored again at −80 °C until use.

Monoclonal antibodies

To assess epidermal differentiation we used a monoclonal antibody against filaggrin (monoclonal mouse anti-filaggrin, BTI, BT576) and involucrin MON-150 (monoclonal antibody

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Table I. *Epidermal proliferation and differentiation in normal, psoriatic and stripped skin*
Comparison of the expression of filaggrin, involucrin and Ki-67 positive nuclei in normal skin, psoriatic uninvolved skin (PUI) after tape stripping (PS) before cyclosporin A (-CsA), during CsA (+CsA), in the psoriatic lesion (PL)(previously presented results: *:, **:, ***:) without acitretin (-Ac) and during acitretin (+Ac). Data expressed as mean ± SEM and quartiles.

	Normal skin*	PUI(- CsA)** 48 h PS	PUI(+CsA)** 48 h PS	PL***	PUI 48 h PS	PUI(-Ac) 48 h PS	PUI(+Ac) 48 h PS
Filaggrin str.cor.							
M ± SEM	100 ± 0	15.0 ± 10.7	10.0 ± 10.0	26 ± 11.3	5.8 ± 4.9	14.0 ± 8.7	34 ± 21
100-%tile	100	100	100	100	30	40	100
75-%tile	100	0	0	70	5	30	70
50-%tile	100	0	0	25	0	0	0
25-%tile	100	0	0	0	0	0	0
0-%tile	100	0	0	0	0	0	0
str.gran.							
M ± SEM	100 ± 0	94.0 ± 6.0	80.0 ± 13.4	55 ± 11.6	80 ± 10.5	64.0 ± 19.4	86 ± 11.5
100-%tile	100	100	100	100	100	100	100
75-%tile	100	100	100	90	95	90	100
50-%tile	100	100	100	75	90	50	95
25-%tile	100	100	100	40	80	34	95
0-%tile	100	40	0	0	30	0	40
Involucrin tip							
M ± SEM	25.4 ± 1.2	58.7 ± 3.3	53.4 ± 2.8	63.8 ± 3.5	71.2 ± 2.8	69.8 ± 3.4	52.5 ± 4.2
100-%tile	33	71	66	75	83	83	71
75-%tile	25	66	60	70	75	75	57
50-%tile	25	60	57.5	56	71	71	50
25-%tile	25	50	43	50	66	67	44
0-%tile	20	43	42	50	66	60	43
interpap.							
M ± SEM	20.9 ± 2.7	37.0 ± 2.8	35.3 ± 2.6	31.0 ± 2.1	54.8 ± 2.6	51.7 ± 3.5	41.0 ± 3.3
100-%tile	36	50	53	40	65	60	50
75-%tile	25	43	40	31	62	60	47
50-%tile	18	35.5	33	30	60	53	42
25-%tile	18	33	30	25	50	50	38
0-%tile	13	22	23	22	45	37	27
Ki-67 pos.nucl.							
M ± SEM	10.5 ± 2.5	86.4 ± 15.7	89.4 ± 21.3	119.0 ± 22.1	106.3 ± 15.0	99.6 ± 14.8	76.2 ± 15.2
100-%tile	20	160	200	260	152	152	140
75-%tile	14	128	128	176	138	108	132
50-%tile	12	82	81	112	126	94	73
25-%tile	4	40	40	100	84	72	54
0-%tile	0	16	4	32	66	62	44

against involucrin) (12). To characterize the epidermal proliferation we used a monoclonal antibody against a nuclear antigen present in the cycling cells (MIB 1 (cellprol. assoc. nucl. antigen (Ki-67), Immunotech SA AMAC Inc) (13).

Staining procedures

For the staining with anti-filaggrin, anti-involucrin, and Ki-67 an indirect immunoperoxidase technique was used. The slides were put in a phosphate-buffered stock solution (PBS solution: 360 ml Na_2HPO_4 (7.9 g Na_2HPO_4, Merck in 500 ml demineralized water) plus 70 ml NaH_2PO_4 (13.8 g NaH_2PO_4, Merck, in 500 ml demineralized water) plus 70 ml demineralized water). For 60 min the slides were incubated with the monoclonal antibodies MIB-1 (1:50 diluted in PBS solution + azide + 1% BSA), anti-filaggrin and anti-involucrin (1:500 and 1:50 diluted in PBS solution). After washing with phosphate buffer the slides were incubated with rabbit anti-mouse immunoglobulin conjugated with peroxidase (1:25, RAMPO). After washing in the PBS solution and pre-incubation with sodium-acetate buffer (pH 4.9), a solution of 3-amino-9-ethylcarbazole (AEC) in sodium-acetate buffer containing 0.01% H_2O_2 was added for 10 min.

All slides were counterstained with Mayer's haematoxylin (Sigma, St. Louis MO USA) and mounted in glycerol gelatin.

Histological examination

Epidermal proliferation was measured by counting the number of Ki-67 positive nuclei per mm length of the section. The filaggrin expression was assessed by measuring the percentage of the length of the stratum corneum and stratum granulosum which was stained. The involucrin expression was assessed by calculating the ratio positive cell layers/total cell layers of the living epidermis. This was performed at two sites: at the tip of a dermal papilla and between two dermal papillae (14).

Statistical evaluation

For statistical analysis the Wilcoxon test for matched pairs and the Mann-Whitney test were used.

RESULTS

None of the patients showed a Koebner response.

Histological results

The results appear in Table I. After tape-stripping anti-filaggrin was absent or showed discontinuous expression in both layers. The filaggrin expression in the stripped areas during acitretin treatment did not show a significant change, compared to filaggrin in the stripped areas after discontinuation of acitretin ($p_{str.gran} \geq 0.11$, $p_{str.corn} > 0.15$).

Whereas involucrin expression in normal human skin is present in the stratum granulosum and upper third of the stratum spinosum (15–16), after tape-stripping the involucrin expression is also observed in the lower region of the epidermis (17). This induction of involucrin decreased significantly during acitretin treatment ($p_{tip} \leq 0.02$, $p_{interpap.} \leq 0.04$).

The degree of stripping induced recruitment was significantly reduced during acitretin treatment, compared to the stripping induced recruitment 3 weeks after discontinuation of acitretin ($p \leq 0.04$; Fig. 1).

The histological results of biopsies taken from the control group did not show a significant difference compared to the observation in the group of patients who stopped the acitretin for 3 weeks.

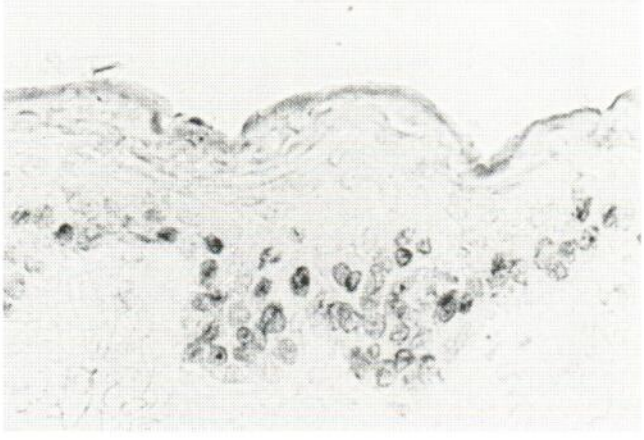

Fig. 1. Ki-67 positive nuclei in the tape-stripped uninvolved skin 3 weeks after withdrawal of acitretin.

Fig. 2. Ki-67 positive nuclei in the tape-stripped uninvolved skin during acitretin.

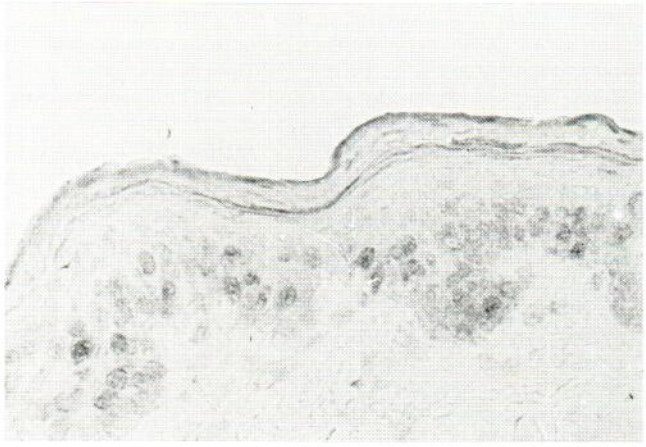

Fig. 3. Ki-67 positive nuclei in the tape-stripped uninvolved skin of the control group.

DISCUSSION

In 1951 Pinkus made the discovery that following the application of multiple strips of cellophane tape to normal skin, a predictable mitotic response of epidermal cells occurs. After multiple strips of tape had been successfully applied to the same area the corneocytes were removed, layer by layer. Within 48–72 h after tape stripping he observed a burst of mitoses in the basal layer and in the lower region of the stratum spinosum (5). The peak of the mitotic response is usually after 48 h (18). The stratum granulosum is lost after 12 h (6). After 24 h, a parakeratotic layer starts to form and the stratum granulosum starts to regenerate beneath the parakeratotic cells (18).

The present investigation confirms earlier observations of our group that epidermal dysregulation after tape-stripping and epidermal characteristics of the psoriatic plaque have important similarities with respect to recruitment of cycling epidermal cells, filaggrin and involucrin expression (Table I) (17).

Following tape stripping, filaggrin expression had decreased markedly. The observation that filaggrin expression remains decreased during acitretin treatment is in line with the previous in vitro findings that retinoic acid can inhibit profilaggrin synthesis and prevent the conversion of profilaggrin into filaggrin in cultured human epidermal keratinocytes (1, 2). Our results are also in agreement with the observation of Eichner et al., who demonstrated a reduction of filaggrin expression in epidermal cells of rhino mice after topical application with retinoic acid (19). In contrast to the single strip model, following repeated stripping we observed a substantial increase of filaggrin and epidermal cells, which implies that the superficial layers of the epidermis remain to be biochemically active (20). On the other hand, Rosenthal et al. noted an increased number of cell layers with involucrin and filaggrin expression after topical retinoic acid treatment in healthy volunteers (21). However, the irritation of the skin following topical retinoid might be responsible for this effect. It is known that retinoids exert a different effect in normal human epidermis compared to psoriatic epidermis. Gillenberg

et al. observed an increase of epidermal proliferating cells in normal human epidermis during therapeutic doses of oral aromatic retinoids (22). The reports of Lowe et al. and Dierlich et al. definitely demonstrate the antiproliferative effect of retinoids in psoriatic skin (9, 23). In the present investigation using an in vivo model for epidermal proliferation, an inhibition of the recruitment of cycling cells could be substantiated. The results confirm that the 3-week interval between the two biopsies was correct.

The observation that the stripping-induced recruitment of cycling epidermal cells and involucrin expression are both reduced during systemic acitretin treatment ($\geq$ 35 mg daily) supports the hypothesis that involucrin synthesis is related to the proliferation rate of epidermal keratinocytes. It has been reported that the number of T-cells is not induced by tape stripping in patients who do not show a positive Koebner response (17). Indeed, only sporadically distributed infiltrate cells were observed following tape stripping in our patients. Therefore, the modulation of epidermal changes following tape stripping by acitretin is unlikely to be secondary to immune modulatory effects of the drug.

REFERENCES

1. Fuchs E, Green H. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell 1981; 25: 617–625.
2. Asselineau D, Dale BA, Bernard B. Filaggrin production by cultured human epidermal keratinocytes and its regulation by retinoic acid. Differentiation 1990; 45: 221–229.
3. Floyd EE, Jetten AM. Regulation of Type I (epidermal) transglutaminase mRNA levels during squamous differentiation. Mol Cell Biol 1989; 9: 4846–4851.
4. Thacher SM, Rice RH. Keratinocyte-specific transglutaminase of cultured human epidermal cells: relation to cross-linked envelope formation and terminal differentiation. Cell 1985; 40: 685–695.
5. Pinkus H. Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping. J Invest Dermatol 1951; 16: 383–386.
6. Pinkus H. Examination of the epidermis by the strip method. II. Biometric data on regeneration of the human epidermis. J Invest Dermatol 1952; 19: 431–446.
7. Rijzewijk JJ, Boezeman JBM, Bauer FW. Synchronized growth in human epidermis following tapestripping: its implication for cell kinetic studies. Cell Tissue Kinet 1988; 21: 227–229.
8. Kerkhof PCM van de, Chang A, Dooren-Greebe R van, Geiger JM, Happle R. Intra-epidermal accumulation of polymorphonuclear leukocytes in persistent palmoplantar pustulosis during treatment with acitretin. Acta Derm Venereol (Stockh) 1988; 68: 499–503.
9. Dierlich E, Orfanos CE, Pullmann H, Steigleder GK. Epidermale Zellproliferation unter oraler Retinoid-Therapie bei Psoriasis. Arch Dermatol Res 1979; 264: 169–177.
10. Boezeman JBM, Bauer FW, Grood RM de. Flow cytometric analysis of the recruitment of G_0 cells in human epidermis in vivo following tape stripping. Cell Tissue Kinet 1987; 20: 1–10.
11. Kerkhof PCM van de, Rennes H van, Grood RM de, Jongh GJ de, Bauer FW, Mier PD. Response of the clinically uninvolved skin of psoriatic patients to standardized injury. Br J Dermatol 1983; 109: 287–294.
12. Duijnhoven HLP van, Schalkwijk J, Kranenborg MHGC, Vlijmen-Willems IMJJ van, Groeneveld A, Erp PEJ van, et al. MON-150, a versatile monoclonal antibody against involucrin: characterization and applications. Arch Dermatol Res 1992; 284: 167–172.
13. Cattoretti G, Becker MHG, Key G, Duchrow M, Schlüter C, Galle J, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992; 168: 357–363.
14. Gerritsen MJP, Rulo HFC, van Vlijmen I, Kerkhof PCM van de. Topical treatment of psoriatic plaques with 1,25-dihydroxyvitamin D_3: a cell biological study. Br J Dermatol 1993; 128: 666–673.
15. Watanabe S, Wagatsuma K, Ichikawa E, Takahashi H. Abnormal distribution of epidermal protein antigens in psoriatic epidermis. J Dermatol 1991; 18: 143–151.
16. Murphy GF, Flynn TC, Rice RH, Pinkus GS. Involucrin expression in normal and neoplastic human skin: a marker for keratinocyte differentiation. J Invest Dermatol 1984; 82: 453–457.
17. Gerritsen MJP, Rulo HFC, Arnold WP, Kerkhof PCM van de. Response of the clinically uninvolved skin of psoriatic patients to repeated tape stripping during cyclosporin A treatment. Br J Dermatol 1994; 130: 181–188.
18. Williams MG, Hunter R. Studies on epidermal regeneration by means of the strip method. J Invest Dermatol 1957; 29: 407–413.
19. Eichner R, Kahn M, Capetola RJ, Gendimenico GJ, Mezick JA. Effects of topical retinoids on cytoskeletal proteins: implications for retinoid effects on epidermal differentiation. J Invest Dermatol 1992; 98: 154–161.
20. Gerritsen MJP, Erp PEJ van, Vlijmen-Willems IMJJ, Lenders LTM, Kerkhof PCM van de. Repeated tape stripping of normal skin: a histological assessment and comparison with events seen in psoriasis. Arch Dermatol Res 1994; 286: 455–461.
21. Rosenthal DS, Griffiths CEM, Yuspa SH, Roop DR, Voorhees JJ. Acute or chronic topical retinoic acid treatment of human skin in vivo alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10 and 14. J Invest Dermatol 1992; 98: 343–350.
22. Gillenberg A, Immel C, Orfanos CE. Retinoid-Einfluss auf die Zellkinetik gesunder menschlicher Epidermis. Arch Dermatol Res 1980; 269: 331–335.
23. Lowe NJ, Kaplan R, Breeding J. Etretinate treatment for psoriasis inhibits epidermal ornithine decarboxylase. J Am Acad Dermatol 1982; 6: 697–698.