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Phase I clinical trial of all-trans-retinoic acid with correlation of its pharmacokinetics and pharmacodynamics

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Abstract A phase I trial of all-trans-retinoic acid (ATRA) was conducted to establish the maximum tolerable dose (MTD) of ATRA given once daily to patients with solid tumors. Cancer patients for whom no standard therapy was available were treated with ATRA once daily. Doses were escalated in cohorts of at least three patients. The pharmacokinetics of ATRA were assessed on day 1 for all patients and weekly for 31 patients who received doses of ≥ 110 mg/m^2 per day. Patients were followed for toxicity and response. Correlations of toxicity frequency and grade with pharmacokinetic parameters were sought. In addition, correlation of changes in ATRA pharmacokinetics with the concentration of ATRA metabolites in plasma were sought. A total of 49 patients received ATRA at doses ranging from 45 to 309 mg/m^2 per day. Hypertriglyceridemia was dose-limiting at 269 mg/m^2 per day. Other frequent toxicities included mucocutaneous dryness and headache. With chronic dosing, plasma ATRA concentrations fell in 59% of patients. Stable, low, or variable [ATRA] were seen in 16%, 6%, and 16% of patients respectively. Age, gender, smoking, or concurrent medication did not correlate with the pharmacokinetic pattern. Severe toxicities tended to occur with initial peak [ATRA] of ≥ 0.5 μg/ml (1.7 μ*M*), and the toxicity frequency did not change if [ATRA] decreased with continued dosing. No consistent change in 4-oxo-ATRA or retinoid glucuronide concentrations was observed with decreases in plasma [ATRA]. The recommended once-daily ATRA dose is 215 mg/m^2, although significant interpatient variability is observed in toxicity and plasma retinoid concentrations. Although not statistically significant, more frequent and severe toxicity tended to occur in patients with higher plasma peak ATRA concentrations. Other factors, such as responses at target tissues, may be at least as important as the plasma ATRA concentration in predicting toxicity and/or response.

Key words Retinoids · Cancer · Phase I trial

Abbreviations *ATRA* All-trans-retinoic acid · *ECOG* Eastern Cooperative Oncology Group · *WBC* white-blood-cell count · *PLT* platelet count · *NCI* National Cancer Institute · *CBC* complete blood count · *BUN* blood urea nitrogen · *4-oxo-ATRA* 4-oxo-all-trans-retinoic acid · *HPLC* high-performance liquid chromatography · *CV%* percentage of coefficient of variation · *AUC* area under the plasma concentration-time curve

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Introduction

All-trans-retinoic acid (ATRA) has generated intense interest as an anticancer agent because it has produced

complete remissions in the majority of patients with acute promyelocytic leukemia to whom it has been given [4, 5, 10, 20]. In addition, ATRA has shown activity in a number of premalignant conditions [8, 12, 13, 18]. Retinoids are known to be necessary for normal development and maintenance of epithelial tissues [6, 12]. It is unknown at present whether epithelial malignancies can differentiate to a more normal phenotype with retinoid treatment. Furthermore, the optimal plasma concentration and duration of exposure to a given ATRA concentration remains to be defined for both malignant and premalignant conditions. In treatment of acute promyelocytic leukemia, relapse occurs almost universally, even if treatment with ATRA is continuous [4, 5, 10, 20].

The mechanism for this development of resistance to ATRA remains undefined. One postulated mechanism of resistance is decreased exposure to ATRA due to an up-regulation of ATRA metabolism, resulting in decreased plasma concentrations of ATRA despite continued dosing. Decreased ATRA concentrations with continued dosing have been reported [1, 11, 14, 15], as has the ability of retinoids to induce their own metabolism [9]. However, the relationship of such decreases in ATRA plasma concentration over time to clinical events (response or toxicity) remains to be defined. In addition, although most studies have reported decreased mean plasma ATRA concentrations in a population receiving continuous ATRA treatment, there has been one report of a subpopulation of patients that does not achieve high plasma ATRA concentrations [16]. We performed a phase I trial of daily administration of ATRA to define its toxicity, maximum tolerable dose, and pharmacokinetics and the relationship of its pharmacokinetics to its toxicity. In addition, we searched for subsets of the population in whom ATRA pharmacokinetics differed substantially.

Patients and methods

Patients

Eligible patients were those with a histologically proven malignant tumor who were ≥ 18 years of age and for whom no more effective standard treatment was available. Patients had recovered from all toxicity of previous treatment and had undergone no radiation therapy or chemotherapy within the 4 weeks preceding study entry [8 weeks for drugs with delayed toxicity, such as carmustine (BCNU) or mitomycin C]. Patients had a life expectancy of ≥ 12 weeks and an ECOG performance status of < 3. Adequate bone marrow function (WBC $\geq 3{,}500$ cells/μl, PLT $\geq 100{,}000$/μl), liver function (bilirubin ≤ 1.5 mg/dl), and renal function (serum creatinine ≤ 1.5 mg/dl) were required. Patients were not eligible if they had uncontrolled medical illness, psychotic illness, a history of seizures or brain tumor, or hypertriglyceridemia or hypercholesterolemia of > 300 mg/dl. Pregnant or nursing patients were not eligible. All sexually active, fertile patients agreed to use effective birth control, and sexually active, fertile women had a negative serum pregnancy test prior to going on study. All patients gave written informed consent, which had been approved by the Institutional Review Board at the University of Maryland at Baltimore and the National Cancer Institute (NCI).

Treatment plan

Cohorts of at least three patients each were treated with ATRA, with the dose beginning at 45 mg/m^2 per day and being escalated in a modified Fibonnaci scheme to 309 mg/m^2 per day. ATRA was given as a once-daily dose, rounded to the nearest 10 mg, after an overnight fast. The formulation consisted of an oval, soft gelatin capsule supplied by the NCI, which was responsible for its stability and purity. Each capsule contained 10 mg of ATRA as well as butylated hydroxyanisole, disodium ethylenediaminetetraacetic acid (EDTA), refined soybean oil, and a mixture consisting of purified beeswax, hydrogenated soybean-oil flakes, and hydrogenated vegetable oil. The capsules were stored at room temperature and protected from light. Dosing continued daily until the development of unacceptable toxicity, progression of disease, or the patient's refusal to continue. An evaluable course was defined as 28 days of drug treatment. Doses were not escalated in a given patient, but dose reductions were allowed as follows: (1) if grade 3 or 4 toxicity was encountered after the initial 28-day evaluation period the drug was temporarily discontinued and the dose was reduced by 25% following resolution of the toxicity, (2) if grade 3 or 4 toxicity occurred during the initial 28-day evaluation period the drug was discontinued, and (3) if toxicity exceeding grade 3 occurred it was treated symptomatically. Patients were given lip- and skin-moistening agents at the initiation of retinoic acid treatment.

Toxicities were graded with the Common Toxicity Criteria of the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI (Bethesda, Md.). In addition, skin toxicity was graded as follows: grade 1 – dryness, asteatosis, pruritis, xerosis; grade 2 – erythema with mild eczematous changes; grade 3 – severe eczematous changes; and grade 4 – desquamation with erythema. Elevations of cholesterol and triglyceride were graded as follows: grade 1 – 201–300 mg/dl; grade 2 – 301–400 mg/dl; grade 3 – 401–500 mg/dl and grade 4 – > 500 mg/dl. The scale for cholesterol and triglyceride toxicity was based on acceptable chronic levels of cholesterol and triglyceride in plasma. In general it is recommended that patients with plasma cholesterol levels in the upper 25th percentile of the normal distribution be treated via the diet and that patients with low-density-lipoprotein cholesterol in the upper 5th to 10th percentile be treated with medication. Although medical treatment for hypertriglyceridemia is less satisfactory, it is generally thought that chronic mild to moderate elevations in triglyceride levels are matters of concern in terms of development of atherosclerosis [2a]. The upper limit of normal for cholesterol and triglyceride concentrations is 200 mg/dl. Consequently, we defined arbitrarily that twice-normal concentrations of triglycerides and cholesterol would be unacceptable on a chronic basis and, thus, would warrant a grade-3 designation.

The maximal tolerable dose was defined as the dose at which 30% of at least six patients in the cohort experienced at least grade 3 toxicity of the same nature in the first 28 days of treatment.

Follow-up

The patients' history and physical examination, weight, performance status, toxicity evaluation, CBC, differential count, PLT, urinalysis, BUN, creatinine, liver-function studies (alkaline phosphatase, bilirubin, aspartate aminotransferase and alanine aminotransferase, albumin), and chemistry studies (electrolytes, uric acid, calcium, phosphorus, total protein) were obtained prior to treatment, weekly for the initial 2 months on study, and then every other week during treatment. Fasting values for cholesterol and triglycerides were obtained every other week. A chest radiograph and an electrocardiogram were performed prior to study entry and afterward as needed.

Tumor measurements were repeated every 2 months for measurable disease. Initial assessment also included an eye examination and a Schirmer test, which were repeated as needed.

Pharmacokinetics

Blood for determination of ATRA pharmacokinetics was collected in heparinized tubes before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, and 8 h after the first dose of ATRA. When reports from investigators studying leukemia noted a decrease in plasma ATRA concentrations with continuous dosing [1, 11, 14, 15], subsequent patients entered into the trial had plasma collected for pharmacokinetics studies on days 1, 8, 15, and 22 of their course, if possible. In addition, one patient who remained on study at 45 mg/m^2 per day and some who had had dose reduction agreed to have plasma collected again for determination of pharmacokinetics. Plasma was immediately prepared from whole blood by centrifugation at 1,000 *g* for 10 min at 4 °C. Urine was collected at 4-h intervals for the first 24 h. The amount of urine in each collection was recorded, and an aliquot was saved for future analysis. Collection tubes were wrapped in foil to protect the sample from light, and samples were stored at − 70 °C until analysis.

ATRA, 4-oxo-all-trans-retinoic acid (4-oxo-ATRA), 9-cis-retinoic acid, 13-cis-retinoic acid, and internal standard (authentic retinoid standards and the internal standard, Ro-11-5036, were kindly provided by Hoffmann-LaRoche, Inc., Nutley, N.J.) were extracted from plasma under dim light with 0.7 vol. of acetonitrile:butanol (50:50, v/v) in the presence of 0.3 vol. of K_2HPO_4 (1 kg/l, pH 11.08). The organic extract was analyzed with reverse-phase gradient high-performance liquid chromatography (HPLC; Beckman Instruments, Inc., San Ramon, Calif., USA) [3]. Mobile phase A consisted of acetonitrile: 0.02 *M* ammonium acetate: acetic acid (50:50:0.5, by vol.; pH 4.6), and mobile phase B comprised acetonitrile: 0.2 *M* ammonium acetate: acetic acid (95:5:0.04, by vol.; pH 8.41), pumped in a 10-min linear gradient from 30% B to 100% B at a flow rate of 1.5 ml/min. A Zorbax ODS analytical C_{18} column (25 cm × 4.6 mm inside diameter) with no end capping and with 5-μm spherical particles (MacMOD Analytical, Inc., Chadds Ford, Pa.) was used. Retinoid peaks were detected with a Beckman model 406 variable-wavelength UV absorbance detector set at 360 nm. The sample injection volume was 100 μl.

With these conditions, good baseline separation of 4-oxo-ATRA, internal standard, 13-cis-retinoic acid, 9-cis-retinoic acid, ATRA, and retinol was achieved, with retention times being approximately 6, 11, 13.3, 14, 14.5, and 15 min, respectively. The lower limit of quantitation for ATRA was 20 ng/ml, and the assay was linear between 20 and 500 ng/ml ATRA. ATRA in plasma matrix was stable for at least 1 year when stored at − 70 °C. Plasma samples that were expected to contain ATRA concentrations higher than 500 ng/ml were diluted prior to analysis. The assay CV% obtained for ATRA was 8% at 35 ng/ml and 3% at 280 ng/ml. The assay for 4-oxo-ATRA was linear between 25 and 100 ng/ml, and the lower limit of quantitation was 25 ng/ml. The CV% recorded for 4-oxo-ATRA was similar to that noted for ATRA.

Assessment of glucuronidation

In vitro hydrolysis of glucuronides was accomplished by incubation of plasma or retinoid glucuronides with beta glucuronidase. In brief, 100 μl of plasma or pooled, concentrated urine was added to 100 μl of 0.5 *M* sodium acetate buffer (pH 5.0) in an amber microcentrifuge tube. Beta glucuronidase at 120 μl (125 units/ml, from bovine liver, purchased from Sigma Chemical Co., St. Louis, Mo.) was added to the mixture, and tubes were placed in a shaking water bath at 37 °C for 16 h. Control samples did not contain beta glucuronidase. The reaction was stopped by the placement of tubes in a − 70 °C freezer until analysis by HPLC as described above. The concentration of various glucuronides was obtained as the difference between the concentration of analyte measured in control tubes and that determined after incubation with beta glucuronidase.

Synthesis of glucuronides of 4-oxo-ATRA and 9-cis-retinoic acid was accomplished in amber microcentrifuge tubes by the addition of 40 μl of 25 m*M* TRIS-HCl buffer (pH 7.4) to 40 μl of 9-cis-retinoic acid (20 μg/ml) or 4-oxo-ATRA (20 μg/ml), 400 μl of uridine-5'-diphosphoglucuronyltransferase (0.072 units/ml, from bovine liver, Sigma Chemical Co.), 200 μl of 0.01 *M* uridine-5'-diphosphoglucuronic acid (Sigma Chemical Co.), and 120 μl of TRIS buffer (pH 7.4). Tubes were incubated in a shaking water bath at 37 °C for 30 min, and the reaction was terminated with the addition of 800 μl of 1:1 glycine: trichloroacetic acid (pH 2.8). Reaction products were then extracted for HPLC as described above.

Pharmacokinetic data were modeled as a one-compartment, open linear model, assuming first-order absorption and elimination and a lag time (PCNONLIN, Statistical Consultants Inc., Lexington, Ky.).

Relationships between pharmacokinetics and pharmacodynamics were sought by comparison of the frequency and severity of toxicity with the peak plasma ATRA concentration or the area under the plasma concentration-time curve (AUC) as well as by searches for evidence of diminished grade of toxicity with diminished plasma ATRA concentration. Relationships between changes in pharmacokinetics and patients' characteristics such as gender, age, smoking habit, and previous anticancer treatment were also sought using two sample *t*-tests for age and Fisher's exact test for the other categorical characteristics.

Results

Patients' characteristics

A total of 49 patients were entered into the trial. Their characteristics are shown in Table 1. The numbers of patients treated and courses completed at each dose

Table 1 Patients' characteristics (*NSCLC* Non-small-cell lung cancer, *SCLC* small-cell lung cancer)

Number of patients entered	49
Men	25
Women	24
Median performance status (range)	1(0–2)
Median age (range)	60(35–78) years
Previous therapy:	
Chemotherapy only	16
Radiation therapy only	4
Both	27
None	2
Tumor types:	
Colorectal	9
Breast	8
Head/Neck	8
NSCLC	5
Prostate	3
Hepatic	2
Pancreatic	2
Other (1 each: gastric, hodgkin's disease, Melanoma, Schwannoma, Transitional bladder carcinoma, ovarian, Mulaerian duct, uterine leiomyosarcoma, penile, leukoplakia, SCLC, unknown primary)	12

Table 2 Dose escalation of ATRA

Dose (mg/m^2)	Number of patients	Number of courses
45	3	21
56	3	10
70	3	7
88	3	10
110	10	28
138	4	13
172	6	17
215	9	15
269	7	8
309	1	1

level are shown in Table 2. All except four patients had at least 28 days of therapy. Six patients (one each at 45, 56, and 137.5 mg/m^2 per day; two at 110 mg/m^2 per day; and one at 172 mg/m^2 per day) had dose reduction after 28 days due to skin toxicity (two patients); respiratory toxicity consisting of cough, dryness, and shortness of breath on exertion without rales or chest-radiograph changes (three patients); and back pain (one patient). Ten patients were entered at the 110-mg/m^2 dose level because of the observation of grade 3 cough, mandating expansion of the cohort to at least six patients. In addition, grade 3 hypertriglyceridemia, elevation of liver-function values, skin toxicity, and fatigue were observed in one patient each. Because no consistent dose-limiting toxicity was identified with ten patients on the cohort, we decided to continue with dose escalation. A similar expansion of the cohort occurred at the 215-mg/m^2 daily dose level.

Toxicity

The maximum tolerable dose of ATRA was 269 mg/m^2 per day. Although toxicities of at least grade 3 were seen at lower doses, no consistent dose-limiting toxicity occurred at the lower dose levels. The dose-limiting toxicity was hypertriglyceridemia. Grade 3 hypertriglyceridemia was observed in one patient in each cohort receiving doses of 110, 138, and 269 mg/m^2 per day. Grade 4 hypertriglyceridemia occurred in one patient who received a dose of 88 mg/m^2 per day, in one patient who received a dose of 215 mg/m^2 per day, and in one patient who received ATRA at a dose of 269 mg/m^2 per day. Grade 3 hypercholesterolemia was seen in one patient treated at the 269-mg/m^2 daily dose. Most patients who developed hypercholesterolemia or hypertriglyceridemia had baseline serum cholesterol and/or triglyceride values of > 200 mg/dl. No clinical complication of hyperlipidemia was observed over the short period during which patients were on the study drug.

Other grade 3 and 4 toxicities occurred sporadically: a localized desquamative scrotal rash occurred in two patients (at doses of 45 and 138 mg/m^2 per day); staphylococcal bacteremia occurred in one patient receiving a dose of 110 mg/m^2 per day; one patient receiving a dose of 110 mg/m^2 per day and one patient treated at a dose of 309 mg/m^2 per day developed probable pseudotumor cerebri; shortness of breath on minimal exertion occurred in one patient treated at a dose of 110 mg/m^2 per day and in one patient receiving a dose of 138 mg/m^2 per day, and severe cough developed in two patients treated at the same doses. Dehydration and transient renal failure developed in one patient (described below) treated at a dose of 56 mg/m^2 per day. Grade 3 myalgias occurred in one patient in each cohort receiving doses of 172 and 215 mg/m^2 per day. One patient who had previous severe osteoarthritis and kyphosis experienced a severe exacerbation of neck pain requiring a neck brace and interruption of treatment. Grade 3 headache, not classified as pseudotumor cerebri, was observed in two patients treated at a dose of 110 mg/m^2 per day and in one patient receiving a dose of 215 mg/m^2 per day. The following toxicities were observed in one patient each, without relationship to dose: grade 3 anorexia, fatigue, dysphagia, scleral hemorrhage, thrombocytopenia, anemia, nausea, and vomiting. Grade 3 transient elevations of transaminases were seen in four patients treated at doses of ≥ 56 mg/m^2 per day.

Frequent grade 2 toxicities included anemia in eight patients, anorexia in six patients, dyspnea in four patients, depression in four patients, fatigue in eight patients, dry skin and/or eyes in the majority of patients, "stuffy ears" in several patients, fever in four patients, transient headache in nine patients, hypertriglyceridemia in four patients, transaminasemia in four patients, nausea in seven patients, and vomiting in six patients. Grade 2 infections included one patient with a paronychia, two patients with urinary tract infections, and one patient with vaginal candidiasis.

One patient had acidosis, skin burning (in metastatic breast-cancer lesions), acute transient renal failure (secondary to dehydration), and shortness of breath. Treatment was discontinued. Symptoms resolved, and the patient refused further treatment. All toxicities were reversible upon cessation of drug administration. Other than the patient with scleral hemorrhage, no ocular abnormality other than conjunctivitis or dry eyes was observed. The frequency of toxicities encountered at each dose level is shown in Table 3.

Responses

One patient with hepatocellular carcinoma had stable disease for 14 months on study. One patient with severe leukoplakia and a history of several previous oral cancers remained stable for 7 months. A third patient, who had squamous cancer of the penis metastatic to skin of the inguinal area, remained stable for 2–3 months, but

Table 3 Number of toxicities observed according to dose and grade

Dose (mg/m^2)	Number of patients	Grade			
		1	2	3	4
45	3	23	7	2	0
56	3	23	7	5	0
70	3	27	9	0	0
88	3	18	6	0	0
110	10	51	28	10	0
138	4	13	9	9	0
172	6	23	18	3	0
215	9	50	19	9	2
269	7	44	15	2	1
309	1	0	0	1	0

Table 4 Pharmacokinetics of ATRA, Day 1[a]

Dose (mg/m^2)	Peak [ATRA] (μg/ml)	Peak time (h)	AUC ATRA (μg ml^{-1} h)	$t_{1/2}$ (h)
45	0.52(0.34–0.8)	1.5(1–2)	1.3(1–1.85)	0.9(0.7–1.1)
56	0.48(0.14–0.96)	2.5(1–5)	1.3(0.6–2.3)	1.4(0.9–1)
70	0.38(0.14–0.71)	2.2(1.5–3)	0.8(0.6–1.2)	1.4(0.7–2.2)
88	0.7(0.34–0.9)	3.7(2–5)	1.8(0.9–3)	0.7(0.4–1)
110	0.93(0.12–2.32)	2.6(1.5–5)	2.4(0.3–4.1)	1.2(0.6–2.1)
138	1.1(0.48–2.6)	2.4(1.5–3)	3.2(1.2–7.6)	0.6(0.2–1)
172	0.9(0.2–1.72)	3.3(2–6)	2.8(0.4–5.8)	0.9(0.1–1.5)
215	1.8(0.11–3.4)	2.2(1–5)	6.2(0.6–17.4)	1.2(0.6–2)
269	1.5(0.8–2.5)	2.9(2–7.5)	4.6(1.5–7.6)	0.7(0.5–1.1)
309[b]	1.6	2.5	5.9	0.9

[a] Data represent mean values (ranges)
[b] 7 patient only

when the drug was discontinued due to severe myoskeletal pain the tumor resumed rapid growth.

Pharmacokinetics

Pharmacokinetics were obtained on day 1 for 48 of the 49 patients and on both day 1 and weekly for 31 patients. An additional patient had plasma pharmacokinetics repeated after 8 months of daily treatment at the same dose. Pharmacokinetic parameters for day 1 are shown in Table 4. There was considerable interpatient variability in the peak plasma concentration and AUC achieved for any given dose. The terminal half-life was approximately 0.7 h, but outliers were evident. Both the peak plasma ATRA concentration (Fig. 1, $r = 0.5$) and the AUC (data not shown) increased with dose (Fig. 1).

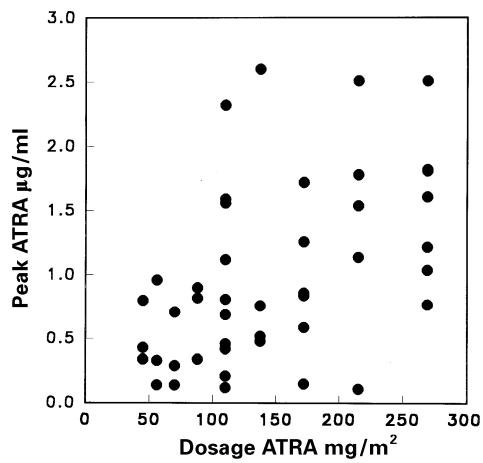

Fig. 1 Relationship of ATRA dosage to peak plasma ATRA concentration

In all, 32 patients were studied on more than one occasion or on a weekly basis (Table 5). Although more than half of the patients (19/32) had a consistent decrease of $>25\%$ in peak plasma ATRA concentration with continued dosing, other pharmacokinetic patterns were observed (Fig. 2). These included 5 patients (16%) whose peak plasma ATRA concentration remained unchanged, 5 patients with peaks that were variable weekly (16%), as well as 2 patients (6%) who never achieved high ATRA concentrations and 1 patient who had increasing peak ATRA concentrations and AUCs with time. Calculated AUCs revealed that the majority of patients had a decrease of at least 25% in ATRA AUC with time, even if the peak plasma ATRA concentration increased. However, 11 patients had variable AUCs, and 1 patient each had consistent, low, or increased AUCs over time (data not shown).

Table 5 also reveals heterogeneity in the time it took to develop maximal changes in plasma ATRA concentration. Some patients showed decreased plasma ATRA concentrations by day 8, without further reduction. Others showed a continued decrease over the study period or stable plasma ATRA concentrations until a sudden drop occurred on day 22 or 29. The percentage of decrease in plasma peak ATRA concentration and AUC was also variable. Age, gender, smoking habit, concurrent medication, or previous chemotherapy did not predict the stability of ATRA pharmacokinetic parameters over time in our patient population. All but a few patients had received prior anticancer therapy, and 12 of the 32 patients with repeated pharmacokinetic determinations were smokers. The most frequently observed concomitant medications were morphine, oxycodone, codeine, ibuprofen, acetaminophen, ranitidine, triazolam, alprazolam, and megestrol acetate.

In general, very low plasma concentrations of 4-oxo-ATRA were observed, comprising 0–36% of the parent compound at the peak plasma ATRA concentration. In most patients in whom peak plasma ATRA concentrations decreased over time, the ratio of peak 4-oxo-ATRA to peak ATRA remained constant. However, three patients showed increasing ratios of metabolite to parent compound as peak plasma ATRA concentrations decreased. Patients with consistently low plasma

Table 5 Pharmacokinetics of continued-dosing ATRA (*ND* Not detectable)

Patient	Dose (mg/m^2 daily)	Peak [ATRA] (μg/ml) day 1	Peak [ATRA] (μg/ml) max. change	Day of maximal change	Pattern
1	45	0.34	0.43	8.5 months	Stable
2	110	1.12	0.49	15	Decrease
3		2.32	0.42	22	Decrease
4		0.69	0.22	8	Decrease
5		0.12	0.2	15	Low
6		0.46	0.2	22	Decrease
7		1.59	0.33	22	Decrease
8		0.81	0.29, 1.0	8, 29	Variable
9	138	0.52	0.9–1.44	8–22	Variable
10		0.76	0.5–1.39	8–22	Variable
11		0.48	0.59	15	Stable
12	161	1.04	0.97	22	Stable
13	172	0.59	0.09	8	Decrease
14		0.86	0.35, 0.76	8, 15	Variable
15		0.84	0.52	8	Decrease
16		0.15	0.73	22	Increase
17		1.26	ND	15	Decrease
18		1.72	0.87	15	Decrease
19	215	1.14	0.08	8	Decrease
20		1.78	0.31	15	Decrease
21		1.54	0.61	22	Decrease
22		0.11	0.23	15	Low
23		2.51	1.97	22	Decrease
24		2.64	2.77	22	Stable
25		1.63	0.48	8	Decrease
26	269	2.51	1.69	22	Decrease
27		1.82	1.79	22	Stable
28		1.04	0.55	22	Decrease
29		1.22	0.53	15	Decrease
30		1.8	1.3–2.3	15, 22	Variable
31		1.6	0.25	22	Decrease
32		0.77	0.56	22	Decrease

ATRA concentrations did not have measurable 4-oxo-ATRA concentrations (data not shown).

Treatment of plasma samples with beta glucuronidase revealed the presence of two new peaks, one of which cochromatographed with 9-cis-retinoic acid. The second new peak eluted at a retention time between that of 13-cis-retinoic acid and 9-cis-retinoic acid. The glucuronides of 4-oxo-ATRA and 9-cis-retinoic acid eluted at 1.5 and 2.9 min, respectively, corresponding to peaks present in plasma before treatment with beta glucuronidase. Concentrations of 9-cis-retinoic acid measured in plasma after incubation with beta glucuronidase were 0.2–0.9 μM and represented 9–19% of total plasma ATRA concentrations. Small amounts of the glucuronides of ATRA were also present. There was no consistent increase or decrease in the concentration of retinoid glucuronides found in the plasma of patients who had decreased peak plasma ATRA concentrations or AUCs with continued dosing (data not shown).

Up to the dose of 269 mg/m^2 per day, no ATRA was detected in urine. The only detectable glucuronide in urine was that of 4-oxo-ATRA. Because pooled urine from several patients was analyzed, it is not possible to correlate urinary 4-oxo-ATRA concentrations with plasma ATRA concentrations in individual patients. Although not quantified, there was no obvious change in the peak height ratios of retinol and internal standard. Minor changes in retinol concentration may be difficult to discern, as the optimal absorbance for retinol is at approximately 325 nm rather than the 360 nm that was monitored by the HPLC system.

Pharmacokinetic-pharmacodynamic relationships

The frequency and severity of toxicity associated with ATRA tended to increase with initial peak plasma ATRA concentrations (Table 6), although this trend did not reach statistical significance. In our study the frequency or severity of toxicity did not decrease when plasma ATRA concentrations decreased to below 0.5 μg/ml with continued dosing. Patients who had consistently low plasma ATRA concentrations did not experience severe toxicity.

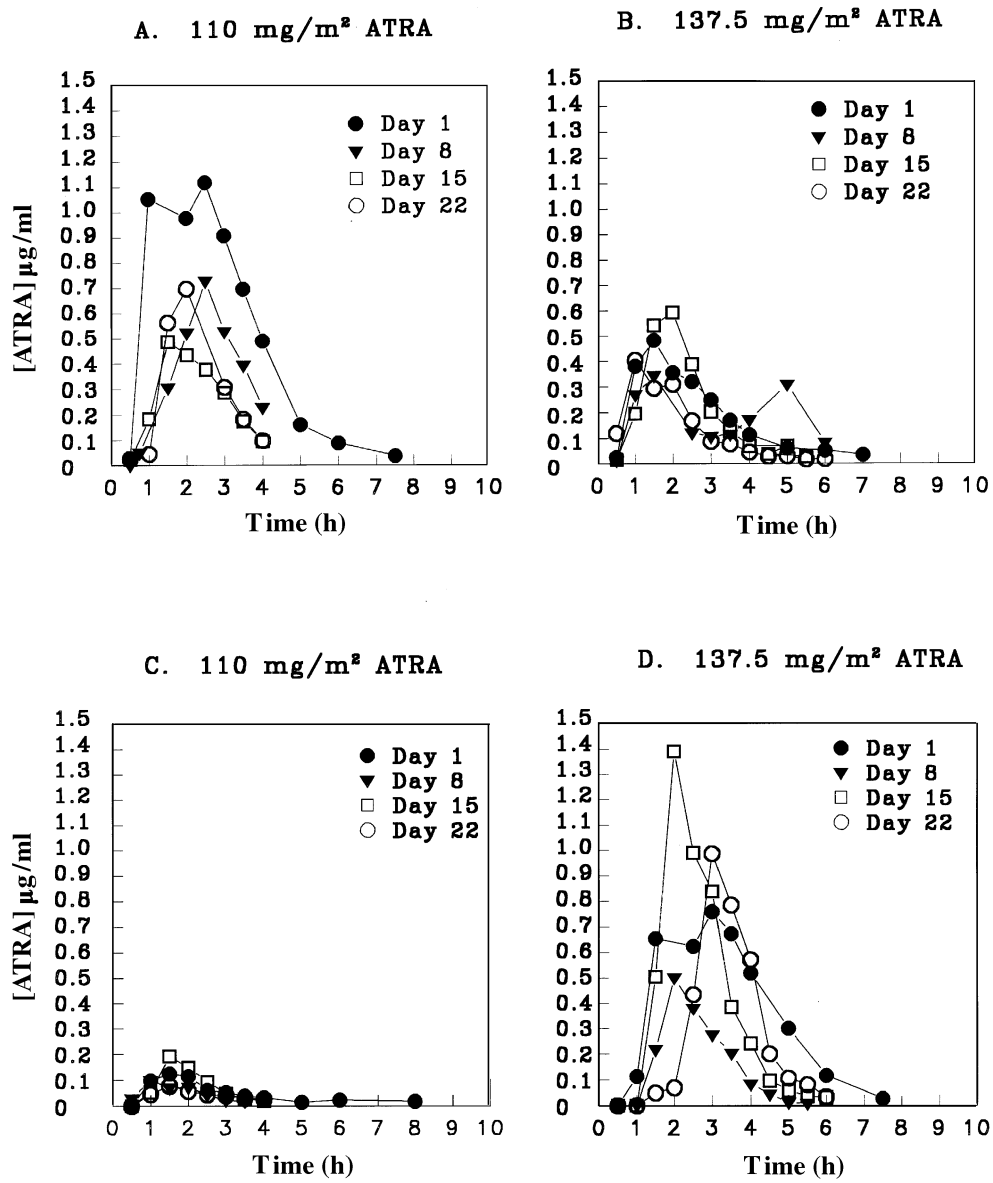

Fig. 2 A–D Representative pharmacokinetic patterns obtained with continued dosing. **A** Consistent decrease in plasma ATRA concentration with continued dosing (59% of patients). **B** Stable pharmacokinetics with continued dosing (16% of patients). **C** Consistently low plasma ATRA concentrations (6% of patients). **D** Variable pharmacokinetics with continued dosing (16% of patients). Note the wide variability in plasma concentrations with dose

Table 6 Relationship of toxicity grade to initial peak plasma ATRA concentration

	Peak [ATRA] (µg/ml)	
	< 0.5 ($n = 24$)	≥ 0.5 ($n = 71$)
Number of toxicities		
Grade 1–2	23	57
Grade 3–4	1	14

Fisher's exact test: $P < 0.06$, 1 Tail

Discussion

The recommended phase II dose of ATRA is 215 mg/m^2 per day given once daily. Dose modifications may need to be made in patients who have hyperlipidemia of > 200 mg/dl (cholesterol or triglycerides). We did not observe any instance of retinoic acid syndrome [7] in our patients, i.e., severe respiratory distress and capillary leak. However, several patients developed shortness of breath or cough, sometimes necessitating dose reduction.

Analysis of ATRA pharmacokinetics revealed heterogeneity in the population with respect to the apparent increased clearance of ATRA. It has been shown that ATRA and other retinoids can up-regulate their own metabolism through the cytochrome P450 enzyme system, although the major cytochrome P450 enzyme responsible for ATRA metabolism has not yet been defined [17]. Because many medications, as well as tobacco constituents and endogenous steroids, are also metabolized by cytochrome P450s, we sought correlations between gender, smoking status, and medication

history and a pharmacokinetic pattern consistent with an increase in apparent clearance of ATRA. However, we did not find any correlation between pharmacokinetic patterns and the above-mentioned patients' characteristics. It should be stressed, however, that most of the patients we studied had received previous anticancer treatment and were on several concurrent medications. A history of smoking was obtained for 12 of the 32 patients who had repeated pharmacokinetic assessments, but these data were obtained retrospectively from the medical record and were not consistently sought prior to treatment. Consequently, the lack of relationship between patients' characteristics and changes in ATRA pharmacokinetics over time in our study should be interpreted with caution.

We did not observe significant increases in 4-oxo-ATRA, which would be the ultimate metabolite of a cytochrome P450 metabolic process. Rapid elimination of this metabolite could be the cause. Glucuronidation is another elimination mechanism for retinoids and their metabolites [2, 19, 21]. Our studies of glucuronides, however, did not reveal consistent up-regulation of this elimination mechanism in patients with decreasing plasma ATRA concentrations over time. The only glucuronide found in urine was that of 4-oxo-ATRA.

We also confirmed the observation of Rigas et al. [16] that there is a small population of patients who achieve only low ATRA concentrations in plasma after ATRA administration. Whether this is due to poor absorption or to rapid metabolism is not known. However, the observation that plasma ATRA concentrations do not increase in these subjects with administration of cytochrome P450 inhibitors [16] and the small amount of glucuronidation observed in our patients indicate that the reason for the low plasma ATRA concentrations may well be poor absorption. Perhaps the most intriguing population in our study comprised those patients who had variable pharmacokinetics of ATRA over time as well as the single patient who had increasing peak plasma ATRA concentrations over time. All patients kept a diary of ATRA dosing and side effects; thus, noncompliance, i.e., a drug holiday with return of day-1 pharmacokinetics, is not a likely explanation. In addition, there was no obvious difference in oral intake during the collection of blood for pharmacokinetics between these subjects and others.

The present study does not show that a decreased plasma ATRA concentration correlates with decreased toxicity. In our population, severe (grade ≥ 3) toxicities tended to occur more frequently in patients who had an initial peak ATRA concentration of ≥ 0.5 μg/ml (1.7 μ*M*). The frequency of toxicity of any grade was also greater in the group with high initial peak ATRA concentrations. However, in our patients, decreased plasma ATRA concentrations over time were not associated with a decrease in initial toxicity. Perhaps this is not surprising, in that plasma ATRA concentrations decline quickly due to the drug's short half-life and are not measurable by 8 h after a dose, although toxicity is constant. This implies that the target (presumably tissue) can respond to the agent in the absence of significant plasma concentrations. The mechanism for these pharmacodynamics is unknown at present but, conceivably, may be related to either initiation of a cascade of genetic events by the initial (brief) exposure or to a storage of ATRA for later use. Given that ATRA is an antiproliferative agent, it is interesting that we observed stable disease in two patients, one with hepatoma and one with a rapidly growing, metastatic squamous-cell carcinoma of the penis. In addition, a breast cancer patient had a response in her metastatic skin lesions, although no response was observed in other organs. Both skin responses (breast and penile) were accompanied by severe toxicity.

Retinoid glucuronides have been reported to have retinoid activity ([2] and references therein). This is the first report of 9-cis-retinoic acid glucuronide in the plasma of patients treated with ATRA. 9-Cis-retinoic acid is a ligand for both retinoic acid receptors and retinoid X receptors, and this compound may have been responsible for some of the side effects observed in our study. Small amounts of retinoic acid glucuronides were also observed in plasma.

In summary, ATRA can be given once daily to cancer patients, with the recommended phase II dose being 215 mg/m^2 per day. The dose-limiting toxicity in our study was hypertriglyceridemia. Severe toxicity tended to occur more frequently at higher initial peak plasma ATRA concentrations, and there was no associated decrease in toxicity with a decrease in the peak plasma ATRA concentration below 0.5 μg/ml. Different pharmacokinetic patterns were observed in the population, including apparent increased ATRA clearance, stable pharmacokinetics, consistently low plasma concentrations of ATRA, and variable plasma ATRA concentrations with continued dosing. No obvious increase in ATRA metabolites or in glucuronidation was observed in patients with decreased ATRA concentrations over time.

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