Phase I Trial of 9-*cis* Retinoic Acid in Adults with Solid Tumors

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ABSTRACT

Retinoids have been shown to be potent inhibitors of epithelial carcinogenesis. Recent evidence has demonstrated that retinoid actions are mediated through nuclear receptors, which are proteins encoded by the retinoic acid receptor and retinoid X receptor gene families. These receptors are activated by binding to specific retinoids; of the known naturally occurring retinoids, 9-*cis* retinoic acid is unique in its ability to bind to both receptor families. Because of its unique receptor-binding characteristics, 9-*cis* retinoic acid may have biological activity not possible with other retinoids. For this reason, we conducted a Phase I trial of 9-*cis* retinoic acid in adult patients with solid tumors. Twenty-two patients were treated twice daily with p.o. 9-*cis* retinoic acid at doses ranging from 20 mg/m^2/day to 150 mg/m^2/day. The patients had non-small cell lung cancer (n = 8), breast cancer (n = 5), colorectal cancer (n = 3), head and neck cancer (n = 2), nonmelanoma skin cancer (n = 2), or ovarian cancer (n = 2). The dose-limiting (WHO grade III) toxic effects, which occurred at the 150-mg/m^2/day dose level, were headaches and diarrhea. Less severe (grades I and II) toxic effects included cheilitis, dry skin, conjunctivitis, fatigue, hypertriglyceridemia, alkaline phosphatase elevation, myalgia/arthralgia, and hypercalcemia. Of the 15 patients evaluable for tumor response, no objective responses were observed. Pharmacokinetic analysis revealed a reduction in peak 9-*cis* retinoic acid plasma levels with chronic administration. Based on this study, the recommended Phase II dose of 9-*cis* retinoic acid in adult patients with solid tumors is 100 mg/m^2/day administered in a divided dose twice daily.

INTRODUCTION

Retinoids are a diverse group of compounds which share the ability to bind and activate nuclear receptors that function as transcription factors, regulating the expression of genes which mediate retinoid actions. Two separate families of retinoid nuclear receptors have been discovered, the RARs[3] and the RXRs, and each family consists of three genetically distinct members ($-\alpha$, $-\beta$, $-\gamma$; Refs. 1–6). These receptors can form RAR:RXR heterodimers or RXR homodimers (7–9). RAR:RXR heterodimers bind response elements within gene promoters; these response elements differ from those bound by RXR homodimers (10, 11). Recent studies have shown that these receptor dimers bind different retinoids; of the known naturally occurring retinoids, all t-RA binds only RAR:RXR heterodimers and 9cRA binds both RAR:RXR heterodimers and RXR homodimers (12, 13). Because it has unique binding specificities, 9cRA may have biological activity not observed with other retinoids.

Retinoids have demonstrated potential in the prevention and treatment of cancer. Treatment with 13cRA reverses oral leukoplakia (14). In patients with head and neck cancer who are free of disease following surgery or radiation therapy, treatment with 13cRA reduces the incidence of second primary tumors (15). Similarly, in patients who have undergone complete resection of stage I NSCLC, treatment with retinyl palmitate reduces the incidence of second primary tumors (16). As a treatment of overt cancer, t-RA induces complete remissions in the majority of patients with acute promyelocytic leukemia (17–19). Pilot studies have revealed that 13cRA has activity in the treatment of T-cell lymphoma (20) and juvenile chronic myelogenous leukemia (21). In combination with IFNα-2a, 13cRA has activity in the treatment of renal cell carcinoma and squamous cell carcinoma of the skin and cervix (22–24). These studies have demonstrated enormous potential for retinoids in oncology and illustrate the need to investigate novel retinoids which may have greater efficacy in the prevention or treatment of cancer. Toward this aim, we performed a Phase I trial of 9cRA in adult patients with solid tumors. The objectives of this study were to determine the MTD of 9cRA and to evaluate the toxicities and pharmacokinetics of 9cRA administration.

PATIENTS AND METHODS

Patient Selection. Patients with pathologically confirmed carcinoma were eligible for treatment if they had received at least one prior chemotherapeutic regimen and were not eligible for treatments of known greater efficacy. Patients who required adjuvant therapy were eligible, including those with

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[3] The abbreviations used are: RAR, retinoic acid receptor; RXR, retinoid X receptor; 9cRA, 9-*cis* retinoic acid; 13cRA, 13-*cis* retinoic acid; t-RA, all-*trans* retinoic acid; NSCLC, non-small cell lung cancer; MTD, maximum tolerated dose; AUC, area under the curve; C_{max}, peak plasma concentration; t_{max}, time to reach C_{max}; $AUC_{O\text{-}T}$, time 0 to last measured concentration value.

completely resected squamous cell carcinoma of the skin or locally advanced or incompletely resected NSCLC or head and neck cancer following radiation therapy to the primary tumor. Other eligibility criteria included age between 18 and 80 years, Karnofsky performance status ≥70%, and a life expectancy of ≥3 months. Women of childbearing potential were required to practice adequate contraceptive methods. Patients were excluded from treatment if they had inadequate renal function (serum creatinine >1.5 mg/dl), hepatic function (alanine transferase or aspartate aminotransferase >1.25 × normal or bilirubin >1.25 × normal), bone marrow function (hemoglobin <10 g/dl, leukocyte count <4000 × 10^9/liter, or platelet count <100,000 × 10^9/liter), or cardiac function (New York Heart Association grade III–IV). Patients who had received 13cRA or t-RA within the previous 2 months were ineligible. Patients must not have received systemic treatment for their cancer within the previous 4 weeks and must not have had symptomatic central nervous system metastases. Patients with uncontrolled metabolic disorders, intercurrent infections, or known hypersensitivity to retinoids were ineligible.

Prior to treatment, patients underwent a complete medical history, physical examination, and appropriate laboratory and radiological studies. These included electrocardiogram, chest and cervical spine X-rays, complete blood count, SMA-12, β_2-microglobulin, serum triglycerides, lipoproteins, cholesterol, urinalysis, pregnancy test (if appropriate), prothrombin time, partial thromboplastin time, serum electrolytes, serum creatinine, and radiological studies to evaluate the extent and severity of existing or suspected malignant and nonmalignant conditions. In addition, patients underwent baseline ophthalmologic and auditory examinations prior to treatment. The nature and purpose of this study were discussed with each patient, and written formal consent was obtained. This trial was conducted under the recommendations for the protection of human rights made by the U. S. Department of Health, Education, and Welfare and was reviewed and approved by the Institutional Review Board of the University of Texas M. D. Anderson Cancer Center.

Treatment Plan. A minimum of three patients were treated at each dose level at The University of Texas M. D. Anderson Cancer Center. Capsules containing 5 or 20 mg 9cRA provided by Hoffman LaRoche Ltd. [Basel, Switzerland (CH)] were p.o. administered in a divided dosage twice a day (in the morning and evening) within 30 min after a meal. The first cohort received 20 mg/m^2/day, and the calculated dosage was rounded up to the closest multiple of 5 mg. Planned treatment duration was 4 weeks, but the treatment continued without interruption as long as there was no tumor progression and toxicity was acceptable. If toxic effects within a cohort were of less than grade III severity during the first month of treatment, the dose was escalated in the next cohort of patients until the MTD was determined. The dose escalation scheme involved increasing levels in subsequent cohorts by 100% until grade I toxic effects occurred, at which point doses were escalated by 50% in subsequent cohorts. If one patient within a cohort developed a toxic effect of grade III or greater severity in the first month of treatment, then three additional patients were enrolled at that dose level to enlarge the cohort to six patients. If two or more patients at any given dose developed grade III or IV toxic effects in the first month, the trial was stopped and that dose level considered the MTD.

Patients were examined weekly during the first month of treatment. Blood samples were collected weekly to evaluate toxicity, and pharmacokinetic analysis was performed on days 1 and 22. Radiographic tests were performed at the completion of each month of treatment to determine tumor response or progression.

Drug Assay. Blood samples (10 ml) were collected for pharmacokinetic purposes at various time points after the first dose (day 1) and at steady state (day 22). On these days, the patients fasted prior to taking the morning dose. On the evening prior to day 22, the evening dose was taken after the evening meal. The samples were collected into cooled brown vacutainer tubes containing EDTA as anticoagulant. Within 30 min after collection, the sample was centrifuged at 4°C, plasma was transferred into brown glass tubes, and immediately stored in the dark at −20°C. Blood and plasma samples were always protected from light during the collection, handling, shipping, and analytical procedures.

Plasma concentrations of 9cRA were determined by a specific reverse-phase high-pressure liquid chromatography method with UV detection at 350 nm. In brief, 9cRA and the internal standard Ro 10–1670 were extracted from buffered plasma (KH_2PO_4, pH 3.5) using a mixture of ethanol and hexane. The organic (hexane) extract was evaporated to dryness at 25°C under a stream of nitrogen, redissolved in an aliquot of 0.1% ammonium acetate/1% glacial acetic acid/80% acetonitrile and injected onto the high-pressure liquid chromatography column. Chromatography was performed using a 5 μ Spherical C18 (3.9 × 300-mm) column. The mobile phase consisted of an acetonitrile gradient 62.6–95% in 0.04% ammonium acetate/3% glacial acetic acid.

The performance of all chromatographic runs was guaranteed by simultaneous analysis of independent quality control samples of various concentrations covering the whole calibration range. The interassay coefficients of variation for these quality control samples were between 5 and 10%, the imprecision between −0.5 and +7%. The limit of quantification was 2 ng/ml.

Pharmacokinetic Evaluation. Pharmacokinetic evaluation was performed using model independent methods. The C_{max} and the time to reach C_{max} (t_{max}) were taken directly from the observed plasma concentration-time data. The area under the plasma concentration-time curve from time 0 up to the $AUC_{O\text{-}T}$ during one dosing interval was estimated using the linear trapezoidal rule. The apparent elimination $t_{1/2}$ was estimated by dividing ln2 by the terminal elimination constant which was estimated by performing standard unweighted log linear least-squares regression analysis of the apparent terminal phase.

Statistical Methods. To examine whether the pharmacokinetics of 9cRA was dependent on the dose, a linear regression analysis was performed for $AUC_{O\text{-}T}$ and C_{max} values for day 1 and day 22, separately. Dependency on doses was tested by comparing the slopes of the regression lines to zero. To compare both parameters between days 1 and 22, a Student's t test for paired samples was performed. A P value of <0.05 was considered as statistically significant.

Table 1 Patient characteristics

		n	%
Patients treated		22	
Assessable for response and toxicity		15	68
Toxicity only		7	32
Age (yr)			
Median	61		
Range	35–82		
Karnofsky performance status			
70		3	14
80		11	50
90		5	22
100		3	14
Sex			
Female		16	73
Male		6	27

Assessment Criteria and Statistical Methods. Response was assessed after each month of treatment by chest radiograph, physical examination, and other radiological studies as indicated. Responses and toxic effects were assessed by using the WHO grading system. For hyperlipidemia, the WHO grading criteria are: grade I, triglyceride levels >2.5–5 times the upper limits of normal or cholesterol >1.25 times normal; grade II, triglyceride levels 5.1–10 times normal or cholesterol 1.26–1.5 times normal; grade III, triglyceride levels >5 times normal and uncontrolled by gemfibrozil (Lopid; Parke-Davis, Morris Plains, NJ) or cholesterol >1.5 times normal; grade IV, triglyceride levels >5 times normal and uncontrolled by medication, leading to pancreatitis.

RESULTS

Patient Characteristics. The study lasted from January 1994 to March 1995, and a total of 23 patients were registered. Patient characteristics are listed in Table 1. One patient (patient 10) did not receive treatment because of deteriorating hepatic function. NSCLC was the most common type of tumor included in this study (eight patients), followed by breast cancer (five patients), colorectal cancer (three patients), head and neck cancer (two patients), nonmelanoma skin cancer (two patients), and ovarian cancer (two patients).

Toxic Effects. There were no hematological toxic effects attributable to 9cRA. Nonhematological effects reported during the first 4 weeks of treatment are listed in Tables 2 and 3. The dose-limiting effects were headache and diarrhea. The grade III headaches began during the first day of treatment, were unrelenting, did not respond to nonsteroidal anti-inflammatory drugs, necessitated discontinuation of treatment within 2–4 days, and resolved within 1 day following treatment cessation. The grade III diarrhea occurred in two patients treated at 150 mg/m^2/day, both with colorectal cancer, began within the first 2–3 days of treatment, did not respond to treatment with diphenoxylate hydrochloride with atropine sulfate (Lomotil) and Kaopectate, necessitated discontinuation of treatment after 6 days, and resolved within 1 day following discontinuation of 9cRA treatment. Neither of these colorectal cancer patients tested at this dose level had experienced significant diarrhea during the course of their disease prior to treatment with 9cRA. The only other colorectal cancer patient enrolled in this trial was treated at a lower 9cRA dose (30 mg/m^2/day) and experienced no significant diarrhea during treatment. These three colorectal cancer patients had had similar treatments prior to initiating 9cRA. All had undergone hemicolectomy and treatment with 5-fluorouracil and leucovorin. The possibility that the diarrhea observed at the 150-mg/m^2/day dose level was related to colorectal cancer or to prior treatment for colorectal cancer cannot be excluded.

Grade III lipid abnormalities occurred in two patients who had diabetes mellitus: one developed grade III hypertriglyceridemia (peak value, 1045 mg/dl) and the other developed hypercholesterolemia (peak value, 455 mg/dl). Lipid abnormalities were graded according to WHO criteria. In these two diabetic patients, lipid abnormalities occurred rapidly (within the first week) and Lopid was not effective in reversing these abnormalities. Withdrawal of 9cRA normalized serum lipid values within 1–2 weeks.

The predominant grade I or II toxic effects which occurred during the first month of treatment included cheilitis (16 patients), dry skin (16 patients), headache (13 patients), fatigue (11 patients), hypertriglyceridemia (9 patients), conjunctivitis (7 patients), alkaline phosphatase elevation (7 patients), myalgia/arthralgia (8 patients), nausea (5 patients), and anorexia (4 patients). The alkaline phosphatase abnormalities occurred without associated aminotransferase abnormalities, except in one patient with colorectal cancer and extensive liver metastasis, who developed a grade IV alkaline phosphatase elevation.

Response and Response Duration. Of 22 patients treated in this study, 15 were evaluable for tumor response. There were no objective responses. Of the five breast cancer patients, three were evaluable for response. These three patients had stable disease after 6 weeks of treatment. One patient experienced a transient shrinkage of lymph node metastases but this did not qualify as a response because of its brief duration. Prior to 9cRA treatment, this patient had disease which was estrogen receptor negative, and examination revealed that she had a right-sided pleural effusion, skin and soft tissue metastases, and bilateral lymph node involvement including measurable disease in the left subpectoral and axillary regions. After 2 weeks of treatment, she had objective improvement in facial and left arm edema. After 4 weeks of treatment, ultrasound examination documented shrinkage of the left subpectoral and left axillary lymph nodes. No other enlarged lymph nodes were measurable because of confluence or previous radiation treatment. Two weeks later, this patient developed increasing shortness of breath and generalized weakness. Treatment was discontinued, and her respiratory function continued to deteriorate. She died approximately 2 weeks following discontinuation of the treatment. The cause of death was thought to be respiratory insufficiency due to progressive disease.

Pharmacokinetics. The pharmacokinetic parameters are summarized in Table 4. The apparent $t_{1/2}$ could be estimated only in very few subjects. This is due to the fact that at the lower dose levels plasma concentrations fell below the limit of quantification before the terminal phase was reached and also due to the fact that in some individuals at the higher dose levels C_{max} was achieved late (4–8 h), and the terminal phase was therefore not reached until 12 h postdose when the last sample was taken.

Table 2 Graded overall toxicity

	Dose level (mg/m²/day)																				
	20 (n = 3)			30 (n = 3)			45 (n = 3)			70 (n = 4)			100 (n = 6)			150 (n = 3)			All cases (n = 22)		
Toxicity	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Cheilitis	2	0	0	2	0	0	2	0	0	3	0	0	5	0	0	2	0	0	16	0	0
Skin reaction	1	0	0	2	0	0	2	0	0	3	0	0	5	0	0	3	0	0	16	0	0
Headache	2	0	0	0	0	0	2	0	0	2	1	0	3	1	1	0	2	1	9	4	2
Fatigue	2	0	0	1	0	0	1	0	0	3	0	0	2	0	0	2	0	0	11	0	0
Arthralgia	1	0	0	2	0	0	0	0	0	0	0	0	4	0	0	1	0	0	8	0	0
Conjunctivitis	1	0	0	0	0	0	1	0	0	2	0	0	3	0	0	0	0	0	7	0	0
Nausea/vomiting	1	0	0	1	0	0	0	0	0	2	0	0	1	0	0	0	0	0	5	0	0
Anorexia	1	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0
Insomnia	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	4	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	1	0	2
Hypercalcemia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	2	1	0
Mucositis	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Blurry vision	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0

Table 3 Graded hepatic toxicity

	Dose level (mg/m²)																					
	20 (n = 3)			30 (n = 3)				45 (n = 4)			70 (n = 4)			100 (n = 6)			150 (n = 3)			All cases (n = 22)			
Toxicity	1	2	3	1	2	3	4	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	4
Triglyceride	1	0	0	0	0	1[a]	0	0	0	0	0	2	0	2	2	0	2	0	0	5	4	1	0
Cholesterol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Alkaline phosphatase	0	0	0	0	0	0	1[a]	1	0	0	0	0	0	4	0	0	2	0	0	7	0	0	1
AST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0

[a] These patients had abnormal levels at registration.

Table 4 Pharmacokinetic parameters

		Day 1			Day 22				
Dose (mg/m²/day)		t_{max}[a] (h)	C_{max} (ng/ml)	AUC_{O-T} (ng*h/ml)	t_{max}[a] (h)	C_{max} (ng/mL)	% of Day 1[b]	AUC_{O-T} (ng*h/ml)	% of Day 1[b]
20[c]	Mean[a]	6.1	60.4	165.1	3.6	28.5	92.9	83.5	48.9
	SD		56.5	55.7		32.4	98.7	102.8	54.4
	n	3	3	3	2	2	2	2	2
30	Mean	1.1	59.5	128.4	3.1	54.8	170.0	133.2	254.1
	SD		49.7	135.5		15.8	161.3	61.8	271.9
	n	3	3	3	3	3	3	3	3
45	Mean	2.1	161.1	415.5	1.1	33.4	27.0	81.9	25.2
	SD		108.4	200.0		23.8	23.6	53.0	23.6
	n	3	3	3	3	3	3	3	3
67.5	Mean	2.7	247.6	733.7	4.0	60.7	46.8	207.5	50.9
	SD		195.3	476.9		32.5	41.5	24.2	44.1
	n	4	4	4	3	3	3	3	3
101[d]	Mean	2.0	283.0	882.6					
	SD		245.6	671.5					
	n	6	6	6					
151.88[d]	Mean	4.0	409.4	1483.8					
	SD		298.9	888.9					
	n	3	3	3					

[a] For t_{max} the median is reported.
[b] Calculated as (C_{max} day 22/C_{max} day 1) × 100 and (AUC day 22/AUC day 1) × 100, respectively.
[c] For n = 1 of 3 patients all plasma levels on day 22 were below the limit of quantification.
[d] No samples for day 22, except for one patient at the 101-mg/m²/day dose level were collected.

In the six patients where $t_{1/2}$ could be estimated, it ranged between 2.3 and 3.9 h with a mean (SD) of 2.9 (0.6) h. Overall, the plasma concentration-time data showed significant intersubject variability in terms of t_{max} and C_{max} (Fig. 1). C_{max} and AUC_{O-T} increased linearly with dose on day 1 ($P < 0.0005$ for AUC_{O-T} and $P < 0.01$ for C_{max}). This increase was proportional to the increase in dose (Figs. 2 and 3). On day 22 only AUC increased linearly with dose but much less than dose propor-

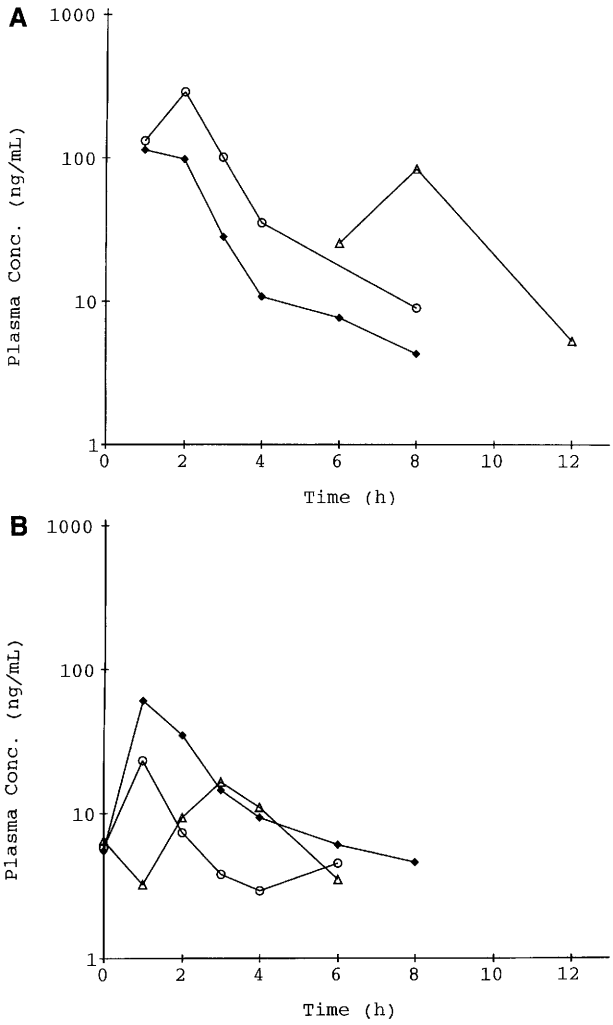

Fig. 1 Plasma 9cRA concentration-time profiles for individuals treated on days 1 (*A*) and 22 (*B*) at the 45-mg/m^2/day dose level. ○, patient 7; △, patient 8; ◆, patient 9.

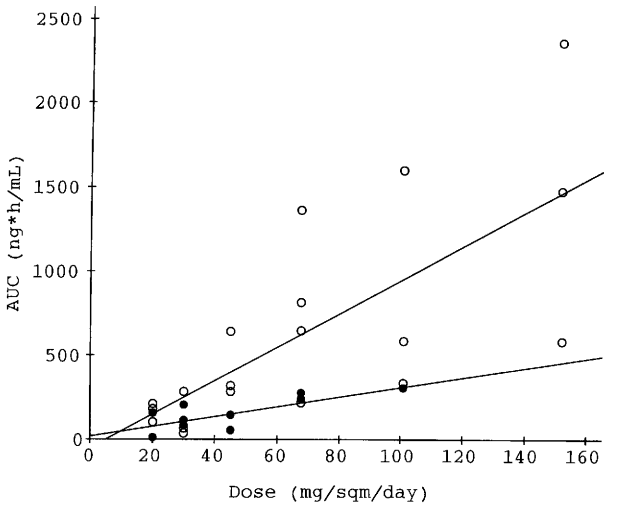

Fig. 2 Correlation of 9cRA dose with AUC at days 1 and 22 of treatment. For day 1 (○), the equation of the regression line is: AUC = 10.07 × dose − 56.35, r^2 = 0.522, $P < 0.0005$. For day 22 (●), the equation of the regression line is: AUC = 2.92 × dose + 16.70, r^2 = 0.544, $P < 0.01$.

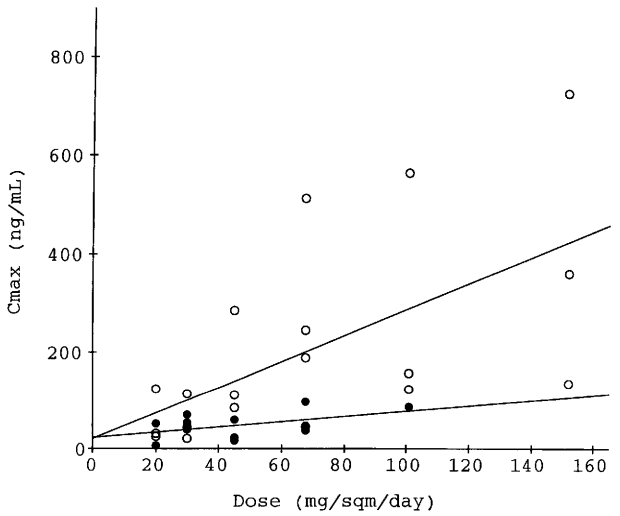

Fig. 3 Correlation of 9cRA dose with C_{max} at days 1 and 22 of treatment. For day 1 (○), the equation of the regression line is: C_{max} = 2.68 × dose + 21.17, r^2 = 0.364, $P < 0.01$. For day 22 (●), the equation of the regression line is: C_{max} = 0.56 × dose + 22.62, r^2 = 0.247, $P = 0.1$.

tional ($P < 0.01$), and C_{max} did not change ($P = 0.1$). This is due to the fact that plasma levels decreased from days 1 to 22. On average the reduction was 23% for C_{max} ($P < 0.03$) and 8% for $AUC_{0\text{-}T}$ ($P < 0.03$) but was more pronounced in the higher dose groups. At dose levels ≥45 mg/m^2/day, the average reductions were 68% for C_{max} and 75% for AUC. Unfortunately, no samples for day 22 were available in five of six patients treated with the two highest dose levels (101 and 151.88 mg/m^2/day).

DISCUSSION

This Phase I trial examined the effects of 9cRA, a naturally occurring retinoid which is unique in its ability to bind both RARs and RXRs (12, 13). This trial complements another ongoing Phase I trial of 9cRA in adult cancer patients (25). 9cRA and t-RA share the ability to bind RARs and differ in that only 9cRA can bind RXRs. Evidence suggests that retinoids mediate their effects through the transcriptional properties of retinoid receptors. For example, NT2/D1 teratocarcinoma cells with defective RARs are refractory to the cytodifferentiating effects of t-RA and reconstitution of the receptors through stable transfection restores retinoid responsiveness (26). Based on the hypothesis that receptors mediate retinoid actions, one might expect 9cRA and t-RA to exhibit effects that are both similar and different.

Findings in this trial support that hypothesis. 9cRA caused toxicity similar to t-RA in a Phase I trial of t-RA in adult patients with solid tumors (27). In that trial, cheilitis, skin

reaction, headache, and hypertriglyceridemia were common, similar to our observations with 9cRA. However, cheilitis was more severe with t-RA, and t-RA-associated toxic effects such as skin desquamation and paronychia were not observed with 9cRA. Although the lips were the major site of mucosal toxicity associated with t-RA, our study suggests that intestinal mucosa may be targeted by 9cRA. Diarrhea was an unexpected finding, and future 9cRA trials will further examine the incidence of diarrhea associated with this drug. In addition, it would be interesting to examine *RXR* gene expression in both tissues involved and not involved with 9cRA-associated toxic effects to further test the hypothesis that receptor activation mediates 9cRA toxicity.

Study data revealed a minor response in one breast cancer patient. Of the four other breast cancer patients entered on this trial, two were not evaluable for response because of discontinuation of treatment prior to completion of the first month, and the other two patients had stable disease after 5 and 6 weeks of treatment, respectively. It is not clear whether this represents the natural history of their disease or an effect of 9cRA. Phase II trials should be considered to test the efficacy of 9cRA in breast cancer patients. *In vitro* data revealed that 9cRA has growth inhibitory effects on breast cancer cell lines, and this effect correlated with estrogen receptor expression (28). One possible mechanism by which retinoids might alter the growth of breast cancer cells is through interactions between estrogen and retinoid receptors. As shown previously, interactions between different members of the steroid receptor superfamily can occur (7), and retinoid receptors can interact with other transcription factors such as AP-1 (29). Data presented here indicate that 9cRA may have activity in estrogen receptor-negative breast cancer patients, suggesting that mechanisms other than steroid receptor interactions play a role in antitumor effects of retinoids on breast cancer cells.

The pharmacokinetics of 9cRA were highly variable between patients and the pharmacokinetic parameters overlapped widely between different dosages. This might be the consequence of differences between patients in absorption and elimination. Similar variability has been observed for t-RA (30). 9cRA plasma levels decreased significantly over time. The degree of decrease varied greatly between patients. Because of the relatively small number of patients in this trial, it was not possible to evaluate whether this reduction was dose dependent. The magnitude of the reduction and its intersubject variability in this trial was similar to that observed in patients treated with t-RA (30, 31). Autoinduction of the metabolic enzyme system contributes to the elimination of t-RA (32–37). Such autoinduction would also be a likely explanation for the phenomenon observed with 9cRA. Studies with cytochrome P450 inhibitors such as ketoconazole and liarazole have shown that t-RA metabolism can be inhibited *in vitro* and *in vivo* (37–40), but these agents have not yet been shown useful in the chronic administration of t-RA (41). It is not yet clear what could be the impact of reduced retinoid plasma levels after long-term administration. As demonstrated for t-RA (42), different schedules might induce a more constant pharmacokinetic profile after repeated dosing, minimizing the AUC reduction after repeated administration.

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