Alterations in Tretinoin Pharmacokinetics Following Administration of Liposomal All-trans Retinoic Acid

By Elihu Estey, Peter F. Thall, Kapil Mehta, Michael Rosenblum, Tim Brewer, Jr, Veronica Simmons, Fernando Cabanillas, Razelle Kurzrock, and Gabriel Lopez-Berestein

We administered liposome-encapsulated all-trans retinoic acid (L-ATRA) to 48 patients with refractory hematologic malignancies using an every-other-day schedule for 28 days and doses of 15 to 175 mg/m². In 19 patients, pharmacology studies were conducted after the first (day 1) and seventh (day 15) doses. In contrast to the decline in tretinoin concentrations seen within 3 to 4 days of administration of daily oral ATRA, there were no differences between the area under the curve (AUC) of tretinoin concentration versus time on day 1 and day 15 (P = .98, Wilcoxon signed-rank test). Peak day 1 concentrations after 15 mg/m² were higher than those reported after 45 mg/m² oral ATRA. Six patients with relapsed acute promyelocytic leukemia (APL) were treated. Three, each in first relapse and at least year from the last chemotherapy, achieved a complete response (CR).

I T IS ESTABLISHED that remissions produced by oral all-trans retinoic acid (ATRA) in patients with acute promyelocytic leukemia (APL) are brief if ATRA is continued without addition of chemotherapy. One possible explanation for this acquired resistance to ATRA is a decrease in the concentration of the drug presented to retinoic acid receptors in the cell nucleus. Consistent with this hypothesis are observations, both in patients with APL and patients with other cancers, that plasma ATRA concentrations start to decline often within 3 to 4 days of beginning oral ATRA.

Encapsulation of ATRA in liposomes (L-ATRA) provides a parenteral ATRA preparation that has been shown in rats to decrease metabolism of ATRA by hepatic microsomes. The liposomal nature of the product may also result in less toxicity seen on a milligram-to-milligram basis in exposure to oral ATRA, achieved a complete response (CR).

Disease recurred in two (one at 3 months despite maintenance L-ATRA and similarity in tretinoin AUC on days 1 and 85, and the other at 5 months, 2 months after discontinuation of L-ATRA) and the third was transplanted 1 month into CR. The three nonresponders were in at least a second relapse and failed to respond to oral ATRA before or immediately after receiving L-ATRA. Severe toxicity developed in three of eight patients treated at 175 mg/m² (joint pains in two, skin in one). The maximum tolerated dose (MTD) was determined to be 140 mg/m², at which dose grade 2 toxicity (primarily headache and skin) occurred in eight of eight patients, but grade 3 to 4 toxicity in none. Compared with oral ATRA, L-ATRA apparently results in greater exposure to tretinoin and for a longer time.

P A T I E N T S AND METHODS

Standard phase 1 entry criteria were used: no known curative treatment for the patient’s malignancy was available, at least weeks had elapsed since the last chemotherapy, serum bilirubin and creatinine each were less than 1.6 mg/dL, and Zubrod performance status was 0 to 3. Pregnant women and patients with circulating blast counts above 5,000/µL were excluded. Forty-eight patients were enrolled beginning in April 1993. Six had relapsed APL, 13 relapsed acute myelogenous leukemia or myelodysplastic syndrome, one relapsed T-cell acute lymphoblastic leukemia, 14 T-cell lymphomas (seven cutaneous and seven systemic), and 14 B-cell lymphomas. The disproportionate number of T-cell lymphomas patients reflected our desire to treat these patients, given the activity of oral ATRA in T-cell lymphomas.

L-ATRA (Aronex Pharmaceuticals Inc, The Woodlands, TX) was given over 1 half-hour every other day for 28 days. The every-other-day schedule was based on animal data indicating that the half-life of L-ATRA in tissue exceeded 39 hours, with concentrations at this time exceeding those necessary to differentiate APL cells in vitro. Responding patients could continue to receive L-ATRA. The dose given to the initial patients was 15 mg/m², and subsequent patients received 30, 60, 75, 90, 110, 140, and 175 mg/m². Given the variable absorption of oral ATRA, it is difficult to translate a given L-ATRA dose into an equivalent oral ATRA dose. Typical phase I guidelines governed dose-escalation: new patients were treated at the next higher dose if grade 3 to 4 toxicity (National Cancer Institute criteria) was observed in none of three or one of six, patients at the previous dose, and if such toxicity was seen in more than two of six patients at a given dose, that dose was considered above the maximum tolerated dose (MTD), with the previous dose called the MTD if toxicity was noted in not more than one of three or two of six patients at that dose. With three exceptions, each patient received only one dose level.

Pharmacokinetics analysis. In 19 patients (three at 15, two at 30, three at 60, three at 75, two at 90, one at 110, three at 140, and two at 175 mg/m²), blood samples were obtained before infusion, 15 and 30 minutes later (end of infusion), and then at 2, 5, 10, 15, 30, 60, 90, 120, 240, 480, 720, and 1,440 minutes on both day 1 and day 15 of the study in 19 of 48 patients. Like oral ATRA, L-ATRA induces differentiation in vitro in cells from patients with APL, and the six patients with relapsed APL who received L-ATRA are thus of particular interest.
and day 15 (after the seventh dose of L-ATRA). In an additional patient with APL, blood samples were obtained at the above times on day 1 and day 85 (after the thirty-second dose).

Analysis of ATRA in whole blood was performed using high-performance liquid chromatography as previously described. The lower limit of detection was 0.2 ng ATRA/mL blood. Standard pharmacokinetic parameters were derived using nonlinear regression analysis (RSTRIP; Micro-Math Inc, Salt Lake City, UT).

Statistical analysis. Confidence intervals for binomial parameters were computed using the method of Ghosh. Day 1 and day 15 values for each pharmacokinetic parameter (concentration of tretinoin at the end of the infusion, terminal-phase half-life [t1/2], area under the curve of [AUC] of tretinoin concentration versus time, clearance of ATRA from blood, and volume of distribution with and without adjustment for body weight) were compared using the Wilcoxon sign-rank test for paired data. Functional relationships were assessed by nonlinear regression, including exploration of various transformations of the variables. Both AUC 1 and AUC 15 showed pronounced heteroscedasticity. The pure error mean squares was used to estimate the variance in computing confidence bands for the predicted line. Model goodness-of-fit was assessed by residual plots on dose and on predicted AUC, Q-Q plots, model R², and individual coefficient P values. All computations were carried out in S plus on a SUN SPARC station 20 desktop computer (Sun Microsystems, Mountain View, CA).

RESULTS

Table 1 shows the number of patients treated at the various doses. With the exception of one patient at 75 mg/m², the same patient after dose-reduction to 60 mg/m², and three patients at 175 mg/m², all patients who did not complete the prescribed 28 days of treatment went off study because of progressive disease (Table 1). Side effects occurred in all 48 patients (95% confidence limit, 93% to 100%). The most common was headache, which occurred in 71% of patients (95% confidence limit, 56% to 81%). Dry skin and nausea/vomiting were reported in 31% (95% confidence limit, 20% to 46%) and 21% (95% confidence limit, 12% to 35%), respectively. The majority of side effects were mild or moderate (with the exception of one case of hypotension at 75 mg/m² and again at 60 mg/m², and one case of severe headache at 110 mg/m²) until a dose of 175 mg/m² was reached (Table 1). Eight patients began treatment at this dose, of whom two were removed from study because of progressive disease without severe toxicity (after two and 10 doses, respectively) and three because of severe toxicity, leaving three patients who completed 28 days without severe toxicity. The dose-limiting side effects in the three who were removed because of toxicity were joint pain in two and exfoliative rash in one. One of the patients who had severe joint pain at 175 mg/m² had therapy resumed at 140 mg/m² 5 days later, after recovery from toxicity. The patient completed 28 days at 140 mg/m² without further grade 3 to 4 toxicity. After the three patients developed grade 3 to 4 toxicity at 175 mg/m², a second cohort (four patients) was treated at 140 mg/m². Although none of the total of eight patients at 140 mg/m² (seven completing 28 days) had dose-limiting
toxicity, all had grade 2 toxicity principally consisting of headache and rash, and in one patient, hearing loss. Based on this experience, we considered 140 mg/m² given every other day for 28 days the MTD.

Pharmacokinetics. Table 2 indicates that AUC values (calculated from the end of infusion until infinity) are relatively similar on day 1 and 15, ie, after the first and seventh doses of the liposomal preparation. Indeed, there is no statistical difference between day 1 and day 15 AUCs (P = .98), with the lower mean day 15 value at 90 mg/m² reflecting one patient whose day 1 and day 15 values were 1,213.5 and 870.3 μg·min/mL, respectively, with the respective values in the second patient at this dose being 259.4 and 207.2 μg·min/mL. A third patient treated at 90 mg/m² had APL in relapse, responded to L-ATRA, and as a result continued to receive 90 mg/m². Blood samples were obtained on day 1 (AUC₁) and day 15 (AUC₁₅) values for apparent volume of distribution expressed on a per-kilogram-body-weight basis (P = .59).

Response. As noted earlier and illustrated in Table 3, we treated six patients with APL in relapse. Disease in the first three, who received relatively low doses, was in second or greater relapse. The second patient had failed to respond to oral ATRA 4 months before receiving L-ATRA, and after failing to respond to L-ATRA, which was given for at least 28 days, both the first and third patients failed to respond to oral ATRA. In contrast, disease in the fourth, fifth, and sixth patients, in each of whom L-ATRA produced a complete response (CR), was at a relatively earlier stage and the inter- vals since last receiving ATRA was at least 12 months. Of the six patients, three had pharmacology studies, including two nonresponders and one responder. Both the lowest and highest AUC values were seen in nonresponders (Table 3).

The pattern of response in all responding APL patients was similar and is illustrated in Fig 3. An initial increase in neutrophils was followed by a decrease and then a second increase coincident with an increase in platelet count. There was a progressive decrease in marrow promyelocytes concomitant with an increase in more mature forms, a pattern similar to that seen after oral ATRA. None of the six patients developed the ATRA syndrome (95% confidence limit, 0% to 39%; reported incidence of ATRA syndrome after oral ATRA, ~25%). All three responding patients were maintained on thrice-weekly (every Monday, Wednesday, and Friday) L-ATRA at their induction dose. Disease in the first patient to receive ATRA 4 months before receiving L-ATRA was at a relatively earlier stage and the interval since last receiving ATRA was at least 12 months. Of the six patients, three had pharmacology studies, including two nonresponders and one responder. Both the lowest and highest AUC values were seen in nonresponders (Table 3).
reappeared during maintenance treatment after a CR of 3 months, despite the similarity in day 1 and day 85 tretinoin AUC values noted earlier. The second received 3 months of maintenance L-ATRA; 2 months after discontinuation of L-ATRA, his disease reappeared and an allogeneic marrow transplant was performed. The third remained in remission for 1 month, at which time she received an allogeneic marrow transplant.

Six minor, transient responses have occurred among 14 patients with T-cell lymphomas, most often (five of five, two not assessable for response because of toxicity at 175 mg/m²) in patients with cutaneous T-cell lymphoma. No other responses have occurred while 11 of 14 patients with non-T-cell lymphomas have had progressive disease.

DISCUSSION

Our results indicate that liposomal encapsulation of ATRA appears to permit maintenance of tretinoin concentrations longer than is currently possible with standard oral preparations of ATRA (compare Table 2 with data in Muindi et al). This result, together with the data in Table 4 indicating that the peak concentration at the lowest L-ATRA dose administered (15 mg/m²) averaged two to three times higher than peak concentrations reported after 45 mg/m² oral ATRA, reflecting, at least to some extent, the limited bioavailability of the oral preparation, suggests that exposure to ATRA is likely to be greater after L-ATRA than after oral ATRA, certainly at the usually used dose of the latter, 45 mg/m² daily. Furthermore, the quadratic relationship between AUC and dose suggests that accumulation of ATRA occurs, although we have no direct evidence that this takes place at any site, for example, the bone marrow.

The mechanism underlying the stability in AUC between day 1 and day 15 is the presumed ability of the liposomal preparation to bypass metabolism by hepatic microsomes. Although no metabolites of ATRA, eg, 4-oxo-retinoic acid glucuronide, were detected in blood, a failure to detect such metabolites does not imply that L-ATRA is not metabolized, given that we did not assay urinary metabolites of L-ATRA.

It should also be noted that whereas tretinoin concentrations after oral ATRA are measured in plasma, tretinoin concentrations after L-ATRA were measured in blood. The reason is as follows: hydrophobic drugs injected intravenously must be delivered in a detergent, or a vehicle such as liposomes in the case of L-ATRA or chylomicrons (after absorption) in the case of oral ATRA. Interactions then occur between the drug and blood compartments such as (1) lipoproteins, (2) cell membranes, and (3) phagocytes after ingestion. Therefore, to assess the total available drug, we measure the drug level in whole blood. It should be noted that hydrophobic drugs like ATRA do not leak out of liposomes, but instead exchange as described earlier, and because of its hydrophobic nature, ATRA is technically not "free" in the circulation. Clearly, what is critical is the amount of intracellular ATRA, and correlations remain to be established

Table 3. Liposomal Tretinoin in Six Patients With APL in Relapse

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Liposomal Tretinoin (mg/m²)</th>
<th>Relapse No.</th>
<th>Months Since Last Oral Tretinoin</th>
<th>CR After L-ATRA</th>
<th>AUC (µg·min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>2</td>
<td>9</td>
<td>No</td>
<td>74.1</td>
</tr>
<tr>
<td>2</td>
<td>30, 60*</td>
<td>4</td>
<td>4</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>2</td>
<td>14</td>
<td>No</td>
<td>798.7</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>1</td>
<td>12</td>
<td>Yes</td>
<td>142.3</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>1</td>
<td>21</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>110</td>
<td>1</td>
<td>14</td>
<td>Yes</td>
<td>ND</td>
</tr>
</tbody>
</table>

AUC is the mean of day 1 and day 15 values for patients no. 1 and 3, and the mean of day 1 and day 85 values for patient no. 4. Abbreviation: ND, not determined.

* 30 mg/m² every other day for 28 days, and then 60 mg/m² every other day for 8 days.

Fig 3. Response of APL patient no. 4 (Table 4) to L-ATRA. bmprogran, % promyelocytes in the bone marrow as read on the right-hand scale.

Table 4. Peak Day 1 Tretinoin Concentration After Administration of Liposomal or Oral Tretinoin

<table>
<thead>
<tr>
<th></th>
<th>Liposomal</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mg/m²</td>
<td>90 mg/m²</td>
</tr>
<tr>
<td></td>
<td>45 mg/m²†</td>
<td></td>
</tr>
<tr>
<td>Patients (N)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mean peak concen.</td>
<td>1.0</td>
<td>6.0</td>
</tr>
<tr>
<td>(µg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td>0.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* See Muindi et al.† See Lefebvre et al.
between the amount as measured in either blood or plasma and the amount available to tumor cells; for example, it is unknown if ATRA bound to membranes or ingested by phagocytes is available to tumor cells. It should also be noted that with both liposomal and standard oral preparations there is considerable patient-to-patient variation in pharmacokinetics. However, with L-ATRA, the significant variation in bioavailability seen with the oral preparation\textsuperscript{11,12} is obviously not an issue. The lowest tretinoin concentration (~2.3 \( \mu \)mol/L) observed at the lowest L-ATRA dose (15 mg/m\(^2\) daily for 6 weeks)\textsuperscript{15} is similar (with headache, dry skin, and nausea/vomiting being the prominent side effects with both preparations), and it is interesting that the much greater AUC after administration of L-ATRA does not result in much greater toxicity.

This raises the question of whether the greater exposure to ATRA presumably afforded by the use of L-ATRA will have clinical benefit relative to oral ATRA. A major impetus for the development of L-ATRA was the observation that at disease relapse patients with APL had lower serum tretinoin concentrations than when initially treated with oral ATRA,\textsuperscript{11} together with observations that at relapse the APL cells of these patients remained responsive to tretinoin in vitro,\textsuperscript{16} although this latter finding has been questioned.\textsuperscript{16} If both observations were correct, maintenance of tretinoin concentrations for longer periods (eg, with L-ATRA) might avert relapse. Our results indicate that L-ATRA has activity in APL. However, it should be noted that the three patients in whom L-ATRA produced a CR were perhaps also likely to have responded had they received oral ATRA, given the relatively early stage of their disease together with the relatively long interval since last receiving oral ATRA (Table 4).\textsuperscript{1} In contrast, disease in the three patients who did not respond to L-ATRA was more advanced. Furthermore, given the small number of patients, there was no strict relation between AUC and response (Table 3), and a relapse occurred despite similar AUC values on day 1 and day 85. It would not be surprising if the failure to maintain tretinoin concentrations were only one (if one) mechanism underlying the development of resistance to ATRA. Although the future utility of L-ATRA in APL is thus uncertain, the favorable pharmacologic profile of the material will permit determination of whether a change in pharmacologic properties results in more effective treatment of APL.

ACKNOWLEDGMENT

The authors acknowledge the dedicated data-collection efforts of Jan Bole, RN, the pharmacokinetic analyses of Joe Wyse, PhD, Thomas Wallace, PhD, Dawn Cardoza, Debbie Allen, and Chris Wilson, and the expert secretarial assistance of Soon Woo.

REFERENCES

Alterations in tretinoin pharmacokinetics following administration of liposomal all-trans retinoic acid

E Estey, PF Thall, K Mehta, M Rosenblum, T Jr Brewer, V Simmons, F Cabanillas, R Kurzrock and G Lopez-Berestein