Phase I Study and Pharmacokinetics of Weekly High-Dose 13-cis-Retinoic Acid

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ABSTRACT

In an attempt to increase the peak plasma levels of 13-cis-retinoic acid (13-cis-RA) and its efficacy in vivo, a Phase I study and pharmacokinetics of weekly high-dose, oral 13-cis-RA was conducted in 23 cancer patients who were refractory to conventional treatments. At 200 mg/sq m, the mean peak plasma level of 13-cis-RA was 1.5 ± 0.1 (SE) µg/ml; at 400 mg/sq m, the mean peak plasma level increased to 3.8 ± 0.7 µg/ml. Further increases of the 13-cis-RA dose up to 1800 mg/sq m did not lead to proportional increases in either the mean peak plasma levels or area under the curve, indicating a saturable absorption phenomenon. The terminal half-life was highly variable (range, 2.8 to 101.3 h) and was not related to the dose given. A secondary peak plasma concentration was seen in five patients, suggesting enterohepatic circulation. The toxicities such as headache, cheilitis, dry skin, and dry eyes were frequent on the weekly schedule but were not dose-limiting. One patient had an elevation of the triglycerides of 2 to 5 times the upper limit of normal; five patients had an elevation of 1.1 to 2 times normal. No objective responses were observed to treatment with 13-cis-RA. Of 20 patients receiving an adequate trial of the drug, 18 showed progression of their cancer, and two had stable disease.

INTRODUCTION

Retinoids (Vitamin A and its analogues) have demonstrated antineoplastic activity in vitro (2, 18, 19, 23, 28). In vivo, retinoids have shown promise in a number of human proliferative dermatological lesions (7, 25, 26), as well as malignant diseases such as mycosis fungoides (15), skin carcinomas (8), melanoma, ovarian cancer, head and neck cancer (22), chronic myelomonocytic leukemia, and refractory anemia with excess blasts (10).

Despite sensitivity of a wide range of human malignant cell lines in vitro (2, 18, 19, 23, 28) to 13-cis-RA,4 clinical results have been less impressive (10, 22). For example, no activity has been shown in breast cancer (4), a cancer which is sensitive in vitro (18). Among the reasons for this disparity between in vitro and in vivo results, pharmacological reasons should be considered. Antineoplastic activity might depend upon peak drug level obtained if 13-cis-RA acts as a cytotoxic agent; a constant blood level might be optimum if 13-cis-RA works as a differentiating agent or as a hormonal agent.

Previous studies using 13-cis-RA to treat neoplasia have used daily oral doses up to acceptable toxicity, with the maximum tolerable plasma level being about 10⁻⁶ M or about 0.3 µg/ml (13, 14). This is at the lower level of the concentration required to control tumor growth in vitro (19, 28). Due to the half-life of 13-cis-RA of about 25 h or more (11, 16), daily chronic administration might lead to accumulation of either parent drug or metabolites. With daily administration, the dose-limiting toxicities have been dryness and painful fissures of the lips, hepatotoxicity, headaches, hypercalcemia, earaches, nausea, vomiting, or abdominal pain (4, 7).

There has been no clinical trial of large single doses given intermittently. Such pulse therapy might allow higher peak levels of the drug to be obtained. Furthermore, by allowing for clearance of the drug and its metabolites before the next dose is given, toxicity might be diminished. In order to investigate the feasibility of such intermittent therapy, we have entered 23 patients with advanced incurable cancer in a Phase I trial of weekly, oral, high-dose 13-cis-RA. We report here on the pharmacokinetics and the toxicity of this regimen.

MATERIALS AND METHODS

Study Design. The study was designed as a classic Phase I trial starting at a dose "n" and escalating the dose according to the modified Fibonacci method outlined by Carter (3). The initial starting dose of 13-cis-RA was 200 mg/sq m; it was given as a single p.o. dose weekly for 6 weeks. Three patients were to be entered at each dose level. If dose-limiting toxicity did not occur, 3 new patients were entered at the next higher dose. If Grade 2 or more toxicity was found, then 3 more patients were to be entered at the same dose level, before entry of new patients at the next level. For Grade 2 toxicity, the drug was held until recovery and restarted at the immediately lower dose level. If toxicity recurred, the drug was discontinued. If Grade 3 or 4 toxicity occurred, the patient was removed from the study. Patients experiencing disease progression after 3 weeks were removed from the study.

Patient Eligibility. Patients had to have a histopathological diagnosis of cancer and be refractory to all standard therapy and to other experimental therapies commonly thought of as effective. Patients refusing all other cytotoxic therapy were allowed to be considered for the study if there was no existing alternative therapy thought to be potentially curative or which might prolong life. Measurable lesions were not required. However, if measurable disease was present, it was measured and followed for response or progression. A Karnofsky performance status of 60% or better was required. At least 2 weeks were to have elapsed from any prior chemotherapy, and patients were to have an anticipated survival of more than 6 weeks. Patients had to be 18 years of age or older, had to be capable of giving informed consent for...
transaminase and alkaline phosphatase less than 1.1 x normal, and triglycerides had to be normal. Alcohol use was not allowed during the period of the study.

Criteria of Response. Complete response was defined as complete disappearance of all objective evidence of disease. Partial response was defined as decrease of 50% or more in the sum of the products of diameters of all measurable disease. Stable disease was defined as changes less than partial response or progression. Progression was defined as an increase of 25% or more in the sum of the products of diameters of all measurable lesions or development of any new lesions. Standard Southeastern Study Group criteria were used to assess toxicity.

Chemicals. Standard 13-cis-RA (isotretinoin) and 13-cis-RA capsules (Accutane) containing 10 or 40 mg of the drug were provided by Hoffman La-Roche Inc., Nutley, NJ. Ethyl acetate and water were high-pressure liquid chromatography grade. Ammonium acetate was reagent grade.

Determination of Plasma Levels of 13-cis-RA. Plasma levels were taken at a time prior to therapy and 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after the drug was administered. In addition, six patients were sampled at 168 h, 3 were sampled at 192 h, and one patient (K. E.) was sampled hourly until 12 h, then every 2 h until 32 h, and at 48, 72, 96, and 168 h. Levels of 13-cis-RA were only determined after the first dose of the drug. Approximately 10 ml of blood were collected in Vacutainer tubes containing 200 units of sodium heparin. Samples were kept in the dark prior to centrifugation at 2000 x g for 10 min at 4 °C. The plasma was removed, placed in propylene freezer tubes, and stored at −25 °C until analysis. Plasma samples were allowed to thaw at room temperature in the dark. All subsequent manipulations of the samples were done with minimal exposure to subdued light. Standard 13-cis-RA solutions in ethyl acetate or in plasma processed under these conditions did not show any detectable photodegradation. The extraction procedure was done according to the method of Goodman et al. (11). Samples of 50 μl of plasma were placed into polypropylene tubes, and 100 μl of cold 5% perchloric acid were added. They were then vortexed for 10 s. Ethyl acetate (500 μl) was added, and the samples were vortexed for a further 75 s. After centrifugation (13,000 x g, 5 min), the supernatant was removed and kept on ice and in the dark. Fifty μl of the organic layer were injected into a high-pressure liquid chromatograph (Model 5000, Varian Instruments) equipped with a reverse-phase column (30 cm x 4 mm, MCH-5 Varian Micro Pak) protected by a precolumn. Isocratic elution was done at room temperature with 75% acetonitrile and 25% of an ammonium acetate (1%) solution in water at a flow rate of 1 ml/min. Detection was carried out with a Varian UV-50 detector at a wavelength of 340 nm. Retention time for 13-cis-RA was 10 min. The quantitation of 13-cis-RA in plasma was done by comparing the peak height of the unknowns with standard curves prepared in plasma. A standard curve in plasma was prepared for every analysis by adding standard solutions of 13-cis-RA to blank plasma. The standard curves were linear over the concentration range used (0 to 10 μg/ml) and had coefficients of correlation near unity.

Stability Studies and Capsule Content. Standard solutions of 13-cis-RA in propylene tubes were exposed to 4 fluorescent tubes (40 W) at a distance of 6 feet either at room temperature or on ice. 13-cis-RA decomposed very rapidly under this normal laboratory lighting, with a half-life of about 39 min at room temperature; the light-exposed drug kept on ice decomposed almost as rapidly as did the drug kept at room temperature. At 200 min, the drug kept on ice showed only 12% less decomposition than did the drug kept at room temperature. On the other hand, the 13-cis-RA solutions kept in the dark were very stable either at room temperature or on ice. At 200 min, the sample kept in the dark and at room temperature showed only a 7% decomposition compared to the sample kept in the dark and on ice. Consequently, all patient samples were kept in the dark and on ice for laboratory work. The 13-cis-RA capsules showed a content slightly higher than their label. They contained 101 ± 0.15% (3 independent determinations) of 13-cis-RA when compared to standard 13-cis-RA.

Pharmacokinetic Analysis. The 13-cis-RA concentration-time data were fitted to a multieponential equation using the decision-making pharmacokinetic program AUTOAN (29). This program chooses the best number of exponentials according to the Boxenbaum method (1) and uses the nonlinear regression program NONLIN (21) to fit the curves. Elimination kinetics were assumed to be first-order. A weighting factor of the reciprocal concentration was used for the analyses. The plasma concentration-time data were fitted to a bi- or triexponential equation:

\[ C_p(t) = B e^{-\alpha t} - A e^{-\beta t} \]

or

\[ C_p(t) = B e^{-\alpha t} + A e^{-\beta t} - (A + B)e^{-\gamma t} \]

where \( C_p \) is the plasma concentration, \( A \) and \( B \) are coefficients, \( \beta \) and \( \kappa_k \) are the elimination and absorption rate constants, respectively, for the one compartment model, and \( \alpha \) and \( \beta \) are the distribution and elimination rate constants for the two compartment model. The time after ingestion of the drug is \( t \). The area under the curve from Time 0 to infinity (AUC\(_{0-\infty}\)) was estimated by:

\[ \text{AUC}_{0-\infty} = B/\beta - A/\kappa_k \]

or

\[ \text{AUC}_{0-\infty} = A/\alpha + B/\beta - (A + B)/\kappa_k \]

The volume of distribution was calculated by the area method as

\[ V_{\text{ss}} = (D - f)/(\text{AUC}_{0-\infty} \times \beta) \]

where \( f \) is considered to be unity. The plasma clearances were calculated as

\[ CL = K_e \times V_e \]

or

\[ CL = K_e \times V_e \]

for the bi- or triexponential equation, respectively.

RESULTS

Patients' Characteristics. Twenty-three patients were entered on the Phase I trial. The patient's pretreatment characteristics are listed in Table 1. Two patients with elevated alkaline phosphatase felt to be of bone origin were included. Patients who received less than the full six doses were included in the analyses as far as any toxic reactions noted. Six patients without significant toxicity were removed from the study due to rapid progression of their cancer. One patient received simultaneous radiation therapy to the chest, and it was not possible to discern whether some toxicities were due to the radiation therapy or the 13-cis-RA.

13-cis-RA Plasma Pharmacokinetics. The 13-cis-RA pharmacokinetic parameters derived from nonlinear regression fitting of the plasma concentration versus time data are summarized in Table 2. The curve fitting was generally good, as shown by the correlation coefficient \( r \). The time required to attain the actual peak plasma concentration varied from 2 to 24 h and did not seem related to the dose given. The mean plasma concentration was increased when the dose was increased from 200 to 400 mg/sq m. Any further dose increase did not lead to higher peak plasma concentrations.

Nine of 21 patients showed a biphasic decline (Chart 1A) of 13-cis-RA plasma concentration with rapid phase (\( \alpha \)) disposition.

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Half-lives ($t_{1/2}$) ranging from 2.5 to 9.1 h, followed by a slower disposition phase ($\beta$), with $t_{1/2}$ ranging from 11.7 to 101.3 h. For the other 12 patients, a single disposition phase was seen (Chart 1B), with $t_{1/2}$ ranging from 2.8 to 12.4 h. Half-lives were highly variable from one patient to another and were not related to the dose of 13-cis-RA given.

Four patients (J. C., J. K., C. M., H. C.) showed a secondary peak plasma concentration 24 to 72 h after the drug administration (Chart 1C). To try to ascertain if enterohepatic recirculation occurs, another patient (K. E.) was sampled more frequently. The resultant 13-cis-RA plasma concentration showed clearly the presence of a reabsorption phenomenon taking place at 30 h (Chart 1D). One patient (M. K.) with intestinal obstruction showed a prolonged absorption phase reaching a peak of 6.6 $\mu$g/ml at 24 h after the ingestion of the capsules.

The volume of distribution calculated by the area method, as well as the total plasma clearance (Table 2), showed a tendency to increase with the dose of 13-cis-RA. The areas under the curves were variable and did not increase in proportion to the dose given.

Toxicity and Response to Treatment. No dose-limiting toxicity was encountered. The toxicities observed over the dose range of 200 to 1800 mg/sq m are recorded in Table 3. As presented above, a saturable limit of absorption was observed such that no increase in plasma levels were observed at doses over 400 mg/sq m. In parallel with that saturable limit, the toxicity did not increase with further dose escalations. Headache, cheilits, and dry skin were frequent on the weekly schedule but not of such severity that they would have been dose-limiting. Headache and dry skin uniformly began with the first dose if they

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### Table 1
Pretreatment characteristics

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Median</th>
<th>Range</th>
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<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Performance status (Kamofsky):</td>
<td>60%</td>
<td>70%</td>
</tr>
<tr>
<td>Prior cytotoxic chemotherapy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Malignant diagnoses</td>
<td>Adenocarcinoma of the colon</td>
<td>Non-small cell lung cancer</td>
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</table>

### Table 2
13-cis-RA pharmacokinetic parameters in cancer patients receiving a single dose p.o.

Patients received p.o. the indicated dose of 13-cis-RA and blood samples were collected at various times thereafter. The 13-cis-RA plasma concentrations were determined by HPLC.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Time to peak plasma concentration (h)</th>
<th>Peak plasma concentration (µg/ml)</th>
<th>$t_{1/2a}$ (h)</th>
<th>$t_{1/2b}$ (h)</th>
<th>$V_{area}$ (liters/sq m)</th>
<th>CL (ml/sq m/min)</th>
<th>AUC$_{0-\infty}$ (µg/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. C. a</td>
<td>200 0.83 4 1.6 7.8 210 309 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. G.</td>
<td>200 0.99 12 1.4 9.1 101.3 716 73 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. S.</td>
<td>200 0.99 4 1.6 4.5 25.8 282 109 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean

A. H. 400 0.99 4 2.9 4.8 15.3 252 184 35
A. D. 400 0.99 2 3.6 4.2 98 263 25
N. K. 400 0.96 2 5.9 3.4 57 161 35
J. K. 400 0.84 2 2.8 11.4 207 195 32
M. K. 400 0.90 24 6.6 9.2 211 52 25
S. T. 400 0.95 4 3.0 2.5 11.7 198 180 34
L. V. R. 400 0.98 12 2.0 8.5 187 191 26

Mean

3.8 ± 0.7 3.7 9.1 ± 1.6 173 ± 26 175 ± 24 30 ± 2
E. G. 660 0.99 4 4.1 3.1 20.1 256 129 75
C. M. d 660 0.97 4 2.8 9.2 281 318 31
Mean

3.5 14.7 269 224 53
R. W. 1000 0.99 2 2.4 4.4 72.7 2360 358 45
N. R. 1000 0.98 6 2.3 11.8 386 361 44
L. D. 1000 0.99 12-24 2.7 7.7 85.5 3257 162 38

Mean

2.5 ± 0.1 6.0 57 ± 23 2001 ± 848 294 ± 66 42 ± 2
O. D. a 1400 8 0.7
R. B. 1400 0.85 4 4.6 12.4 359 319 70
J. B. 1400 0.77 12 0.5 2.8 3443 4951 2
E. K. 1400 0.99 2 4.0 3.2 19.0 782 458 49
K. E. 1400 0.96 5 3.4 3.7 26.6 1487 596 36
H. C. 1400 0.77 8 3.0 4.7 1480 1340 11

Mean

2.7 ± 0.7 3.5 13.1 ± 4.4 1510 ± 529 1533 ± 873 34 ± 12
A. G. 1800 0.91 8 4.6 6.1 628 535 25

a Secondary peak detected at 48 h.
b Mean ± SE.
c Secondary peak detected at 24 h.
d Secondary peak detected at 72 h.
 No curve fitting was done because only 3 time points were available.

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patients. The majority of these elevations were still within the upper limits of normal and were probably not of clinical significance. One patient had a peak elevation of triglycerides of 2- to 5-fold the upper limit of normal after 3 doses. His level started to rise after 1 dose. Five patients had elevations of 1.1 to 2 times normal occurring after the first dose (1 patient), third dose (3 patients), and sixth dose (1 patient). Weight loss was difficult to assess, as most of the patients also suffered progression of their cancer during the trial. Five patients experienced microscopic hematuria (positive dipstick for blood or 1 to 5 RBC/high-powered field) which was otherwise unexplained and could be treatment related.

No objective responses were observed to treatment with 13-cis-RA. Of 20 patients receiving an adequate trial of the drug, 18 showed progression of their cancer, and 2 had stable disease.

**DISCUSSION**

These studies demonstrate a variability in the pharmacokinetics of oral 13-cis-RA in cancer patients, as shown by terminal half-lives ranging from 2.5 to 101.3 h and by the poor correlation between the dose administered and the corresponding area under the curve. It was noteworthy that the mean peak plasma concentrations reached a maximum at the 400 mg/sq m dose levels. The mean peak plasma concentrations were in the range of 2.5 to 4.6 μg/ml for doses of 400 mg/sq m or higher. Further increase of the dose did not lead to higher plasma concentrations, probably reflecting a saturation of intestinal absorption in relation to the transit time of the ingested drug bolus. In one patient in whom partial intestinal obstruction was noted, there was a plateau for over 20 h in the plasma level, probably due to continued intestinal absorption. Therefore, it appears that with p.o. administration, the maximum plasma levels which can be achieved after one dose of 13-cis-RA is in the order of 3 to 4 μg/ml because of saturable absorption.

In four patients, the possibility of enterohepatic recycling was raised by the appearance of a secondary peak plasma concentration. In the extensive study we carried out in one patient, evidence of drug recycling was evident. This phenomenon has been noted by other investigators for retinoids (11, 16, 30).

Higher mean peak plasma levels of 13-cis-RA were obtained in this study for doses of 400 mg/sq m or more as compared
with studies of repeated daily drug exposure (see Table 4). Although peak levels were in the range of 2.5 to 4.6 μg/ml where previous daily doses led to a peak of approximately 0.5 to 1.16 μg/ml, the usual toxicities of headache, chelitis, dry eyes, and dry skin were not dose limiting. The toxicity observed in these studies are consistent with previous reports of 13-cis-RA toxicity in humans (8, 11, 14, 17). The failure to absorb 13-cis-RA with increasing doses beyond 400 mg/sq m precluded evaluation of our hypothesis that greater antineoplastic activity might be obtainable with higher peak levels.

Retinoids remain of promise in the treatment of neoplastic disease because of their demonstrated activity in vitro (24). Further, their activity as immunological stimulants makes them of potential value in reversing the immunological depression caused by antineoplastic therapies (6, 20). However, the optimum dose and schedule of administration remain to be determined. The saturable limit of absorption of 13-cis-RA demonstrated in this study suggests that either a parenteral preparation, alternative synthetic retinoids with greater bioavailability, or modification of the schedule of administration will be necessary to increase the plasma concentration to higher levels.

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