CRABP induction at the level of tRA absorption could lead to a dramatic reduction of tRA bioavailability. Down-regulation of CRABP levels after a drug-free period would then support a discontinuous approach to tRA therapy.

Until now, studies that examined tRA pharmacokinetics have reported induction in terms of AUC with proposed solutions focusing on modulation of hepatic cytochrome P450 enzymes [1-8]. None of these studies reports effects of chronic therapy in terms of $t_{1/2}$ induction nor do they address effects on other pharmacokinetic parameters such as $T_{lag}$ or $T_{max}$. The latter could provide insight into effects at the level of tRA absorption. The purpose of this study is to examine the effects of chronic tRA therapy on tRA absorption and hepatic drug metabolism in human lung cancer patients. We set out to assess the contributions clearance and the overall reduction of tRA bioavailability and to determine whether a seven day discontinuation of tRA therapy would allow the restoration of induced parameters therefore effectivly restoring tRA bioavailability.

**METHOD**

**Patient treatment**

All subjects were diagnosed with advanced non–small cell lung carcinoma and were current or previous smokers. Subjects received 150 mg/m$^2$ of oral tRA (Hoffmann La Roche, Basel, Switzerland) daily for 7 days as part of the cancer therapy protocol. They also received 100 mg/m$^2$ CDDP (Bristol Myers Squibb, Princeton, NJ) IV on day 3 and 100 mg/m$^2$ VP-16 (Bristol Myers Squibb, Princeton, NJ) IV on days 3, 4, and 5. The study protocol was approved by the Human Subjects Committee of the Veterans Administration Hospital in Long Beach, CA. Enrolled patients gave written informed consent prior to treatment. All patient treatment and hospitalization was at the Veterans Administration Hospital in Long Beach, CA and under the supervision of R. Thiruvengadam, M.D. A baseline pharmacokinetic study was conducted prior to the start of drug therapy (day 1), after 7 days of combined therapy (day 7), and 7 days after all drug therapy (day 14). With the exception of smoking history, patients with therapy or behavior (regular alcohol or barbiturate consumption) known to affect hepatic drug metabolism were eliminated from the study.

**Sample processing**

All sample handling procedures were carried out in dim lighting. Patient blood samples were drawn into foil-wrapped vacutainers immediately prior to tRA administration ($t = 0$) and at 0.5, 1, 2, 3, 4, 6, and 8 hours after tRA administration. On day 7, an additional blood sample was drawn 5 hours after tRA administration to compensate for losses of detectable tRA in remaining serum samples due to autoinduction. Blood samples were refrigerated for 30 minutes prior to centrifugation. Serum was separated by centrifugation at 3000 rpm for 10 minutes, transferred to 2 mL microcentrifuge tubes, and stored in small chipboard boxes at $-20^\circ$C.

**Reagents**

High-performance liquid chromatography (HPLC) internal standard (all-trans-3-nosityl-3,7-diethyl-2,4,6,8 nona-tetraenoic acid) was obtained from Dr. P.F. Sorter of Hoffmann La Roche, Nutley, NJ. All-trans retinoic acid was supplied from the National Cancer Institute and manufactured by Hoffman La Roche. All other chemical reagents were of HPLC grade or the highest grade available and were purchased from Fisher Scientific (Tustin, CA).

**HPLC Analysis**

Patient serum samples were extracted and assayed for the presence of tRA by HPLC according to a modified method by Bugge et al. [16]. Under dim lighting, 0.5 mL of patient serum samples were dispensed into 1.5 mL microcentrifuge tubes. Nine standard solutions of tRA in human plasma that had been prepared previously in 0.5 mL aliquots and stored at $-20^\circ$C were thawed, extracted, and analyzed with each patient sample set. We added 20 mL of a 50 $\mu$g/mL internal standard solution in 2.5% methanol/acetonitrile to each of the patient samples and standards; 300 mL 1/1 (v/v) acetonitrile/1-butanol was added to the above mixture as the extraction solvent followed by the addition of 300 mL of 1 kg/L water saturated K$_2$HPO$_4$ and sample centrifugation at 9000 rpm for 10 min. The upper organic layer was removed and 100 mL was injected into a Beckman system GOLD HPLC (Beckman Instrument, Fullerton, CA). The method utilizes a 25 cm x 4.6 mm id Zorbax C-18 column with 5 $\mu$m spherical particles and no end capping. Samples were run on a complex gradient at 1.5 mL/min for 24 minutes per sample. Initial gradient conditions were 30% solvent B: acetonitrile/0.2 M ammonium acetate/acetic acid, pH 4.6 and 70% solvent A: 50/ 50/0.5 (acetonitrile/0.02 M ammonium acetate/acetic acid). This linearly increased to 100% B over 10 minutes. The trial maintains these conditions for seven minutes while sample components are eluted. Initial conditions were then restored with a 2-minute gradient to 30% B. We allowed 5 minute equilibration time
Pharmacokinetic analysis

Serum tRA concentration data were analyzed by extended nonlinear least squares regression analysis using PCNONLIN version 4.0. A best-fit model was selected to minimize values of Akaike information criteria (AIC) and sum of squared residuals for subject serum tRA concentration versus time curves. A visual comparison of residual plots to patient curves was done to ensure suitability of the model. Data were fitted to a one-compartment model with equal first order absorption and elimination with a variable absorption lag time. The expression is written:

\[ C(T) = K^* D(V/T)^* T^n * \exp(-K^* T) \]

where \( D \) = dose, \( V \) = volume, and \( K = K_{10} = K_{01} \) = rate constant. \( t_{1/2} \), \( T_{\text{max}} \), and \( T_{\text{lag}} \) were determined from the estimated values of the rate constant (K) and volume of distribution (V), which were calculated by the curve-fitting program utilizing the above expression. AUC was determined utilizing the trapezoidal rule.

Statistical analysis

Eleven subjects were enrolled in the study, but one subject was dropped from analysis because of high levels of enterohepatic cycling. Some pharmacokinetic data were missing on four other patients. Due to extensive hepatic cycling which prevented accurate modeling, AUC was the only parameter estimated for one patient on day 7. Two other patients on day 7 and one on day 14 exhibited low serum tRA levels that reduced the number of available data points, preventing accurate modeling of their data. The Wilcoxon signed rank test was used to compare changes within study subjects between different days. Median values for pharmacokinetic parameters were used for statistical analysis due to high levels of patient variability and low sample size.

RESULTS

The baseline tRA time course is described by a one-compartment model with equal first order absorption and elimination phase and with a variable absorption lag time. A comparison of median kinetic parameter values on days 1, 7, and 14 is shown in Table 1. In nine of 10 patients chronic dosing with tRA resulted in a reduction of overall drug bioavailability exhibited by statistically significant decreases in both median AUC (\( p = 0.03 \)) and \( C_{\text{max}} \) (\( p = 0.03 \)). The increase in median \( C_{\text{max}} \) values (Table 1) represents a statistical artifact resulting from a missing \( C_{\text{max}} \) value for one patient on day 7. Among patients with both day 1 and day 7 \( C_{\text{max}} \) values, \( C_{\text{max}} \) decreases from 0.51 to 0.48 \( \mu \)g/mL. Three patients decreased by more than 0.9 \( \mu \)g/mL, five decreased by 0.1 or 0.2 \( \mu \)g/mL, and only one patient increased by 0.2 \( \mu \)g/mL. Distributions of serum tRA AUC for days 1, 7, and 14 are displayed in Fig. 1. This figure illustrates the significant increase of day 14 AUC values over day 7 values (\( p = 0.01 \)) that was present in nine of the 10 study subjects.

Half-life responses to chronic tRA therapy were not statistically significant. Four patients showed decreases from baseline of at least 40% while three others showed increases of 15% or less. There was a trend toward decreasing \( T_{\text{lag}} \) with chronic tRA therapy (day 1 to day 7) in six of the seven patients analyzed whose \( T_{\text{lag}} \) increased from day 1 to day 7 (\( p = 0.08 \)). Day 14 values for all parameters were not significantly different from their baseline values.

Figs. 2A and B show time course profiles for two representative study subjects who illustrate the trends in tRA distribution with tRA therapy. Both subjects

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Baseline tRA (day 1)</th>
<th>After chronic tRA therapy (day 7)</th>
<th>After drug holiday (day 14)</th>
<th>Difference: day 1 and day 7 (p values)</th>
<th>Difference: day 1 and day 14 (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (( \mu )g/mL/h)</td>
<td>1.2 (0.7, 2.1)</td>
<td>0.69 (0.09, 1.2)</td>
<td>1.8 (0.8, 2.1)</td>
<td>0.03</td>
<td>0.70</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu )g/mL)</td>
<td>0.47 (0.2, 1.4)</td>
<td>0.48 (0.3, 0.5)</td>
<td>0.6 (0.5, 1.1)</td>
<td>0.03</td>
<td>1.00</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>0.9 (0.6, 1.0)</td>
<td>0.5 (0.4, 0.8)</td>
<td>0.6 (0.5, 0.7)</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>( T_{\text{lag}} ) (h)</td>
<td>0.4 (0, 0.8)</td>
<td>1.8 (0.3, 1.9)</td>
<td>0.4 (0, 1.5)</td>
<td>0.08</td>
<td>0.30</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>1.5 (1.4, 2.1)</td>
<td>2.0 (1.0, 2.5)</td>
<td>2.0 (1.1, 3.0)</td>
<td>0.20</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Parameters are displayed as medians with their corresponding interquartile range shown below. All medians and \( p \) values are based on 9 or 10 observations except for day 7 \( t_{1/2} \) and \( T_{\text{lag}} \) where \( n = 7 \).

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ORAL tRA BIOAVAILABILITY AFTER DRUG HOLIDAY

![Box plot of AUC values](image)

Fig. 1. Box plots of AUC are shown for days 1, 7, and 14. The box gives the range for data between the 25th and 75th percentiles with the median being a white bar. The “bars” extend out to the extreme values except for the outlier on day 1.

...exhibited increased $T_{lag}$ and $T_{max}$ along with the characteristic reduction in tRA bioavailability from chronic tRA dosing with no significant observed effect on $t_{1/2}$. This is followed by increased tRA bioavailability after the drug holiday and is reflected as elevated AUC values.

Figs. 2C and 2D illustrate the time-course profiles of two individuals who exhibited extreme induction of pharmacokinetic parameters upon chronic tRA therapy. Serum tRA levels are reduced below the limits of detection at nearly all day 7 timepoints. The AUC was determined for these subjects with the trapezoidal rule but the remaining day 7 parameters could not be determined. After the drug holiday, their $t_{1/2}$ values were reduced from their baseline values while their AUC values exceeded their baseline levels by a substantial margin.

The time-course profile (Fig. 3A) of one patient exhibits two absorption and elimination phases. Data from this patient were eliminated from statistical analysis due to the apparent existence of enterohepatic cycling. Since the second peak is greater than the first, impaired tRA absorption is implied as well. A time-course profile obtained 1 week after the second tRA course of the third cycle shows a more representative pattern (Fig. 3B).

DISCUSSION

Our data confirm previous reports that there is a substantial level of interpatient variability in tRA pharmacokinetics [1–8]. tRA bioavailability in lung cancer patients, measured as AUC, was significantly reduced after chronic tRA therapy, in accordance with previous reports [1–8]. This reduction, traditionally believed to arise from autoinduction of tRA metabolism leading to reduced tRA $t_{1/2}$, has not been verified to date. Fig. 1 shows patient baseline AUC over a highly variable range that is significantly reduced upon chronic tRA dosing. After the drug holiday, AUC levels increase to values at or slightly above baseline levels. A significant increase in AUC was noted between...
chronic dosing and drug holiday values, further evidence that tRA bioavailability is restored.

The relative contribution of hepatic induction on tRA bioavailability after chronic tRA dosing is unclear from data presented in this paper. The reduction of tRA bioavailability associated with chronic tRA therapy appears to hinge more upon effects at the absorption site than upon induction of hepatic enzymes. Although all study subjects exhibited alterations in tRA $t_{1/2}$ with chronic therapy, neither a significant reduction of $t_{1/2}$ nor a strong correlation between AUC and $t_{1/2}$ was evident in our data. Post-drug holiday data in some individuals (Figs. 2C and D) exhibit reductions in tRA $t_{1/2}$ yet maintain serum levels well above baseline values. This is a direct contradiction of results anticipated from half-life induction. A rapidly adapting interaction at the level of absorption combined with variable responses of metabolic systems could account for such behavior.

A near significant increase in $T_{lag}$ with chronic dosing implies an interaction at the level of absorption. This could result from redistribution of tRA from tissue binding sites, sequestration, or degradation within the gut wall prior to reaching the general circulation or disease progression. The lack of significant differences between baseline and drug holiday in all parameters (including AUC) implies a reversible drug interaction that is restored or down-regulated within a few days of therapy discontinuation. Chronic tRA therapy has been linked to the induction of retinoid binding proteins such as CRABP in cultured stem cells [12] and skin cell biopsies of tRA-treated rhesus monkeys [15]. A substantial induction of such binding proteins at the absorption site could decrease $C_{max}$ and increase $T_{lag}$, thus leading to dramatic reductions in overall drug bioavailability.

Numerous mechanisms exist to regulate and transport cellular retinoids within the body [13]. Such control mechanisms maintain endogenous levels of tRA at nearly undetectable concentrations (4–14 nmol/L in humans) [13]. These mechanisms include direct absorption into the systemic blood system, sequestration
within the gut wall or in adipose tissue, delivery to the bone marrow via chylomicrons or chylomycin remnants, binding to cellular retinoid binding proteins at the absorption site, or enterohepatic cycling in the form of all-trans β-glucuronide [14]. High variability in individual responses of absorption parameters to prolonged tRA exposure combined with variable responses of the cytochrome P450 system would hinder the elucidation of significant response patterns with small sample sizes. Baseline absorbance parameters may vary with many factors and are likely to be influenced strongly by dietary retinoid consumption. Smoking history is also implicated as an inducer of these parameters. Rigas et al. [8] showed that baseline tRA AUC levels are lower in lung carcinoma patients than in patients with acute promyelocytic leukemia. Currently, the relationship of tRA pharmacokinetic variability to the smoking history, dietary and endogenous levels of retinoids, and dietary fat content is unclear.

Regardless of the origin of induction, our studies show that overall tRA bioavailability can be restored effectively in most individuals after a brief interruption of tRA therapy, in our study 7 days. If we assume the loss of tRA efficacy with chronic tRA therapy results primarily from reduced tRA bioavailability and that restoration of tRA bioavailability will restore clinical efficacy, the most effective approach to tRA therapy is the incorporation of tRA drug holidays between weekly cycles of tRA treatment.

ACKNOWLEDGMENT

This study received funding from the PMAF Faculty Development Award in Clinical Pharmacology awarded by University of California, Irvine Clinical Cancer Center.

REFERENCES


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