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Report

Dose-Proportional Absorption of Etretinate After Doses of 25, 50, 75, and 100 mg

Robert J. Wills,[1,2] Lolita C. Rodriguez,[1] Amy H. Lin,[1] Clara Puccini,[1] and Wayne A. Colburn[3]

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Twelve healthy male subjects received single oral doses of etretinate, ranging from 25 to 100 mg (1 to 4 × 25-mg capsules) in an open-label, four-way randomized crossover design. Plasma concentrations of etretinate and two active metabolites were determined by a specific high-performance liquid chromatographic (HPLC) method. Analysis of variance and orthogonal contrasts were used to assess dose proportionality. Mean (± %CV) maximum concentrations after 25- to 100-mg doses were 133 (50), 195 (33), 261 (53), and 446 (65) ng/ml, whereas AUC_{0-12} values were 581 (46), 1090 (39), 1500 (52), and 2440 (63) ng · hr/ml, respectively. The test for proportionality indicated that C_{max} and AUC_{0-12} increased proportionally with an increase in dose ($P > 0.05$).

KEY WORDS: etretinate; pharmacokinetics; dose proportionality.

INTRODUCTION

Etretinate (trimethylmethoxyphenyl analogue of retinoic acid ethyl ester) is an orally active aromatic retinoid indicated in the treatment of severe recalcitrant psoriasis (1). It is currently marketed as 10- and 25-mg capsules (Tegison). The recommended dosing regimen is 0.75 to 1 mg/kg/day in divided doses for the first 8 to 16 weeks of therapy followed by a maintenance regimen of 0.5 to 0.75 mg/kg/day. Depending on the severity of the psoriatic condition, initial therapy may employ single doses as high as 100 mg. Etretinate is not readily soluble in aqueous media (2 μg/ml at 25°C) and is poorly bioavailable (<25%) in dogs (2) and moderately bioavailable (~40%) in humans (3). Plasma concentrations of etretinate following oral administration have been found to be highly variable (3–6). This variability may result from incomplete absorption, an extensive first-pass effect, or the psoriatic condition (3). Therefore, the objective of this study was to evaluate the dose-proportional absorption and disposition of etretinate following 25-, 50-, 75-, and 100-mg oral doses. In addition, an attempt was made to relate serum triglyceride changes to doses and/or plasma concentrations of etretinate, since elevated triglyceride levels are common to etretinate therapy (1).

MATERIALS AND METHODS

Twelve healthy male subjects ranging in age from 20 to 32 years and within ± 10% of ideal body weight participated in the crossover study. The good health of the subjects was determined by a baseline history, physical laboratory examinations consisting of urinalysis, a complete blood count, a platelet count, and 20 serum chemistries. Serum triglyceride concentrations were measured at 0, 4, 8, 12, 24, and 48 hr after each dose. Subjects requiring chronic daily dosing of a drug, who had taken any medication chronically within the 2 weeks before the study, or who had taken any other drug (including alcohol) within 72 hr of the start of the study were excluded. In addition, no medications or other drugs (including alcohol) were allowed during the course of the study. All subjects gave written, informed consent to participate in the study.

Twelve hours prior to the start of the study, all subjects were confined to the study area. Ten hours prior to dosing, a light snack was served, after which an absolute fast, except for water, was maintained. In the morning each subject received either 25-, 50-, 75-, or 100-mg doses as multiples of 25-mg etretinate capsules with 200 ml of water according to a four-way randomized crossover design. Food and water were restricted during the first 4 hr after dosing. Lunch was served after the 4-hr blood sample was drawn and dinner was served after the 10-hr blood sample was drawn. A 2-week washout period separated the treatments.

Blood samples for drug assay were collected in heparinized Vacutainer tubes under yellow light since etretinate and metabolites photoisomerize. Prior to drug administration, a 20-ml blood sample was drawn, then additional 7-ml blood samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, 36, and 48 hr after dosing. For the 0-, 4-, 8-, 12-, 24-, and 48-hr blood samples, an additional 3 ml of blood was collected in Vacutainer tubes containing no anticoagulant for triglyceride determination. All subjects were confined to the study site until the 24-hr blood sample for each phase was obtained. During this time, any adverse effects were recorded. All subjects returned to the study site for withdrawal of the 36- and 48-hr samples. The clinical laboratory

[1] Department of Drug Metabolism, Hoffmann–La Roche Inc., Nutley, New Jersey 07110.
[2] To whom correspondence should be addressed.
[3] Present address: Warner-Lambert/Parke-Davis, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

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tests performed for entry into the study were repeated 5 days after completion of the study. A physician was available during the study.

Plasma concentrations of etretinate and the two major metabolites, the all-*trans* acid (acitretin) and the 13-*cis* isomer of acitretin, were determined by a specific high-performance liquid chromatographic (HPLC) method (7). The calibration range was 10–2000 ng/ml for all three compounds, and 0.5 ml of plasma was analyzed. Calibration samples were prepared from drug-free human plasma. The overall coefficient of variation for interassay precision was 2.6% for etretinate, 3.0% for acitretin, and 3.1% for the 13-*cis* isomer. The mean coefficient of variation for intraassay precision was 1.7, 4.6, and 2.5%, respectively. All assays were conducted under yellow light. Serum triglyceride levels were determined by the Gilford colorimetric method, which has a sensitivity limit of 1 mg/dl (8).

Pharmacokinetic parameters were determined from the etretinate plasma concentration data and used to assess the dose proportionality of etretinate over a range of 25 to 100 mg. The maximum concentration (C_{max}) and the time of maximum concentration (t_{max}) were read directly from the plasma concentration–time data. The area under the plasma concentration–time curve was determined from time zero to 12 hr (AUC_{0-12}) using trapezoidal summation. For comparison across doses, areas were not determined beyond 12 hr since plasma concentrations after 12 hr were nonmeasurable (<10 ng/ml) at the 25-mg dose. The elimination rate constant (β) could not be calculated for etretinate, acitretin, or the 13-*cis* isomer because the terminal log-linear phase could not be ascertained from the available plasma concentration–time data. Previous data generated in our laboratories (6) indicate that only after multiple-dose administration, where terminal plasma concentrations are high enough to be detectable (>10 ng/ml), can a more reliable estimate of the elimination rate constant be determined. In addition, C_{max}, t_{max}, and AUC_{0-12} and AUC_{0-48} were determined for acitretin and the 13-*cis* isomer, respectively.

Each pharmacokinetic parameter was analyzed separately for each of the three compounds. In each case, an analysis of variance of the individual values was performed using a model taken from Hedayat and Afsarinejad (9). The analysis of variance accounted for the effects of subjects, time periods, and treatments and possible carryover effects from the preceding treatments. Sequence effects were not included because no two subjects had the same sequence of treatment administration. To evaluate the effect of dose on the pharmacokinetic parameters, the treatment effect was broken into three orthogonal effects—dose linear, dose quadratic, and dose cubic—which were integrated into the analysis of variance model (see the Appendix for model development).

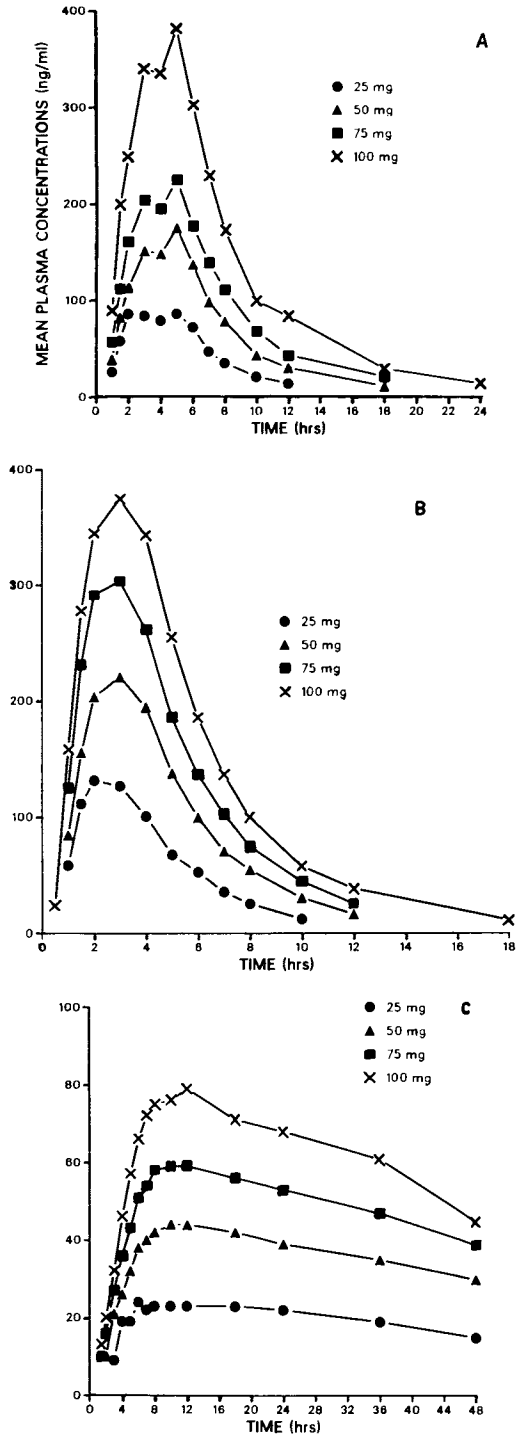

Fig. 1. Mean etretinate (A), acitretin (B), and 13-*cis* isomer (C) plasma concentrations (ng/ml) following single oral etretinate doses of 25 (●), 50 (▲), 75 (■), and 100 (X) mg in 12 subjects.

RESULTS

Three of the 12 subjects treated reported a clinical adverse experience during treatment with etretinate. Each experience was classified as remotely related to therapy. Two of these subjects complained of a mild headache, one after the 100-mg dose and the other after the 75-mg dose. The third subject had a stomachache reported as moderately severe after the 75-mg dose.

All subjects had abnormal serum levels of triglycerides at either 8 or 12 hr after drug administration. These values ranged from 180 to 290 mg/dl and could most likely be attributed to the food intake at 4 and 10 hr after drug administration. In addition, abnormal values were observed at 4, 24, and 48 hr after drug administration. Subjects who had an abnormality at 4 hr also had an abnormally high baseline

Table I. Pharmacokinetic Parameters for Etretinate, Acitretin, and the 13-*cis* Isomer After 25-, 50-, 75-, and 100-mg Oral Etretinate Doses

	Mean (± %CV)			
	25 mg	50 mg	75 mg	100 mg
Etretinate				
C_{max} (ng/ml)*,**,***	113 (50)	195 (33)	261 (53)	446 (65)
t_{max} (hr)	3.5 (45)	3.9 (30)	3.3 (49)	3.8 (35)
AUC_{0-12} (ng · hr/ml)*,**,***	581 (46)	1090 (39)	1500 (52)	2440 (63)
Acitretin				
C_{max} (ng/ml)*,**	134 (37)	230 (35)	319 (47)	397 (29)
t_{max} (hr)	2.5 (29)	2.7 (18)	2.8 (25)	3.0 (25)
AUC_{0-10} (ng · hr/ml)*,**,****	607 (33)	1100 (39)	1550 (50)	1990 (29)
13-*cis* acitretin				
C_{max} (ng/ml)*,**	29 (47)	47 (29)	62 (38)	80 (25)
t_{max} (hr)	8 (23)	14 (78)	11 (24)	14 (75)
AUC_{0-48} (ng · hr/ml)*,**	896 (30)	1700 (32)	2290 (40)	2870 (30)

* Significantly different across treatments: ANOVA (F test), $P < 0.01$

** Significantly different across treatments: linear orthogonal contrast (F test), $P < 0.01$.

*** No significant difference in slopes: $\hat{Y} = \hat{Y}_0$, $P = 0.75$ (C_{max}), $P = 0.85$ (AUC). P values are two sided (see Appendix for details).

**** No significant differences in slopes: $P = 0.13$ (AUC).

value. Five subjects had elevations at 24 hr, with three of these subjects having elevated levels after several doses. There was no record that the 48-hr samples were collected after a fasting state, since the subjects had not spent the previous night in the research unit and they were not instructed to fast. Of all the abnormalities observed at 4, 24, or 48 hr after drug administration, in only two instances, both in the same subject, did the level exceed 300 mg/dl.

The mean etretinate plasma concentration–time profiles for the four dose levels are presented in Fig. 1. Double peaks were observed at approximately 3 and 5 hr after drug administration. Inspection of the individual profiles identified double peaks in only 3 of 12 at 25 mg, 3 of 12 at 50 mg, 0 of 12 at 75 mg, and 3 of 12 at 100 mg. The intersubject variability was large with respect to the pharmacokinetic parameters (Table I). In particular, the wide range in C_{max} and t_{max} among individuals suggested a large intersubject variability in absorption rates. The etretinate individual ranges for C_{max} and t_{max} were 45 to 229 ng/ml at 2 to 6 hr, 111 to 292 ng/ml at 2 to 5 hr, 115 to 491 ng/ml at 2 to 7 hr, and 173 to 1120 ng/ml at 2 to 6 hr following doses of 25 through 100 mg, respectively. The combination of the highly variable C_{max} and t_{max}, the lack of double peaks in the majority of subjects, and the fact that double peaks have not been reported previously (3–6) implies that the double peaks observed using mean data are artifactual. The impact of this is considered negligible because individual data were used in all of the analyses. The individual ranges for AUC_{0-12} were 187 to 1110, 509 to 1730, 544 to 2580, and 1080 to 6150 ng · hr/ml following doses of 25 to 100 mg, respectively. The etretinate values for C_{max} and AUC_{0-12} differed significantly across all doses (Table I). The relationship between the parameters C_{max} and AUC_{0-12} and the administered dose was linear over the dose range of 25 to 100 mg ($P < 0.01$, Table I and Fig. 2). In 8 of the 12 subjects both C_{max} and AUC increased with dose, in 2 subjects both C_{max} and AUC were similar at 50 and 75 mg, and in 2 subjects there was no further increase in C_{max} and AUC after 75 mg. The test for dose proportionality indicated that C_{max} and AUC_{0-12} increased proportionately with an increase in dose ($P > 0.05$).

The acitretin concentration–time profiles were consistent with the etretinate concentration–time profiles (Fig.1). The individual ranges for C_{max} and t_{max} were 52 to 205 ng/ml at 1.5 to 4 hr, 109 to 343 ng/ml at 2 to 3 hr, 130 to 547 ng/ml at 2 to 4 hr, and 226 to 563 at 2 to 4 hr following etretinate doses of 25 through 100 mg, respectively. The individual ranges for AUC_{0-10} were 300 to 831, 504 to 1920, 544 to 2640, and 1050 to 2730 ng · hr/ml following etretinate doses of 25 to 100 mg, respectively. Again, the areas were truncated because the elimination rate constants could not be determined from single-dose data. Similar to etretinate, the acitretin values for C_{max} and AUC_{0-10} differed significantly across all doses and increased linearly with an increase in dose (Table I). However, only AUC_{0-10} increased proportionately with an increase in dose ($P > 0.05$).

The concentration–time profiles of the 13-*cis* isomer of acitretin differed from the etretinate concentration–time profiles (Fig. 1). The concentrations rose and declined slowly. The individual ranges for C_{max} and t_{max} were 13 to 57 ng/ml at 4 to 10 hr, 27 to 69 ng/ml at 7 to 48 hr, 28 to 113 ng/ml at 8 to 18 hr, and 51 to 112 ng/ml at 8 to 48 hr following etretinate doses of 25 to 100 mg, respectively. The individual ranges for AUC_{0-48} were 429 to 1270, 992 to 2520, 912 to 4090, and 1520 to 4370 ng · hr/ml following etretinate oral doses of 25 to 100 mg, respectively. Elimination rate constants could not be determined. The 13-*cis* isomer values for C_{max} and AUC_{0-48} differd significantly across all doses and increased linearly with an increase in dose (Table I). Neither C_{max} nor AUC_{0-48} increased proportionally with an increase in dose.

Attempts to establish a relationship between the administered dose or etretinate concentrations in plasma and elevations in serum triglycerides across the entire group of subjects were unsuccessful. Relationships between AUC_{0-12} and the triglyceride values that exceeded the normal range were not established either (Fig. 3). It should be noted that

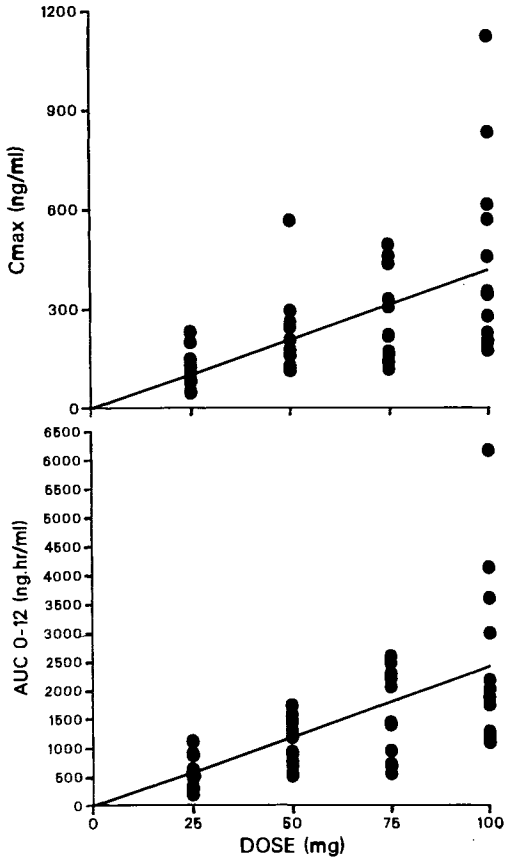

Fig. 2. Relationship of etretinate C_{max} and AUC_{0-12} with the administered dose. The filled circles represent individual data. The solid lines represent the regression on the statistically adjusted means.

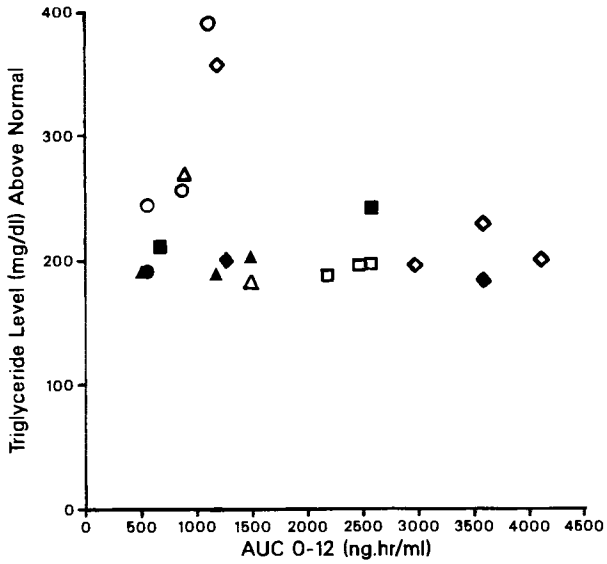

Fig. 3. Relationship between triglyceride values (mg/dl) above normal 24 hr (filled symbols) and 48 hr (open symbols) after dosing (■, □—25 mg; ●, ○—50 mg; ▲, △—75 mg; ◆, ◇—100 mg) and the etretinate AUC_{0-12} (ng · hr/ml).

in most cases, triglyceride values were not above the upper limit of the normal range.

DISCUSSION

Mean C_{max} and AUC_{0-12} values of etretinate increase proportionally with an increase in dose under fasting conditions. Since the elimination rate constants could not be determined from these single-dose data, assessment of saturable elimination processes was not possible. However, a previously reported 6-month multiple-dose study (6) demonstrated linear pharmacokinetics following doses of up to 100 mg/day.

The concentration–time profiles of the two metabolites are consistent with that reported previously (1, 3–5). The first-pass hydrolysis product, acitretin, has been shown to be biologically active and the major metabolite in plasma following single doses (10). The acitretin t_{max} values for each of the four doses are of the same order and the truncated AUC values are proportional, suggesting that there is no saturation of formation of acitretin. The 13-*cis* isomer of acitretin has been shown to be the major metabolite following multiple dosing (4). The concentrations and AUC values of this metabolite increase linearly with increasing doses, suggesting nonsaturable formation even though the increase is not proportional to the dose.

We could not establish any relationship between administered doses or etretinate concentrations in plasma and elevations in serum triglycerides. Since elevated triglyceride levels are common to etretinate therapy, it may be more appropriate to look for a relationship at steady state.

The data presented here support the dose proportionality of etretinate from single oral doses of 25 to 100 mg. Although this study was conducted with single doses, it is anticipated that a similar situation would occur if these doses were administered repetitively. In addition, it appears that elevated triglyceride levels are not a simple function of dose following single doses.

APPENDIX: TEST FOR DOSE PROPORTIONALITY

The treatment effect is broken into three orthogonal effects: dose linear, dose quadratic, and dose cubic. These effects are, according to the Hedayat and Afsarinejad model (9), as follows.

$$\text{Dose linear:} \quad -0.67082\,\hat{\tau}_A - 0.223607\,\hat{\tau}_B + 0.223607\,\hat{\tau}_C + 0.67082\,\hat{\tau}_D$$

$$\text{Dose quadratic:} \quad 0.5\,\hat{\tau}_A - 0.5\,\hat{\tau}_B - 0.5\,\hat{\tau}_C + 0.5\,\hat{\tau}_D$$

$$\text{Dose cubic:} \quad -0.223607\,\hat{\tau}_A + 0.67082\,\hat{\tau}_B - 0.67082\,\hat{\tau}_C + 0.223607\,\hat{\tau}_D$$

If we assume that the dose effect is linear, i.e., $\tau_A = 25\,Y$, $\tau_B = 50Y$, $\tau_C = 75Y$, and $\tau_D = 100Y$, where Y is the slope of the regression line, then the linear contrast is $55.9\hat{Y}$. A significant positive (negative) dose linear effect in C_{max}, for example, indicates that C_{max} increases (decreases) as the dose increases.

The four adjusted treatment means are defined as $G_1 = \hat{\mu} + \bar{\alpha} + \bar{\beta} + \hat{T}_1$, where $\bar{\alpha}$ and $\bar{\beta}$ are the average period and subject effects, and 1 = A, B, C, or D. If the dose effect is linear, then the treatment mean is also predicted as an intercept plus the slope times the dose of that treatment, i.e., $G_1 = \mu^* + \hat{Y}\,\text{dose}_1$. The definition of dose proportionality is that the ratio of the responses (say G_m to G_1) is equal to the

ratio of the doses ($dose_m$ to $dose_1$), i.e., $dose_m/dose_1 = G_m/G_1$, and G_m/G_1 can be expressed as $(\mu^* + \hat{Y}\, dose_m)/(\mu^* + \hat{Y}\, dose_1)$.

This holds if and only if the intercept $\mu^* = 0$, i.e., $\hat{Y} = G_1/dose_1$, for 1 = A, B, C, or D. We can estimate $\overline{G}$, the average adjusted treatment mean (which is identical to the overal pooled raw mean), with $\overline{G} = (25\hat{Y} + 50\hat{Y} + 75\hat{Y} + 100\hat{Y})/4$ if proportionality exists. This is further expressed as $\overline{G} = 250\hat{Y}/4$ or $\hat{Y} = 4\overline{G}/250 = \hat{Y}_0$. This, therefore, allows a test for dose proportionality via testing H_0: $\hat{Y} = \hat{Y}_0$, i.e., testing the equality of slopes obtained without and with the dose proportionality assumption.

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