Skin, Adipose Tissue and Plasma Levels of Acitretin with Rare Occurrence of Esterified Acitretin during Long-term Treatment

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In a previous study acitretin and its 13-*cis*-metabolite were monitored in the plasma and epidermis of healthy volunteers. They were given 50 mg of *trans*-acitretin daily. No drug accumulation was observed in the skin, nor in the plasma. The purpose of the present study was to extend the data from non-psoriatic to psoriatic ($n = 11$) subjects, treated for at least 1 month with 25 mg acitretin. Plasma, skin biopsies and subcutaneous fat samples were analysed using HPLC. Trough levels of acitretin in skin were below the quantification limit, increasing to 28 ± 16 ng/g within 5 h after dosing. Fat tissue levels exceeded those of skin, with values of 98 ± 71 ng/g within 5 h after drug intake. In 2 patients, additional samples were taken 3 days post-therapy. Here, concentrations were below the quantification limit in adipose tissue, confirming that acitretin is not stored in subcutaneous fat. Esterification of acitretin into etretinate was observed in 2 subjects. This observation illustrates the recently described new metabolic pathway for acitretin. On both occasions, the unexpected ethylester metabolite was extensively stored in fat tissue. *Key words: Etretinate; Body storage; HPLC.*

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Acitretin and its ethylester etretinate are used to treat various dermatological disorders, e.g. psoriasis. Because of its high lipophilicity etretinate accumulates in adipose tissue. It is slowly released from its storage compartment and further hydrolysed to the active free acid form (1). Both compounds are teratogenic. The terminal elimination half-life of etretinate is about 105 days (2), while acitretin is cleared from the body with a half-life of approximately 48 h. Thus, the use of acitretin appears a preferable alternative to that of its prodrug.

Data on concentrations of acitretin in skin are limited (3–5). The objective of a previous study (3) was to determine epidermal concentrations of acitretin and its 13-*cis*-metabolite in healthy volunteers following single and multiple dosing. Short-term variations (order of hours) in epidermal levels of the drug were assessed. In the current study both total skin, subcutaneous fat and plasma samples from psoriatic patients were analysed. Again samples were taken for each subject with short time intervals.

PATIENTS AND METHODS

Twelve patients participated in this study: 3 women (aged 40 to 69 years) and 9 men (aged 33 to 71 years). Nine patients had psoriasis vulgaris, 2 patients had psoriasis arthropathica and 1 patient had premalignant keratosis actinica. They were given acitretin only, as systemic medication for 2 months up to 3.5 years. Patient no. 10 is an exception: he had received previous treatment with 25 mg etretinate but changed to acitretin therapy 3.5 years before sampling. This is more than 10 times the terminal elimination half-life of etretinate. The etretinate concentration, when changing to acitretin, was about 100 ng/ml plasma. Four patients received topical dithranol treatment. All patients took 25 mg *trans*-acitretin in capsules at breakfast (Neotigason®, Hoffmann-La Roche, Basle, Switzerland). Full thickness elliptical skin biopsies (10 mm–5 mm, mean weight 75 mg) and subcutaneous fat (mean weight 124 mg) were obtained from uninvolved abdominal skin under xylocaine anesthesia.

Samples were blotted, rinsed and submitted to freeze-thaw cycles in liquid nitrogen as described previously (3). Instead of ultrasonic treatment, mechanical homogenization with a Polytron on ice was used. An essential aspect is the nonhydrolytic extraction procedure. In short, 0.5 ml 2N HCl and 5.0 ml water are added to the ethanol-DMSO homogenate. After vortex-mixing (30 s), 7.5 ml *n*-hexane is added and the retinoids are extracted by mixing on a rotating device (15 min). Then, a centrifugation step is performed (8 min, 2500 g), followed by isolation of the organic layer, which is evaporated under N_2. Retinoids were analysed by HPLC as reported (6, 7). Etretinate and both acitretin isomers could be quantitated simultaneously. The detection limits for acitretin were 10 ng/g (skin), 7.5 ng/g (adipose tissue) and 2 ng/ml (plasma), respectively.

RESULTS

Table I shows the individual *cis*- and *trans*-acitretin levels in skin, adipose tissue and plasma at pre-dose ($n = 10$), 5 h after dose intake ($n = 12$) and 3 days after the treatment ($n = 2$). For *trans*-acitretin, pre-dose levels showed minor variations: from BQL (below the quantification limit) to 15 ng/g in skin and from BQL to 39 ng/g in subcutaneous fat. Five hours after drug intake these concentrations had increased significantly, ranging from 10 to 58 ng/g (28 ± 16 ng/g) and from 26 to 273 ng/g (98 ± 71 ng/g), respectively. In 2 patients (nos. 6 and 11) additional samples were taken 3 days post-therapy. All tissue levels had dropped below the quantification limit.

The individual trough levels of 13-*cis*-acitretin ranged from BQL to 43 ng/g in skin and from BQL to 52 ng/g in adipose tissue. Five hours after drug intake these levels increased slightly, varying from BQL to 60 ng/g and from BQL to 83 ng/g, respectively. The corresponding plasma concentrations again showed major interindividual differences (between 20 and 287 ng/ml) but had reached steady-state levels for each individual.

Unexpectedly, a compound coeluting with authentic etretinate was detected in the plasma samples from 2 acitretin-treated patients (nos. 10 and 12). The corresponding fat samples contained large amounts of a compound with a retention behaviour slightly different from etretinate. In the skin biopsies much smaller peaks were observed, also displaying a minor shift in retention time compared to the standard. This

Table I. *Acitretin concentrations in skin (a), adipose tissue (b) and plasma (c); in ng/g wet weight and ng/ml respectively*

Trans- and 13-*cis*-values are given (top and bottom values). BQL = below the quantification limit; – = no sample available.

Patient no.	Pre-dose			5 h after dose intake			3 d post-therapy		
	a	b	c	a	b	c	a	b	c
1	15	–	8	40	–	100	–	–	–
	BQL	–	66	BQL	–	68			
2	BQL	–	7	12	–	–	–	–	–
	4	–	61	6	–	–			
3	BQL	11	12	14	69	143	–	–	–
	BQL	14	111	BQL	83	143			
4	–	–	–	16	49	101	–	–	–
				BQL	43	52			
5	–	–	–	10	26	43	–	–	–
				12	6	119			
6	BQL	39	20	20	92	159	BQL	BQL	6
	BQL	BQL	164	BQL	10	180	BQL	BQL	122
7	14	BQL	4	42	47	41	–	–	–
	43	BQL	21	BQL	BQL	20			
8	BQL	27	37	58	153	218	–	–	–
	35	BQL	287	18	BQL	202			
9	BQL	BQL	17	12	88	93	–	–	–
	25	BQL	103	28	BQL	157			
10	BQL	18	17	29	101	138	–	–	–
	10	17	77	4	22	74			
11	15	BQL	5	47	273	152	BQL	BQL	2
	16	BQL	57	60	20	70	BQL	BQL	28
12	BQL	8	25	38	78	188	–	–	–
	18	52	141	26	19	130			

shifting phenomenon was probably caused by matrix effects combined with peak overload. Re-analysis of the plasma and fat samples with a different HPLC system (8) provided additional information. A second rechromatography, using capillary GC (9) without prior derivatization, was performed on an aliquot of the HPLC peak collectate. Finally, another aliquot of this collected HPLC eluate was used in an alkaline hydrolysis step to form acitretin. All experiments confirmed that this compound is etretinate. Experiments in skin, however, could not be repeated due to lack of sample amount. Concentrations were measured at pre-dose and 5 h after drug intake. Plasma levels increased from 14 to 21 ng/ml and from 28 to 56 ng/ml in both patients. The corresponding skin levels were BQL to 76 ng/g and 118 to 290 ng/g, respectively. All calculated fat tissue amounts (extrapolation) varied between 1 and 2 μg/g.

DISCUSSION

Tissue levels of acitretin and 13-*cis*-acitretin often approach the detection limit of the assay. When compared to previous studies (3–5), our values are lower but in the same order of magnitude in both skin and adipose tissue. Differences accounting for these lower values probably are: the use of epidermis instead of total skin samples in studies (3) and (4) and/or the use of a different sample clean-up (4, 5). In analogy with the varying retinoid concentrations observed in different skin cell-layers (10), acitretin will not be equally distributed throughout epidermis and dermis. The use of epidermal samples (suction blister technique) is advantageous, because the obtained data do not represent means of the epi- and dermal levels. Moreover, the technique is not invasive, allowing sampling with short time intervals. Drawbacks are that the procedure is both lengthy (at least 1.5 h) and artificial. Total skin biopsies are subject to bigger variations and invasive, limiting the number of samples. On the other hand, the procedure is faster and thus more elegant to perform on a routine basis. Patients in other studies received 30 mg (4, 5) and even 50 mg (3), instead of 25 mg of the drug per day. Finally, the use of a non-hydrolytic extraction procedure eliminates artefactual ester hydrolysis (4), and supplementary rinsing combined with blotting results in a more extensive removal of adhering blood.

The obtained data display considerable interindividual variations. Nevertheless, two common trends can be observed. Firstly, tissue *trans*-acitretin levels increase within 5 h following drug intake. This suggests rapid penetration of the drug into skin and adipose tissue. However, the individual plasma and tissue values do not correlate. This observation confirms the findings of Grønhøj Larsen *et al.* (4) in subcutis and epidermis after several months of treatment. On the other hand, they found a significant correlation (0.89, $p < 0.01$) after 1 month of therapy. Secondly, pre-dose levels are consistently at the limit of quantification in both adipose tissue and skin. Levels in adipose tissue were even below the quantification limit within 3 days post-therapy. These findings strongly indicate that neither skin nor adipose tissue functions as a body storage compartment for acitretin. In our opinion the significant amounts of acitretin found in the wash-out samples by Grønhøj Larsen *et al.* originate from esterified acitretin (etretinate) and are artefactually generated in the hydrolysis step of their extraction procedure. This exceptional esterification phe-

nomenon was recently commented on by Chou *et al.* (11, 12). They concluded that ethanol intake enhances, but is not essential for, ethyl ester formation of acitretin. Moreover, if ethanol is present, this etretinate formation is ethanol dose-dependent. Our samples have been simultaneously monitored for etretinate peaks as described by Lambert *et al.* (7). In 2 out of 12 patients etretinate appeared in the plasma traces. Both patients later admitted to be regular beer drinkers. Although the etretinate concentrations in adipose tissue were as high as 1 μg/g wet weight, the acitretin levels remained normal in all samples. Our data confirm that due to the potential esterification of acitretin into etretinate, a non-hydrolytic extraction procedure should be used in order to allow differentiation between free and esterified forms of the drug.

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