Inability to detect plasma etretinate and acitretin is a poor predictor of the absence of these teratogens in tissue after stopping acitretin treatment

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- 1 Concentrations of etretinate, acitretin and its main metabolite 13-*cis*-acitretin were measured in plasma and subcutaneous fat samples from 37 women of childbearing age exposed to acitretin before November 1990. Twenty of the women still used acitretin and 17 had stopped therapy for a period ranging from 1 to 29 months.
- 2 The prevalences of detectable etretinate concentrations were 45% and 83% in plasma and subcutaneous tissue, respectively, among current acitretin users and 18% and 86% among those who had stopped acitretin therapy. Thus, inability to detect plasma etretinate is a poor predictor of the absence of etretinate in fat.
- 3 Acitretin and/or etretinate were detectable in fat and in some cases in plasma from women who had ceased acitretin therapy for up to 29 months.
- 4 We suggest that (cis)-acitretin and etretinate should be monitored in subcutaneous tissue when plasma measurements are negative. The recommended contraception period of 2 years after cessation of acitretin therapy should be reconsidered to avoid the risk of teratogenicity.

Keywords etretinate acitretin sensitivity monitoring postmarketing surveillance teratogenicity risk

Introduction

Acitretin (Neotigason®, Soriatane®) and its prodrug the ethylester etretinate (Tegison®, Tigason®) were discovered in 1972 and found to have a high therapeutic index in a mouse model of papilloma [1]. Etretinate was selected initially for further development as its absorption and efficacy in mice were better [1]. Since 1980, etretinate has been used successfully for the systemic treatment of various dermatological disorders. Owing to its lipophilicity, etretinate is retained in adipose tissue, resulting in a highly variable elimination half-life of up to 175 days [2]. Both etretinate and acitretin are teratogenic retinoids [3]. Reports of teratogenicity occurring up to 1 year after cessation of etretinate therapy have been published [4]. In most countries where etretinate was marketed, women of childbearing potential were

advised to preclude conception until 2 years after cessation of therapy. However, the period of time during which pregnancy must be avoided after etretinate treatment has not been determined [5]. Etretinate is readily metabolized to acitretin and its isomer 13-*cis*-acitretin. In contrast to etretinate, acitretin has been shown not to accumulate in fatty tissue and is cleared from the body with a half-life of approximately 50 h [6, 7]. Acitretin has a clinical efficacy comparable with that of etretinate [8]. Thus, the use of acitretin appeared to be a preferable alternative, especially for women of childbearing potential, as the contraception period after therapy could be shortened to 2 months. In October 1989 acitretin replaced etretinate in the Netherlands. However, in October 1990 it was reported that etretinate was unexpectedly present

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in the plasma of 17 of 30 patients who were being treated with acitretin [6]. This finding was confirmed by Larssen *et al.*, Lambert *et al.* and Meyer *et al.* [7, 9, 10]. Thus, health authorities decided to extend the recommended contraception period after stopping acitretin therapy from 2 months to 2 years. Currently, monitoring and counselling of women who want to get pregnant and who have been treated with acitretin or etretinate is based mostly on a contraceptive period of 2 years. Sometimes plasma concentrations of acitretin and its metabolites are measured. However, the minimum plasma concentration associated with teratogenicity is unknown [4, 5].

We are currently performing a follow up study in women of childbearing age who have been treated with acitretin. In our cohort the presence or absence of acitretin, 13-*cis*-acitretin and etretinate in plasma varied greatly between patients. In addition, 13-*cis*-acitretin was detectable in plasma up to 1.5 years after cessation of acitretin therapy. Most striking was the finding that the absence of acitretin, 13-*cis*-acitretin and etretinate in plasma did not preclude the presence of 13-*cis*-acitretin in plasma from the same patient 3 months later, without intercurrent intake of the drug. Since it is known that etretinate is stored predominantly in fatty tissue, it was decided to perform a cross-sectional investigation among women of childbearing age with variable cessation times of acitretin therapy to compare plasma and fat concentrations of the teratogens acitretin, 13-*cis*-acitretin and etretinate. The study was conducted without interference with diet or medication.

Methods

This cross-sectional study was part of a follow-up study in 62 out of a cohort of 199 female patients of childbearing age who had been treated with or were still being treated with acitretin. The women were identified anonymously in 1990 following the *ad-hoc* cohort tracing conducted by the Netherlands Centre for Monitoring of Adverse Reactions to Drugs [11]. During the follow-up study blood samples were collected every 3 months to determine the plasma concentrations of acitretin, 13-*cis*-acitretin and etretinate.

Patients

A total of 37 patients (mean age $\pm$ s.d. 33.8 ± 9.6 years) participated in this study. Two patients took part at their own request. Thirty-five of the 62 patients followed as from November 1991 agreed to participate in the study at our request. The 35 patients were a selected group as at the start of the cohort study (13 months earlier) they did have detectable concentrations of either acitretin, 13-*cis*-acitretin or etretinate in plasma.

Twenty-one patients had psoriasis, eight patients had ichthyosis, seven patients had Darier's disease and one suffered from warts. Twenty-seven of the 37 participating women had been treated with etretinate; all

of them switched to acitretin between 33-90 months prior to the study. Eventually, 11 of these 27 initial etretinate users stopped retinoid therapy entirely; 16 of them still use acitretin. The time elapsed since etretinate was stopped did not differ significantly between women who subsequently also stopped acitretin (43 ± 14 months) and those who continued that drug (45 ± 17 months) (Table 1). The remaining 10 patients and both their dermatologists and general practitioners indicated in concordance that these patients never used etretinate, but only acitretin. Four continued this medication and six stopped. Thus, overall, 20 women still used acitretin in different dosages ($10-40$ mg day⁻¹) and 17 had stopped the drug for a period ranging from 1-29 months (Table 1). All hepatic, renal and haematological functions were normal.

The cumulative amount of acitretin ingested was approximated by multiplying the average prescribed daily dose (derived from a 3 year medication history from pharmacy records) with the total duration of acitretin treatment.

The percentage of fat per body weight was measured using a skin fold technique. Body weight was measured, and information on height and the time of last intake of acitretin were provided by the patients. The study was approved by the ethics committee of the University Hospital of Groningen.

Sample collection

Blood and fat samples were collected under fasting conditions from all 37 patients, and before dosage (10-24 h after last dose) from the 20 patients who were still under treatment. Full thickness cutaneous punch biopsies (3-4 mm) plus subcutaneous fat (mean wet weight $\pm$ s.d.: 40.2 ± 14.9 mg) were obtained from non-affected skin of the buttock under local anaesthesia. The samples were blotted to remove blood and excess fluid, weighed and stored in the dark at -20°C until assayed. Immediately after subcutaneous tissue sampling, 8 ml blood was drawn from an antecubital vein into oxalated Vacutainers®. Plasma was separated immediately under minimal light and stored in the dark at -20°C until analysed. Processing of samples was always done by the same investigator (MS) and the analyses were performed under single-blind conditions.

Analytical methods

Subcutaneous tissue The concentrations of acitretin, 13-*cis*-acitretin, etretinate and 13-*cis*-etretinate in subcutaneous tissue were measured by h.p.l.c. at Roche (Basel), according to a slightly modified, fully-automated h.p.l.c. column switching method using direct injection of plasma samples, on line solid-phase extraction, and reversed-phase chromatography with u.v. detection [12]. Frozen samples (maximum amount 50-70 mg weight) were weighed directly into glass tube homogenizers and 1 ml of ethanol containing the internal standards (Ro 11-6738: all-*trans*-9-(4-methoxy-3,5-diethyl-2,6-dimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetranoic acid, and

Ro 12-7554: ethyl all-*trans*-9-(2,6-dichloro-4-methoxy-3-methylphenyl)-3,7-dimethyl-2,4,6,8-nonatetranoate) was added. The samples were homogenized with a motor driven pestle while cooled in ice. After centrifugation 0.6 ml of the supernatant was injected directly into the h.p.l.c. column-switching system without any further purification or solvent evaporation. The gradient mobile phase, M2, consisted of the following components: (A) 1% w/v ammonium acetate/acetonitrile/acetic acid (30:70:3), (B) 1.7% w/v ammonium acetate/acetonitrile/acetic acid (6:95:3), (C) acetonitrile/tetrahydrofuran/water (80:20:2). The total sequence of automated sample analysis required 35 min and included the following steps, modified from the previously reported method [12].

Step A: Injection of the sample on the precolumn (14 × 4.6 mm, Bondapak C18 Corasil, 37–50 µm). Polar components were washed out using ethanol as a mobile phase, which was diluted on-line with an eluent consisting of 1% w/v ammonium acetate and acetic acid/acetonitrile (100:2). The guard column (14 × 4.6 mm, Bondapak C18 Corasil, 37–50 µm) and analytical column (Spherisorb ODS 1.5 µm) were meanwhile equilibrated with mobile phase M2 (100% A).

Step B: The precolumn was purged in the backflush mode.

Step C: While retained components were transferred from the precolumn to the analytical column and detector D2 (360 nm) in the backflush mode by a gradient of mobile phase M2 (100% A to 100% C), the capillaries between the autosampler and u.v. detector D1 (230 nm) were purged with ethanol (mobile phase M1A) and ethanol/water (8:2). After transfer of the retained components over 6 min, the precolumn was disconnected from the analytical column.

Step D: Mobile phase 2 consisting of 100% C until 35 min was changed from 100% C through B to 100% A within 0.1 min and the precolumn was re-equilibrated with mobile phase M1 (ethanol and 1% w/v ammonium acetate and acetic acid/acetonitrile (100:2)).

The limit of quantification was 8 ng/g for a 50 mg tissue sample but increased if the sample weight was less than 50 mg as can be seen in Table 1. Mean recoveries of acitretin, 13-*cis*-acitretin, etretinate and 13-*cis*-etretinate were 98.7%, 91.6%, 94.2% and 94.2%, respectively, for 5–15 mg samples. For 30–70 mg samples, mean recoveries were 90.8%, 80.6%, 73.9% and 73.1%, respectively. Although 13-*cis*-etretinate was measured in subcutaneous tissue, the concentrations are not reported since, for analytical reasons, plasma concentrations were not measured. As most of the patients with high fat concentrations of etretinate have 13-*cis*-etretinate in fat also, the total retinoid concentrations reported will be underestimated.

Since the sample size of tissue was inversely related to the quantification limit, very small samples with quantification limits exceeding the mean quantification limit of all samples by twice the standard deviation were excluded from all data analyses. Samples in which traces below the quantification

limit were found were considered to contain 0.5 x the quantification limit of the substance concerned. Samples in which no observable chromatographic peak for the compound of interest was present were considered as zero-values.

The concentration of acitretin equivalents in subcutaneous tissue was calculated as the sum of nmol g⁻¹ concentrations of acitretin, 13-*cis*-acitretin, and etretinate. Conversion to ng g⁻¹ acitretin was done by multiplying by the molecular weight (M) of acitretin (M = 326.44).

Plasma

Concentrations of acitretin, 13-*cis* acitretin and etretinate in plasma were determined using two different assays. Both were based on an automated column switching h.p.l.c. method with u.v. detection which has been described previously [12]. The second assay has been developed recently.

Assay (1) (detection limit 1.0 ng ml⁻¹) Plasma (0.5 ml) was deproteinized by adding two volumes of ethanol which contained 100 ng ml⁻¹ of isotretinoin as internal standard. After centrifugation, 0.5 ml of the supernatant was injected into the chromatograph. The recoveries of the three compounds were between 63–80%. The limit of quantification was 5 ng ml⁻¹ for acitretin, 13-*cis*-acitretin and etretinate, and the limit of detection was 1 ng ml⁻¹ assuming a 3:1 signal-to-noise ratio. The inter-assay precision (defined as the coefficient of variation of replicate analyses) in the concentration range 20–1000 ng ml⁻¹ was between 0.9 and 4% for all three compounds [12].

Assay (2) (detection limit 0.1 ng ml⁻¹) Plasma (0.4 ml) was deproteinized by adding three volumes of ethanol which contained 20 ng ml⁻¹ of Ro 11-6738 as internal standard. After centrifugation, 1.4 ml of the supernatant was injected into the chromatograph. The recoveries of the three compounds were 85–89%. The limit of quantification was 0.3–0.5 ng ml⁻¹ for acitretin, 13-*cis*-acitretin and etretinate, and the limit of detection was 0.1 ng ml⁻¹ assuming a 3:1 signal to noise ratio. The inter-assay precision in the concentration range 0.3–500 ng ml⁻¹ was between 3.3 and 5.8% for all three compounds. Concentrations between the limit of quantification and detection were defined as traces. For arithmetic reasons all traces were set at 0.2 ng ml⁻¹.

Statistical analysis

Student's *t*-test was used for comparison of continuous variables. For data which were not normally distributed, the Mann-Whitney test was used. For categorical data and differences in proportions χ^2 statistics with Yates correction were applied. Correlation coefficients were calculated with 95% confidence interval (CI). Differences were considered to be significant if *P* was <0.05 (two sided).

Sensitivity values were calculated by dividing the number of patients positive for the compound in both plasma and tissue (true positives) by the total number of patients with detectable compounds in subcutaneous tissue. Specificity was calculated as the ratio

of the number of patients who did not have detectable compounds in either plasma or tissue (true negatives) by the number who did not have the compound in subcutaneous tissue.

Results

Plasma concentrations

Individual plasma concentrations of acitretin, 13-*cis*-acitretin and etretinate are listed in Table 1. Concentrations refer to the most sensitive assay, with a detection limit of 0.1 ng ml⁻¹. In users of acitretin, predose plasma acitretin concentrations ranged from 1.4 ng ml⁻¹ to 83.4 ng ml⁻¹. The correlation coefficient between acitretin concentrations (ng ml⁻¹) and current daily dosage of acitretin (mg) was 0.46 (95% CI: 0.02 < *r* < 0.75). Plasma concentrations of 13-*cis*-acitretin among users ranged from 2.1 to 159.1 ng ml⁻¹. The correlation coefficient for plasma concentrations of 13-*cis*-acitretin and current daily dosage of acitretin was 0.50 (95% CI: 0.07 < *r* < 0.77). As indicated in Table 1, nine out of the 20 acitretin users (45%) had detectable plasma concentrations of etretinate ranging from traces to 1.7 ng ml⁻¹. Of these nine patients, two had never been treated with etretinate. The other seven women with detectable plasma etretinate concentrations had ceased etretinate therapy for more than 3 years. Among those patients who had stopped acitretin therapy, 10 had detectable plasma concentrations of acitretin and/or 13-*cis*-acitretin up to 29 months after cessation of therapy. Three of these patients had plasma etretinate concentrations ranging from 0.4 to 1.1 ng ml⁻¹. Of the total group (*n* = 37), 12 patients (32%) had etretinate in their plasma and three of these had never been treated with etretinate.

Tissue concentrations

The average limit of quantification ± s.d. was 13.1 ± 8.9 ng g⁻¹ for all subcutaneous tissue samples. Those patients in whom small tissue samples were obtained, which resulted in limits of quantification above the mean quantification limit + 2 × s.d. (30.9 ng g⁻¹), are indicated in italics and brackets (*n* = 5) in Table 1 and were excluded from all data analyses. In the users of acitretin, predose concentrations in subcutaneous tissue samples ranged from non-detectable to 31.5 ng g⁻¹ for acitretin, from traces to 40.6 ng g⁻¹ for 13-*cis*-acitretin and from non-detectable to 628.9 ng g⁻¹ for etretinate. Although three acitretin users had no detectable acitretin in their tissue, all had 13-*cis*-acitretin in quantifiable amounts. Fifteen of the remaining 18 current acitretin users (83%) had etretinate in their tissue. Three of the four current acitretin users without prior etretinate treatment had tissue etretinate concentrations ranging from 20.9 up to 513.3 ng g⁻¹. Concentrations of acitretin (*P* = 0.29), 13-*cis*-acitretin (*P* = 1.0) or etretinate (*P* = 0.83) in the tissue of current acitretin users with as compared

to without a history of etretinate use did not differ significantly according to non-parametric testing.

In stoppers of acitretin therapy, acitretin concentrations ranged from non-detectable to 24.0 ng g⁻¹, 13-*cis*-acitretin concentrations from traces to 37.9 ng g⁻¹ and etretinate concentrations from non-detectable to 1029 ng g⁻¹. No correlations were apparent between cessation time of therapy and tissue acitretin or 13-*cis*-acitretin concentrations. Etretinate, acitretin or 13-*cis*-acitretin were detected in samples from all patients who had ceased acitretin for more than 2 years. Importantly in two patients who had ceased acitretin therapy for 26–29 months and etretinate therapy for 33–40 months, all three compounds were found in subcutaneous tissue. In stoppers of acitretin without prior exposure to etretinate and included in the analysis, acitretin cessation times ranged from 7–26 months. All of these four women had detectable levels of one or more of the three compounds.

No significant differences in tissue acitretin (Mann-Whitney: *P* = 0.17) or etretinate concentrations (Mann-Whitney: *P* = 0.96) were found between current users and stoppers of acitretin therapy; only 13-*cis*-acitretin concentrations were different (Mann-Whitney: *P* = 0.046).

Total acitretin equivalents in tissue ranged from 18.7–618 ng g⁻¹ (mean ± s.d.: 142 ± 187; median 54) in acitretin users who had been treated with etretinate in the past, from 28.4 to 534.1 ng g⁻¹ (189 ± 237; 96) in users without etretinate treatment, from 17.8 to 176 ng g⁻¹ (80 ± 52; 71) in stoppers of acitretin with previous etretinate treatment, and from 8.4 to 950 ng g⁻¹ (248 ± 468; 17) in stoppers who had never been treated with etretinate. No significant difference in total ng g⁻¹ acitretin equivalents was found between users and stoppers of acitretin therapy (Mann-Whitney: *P* = 0.45) or between stoppers having been treated with etretinate or not (Mann-Whitney: *P* = 0.20).

No correlation was found between months of cessation of treatment and the sum of acitretin equivalents in tissue, even after stratification for history of exposure to etretinate.

Sensitivity/specificity of plasma measurements

Table 2 shows sensitivity and specificity values for the detection of the various compounds in plasma using the presence or absence of etretinate in subcutaneous tissue as the gold standard. Results according to both the established, and the newer assay are presented. Amongst users and stoppers of acitretin 33% and 8%, respectively, of the women with etretinate in their subcutaneous tissue had detectable concentrations of etretinate in plasma according to the less sensitive assay. Corresponding results with the new assay were 53% and 25% for users and stoppers, respectively. Table 2 also shows that the detection of any of the compounds with respect to presence of etretinate in tissue for stoppers increases from 17% to 83% with the tenfold decrease in assay detection limit. Specificity values are not reliable because only a few women had no etretinate in their tissue.

Table 1 Concentrations of acitretin, 13-*cis* acitretin and etretinate in plasma and subcutaneous tissue from users and stoppers of acitretin therapy stratified according to their history of etretinate intake

								Plasma		Subcutis				

#: dose (g): accumulated dose of acitretin @: BMI = Body Mass Index

a: acitretin b: 13-*cis*-acitretin, c: etretinate

n.a: not applicable nop: no observable peak traces: detectable peak, but below limit of quantification

(italics): patients with limit of quantification ≤ 30.9 ng g⁻¹, not included in analysis* adjusted: traces quantified as 0.50xlimit of quantification (limit of quant.) for limits of quantification ≤ 30.9 ng g⁻¹

Table 2 Sensitivity and specificity for the determination of etretinate, or any of the three compounds (acitretin, 13-*cis*-acitretin and etretinate) in plasma by two assays with different detection limits (1 ng ml⁻¹ and 0.1 ng ml⁻¹) compared with presence of etretinate in the subcutis. Only patients with limits of quantification ≤ 30.9 ng g⁻¹ tissue were included in the analysis

	<i>Etretinate in subcutaneous tissue</i>			
	<i>Users of acitretin (n = 18)</i>		<i>Stoppers of acitretin (n = 14)</i>	
	<i>Assay detection limit</i>			
	<i>1.0 ng ml⁻¹</i>	<i>0.1 ng ml⁻¹</i>	<i>1.0 ng ml⁻¹</i>	<i>0.1 ng ml⁻¹</i>
<i>Etretinate in plasma</i>				
sensitivity	5/15 (33%)	8/15 (53%)	1/12(8%)	3/12(25%)
specificity	3/3	3/3	2/2	2/2
<i>Any of the three compounds in plasma</i>				
sensitivity	15/15	15/15	2/12(17%)	9/12(83%)
specificity	0/3	0/3	2/2	2/2

Discussion

Our study has yielded four important results. Firstly, it is clear that female users of acitretin may have substantial etretinate concentrations in their subcutaneous tissue, even if they have never used preparations containing etretinate. This is compatible with the previous observation that acitretin is converted to etretinate in humans [7, 9, 10]. However the estimation of the prevalence of individuals effecting this conversion depends highly on the type of sample used. In our study the estimated prevalence was higher when tissue measurements of etretinate were used (> 80%) rather than plasma, especially among stoppers of acitretin therapy. The prevalence of current acitretin users with etretinate in their plasma was 45% when measurements were made with the more sensitive plasma assay. The prevalence measured in plasma was within the range reported in acitretin users by Meyer *et al.* [10], Larsen *et al.* [7], and Lambert *et al.* [9], who reported mean values of 18%, 70% and 60% [7, 9, 10]. Secondly, our study shows that the absence of etretinate or (13-*cis*-)acitretin in the plasma of female acitretin users does not preclude the presence of often substantial amounts of the teratogen etretinate (or its metabolites) in tissue. Thus, plasma assay is a poor predictor of the absence of etretinate and its metabolites in fatty tissue. This is especially true for the widely used assay with a detection limit of 1.0 ng ml⁻¹. Prediction of the presence of etretinate in tissue is improved with the new assay. Although our assessments were done using sub-cutaneous fat, it is likely that the measured concentrations also reflect those of retinoids in other fatty tissues, as results from autopsy cases revealed that etretinate is distributed homogeneously in different types of fatty tissue [13]. Thirdly, in patients who had discontinued acitretin intake for more than 24 months, etretinate or 13-*cis*-acitretin was present in subcutaneous tissue in concentrations comparable to those in tissue from women on acitretin therapy. Fourthly, in plasma from 10 of the stoppers of acitretin therapy, acitretin and 13-*cis*-acitretin were detectable up to 29 months after cessation of drug administration. This is much longer than might be

expected. Therefore, the currently recommended posttherapy contraception period of 2 years seems insufficient, especially for those patients who have a history of prior use of etretinate.

Several investigators have advised monitoring of plasma concentrations of etretinate in women who want to become pregnant after having been treated with etretinate [4, 7, 14]. Rollmann & Vahlquist [15] have shown that in psoriatic patients 7 weeks after the end of etretinate treatment, etretinate concentrations in subcutaneous fat are approximately 100 fold higher than those in serum and that etretinate can be present in subcutaneous tissue while it is undetectable in plasma. Despite the long history of etretinate use, little is known about the relationship between etretinate concentrations in plasma and adipose tissue. The minimum teratogenic concentration of etretinate and acitretin is unknown. Experimental proof that concentrations in the subcutis, while undetectable in plasma, are not associated with teratogenicity is difficult. In humans provision of this information may be complicated further by the possibility of redistribution of fatty tissue during pregnancy as a result, for example, of hyperemesis. A conservative assumption is that even plasma drug concentrations below the limit of detection may constitute a risk [4, 5]. Using the less sensitive assay only 17% of the stoppers with etretinate in the subcutis were found to have detectable concentrations of the compounds (etretinate, acitretin) in plasma, whereas a tenfold decrease in the detection limit revealed that 83% of them had one or more of the compounds in plasma (Table 2).

Löfberg *et al.* [16] suggested that the area under the concentration-time curve is important with respect to the teratogenicity of retinoids. As long as experimental proof of a minimum teratogenic concentration of etretinate and acitretin in the subcutis and plasma is lacking the safest position would be to avoid pregnancy as long as etretinate or acitretin is present in the subcutis. The optimal measurement site for monitoring women who wish to become pregnant following acitretin therapy should be a storage site, namely fatty tissue. This is especially so with respect to women monitored using the less sensitive plasma

assay. As collection of subcutaneous tissue is an invasive procedure monitoring should be initiated with repetitive measurement of plasma drug concentrations followed by monitoring of tissue concentrations after the former became undetectable, preferably using the more sensitive plasma assay.

Our estimate of the prevalence of etretinate in the subcutis could be low for two reasons: Firstly, the four patients who stopped acitretin therapy for more than 24 months ingested far less acitretin (cumulative dose) than the average patient in our study. The concentrations of the compounds in subcutaneous tissue after 2 years will probably be even higher in patients who ingested larger amounts of acitretin. Secondly, the concentration of acitretin equivalents in the subcutis may be higher than reported since 13-*cis*-etretinate was not included in the calculation.

In conclusion, our study confirms that female users of acitretin may have substantial concentrations of etretinate in their fatty tissue, probably due to conversion of acitretin into etretinate. Hence a post-therapy contraception period of 2 years may be too short, especially in patients with a history of prior

etretinate use. It may be important for regulatory authorities to reconsider this period. As long as no experimental indication of a minimal teratogenic concentration of acitretin or etretinate in humans is available, we recommend monitoring of females treated with acitretin and wishing to become pregnant by plasma measurements first. As soon as plasma (13-*cis*-)acitretin concentrations become undetectable, monitoring should be continued in fatty tissue. In the case of an existing pregnancy, risk assessment is necessary on an individual basis. Physicians should be encouraged to report any pregnancy after exposure to etretinate or acitretin independent of the post-therapy time.

We are grateful to our dermatologist colleagues P. J. M. Berretty and P. Meijer, who kindly took the biopsies, to A. Middelbeek for her assistance and most of all to the women participating in this study. Thanks are due to S. Mannino and R. Hoskins for their collaboration and to R. Wyss and A. Busslinger for the analysis of the samples. This study was supported by Hoffmann-La Roche.

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(Received 22 February 1994,
accepted 12 May 1994)