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Oral Acitretin in Psoriasis: Drug and Vitamin A Concentrations in Plasma, Skin and Adipose Tissue

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The purpose of the present study was to determine the concentrations of acitretin and its main metabolite, 13-cis acitretin, in epidermis, subcutis and plasma in twelve psoriatic patients treated with 30 mg acitretin orally daily for 6 months. In addition, endogenous concentrations of vitamin A were monitored. Blood samples and biopsies from normal appearing skin were obtained prior to therapy, after 1 and 6 months of treatment and finally 1 month after cessation of therapy. Using a highly sensitive liquid chromatography method concentrations of synthetic retinoids and endogenous retinoid (retinol, 3,4-didehydroretinol) were analysed in hydrolyzed tissue samples and plasma. Steady-state concentration of acitretin in epidermis (17±9 ng/g) was reached within 1 month of therapy. There was a significant correlation between the individual plasma trough value and the epidermal concentration of acitretin after 1 month of therapy. The acitretin concentrations in subcutis varied from 15 to 1437 ng/g, but the mean values at 1 and 6 months of therapy were similar (177 and 227 ng/g, respectively). After stopping therapy the acitretin level was below the detection limit in both epidermis and serum within 1 month in 9 out of 12 patients. In contrast, only 3 of the patients were negative for acitretin in subcutis biopsies obtained 1 month after stopping therapy. The occurrence of a presumed tissue contaminator with characteristics similar to 13-cis acitretin prevented quantitation of this metabolite in many subcutis samples. The epidermal, subcutis and serum composition of retinol and 3,4-didehydroretinol remained unchanged during therapy, indicating no or only minimal interaction between acitretin and endogenous vitamin A metabolism. ***Key words: Retinoids; Vitamin A metabolism; Skin concentrations; 3,4- Didehydroretinol.***

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Acitretin has recently been introduced for the treatment of severe psoriasis (1–3). Hitherto, the use of its carboxylic acid ester analogue, etretinate, has been hampered by an extremely long elimination half-life of up to 120 days after repetetive dosing (4,5). This is due to accumulation in the adipose tissue (6). In contrast, acitretin has been shown to be eliminated from the body considerably faster (terminal elimination half-life in the order of about 2 days) than etretinate (7–9), presumably because of lack of ability of binding to adipose tissue for this drug. Whereas concentrations of etretinate during long-term therapy have been investigated rather intensively in skin (6) as well as in a variety of organs (10), only a single preliminary study from psoriatic patients has been performed on acitretin (11).

Another issue of concern is how acitretin affects the endogenous vitamin A metabolism. The two predominant forms of vitamin A in human skin are retinol and 3,4-didehydroretinol. The latter compound, which is a metabolite of retinol, is markedly increased in lesional psoriatic skin (12).

Isotretinoin has proved to increase the epidermal retinol concentration and decrease the 3,4-didehydroretinol concentration in normal appearing skin (13). In contrast, etretinate representing a second generation retinoid has only little influence on endogeneous vitamin A levels (6). The effect of acitretin, also a second generation retinoid, has not been studied in this respect.

The major aim of the current study was to determine the concentrations of acitretin and its main metabolite, 13-cis-acitretin, in epidermis and subcutis from psoriatic patients during prolonged multiple dose administration of the drug. Furthermore, we have addressed the question whether acitretin therapy interferes with the plasma and tissue concentrations of retinol and 3,4-didehydroretinol.

MATERIAL AND METHODS

Patients

The study was approved by the local Ethics Committee and the National Health Service of Denmark according to the Declaration of Helsinki II. A total of 12 patients participated in the study: 8 women, aged 36–63 years (mean 48 years) and 4 men, aged 45–60 years (mean 51 years). Eleven patients had psoriasis vulgaris and 1 palmoplantar pustular psoriasis (no. 2). All patients were otherwise healthy judged by clinical examination and laboratory tests for haematologic, hepatic and renal function. They had never received any systemic retinoid medication except for patient no. 3 (3 months of treatment with acitretin 24 months before initiation of the present study) and patient no. 7 (3 months of treatment with etretinate 18 months before initiation of the present study). Five of the patients were taking regular or as needed systemic medications, primarily ibuprofen (n = 5). This medication was kept unchanged throughout the study. Topical therapy included corticosteroids (n = 2) and bland emollients. Combined regimens with ultraviolet B or photochemotherapy (Psoralens with ultraviolet A) were not allowed. One patient was excluded from the study due to excessive alcohol consumption during the 6-months drug treatment period leading to a significant increase in S-GOT and alkaline phosphatase. Females of childbearing potential used an effective form of contraception for 2 months before the trial and for at least 3 months following cessation of therapy. Our patients took part in a multicenter trial, the pharmacokinetic results of which are presented elsewhere (14).

Study design

Capsules containing 10 mg of acitretin in oil were supplied by Hoffmann-La Roche & Co AG, Basel, Switzerland. All patients were treated with acitretin for a period of 6 months. The daily dose was administered as three 10 mg capsules taken together with breakfast.

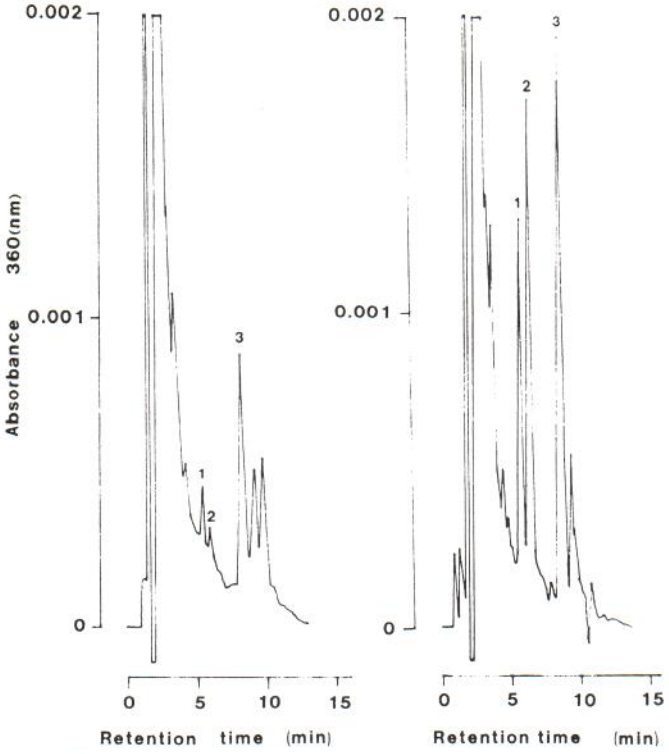

Fig. 1. HPLCs of acidic retinoids extracted from an epidermal shave biopsy (*left*) and subcutis (*right*) from an acitretin-treated patient (no. 1). Conditions as described under Retinoid analysis. The figures denote the elution positions of 13-cis acitretin (1), acitretin (2) and internal standard isotretinoin (3).

Each subject was instructed to maintain normal eating habits throughout the study period.

Tissue collection

Epidermal shave biopsies (10–25 mg) and subcutaneous fat samples (5–15 mg) were obtained from uninvolved skin on the buttock as described earlier (6, 15). The samples were blotted to remove blood, rapidly frozen and stored in plastic capsules at −70°C until assayed. The biopsies were taken immediately before start of treatment and after treatment for 1 and 6 months and finally 1 month after cessation of drug treatment.

Plasma

Six ml of blood were obtained by venepuncture from an antecubital vein into oxalated vacutainers on the occasion of biopsy. All samples were immediately processed under minimal light, and after centrifugation the plasma samples were stored at −70°C until analysed.

Retinoid analysis

Aromatic retinoids (acitretin and 13-cis acitretin) and endogenous vitamins A (retinol and 3,4-didehydroretinol) were analysed by HPLC as previously described (16, 17), except that, owing to interference problems, tretinoin could not be used as internal standard. Briefly, the samples were hydrolyzed in the presence of two internal standards (Ro 12–0586 and isotretinoin), and the water phase extracted twice with hexane before and after titrating the alkaline mixture to pH 4–5. The evaporated hexane extracts were redissolved in methanol and separately injected on an ODS column (5×200 mm) eluted with acetonitrile:water:acetic acid (82:17.9:0.1) at a flow rate of 1.2 ml/min. In one experiment, a straight-phase HPLC system consisting of a silica column eluted with dichloromethane:acetic acid (99.7:0.3) was used. The eluate was monitored at 325 and 360 nm, respectively, using serially connected, fixed wavelength UV-detectors. Calibration curves (peak height vs mass) were established by applying varying volumes of standard drug solutions (Hoffmann-La Roche, Basel) and a fixed amount of internal standards to identically sized blank samples. The detection limits for acitretin were 2 ng/g (epidermis), 10 ng/g (subcutis), and 2 ng/ml (plasma), respectively. Quantitation of retinol and 3,4-didehydroretinol was accomplished as previously described (17).

RESULTS

Identification of acitretin in biopsy material

Fig. 1 shows the chromatograms obtained with acidified extracts of hydrolyzed epidermis and subcutis from an acitretin-treated patient. In both chromatograms, distinct peaks can been seen at the positions of 13-cis acitretin and acitretin. The 325/360 nm absorption ratio of these peaks was between 0.8 and 0.9, i.e. close to the value of 0.7 observed for authentic 13-cis acitretin and acitretin. However, in some samples the peaks had a 325/360 ratio clearly exceeding 1.0, thus indicating impurity. The problem was greatest with 13-cis acitretin in subcutis. To characterize the peak content further, fractions corresponding to the elution position of 13-cis acitretin were collected from a pooled subcutis sample. Two peaks appeared when this material was rechromatographed in a straight-phase HPLC system (not shown). The smaller peak, which eluted last, corresponded to 13-cis acitretin both with regard to retention time and absorption ratio. The larger peak did not co-elute with any of the available retinoid standards; paucity of material precluded further characterization. Unfortunately, the straight-phase HPLC system which separated 13-cis acitretin from the contaminations did not work for routine acitretin determination. To avoid spuriosly high values in the standard HPLC assay, only peaks with a 325/360 ratio < 1.0 were quantitated.

Drug concentrations in epidermis and plasma

Table I shows the individual acitretin values in epidermal shave biopsies obtained before, during and after treatment. Pre-treatment samples were negative for acitretin in all but 3

Table I. *Acitretin concentrations in epidermis (ng/g wet weight)*

Pat. no	Before	1 month	6 months	Wash-out
1	0	5.6	17.0	0
2	0	23.1	3.2	0
3	0	14.0	13.9	0
4	0	18.6	13.8	0
5	0	28.1	21.0	0
6	0	3.5	11.2	0
7[a)]	6.0	10.9	8.7	0
8	0	30.1	7.5	0
9	12.8	10.6	4.4	0
10	4.1	16.8	15.0	0
11	0	12.7	6.9	≤2
12	0	18.8	11.6	0
Mean±SD		17.2±8.7	11.8±5.1	
n		9[b)]	9[b)]	

a) The patient had received etretinate 18 months before. Etretinate is converted to acitretin during assay.

b) Only patients with zero pre-treatment values and unequivocal 325/360 nm values are included in the mean±SD values.

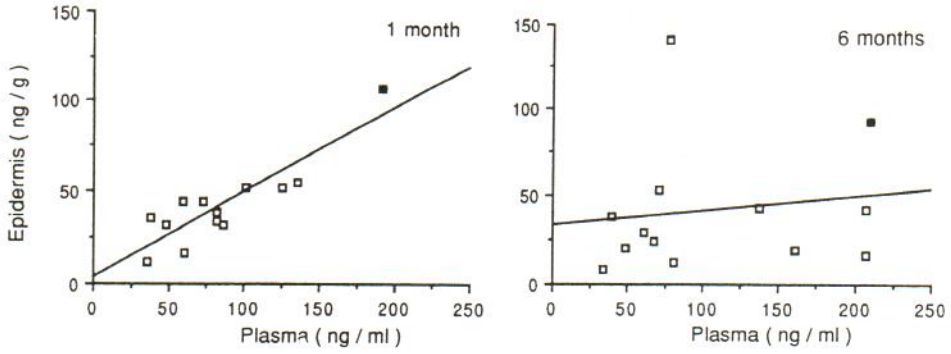

Fig. 2. Correlation between the plasma and epidermis concentrations of aromatic retinoids (sum of acitretin and 13-cis acitretin) after 1 (*left*) and 6 (*right*) months of therapy. The filled symbol represents the values for one patient excluded from other calculations because of a liver disease appearing during the study.

patients. Patient no. 7 had received etretinate 18 months earlier and significant amounts of the drug were still present in both epidermis and subcutis (see below). The reason for the acitretin peaks in the two other pretreatment samples (pat. no. 9 and 10) is not clear; the patients had not received aromatic retinoids before. The epidermal acitretin concentration during therapy varied between 3 and 30 ng/g. However, the mean values at 1 and 6 months were rather similar and virtually unaffected by whether or not only patients with zero pre-treatment levels were included. One month after stopping therapy the acitretin level was below detection limit in all 12 samples. The individual concentrations of 13-cis acitretin ranged from 0 to 134 ng/g during therapy and the mean ±SD values at 1 and 6 months were 21 ± 11 and 27 ± 36 ng/g, respectively. Nine of the pre-treatment and 3 of the wash-out samples were negative for 13-cis acitretin. In the remaining 9 wash-out samples the mean 13-cis concentration was 14 ± 12 ng/g but several of the corresponding HPLC peaks had questionably high 325/360 nm absorption ratio.

Fig. 2 shows the covariation of individual drug concentrations (sum of acitretin and 13-cis acitretin values) in plasma and epidermis at 1 and 6 months, respectively. For comparision, the high values observed in a patient excluded for medical reasons (see under Patients) are depicted. The correlation coefficient for the 1 month values (n = 13) was 0.89 ($p < 0.01$) and for the 6 months values 0.05 ($p > 0.05$). The reason for this discrepancy is not known. The correlation coefficient for the total drug values in plasma and epidermis was generally higher than for the acitretin and 13-cis acitretin values per se, indicating that the isomerization equilibrium may differ in plasma and epidermis.

Table II. *Acitretin in subcutis (ng/g wet weight)*

Pat. no.	Before	1 month	6 months	Wash-out
1	0	36	69	30[d]
2	0	154	211	226[d]
3	0	72	164	0
4	0	56	58	0
5	0	62	15	0
6	0	199	1032	(702)[b]
7[a]	1078	1115	499	433
8	0	284	292	219
9	23	44	27	24
10	28	103	1437	(1046)[b]
11	0	674	120	215
12	0	55	83	169
Mean±SD		177±204	227±314	107±109
n		9[c]	9[c]	8[c]

a) See Table I.
b) Value excluded because of an 325/360 nm absorption ratio >1.2 (see Methods).
c) Only patients with zero pre-treatment values and unequivocal 325/360 values are included in the mean ± SD values.
d) A repeated biopsy 6 months after stopping therapy was negative for acitretin.

Drug concentrations in subcutis

Table II shows the concentrations of the parent drug in subcutis before, during and after therapy. As noted above, patient no. 7 had a high pre-treatment level of acitretin consistent with a prior intake of etretinate; his values declined over the study period in accordance with a terminal elimination half-life of 3 months previously reported for etretinate in subcutis (5, 6). Again for unknown reasons patients no. 9 and 10 had small, but detectable peaks of acitretin in their pre-treatment samples. During therapy the acitretin values varied markedly both inter- and intraindividually. Furthermore, patients nos. 6, 10 and 11 occasionally showed high values; however, the 325/360 ratios of the corresponding peaks were questionably high. The mean acitretin concentrations in subcutis at 1 and 6 months of therapy, calculated for patients with zero pre-treatment values, were an order of magnitude higher than in epidermis. There was no correlation between the individual plasma and subcutis concentrations of acitretin (data not shown). It is a

Table III. *Vitamin A concentrations in plasma, subcutis and epidermis of patients undergoing acitretin treatment*

	Pretherapy	Acitretin		Wash-out
		1 month	6 months	
Plasma (ng/ml)[a]	591±220[b]	523±164	602±159	613±161
Subcutis (ng/g)[a]	1472±661	1411±718	1600±748	1675±685
Epidermis (ng/g)				
Retinol	93.3±31.6	78.8±32.8	81.9±23.0	121±34.2*
3,4-Didehydro-retinol	78.8±50.2	84.0±38.0	62.8±19.3	48.1±17.9

*) Difference vs pretherapy value statistically significant ($p<0.05$, students t-test).
a) The concentrations of 3,4-didehydroretinol in plasma and subcutis were below the detection limit of the assay.
b) Values denote mean±SD (n = 12).

disturbing fact that several of the wash-out samples contained significant amounts of a compound indistinguishable from acitretin in the HPLC assay. However, in some of these cases a new biopsy obtained 6 months posttherapy was negative for acitretin.

As noted earlier, the 13-cis acitretin peak is difficult to evaluate in subcutis. Thus, whereas the pretreatment samples were usually negative for the metabolite, many samples obtained during and after therapy contained a prominent peak at the position of 13-cis acitretin with a 325/360 ratio > 2 making it impossible to quantitate. As a whole, no meaningful data could be extracted from the 13-cis acitretin analysis of subcutis.

Effect on endogenous vitamin A levels in blood and tissue

Table III shows the mean concentrations of vitamin A in plasma, subcutis and epidermis of all 12 patients before, during and after acitretin therapy. The patients pre-treatment retinol values are in the normal range in serum and subcutis, but slightly below the normal range of 100–150 ng/g in epidermis. 3,4-didehydroretinol was present only in epidermis; the pre-treatment value was slightly above the normal range of 30–70 ng/g (15). Acitretin therapy did not induce any statistically significant change in the retinol values in plasma, subcutis and epidermis. After cessation of therapy the retinol concentration tended to rise and the epidermal mean value was significantly higher in the wash-out samples than in the pre-treatment samples. The epidermal 3,4-didehydroretinol values were virtually unaffected by therapy but tended to decrease after cessation of therapy.

DISCUSSION

The therapeutic concentrations of acitretin in blood and epidermis as observed in this study are generally only 1/4 those of etretinate (3, 6). The low acitretin concentration creates methodological problems as the amount of drug in the skin biopsies approaches the detection limit of the assay. Because most of the analyses could not be repeated, the individual values should be regarded as approximates. Nevertheless, we are confident that the total drug concentration (sum of acitretin and 13-cis acitretin) during oral dosing with 30 mg acitretin daily averages 40–50 ng/g in epidermis as compared to 80 ng/ml in plasma (pre-dose values). Plasma and epidermal values appeared to be correlated, at least during the initial phase of therapy, suggesting that penetration of the drug into the skin is efficient.

In a recent publication Laugier et al. (11) reported on the skin concentrations of acitretin in 4 psoriasis patients receiving 30 mg of the drug per day. They studied full-thickness skin biopsies and used a non-hydrolytic extraction procedure without internal standardization of the HPLC assay. Nonetheless, their values are of the same order of magnitude as the ones we observed in epidermal biopsies. Our analytical approach, which involves tissue hydrolysis in the presence of an internal standard, yields a high and reproducible recovery of retinoids (17). However, esterified compounds, such as etretinate and retinyl esters, are completely converted to the free congeners, acitretin and retinol, respectively, and are quantitated as such. Also, some artifactual isomerization of acidic retinoids inevitably occurs during tissue hydrolysis, but the 13- cis/all-trans isomerization reactions will usually outbalance one another (17), especially when similar amounts of the two acitretin isomers occur in the sample. From an analytical point of view, a far bigger problem was the occurrence of a tissue contaminator with characteristics similar to those of 13-cis acitretin. It appeared during therapy and prevented quantitation of 13-cis acitretin in many subcutis samples. Although the identity of the contaminator(s) has not been established, the possibility that it represents a metabolite of acitretin with high affinity for fat warrants further investigation.

The most notable difference between the tissue distributions of acitretin and etretinate is in subcutis. As previously reported, the etretinate concentration in adipose tissue of patients receiving long-term etretinate treatment is by average 15 μg/g and may reach values as high as 50 μg/g, i.e. two orders of magnitude higher than in epidermis (6, 10). In contrast, the subcutaneous concentration of acitretin (as metabolite) is low in etretinate-treated patients (18), probably reflecting the much lower lipophilicity of the free carboxylic acid. Although the subcutaneous acitretin values were also mostly low (<0.3 μg/g) in acitretin-treated patients, 3 of the patients had unexpectedly high values (0.6–1.4 μg/g) during therapy and many of the wash-out samples still contained significant amounts of a material indistinguishable from acitretin by HPLC. It should be recalled that, since the samples were hydrolyzed, the acitretin-like material may very well represent an esterified compound in vivo, e.g. etretinate, thus explaining its apparent lipophilicity. It is presently uncertain how this finding relates to the appearence of etretinate in the blood of some patients treated with acitretin (U. Wiegand, personal communication). However, the fact that some subcutis samples were negative for acitretin already within 1 month post-therapy whereas etretinate usually persists in subcutis several years after cessation of therapy (ref. 6; see pat. no. 7), corroborates a more rapid plasma clearance of the former compound. Although our study does not allow a final statement about the role played by fat tissue in the body storage of acitretin it points to several possible mechanisms that should be further investigated.

The lack of influence of acitretin therapy on the vitamin A levels in serum, subcutis and epidermis is in line with previous findings in etretinate-treated psoriasis patients but sharply contrasts the marked influence of isotretinoin on epidermal vitamin A levels in acne patients. Thus isotretinoin therapy increases the retinol concentrations by a factor of 1.5 to 2 and virtually depleats the epidermis of 3,4-didehydroretinol without affecting the vitamin level in blood and subcutis (13). These in vivo findings are corroborated by recent in vitro experiments showing that isotretinoin, but not acitretin, markedly inhibits the bioconversion of retinol to 3,4-didehydroretinol in organ cultured human epidermis (19). Interactions with the natural vitamin A metabolism may be of importance for the mechanism of action of synthetic retinoids in various diseases and differences in this respect might help to explain for example why psoriasis patients respond better to acitretin than

to isotretinoin, and why acne patients show the reversed response.

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