

Pharmacokinetics of 13-*cis*-Retinoic Acid in Patients with Advanced Cancer¹

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ABSTRACT

13-*cis*-Retinoic acid (13-CRA) is a synthetic analog of vitamin A effective in reversing preneoplastic lesions in both humans and animals. To study its physicochemical properties and disposition kinetics, we developed a rapid, sensitive, and precise high-performance liquid chromatography assay for 13-CRA in biological samples. This assay system resulted in a clear separation of 13-CRA from all-*trans*-retinoic acid and retinol and had a detection limit of 20 ng/ml plasma. Recovery was $89 \pm 6\%$ (S.D.) at equivalent physiological concentrations with a precision of 8%. To study the disposition kinetics in humans, 13 patients received a p.o. bolus of 13-CRA and had blood samples collected at timed intervals. For the 10 patients studied on the first day of 13-CRA administration, the mean time to peak plasma concentration was 222 ± 102 min. Interpatient peak 13-CRA plasma concentrations were found to be variable, suggesting irregular gastrointestinal absorption. β -Phase $t_{1/2}$ was approximately 25 hr. The prolonged terminal-phase plasma half-life may represent biliary excretion and enterohepatic circulation.

INTRODUCTION

Vitamin A and its synthetic analogs have attracted interest as potential antineoplastic agents. These compounds prevent development of preneoplastic lesions (6, 13, 14) and may inhibit the growth of established cancers (5, 6, 15). Meyskens and Salmon (8) have also shown that several retinoids can inhibit the growth of human melanoma stem cells in soft agar.

13-CRA,⁵ the geometric isomer of the naturally occurring TRA, has been shown *in vitro* and *in vivo* activity in several animal tumor models (6, 14). Because of the relative lack of hepatic toxicity and its significant chemopreventive and anti-tumor activity in laboratory animals, this retinoid has recently been entered into clinical trials. In designing these trials, a basic understanding of the pharmacokinetics of 13-CRA is of importance. However, limited information has been published on the pharmacokinetics and metabolism of 13-CRA in humans. Frolik *et al.* (4) have studied 5 patients after p.o. admin-

istration of 13-CRA, while Puglisi and DeSilva (9) described a single patient receiving chronic p.o. treatment. Neither group published pharmacokinetic parameters. At the University of Arizona Cancer Center, we have completed a broad phase I and II trial of p.o. 13-CRA in patients with advanced cancer.⁶ In parallel with these clinical trials, we have studied the pharmacokinetics of 13-CRA in 13 patients.

MATERIALS AND METHODS

Between October 1979 and April 1981, 105 patients were entered into a Phase I and II trial of 13-CRA.⁶ All patients had advanced cancer and had failed standard therapies. 13-CRA pharmacokinetics was studied in the 13 patients whose clinical characteristics are outlined in Table 1. Informed consent was obtained from all patients prior to drug treatment and pharmacokinetic evaluation.

Drug Administration and Sample Collection. 13-CRA was administered to patients p.o. once daily at a dosage level of 3, 4, or 5 mg/kg. Thirteen of 15 pharmacokinetic studies were performed on the first day of drug administration. Six patients were studied after a dose of 3 mg/kg, 2 patients were studied at 4 mg/kg, and 5 were studied at 5 mg/kg. Two patients were also studied after 12 and 88 days of daily drug administration. Capsules containing 10 and 40 mg of 13-CRA (Hoffmann-La Roche, Inc., Nutley, N. J.) were administered at 8 a.m. as p.o. bolus after an overnight fast. At 30- to 60-min intervals, 7 to 10 ml of blood were collected in foil-wrapped Vacutainers containing 200 units of sodium heparin. Samples were stored on ice in the dark prior to centrifugation at $2000 \times g$ for 10 min at 4°. The plasma layer was then removed, placed in polypropylene freezer tubes (Vanguard International, Inc., Neptune, N. J.), and stored in the dark at -25° until analysis. Fractional urine samples were collected for the first 8 hr and at longer intervals for up to 24 hr. Urine was stored in the dark in polypropylene freezer tubes at -25°. Patients were allowed to resume normal p.o. intake 3 hr after drug administration. One patient with leptomeningeal melanoma had cerebrospinal fluid collected before drug administration and at 6, 24, and 72 hr and 1, 2, and 3 weeks after daily p.o. drug administration (5 mg/kg).

13-CRA Assay Procedure. At the time of analysis, the plasma samples were allowed to thaw in the dark at room temperature (23-26°). One hundred μ l of 5% HClO₄ were added to a 0.5-ml aliquot of plasma in a microcentrifuge tube and rapidly mixed. A 0.5-ml aliquot of ethyl acetate (glass distilled; Burdick & Jackson Laboratories, Inc., Muskegon, Mich.) was added, and the samples were again mixed for 60 to 90 sec and centrifuged at $13,000 \times g$ for 5 min. Fifty μ l of the resulting organic layer were analyzed by HPLC. Urine samples were extracted by adding 0.2 ml of 5% HClO₄ and 0.5 ml of ethyl acetate to 1.0 ml of urine, followed by rapid mixing and centrifugation. After centrifugation, 100 μ l of the organic layer were analyzed by HPLC. Cerebrospinal fluid samples were analyzed directly without extraction.

⁶ F. L. Meyskens, E. Gilmartin, D. S. Alberts, N. Levine, R. Brooks, and E. Surwit. Activity of 13-*cis*-retinoic acid against squamous epithelial premalignancies and malignancies, submitted for publication.

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⁵ The abbreviations used are: 13-CRA, 13-*cis*-retinoic acid; TRA, all-*trans*-retinoic acid; HPLC, high-performance liquid chromatography; C \times t, concentration \times time; 4-*oxo*-CRA, 4-*oxo-cis*-retinoic acid.

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Table 1
Clinical characteristics of 13 patients receiving P.O. 13-CRA

Patient	Tumor type	Sex	Age (yr)	Wt (kg)	Dose (mg/kg)	Serum creatinine (mg/dl)	Billirubin (mg/dl)
1	Squamous cell carcinoma of skin	M	54	71.0	3	1.1	0.3
2	Squamous cell carcinoma of tonsillar pillar	M	54	56.4	3	0.8	0.3
3	Squamous cell carcinoma of cervix	F	52	91.0	3	0.9	1.1
4	Chondrosarcoma	M	53	72.2	3	0.9	0.7
5	Choriocarcinoma	F	36	52.1	3	0.7	0.7
6	Cystadenocarcinoma of ovary	F	53	45.0	3	0.8	0.6
7	Adenocarcinoma of colon	M	75	69.8	4	1.0	0.2
8	Rhabdomyosarcoma	M	65	68.5	4	0.8	0.8
9	Adenocarcinoma of pancreas	F	50	56.0	5	1.2	0.9
10	Adenocarcinoma of colon ^a	M	67	60.3	5	1.3	0.9
11	Chronic myelogenous leukemia blast crisis	M	70	53.0	5	1.5	1.1
12	Chronic erythemic myelosis	F	28	66.2	5	0.7	0.8
13	Leptomeningeal melanoma	M	34	65.0	5	1.0	0.7

^a Patient had liver metastasis and massive ascites.

HPLC analysis was performed using 2 Waters Associates, Inc. (Milford, Mass.) Series M 45 solvent delivery systems, a Model U6K injector, and a Model 440 UV detector. The mobile phase consisted of filtered, degassed, and premixed 75% acetonitrile (Burdick & Jackson; glass distilled) and 25% ammonium acetate (1%) (Fisher Scientific Co., Fair Lawn, N. J.). Flow rate was 2.5 ml/min. Because of the short retention time with the solvent system and flow rate utilized, adequate separation of the *cis* and *trans* isomers was not obtained with a single C₁₈- μ Bondapak column (Waters Associates). Two Bio-Sil ODS-10 columns (150 x 4 mm; Bio-Rad Laboratories, Richmond, Calif.) connected in series provided adequate separation and were used for all analyses. Retinoids were detected at 340 nm. Quantitation of 13-CRA and TRA was done by an external standard method.

Recovery and Precision. 13-CRA or TRA was added to 0.5 ml of pooled human plasma yielding final retinoid concentrations of 50 to 900 mg/ml. Extraction recovery was calculated by comparing the peak height of added retinoid to that of retinoid standards injected directly on the column. All experiments were carried out in triplicate. Precision was determined by assay of patients' samples on 4 separate days in triplicate.

13-CRA Capsule Content. To quantitatively and qualitatively determine the content of the 13-CRA capsules, three 10-mg capsules, and three 40-mg capsules were split, and each was dissolved in 100 ml ethyl acetate. Ten- μ l samples were analyzed by direct HPLC injection and comparison of peak heights to a standard curve.

Stability Studies. To determine the photostability of 13-CRA, 2.5 μ g/ml in either 100% ethyl acetate or pooled human plasma were allowed to stand in glass tubes at room temperature (23–26°) under ambient laboratory fluorescent lighting for a 6-hr period. Retinoid content was determined by HPLC at frequent time intervals by direct injection of the ethyl acetate samples and extraction of the plasma samples.

Data Analysis. Plasma concentration of 13-CRA versus time data was obtained from each patient and fitted to a multiexponential equation using a nonlinear regression computer program (7). Preliminary parameter estimates were obtained with the aid of a previously published computer method (11). Equations used in analysis are identical to those previously published (1).

RESULTS

Assay System. Our HPLC system resulted in a clear separation of 13-CRA, TRA, and retinol with an analysis time of 6 min/sample. Chart 1 shows the extraction of added 13-CRA from human plasma (note endogenous retinol), added TRA extracted from human plasma, an extract of patient plasma

before 13-CRA administration, and an extract of patient plasma 90 min after the p.o. administration of 5 mg of 13-CRA per kg. The recovery of 13-CRA from plasma was linear from 50 to 900 ng/ml and averaged 89.3% \pm 5.9% (S.D.) (Table 2). The assay had a detection limit of 20 ng/ml, determined on the basis of a signal equal to twice the noise level. The precision of the assay for 13-CRA was 8.0%. The recovery of TRA from plasma was also linear from 50 to 900 ng/ml; recovery averaged 94.3 \pm 3.6%, with a detection limit of 20 ng/ml. The precision of the assay for TRA was 6.6%. The collected HPLC peaks corresponding to 13-CRA and retinol were analyzed by gas chromatography-mass spectrometry. The peaks exhibited major mass ions identical to those of 13-CRA and retinol standards.

13-CRA Capsule Contents. Both the 10- and 40-mg capsules were >99.5% 13-CRA, and only trace amounts of TRA were detectable. Retinoid content was 9.77 \pm 0.32 mg for the 10-mg capsules and 38.28 \pm 0.1 mg for the 40-mg capsules.

Stability. 13-CRA was unstable in both ethyl acetate and plasma at 23–26° under standard fluorescent lighting. There was significant isomerization to TRA within 5 min in both media. Equilibrium was reached in 1 to 2 hr with a final CRA:TRA ratio of 75:20.

13-CRA Pharmacokinetics. The pharmacokinetic parameters of p.o. 13-CRA obtained from nonlinear regression fitting of plasma concentration-versus-time data are summarized in Table 3. The mean time to peak plasma concentration of 13-CRA of 5 patients receiving 3 mg/kg was 192.0 \pm 65.7 min. The peak plasma concentration of 13-CRA in these patients varied from 0.20 to 0.86 μ g/ml, with a mean of 0.50 μ g/ml. The mean plasma $t_{1/2}$ β for 13-CRA was 120.9 \pm 43.7 min. Because of an apparent prolonged $t_{1/2}$ γ , the terminal phase half-life could not be determined accurately. Patients studied at 5 mg/kg had mean time to peak plasma concentration of 270 \pm 142 min and a mean peak 13-CRA plasma concentration of 0.74 μ g/ml; however, the peak plasma concentration showed marked variability with a range of 0.19 to 1.5 μ g/ml. The $t_{1/2}$ β of 144.6 \pm 97.3 min was not significantly different from that observed at 3 mg/kg; $t_{1/2}$ γ was again prolonged and could not be determined accurately.

Chart 2 shows the computer-plotted plasma concentration curve of 13-CRA from 2 patients receiving 3 mg/kg. Both

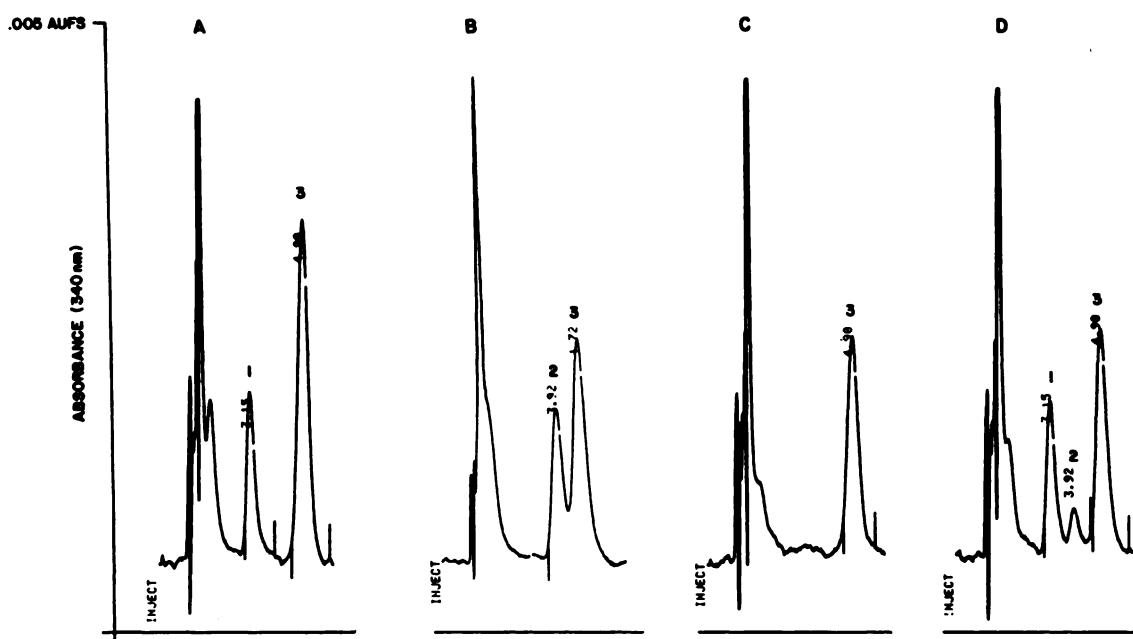


Chart 1. Chromatograms of standard retinoid in plasma and patient samples. A, 300 ng of 13-CRA per ml added to pooled plasma. Peak 1, 13-CRA; Peak 3, endogenous retinol. B, 300 ng of TRA per ml added to pooled plasma. Peak 2, TRA; Peak 3, endogenous retinol. C, extracted plasma of Patient 11 prior to 13-CRA administration; Peak 3, endogenous retinol. D, extracted plasma of Patient 11 90 min after a p.o. dose of 5 mg 13-CRA per kg. Peak 1, 13-CRA; Peak 2, TRA; Peak 3, retinol.

Table 2

Recovery of 13-CRA from plasma

Increasing concentrations of 13-CRA were added to pooled plasma. Samples were extracted as outlined; peak heights obtained were expressed as a percentage of peak heights of equivalent standard 13-CRA. All experiments were run in triplicate.

13-CRA added (ng/ml plasma)	Peak ht	Recovery (%)
50	1.0 ± 0.15 ^a	94.7 ± 3.65
100	2.0 ± 0.10	83.1 ± 4.53
300	5.4 ± 0.25	86.8 ± 3.55
900	15.4 ± 0.25	91.3 ± 3.21
Average		89.3 ± 5.98

^a Mean ± S.D.

patients showed rapid absorption with peak levels reached at 2 to 3 hr. 13-CRA was detected in the plasma of both patients at 24 hr. Although receiving the same dose based on body weight, there was a difference in the $C \times t$ between patients. Chart 3 shows the 13-CRA plasma concentration curves in a patient who was studied on the first and 12th days of daily drug administration (3 mg/kg). Both peak levels and $C \times t$ were increased after chronic p.o. dosing.

Plasma retinol concentrations were followed in all 13 patients. There were no changes in the plasma retinol concentrations during the study period. TRA was detected in the plasma of most patients after the p.o. administration of 13-CRA. Plasma

Table 3

Pharmacokinetic parameters of P.O. 13-CRA (3 mg/kg)

Patient	Dose (mg/kg)	Day of study	Time to peak plasma conc. (min)	Peak plasma concentration (μg/ml)	$t_{1/2} \beta$ (min)	$C \times t$ (μg × min/ml)	$t_{1/2} \gamma$ (min)
1	3	1	180	0.20	114.6	75.3	Indeterminate ^a
2	3	1	300	0.86	180.7	380.5	1225
3 ^b	3	1	180	0.30	169.9	161.0	Indeterminate ^a
	3	12	100	1.10	97.8	503.0	1605
4	3	1	120	0.48	69.3	230.9	716
5	3	88	120	1.15	43.7	409.5	779
6	3	1	180	0.65	90.2	250.9	2094
Mean ^c	3		192.0 ± 65 ^d	0.50 ± 0.26	120.9 ± 43.7	221.5 ± 113.5	
7	4	1	180	0.15	82.5	20.0	Indeterminate ^a
8 ^e	4	1	720	1.06		711.0	
9	5	1	180	0.38	106.8	140.0	4089
10	5	1	360	0.19	815.7	174.8	Indeterminate ^a
11	5	1	240	0.52	96.5	301.7	1889
12	5	1	480	1.15	290.1	760.8	Indeterminate ^a
13	5	1	180	0.93	86.0	271.1	Indeterminate ^a
Mean ^f	5		270.0 ± 142.0	0.74 ± 0.35	144.6 ± 97.3	368.0 ± 271	

^a Half-life appeared very prolonged and could not be accurately determined.

^b Patient 3 was studied on Day 1 and Day 12 of daily drug administration.

^c Mean values are obtained from the 5 patients who were studied on Day 1 only.

^d Mean ± S.D.

^e Patient 8 had 2 peaks in plasma disappearance curve at 1 and 12 hr. Pharmacokinetic parameter could not be calculated.

^f Excludes Patient 10 because of low plasma levels and prolonged $t_{1/2}$. Patient had liver metastasis and ascites.

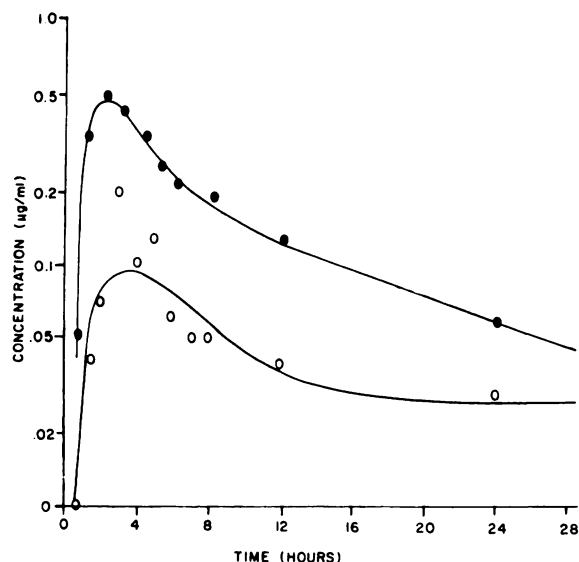


Chart 2. Computer-plotted plasma disappearance curve for 2 patients receiving 3 mg 13-CRA per kg p.o. at Time 0. O, Patient 1; ●, Patient 4.

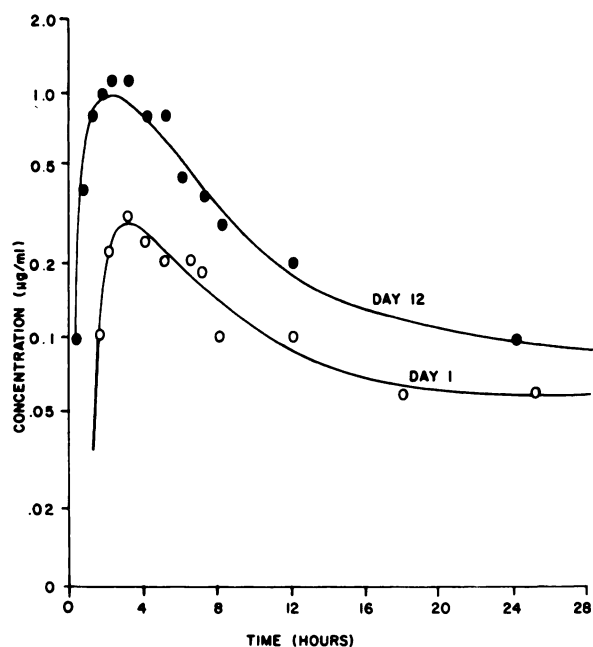


Chart 3. Computer-plotted plasma disappearance curve for 13-CRA after p.o. dose of 3 mg/kg. Patient 3 studied on Day 1 and Day 12 of drug administration.

concentrations of TRA varied from patient to patient and ranged from 0 to 30% of the plasma concentrations of 13-CRA.

Pooled 24-hr urine samples on all 13 patients were assayed for retinoid content. 13-CRA, TRA, and retinol were not detected in any of the collected uring samples. In one patient with leptomeningeal melanoma, 13-CRA was not detected in cerebrospinal fluid samples after up to 3 weeks of daily 13-CRA administration.

DISCUSSION

We have developed a rapid, sensitive, and precise HPLC for the simultaneous determination of 13-CRA, TRA, and retinol in

biological fluids and have shown that this assay can be used to study the *in vitro* stability and the human pharmacokinetics of these retinoids in patients with advanced cancer. This system allowed accurate determination of all 3 retinoids with an analysis time of 6 min, significantly faster than previously published methods (4, 9).

In studying the stability of 13-CRA, we have shown that this compound is very unstable when exposed to fluorescent light. Detectable *in vitro* isomerization occurred within 5 min in both organic and biological fluids (plasma). Equilibrium in plasma occurred within 1 to 2 hr with a resulting 13-CRA:TRA ratio of 75:20. Protection from laboratory fluorescent lighting prevented isomerization for the 6 hr period tested. Our studies also show that *in vivo* isomerization can occur. The majority of patients had TRA levels that were 10 to 20% of the plasma 13-CRA levels. Two patients had no detectable plasma TRA, while one patient's plasma had approximately 30% TRA. Since 2 patients had no detectable TRA in the plasma, it is unlikely that isomerization during handling and storage accounted for the presence of TRA. The factors responsible for the extent of *in vivo* isomerization are unknown but may relate to skin pigmentation, lighting conditions, and body surface area.

When administered as a p.o. capsule, we found the interpatient systemic availability of 13-CRA to be extremely variable. In both dosage groups (3 and 5 mg/kg), plasma $C \times t$ and peak plasma concentration showed a 6-fold range. These results are in contrast to those of Colburn *et al.*,⁷ who observed small interpatient variations in peak plasma concentrations and $C \times t$ values when low p.o. doses were given to normal subjects or to patients who did not have cancer. Because of the relative lipophilic nature of this compound, a functionally normal gastrointestinal tract is probably required for complete absorption. Since patients with advanced cancer frequently have some degree of malabsorption (2, 12), this may have contributed to the variability in peak plasma concentrations and $C \times t$ values observed in our patients.

Biliary secretion and enterohepatic circulation may have also contributed to the observed variation in plasma concentrations. Other investigators have shown that biliary drainage with a T-tube can recover approximately 70% of a p.o. administered dose of ¹⁴C-labeled 13-CRA.⁷ One of our patients (Patient 8) was found to have a 13-CRA plasma concentration at 1 hr of 0.39 µg/ml, followed by decreasing concentrations to a low of 0.08 µg/ml at 7 hr. The 12-hr sample, collected 2 hr after the dinner meal, had a 13-CRA concentration of 1.06 µg/ml. Lipid-induced emptying of the gallbladder and release of 13-CRA may account for this secondary peak. Thus, interpatient variation in 13-CRA biliary secretion may also be responsible for variability in peak plasma concentrations.

The $t_{1/2 \beta}$ for 10 patients studied on the first day of drug administrations (excluding Patient 10, who had low peak levels and greatly prolonged half-lives, and Patient 8, who had 2 peaks in her plasma disappearance curve) was 126.6 ± 66.2 min. The $t_{1/2 \gamma}$, which averaged about 25 hr, could not be precisely determined because all patients received their next dose of retinoid within 24 hr of the start of the pharmacokinetic

⁷ W. Colburn. Pharmacokinetics of 13-*cis*-retinoic acid (Workshop on Oral Retinoids in Dermatology, Iowa City, May 27-29, 1981), submitted for publication.

study. This long terminal-phase plasma half-life may reflect biliary excretion and enterohepatic circulation. Both patients studied after prolonged p.o. administration of 13-CRA (Patient 3, Day 12, and Patient 5, Day 88) showed increased peak plasma concentration and $C \times t$ values, suggesting that 13-CRA progressively accumulates on daily dosing schedules. Because of the prolonged plasma $t_{1/2}$, it may not be necessary to administer 13-CRA on a daily schedule.

Although animal studies have shown that 13-CRA penetrates into brain tissue (16), we found no evidence of 13-CRA in the cerebrospinal fluid in one patient with leptomeningeal melanoma, even after 3 weeks of drug administration. However, since 13-CRA is lipophilic and poorly water soluble, it may concentrate in brain tissue without appearing in the cerebrospinal fluid.

Other than TRA, we did not detect any metabolites of 13-CRA in patients' plasma and were unable to identify any retinoids in the urine. Although our HPLC assay provided a clear separation of 13-CRA, TRA, and retinol, this method will not detect more polar metabolites. Oxidation of 13-CRA to 4-oxo-CRA has recently been described as an important pathway (3, 10).⁷ With the solvent system utilized, 4-oxo-CRA will be excluded in the void volume and will not be separated from our plasma front. Alteration of the mobile phase will be necessary to adequately evaluate the more polar metabolites including 4-oxo-CRA.

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