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13-*CIS*-RETINOIC ACID: PHARMACOLOGY, TOXICOLOGY, AND CLINICAL APPLICATIONS FOR THE PREVENTION AND TREATMENT OF HUMAN CANCER

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I. INTRODUCTION

Vitamin A (retinol, R) is a nutritional principle which is necessary for normal growth, vision, reproduction, and epithelial cell differentiation. Deficiency in vitamin A leads to dysplasia of many tissues; supplementation with vitamin A or its precursor (β-carotene) rapidly reverses these lesions. Early attempts to use vitamin A to mature epithelial tissues led to hepatotoxicity, secondary to accumulation of this compound in the liver. A considerable amount of effort has been devoted to developing vitamin A derivatives (retinoids) with an improved therapeutic/toxicity ratio.[1,2] Early on 13-*cis*-retinoic acid (13cRA), the synthetic isomer of vitamin A acid (β-trans retinoic acid, tRA), appeared quite promising as this derivative was active in in vitro models and did not concentrate in the liver. Subsequent studies of this retinoid in patients with severe cystic and conglobate acne substantiated that the acute side effects of the compound were mild.[3]

Over a decade ago tRA was applied locally to squamous cell carcinomas of the skin and activity was seen.[4] In the past 10 years the appreciable activity of retinoids as antiproliferative and anticarcinogenic agents has become defined in the laboratory[5,6] and enthusiasm for use of these agents as an anticancer modality has increased.[7] The pharmacology, toxicology, and clinical applications of 13cRA will be reviewed, particularly as related to its present and future role in the prevention and treatment of human neoplastic diseases.

II. PHARMACOLOGY

To design clinical trials to examine the efficacy of 13cRA, an understanding of the

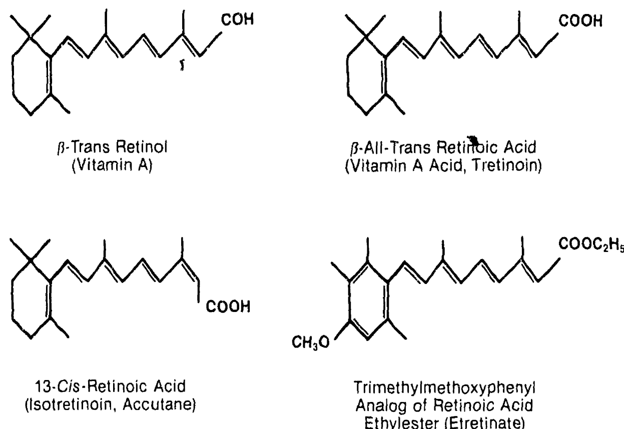

FIGURE 1. Chemical structure of retinoids in clinical use in oncology.

basic physiochemical and pharmacological properties of this retinoid is necessary. This section will summarize our current understanding of the human pharmacology and physiochemistry of this important synthetic analog of vitamin A.

13cRA [3,7-dimethyl-9-2(2,6,6-trimethyl-1-cyclohexene-1-yl)-2-cis-4,6,8-nonatetraenoic acid] is a synthetic member of the family of vitamin A compounds collectively referred to as the retinoids.[7] 13cRA is the stereoisomer of the naturally occurring vitamin A acid. The structures of retinol (R) (the parent retinoid), 13cRA (Isotretinoin, Accutane), tRA (Tretinoin), and an ethyl ester derivative of an aromatic derivative (RO-10-9359, Etretinate, aRA) are illustrated in Figure 1. The common structure of these compounds includes a cyclic end group (the trimethylcyclohexenyl ring) or its aromatic derivative; a dimethyl-substituted, all trans tetraene chain; and a polar terminal group.

A. Analytic Methods

The introduction of high-performance liquid chromatography (HPLC) has greatly improved our ability to analyze and study the in vivo and in vitro biotransformations of the retinoids. These methods allow the separation and quantitation of both the synthetic and naturally occurring retinoids, their polar and nonpolar metabolites, various stereoisomers, and the retinyl esters. We will describe one HPLC method we have used in studying the plasma kinetics of 13cRA.[8] This method has the advantage that it is rapid and can detect 13cRA, tRA, and R in a single plasma sample.

1. Instrumentation

All analyses were performed using two Waters Associates: (Milford, Mass.), series M-45 solvent delivery system, a model UK-6 injector, and a model 440 UV detector. Retinoids were detected at 340 nm. Two Biosil®, ODS-10, 150 × 4 mm columns (Bio-rad Laboratories, Richmond, Calif.) connected in series were used for all analyses. The mobile phase was a mixture of filtered degassed acetonitrile (75%) (Burdick & Jackson,

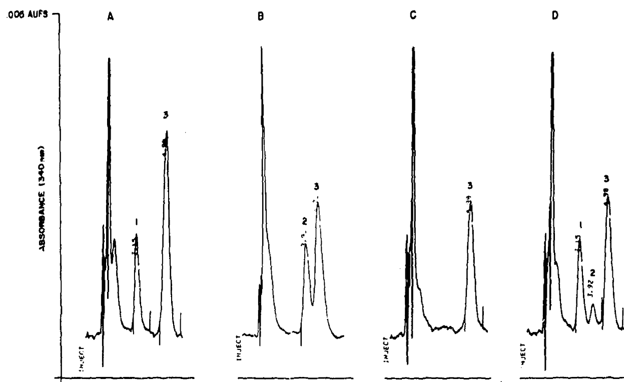

FIGURE 2. Chromatograms of standard retinoid in plasma and patient samples. (A) 300 ng/mℓ 13cRA added to pooled plasma. Peak 1, 13cRA; peak 3, endogenous retinol. (B) 300 ng/mℓ tRA added to pooled plasma. Peak 2, tRA; peak 3, endogenous retinol. (C) Extracted plasma of patient prior to 13cRA administration; peak 3, endogenous retinol. (D) Extracted plasma of patient 90 min after a p.o. dose of 5 mg/kg 13cRA. Peak 1, 13cRA; peak 2, tRA; peak 3, retinol. (From Goodman, G. E., Einspahr, J. R., Alberts, D. S., Davis, T. P., Leigh, S. A., Chen, H. S. G., and Meyskens, F. L., *Cancer Res.*, 42, 2087, 1982. With permission.)

glass distilled) and aqueous ammonium acetate (1.0%); 25% (Fisher Chemical Co., Fairlawn, N.J.,) The flow rate was 2.5 mℓ/min.

2. Extraction Procedure

At the time of analysis, plasma samples were allowed to thaw in the dark at room temperature (23 to 26°C). Added to a 0.5-mℓ aliquot of plasma in a microcentrifuge tube and rapidly mixed in a vortex mixer for 5 to 10 sec was 100 mℓ of 5% $HClO_4$. A 0.5-mℓ aliquot of ethyl acetate (glass distilled, Burdick & Jackson, Muskegon, Mich.) was added, the samples mixed for 60 to 90 sec, and then centrifuged at 13,000 × g for 5 min. Fifty microliters of the resulting organic layer was analyzed by HPLC.

3. Recovery, Precision, and Verification

Quantitation of 13cRA, tRA, and retinol was done by an external standard method. 13cRA or tRA was added to 0.5 mℓ of pooled human plasma yielding a final retinoid concentration of between 50 to 900 ng/mℓ. Recovery was calculated by comparing the peak height of added retinoid to that of retinoids standards. All experiments were carried out in triplicate. Precision was determined by triplicate assay of three different patient samples.

This HPLC system results in a clear separation of 13cRA, tRA, and R. Figure 2 shows chromatograms of an extraction of either exogenous 13cRA or tRA added to pooled human plasma. Also shown is patient plasma prior to and 90 min after the oral administration of 13cRA (5 mg/kg).

The recovery of 13cRA from plasma was linear from 50 to 900 ng/mℓ. Recovery averaged 89.3 ± 5.9%. The assay had a detection limit of 20 ng/mℓ determined on the basis of a signal equal to twice the baseline level. Precision was determined over a wide range of plasma concentrations in patient samples and was 8.0%.

To confirm our HPLC procedure, gas chromatography/mass spectrophotometry

(GC/MS) confirmation of 13cRA and R in patient samples was accomplished using selective ion monitoring. We collected the eluted peak from a patient sample which corresponded to the retention times of standard 13cRA and R. These collected fractions were extracted into ethyl acetate and a small aliquot reinjected into the HPLC to confirm homogeneity. Derivatization of the R fraction was accomplished by reacting the ethyl acetate extract with 25 mcg Derivasil (Regis Chemical Co., Morton Grove, Ill.) at room temperature for 20 min. Of the resulting solution 10 μℓ was then injected onto a 3% OV-101 gas chromatography Q(100/120) 3.5 ft × 2 mm column at a helium flow rate of 20 mℓ/min. The gas chromatograph was interfaced to a Finnigan Model #3300 mass spectrometer. The GC/MS system was run at 70 eV at a sensitivity of 10^{-8} amps/V. The 13cRA fraction was added to 10 μℓ of a 0.2*M* trimethyl anilinium hydroxide solution and injected onto a 4 ft × 2 mm OV-1 column for on-column methylation. The GC/MS was run at the same settings as described above. All patient samples were scanned using the selected ion monitoring mode after selecting four of the most intense and characteristic ions for each of the retinoid standards. R was confirmed using the characteristic ions 91.1, 168.2, 255.3, and a molecular ion for the derivative 358.4. Retinoic acid was confirmed using the ions 105.1, 177.2, 185.2, and the molecular ion of the trimethyl derivative, 314.3.

Vane et al.[9] have described similar methods for assaying both 13cRA and its major metabolites in a 1-mℓ blood sample. This assay can simultaneously measure 13cRA, 4-oxo-13cRA, 4-oxo-tRA, the 5,6 epoxy- and 4-OH metabolites, as well as R. Using these HPLC methods, it has been possible to assay the major retinoids and their metabolites in biologic samples. In general, these two methods or minor variations have been used in the majority of studies that follow.

B. Photoisomerization

Retinoids play a crucial role in the visual cycle. In the retina, the aldehyde 11-*cis*-retinal, complexes with opsin to form the photosensitive pigment rodopsin. Photoisomerization of 11-*cis*-retinal to 11-*trans*-retinal induces electrochemical changes responsible for the initiation of the visual process. The 13-*cis/trans* isomers of retinoic acid also display similar photoinstability. Because photoisomerization of 13cRA may occur both in vitro and in vivo, we determined its photostability in various solvent systems.

Added to either 100% ethyl acetate or pooled human plasma and incubated at room temperature for 6 hr in glass tubes under ambient laboratory fluorescent lighting was 2.5 μg/mℓ of 13cRA. Controls were incubated under similar conditions except that the tubes were protected from light by wrapping with aluminum foil. Retinoid content was determined by HPLC analysis at regular time intervals by direct injection of the ethyl acetate samples and extraction of the plasma samples.

Figure 3 illustrates the photoisomerization of 13cRA in ethyl acetate. There was detectable isomerization to tRA within 5 min of exposure to light. Isomerization between the *cis* and *trans* isomers was rapid with equilibrium achieved by 2 hr. Control samples showed no detectable isomerization to tRA after 6 hr. Figure 4 shows a similar pattern of photoisomerization of 13cRA in human plasma. Again, 13cRA rapidly isomerizes to its *trans*-configuration with a final equilibrium concentration of approximately 75:25, cRA:tRA. There was no detectable isomerization when the sample was protected from light. Because of its extreme photolability, 13cRA should be protected from ambient lighting when working with this stereoisomer in the laboratory setting.

C. Protein Binding

Retinol binding protein (RBP) is a normally circulating plasma protein with a molecular weight of 20,000.[10] It is synthesized in the liver and binds with R on a 1:1 molar ratio. The RBP-retinol complex circulates in the plasma in association with prealbu-

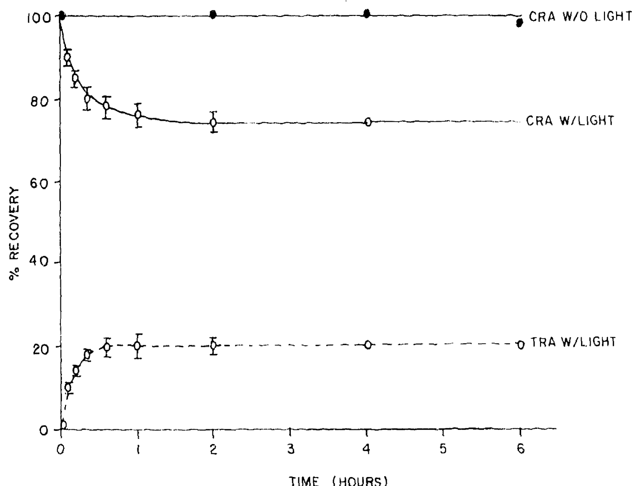

FIGURE 3. Photoisomerization of 13cRA to tRA in ethyl acetate.

min. In contrast to retinol, 13cRA and tRA are transported in the plasma bound almost exclusively to albumin.[11] Binding is approximately 99.9% and is constant over obtainable in vivo plasma concentrations. The binding with albumin is stable even in patients receiving chronic therapy, suggesting that 13cRA is not displaced from its binding sites even by high concentrations of its metabolites.[12]

D. Distribution

The tissue distribution of 13cRA is of considerable importance in particular reference to its clinical and toxicity potential. The major subjective and objective toxicity of R and the retinyl esters are hepatic, reflecting their storage in the liver as retinyl esters.[13-15] On the other hand, 13cRA has a low potential for hepatic toxicity.[16-18]

Wang et al. studied the distribution of intravenously administered 13cRA in mice.[19] Tissue and serum levels of 13cRA are shown in Table 1. In general, serum concentrations are higher than the concentrations in the organs simultaneously examined. Specifically, hepatic concentrations peak and fall with serum concentrations. There is no marked hepatic accumulation. None of the other tissues examined showed significant retention of 13cRA. Zachman et al.[20] administered [^{14}C]-retinol intravenously and found that approximately 25 to 35% of the dose was deposited in the liver as retinyl esters.[20] Other workers have also shown that after the administration of R, retinyl esters accumulate in the liver.[21,22] The difference in hepatic storage likely accounts for the contrast in hepatic toxicity between these two retinoids. 13cRA is known to have a low incidence of hepatic toxicity, whereas R or its fatty acid esters can lead to hepatic toxicity.

E. Metabolism

The use of HPLC to measure retinoid metabolites has made the study of retinoid metabolism possible. Previous to this methodology, assays for the retinoids and their

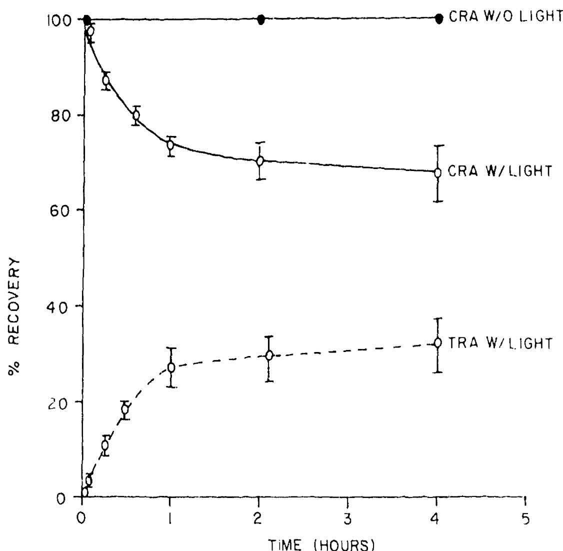

FIGURE 4. Photoisomerization of 13cRA to tRA in human plasma.

Table 1
SERUM AND TISSUE LEVELS OF 13-*cis*-RETINOIC ACID IN MALE DBA MICE AFTER INTRAVENOUS DOSING OF 10 mg/kg

		Tissue levels (μg/g)					
Time (min)	Serum levels (μg/mℓ)	Liver	Kidney	Lung	Brain	Testes	Small intestine
5	16.9 ± 1.1	13.2 ± 1.2	6.0 ± 0.8	8.1 ± 1.1	3.2 ± 0.2	1.3 ± 0.1	1.5 ± 0.2
15	10.6 ± 0.9	10.1 ± 0.7	3.1 ± 0.3	4.6 ± 0.3	3.4 ± 0.2	1.5 ± 0.1	1.1 ± 0.2
30	4.6 ± 0.1	4.7 ± 0.5	1.5 ± 0.0	2.1 ± 0.1	2.1 ± 0.0	1.0 ± 0.0	0.6 ± 0.0
60	2.2 ± 0.2	2.1 ± 0.2	0.7 ± 0.2	1.7 ± 0.6	1.0 ± 0.3	0.5 ± 0.0	0.4 ± 0.1
120	1.0 ± 0.1	1.8 ± 0.3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
240	0.2 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	< 0.1
480	0.1 ± 0.0	0.3 ± 0.1	<0.1	0.2 ± 0.1	<0.1	<0.1	<0.1

Note: Each value represents the average of three groups ± SE; each group contained pooled tissues of three animals.

From Wang, C. C., Campbell, S., Furner, R. L., and Hill, D. L., *Drug Metab. Dispos.*, 8, 8, 1980. With permission.

metabolites were both nonspecific and lacked sensitivity. Using HPLC analysis, we now have a better understanding of the in vitro and in vivo biotransformation and metabolism of 13cRA as well as other retinoids.

Any discussion of the metabolism of 13cRA necessarily includes its *trans*-stereoisomer. As we have shown, isomerization occurs rapidly when 13cRA is exposed to ambient lighting. In vivo isomerization also occurs. We and others have reported tRA plasma concentrations ranging from 0 to 30% of the simultaneous 13cRA levels after the oral administration of 13cRA.[8,23] In vivo isomerization has also been documented by others.[24] Since in vivo isomerization occurs, the metabolic degradation of administered 13cRA necessarily includes the metabolism of tRA.

After the administration of 13cRA to humans, the major metabolite detected in the plasma is 4-oxo-13cRA.[25] Evidence suggests that the first step in this oxidative metabolism is hydroxylation at the C-4 position to form 4-OH-13cRA.[26] This reaction occurs in vitro in liver microsomal preparations and is inducible by retinoic acid.[27] Enzymatic activity requires oxygen and NADPH and is sensitive to carbon monoxide. Further oxidation of the 4-OH compound to the 4-oxo compound required NAD or NADP. This reaction is insensitive to carbon monoxide poisoning. The oxidation of 4-OH-13cRA to 4-oxo-13cRA has an equilibrium constant greater than that of the initial hydroxylation reaction.[28] This may explain why the amount of 4-OH-13cRA detected in plasma is negligible and 4-oxo-13cRA appears as the major plasma metabolite.

Brazzell and Colburn have reported the metabolism of 13cRA to its 4-oxo-metabolite.[12] They administered oral [^{14}C]-13cRA to four normal subjects. The plasma concentrations of 13cRA, 4-oxo-13cRA, and tRA accounted for about 75% of the total [^{14}C] activity in the plasma during the first hour. This fraction declined to approximately one half the total radioactivity between 8 and 12 hr, and by 12 to 24 hr, unknown metabolites accounted for the major fraction of plasma radiolabel.

Utilizing [^{14}C]-labeled retinoic acid with the label located in position C_6 and C_7 as well as in the terminal C_{13} and C_{14} position, DeLuca has described other possible degradation pathways.[29] He administered [^{14}C]-tRA to vitamin A-deficient rats and examined the excretion of expired $^{14}CO_2$ and urinary [^{14}C]. The administered [^{14}C]-retinoic acid was found to be completely metabolized within 48 hr. When the carbon label was located at the C_{15} position, considerable quantities of $^{14}CO_2$ were expired. Retinoic acid labeled in the C_{14} position was also metabolized since 20% was expired as $^{14}CO_2$. Little $^{14}CO_2$ was expired when the label was located in the C_6 and C_7 positions. On the basis of these data, the authors postulated that metabolites with an intact side chain accounted for 65% of the metabolites of retinoic acid in these vitamin A-deficient animals. Sundareson and Sundareson utilizing [^{14}C]-tRA have reported similar findings.[30,31] Rietz et al.[32] and Hanni et al.[33,34] have determined the structure of several urinary and fecal metabolites in rats given large intravenous doses of tRA. However, because high tRA doses were administered, the normally occurring in vivo metabolic pathways may have been overloaded, resulting in unusual routes of metabolism. The contribution of these unusual urinary and fecal metabolites to the overall metabolic degradation of 13cRA remains unknown.

An additional metabolite described by several workers is 5,6-epoxy-retinoic acid. McCormick et al. have found this metabolite in picogram amounts in the intestine and kidney of rats after the administration of intravenous tRA.[35] However, these studies were in animals depleted of vitamin A. McCormick et al. have also reported 5,6 epoxy-retinoic acid to be a normally occurring in vivo metabolite of R.[36] Rats fed a normal diet and given [^{3}H]-retinyl acetate or [^{3}H]-retinol had significant amounts of [^{3}H]-5,6-epoxy-retinoic acid isolated from their kidneys. Hence, although this compound has not been isolated from patients given pharmacologic doses of either tRA or cRA, it may occur as an intermediate metabolic product.

The biologic significance of most of these metabolites is unknown. Both 4-oxo-13xcRA and 4-oxo-tRA in tracheal organ culture have shown less activity than the parent compound.[26] Although these metabolites may have limited biologic function, they could prove chemically important as markers for dosing compliance of 13cRA.

F. Enterohepatic Circulation

In addition to the metabolites described, the glucuronic acid conjugates of 13cRA, tRA, and 4-oxo-RA have been isolated from both stool and the biliary tract. Dunagin et al.[37-39] and Lippel and Olson[40] have shown that after the intraportal injection of [^{14}C]-retinol or [^{14}C]-tRA, over 50% appears in the bile as the glucuronic acid conjugate. These authors have suggested that conjugation of RA with glucuronic acid is a major route of metabolism. In vivo these conjugates are secreted in the bile and pass into the gastrointestinal tract where they undergo deconjugation. Other authors have suggested that the conjugation of RA with glucuronic acid is not significant at physiologic concentrations. Swanson et al. have shown RA to have dose-dependent metabolism and conjugation with glucuronic acid. They have suggested that studies using massive doses of intraportal RA represent metabolism in unphysiologic conditions.[41]

Cotler et al. examined the biliary excretion of 13cRA in dogs after the oral, intraportal, or intravenous administration.[42,43] Using biliary cannulas, they found that 13cRA was excreted in the bile primarily as a conjugate, probably with glucuronic acid. Approximately 27% was excreted in the bile after an intraportal infusion, whereas only 8.5 and 3.3% were excreted after intravenous or oral administration, respectively. The ratios of the concentration × time product for 13cRA in bile-cannulated dogs compared with the uncannulated dogs indicated that the contribution to systemic blood concentration from enterohepatic circulation was the least following intravenous dosing, intermediate following intraportal dosing, and the most following oral dosing. Elimination phase $T_{1/2}$s were shorter in cannulated dogs than in uncannulated dogs. The apparent oral bioavailability of 13cRA was 54% in the intact dog and 14% in the bile-cannulated dog, suggesting that biliary excretion and reabsorption contributed significantly to bioavailability. Wang et al.[19] have also shown in the mouse that significant amounts of 13cRA were found in the small intestines after an intravenous injection, also suggesting biliary excretion. The impact of this recycling phenomenon is most extensive following oral administration and suggests that biliary secretion and enterohepatic circulation play a significant role in the pharmacokinetic profile of 13cRA.

G. Bioavailability

Cotler et al. examined the absolute bioavailability and first pass metabolism of 13cRA in the dog.[43] They simultaneously administered [^{13}C]- and [^{14}C]-labeled 13cRA by the intravenous or oral route and determined an absolute bioavailability of approximately 21%. The first pass effect was 80%. The majority of radiolabeled compounds, approximately 72%, was lost in the gastrointestinal tract. An analysis for [^{14}C] and [^{12}C] content suggested that the majority of 13cRA was biologically or chemically degraded and that many of these degradation products were subsequently absorbed. Khoo et al. conducted a similar study using unlabeled 13cRA in human subjects and found that between 53.2 and 74.3% of an oral dose of 13cRA was recovered as parent drug in the stool within 60 to 72 hr.[23]

H. Overall Scheme

Based on the available information, a general scheme for the in vivo metabolism of 13cRA is outlined in Figure 5. After the administration of 13cRA, about 20 to 30% probably isomerizes to tRA. Conjugation with glucuronic acid, its biliary secretion, and enterohepatic circulation occurs with both parent drug, its stereoisomer, and its oxidized metabolites. Oxidation of the cyclohexenyl ring occurs at either the 4 position or at the 5,6 position with the formation of the epoxide. The 5,6 epoxide and 4-OH compounds are probably short lived. The 4-OH intermediate and perhaps the 5,6 epoxide are further metabolized to the major plasma metabolite, 4-oxo-RA. Further metabolism is not well described, but probably involves oxidation of the side chain.

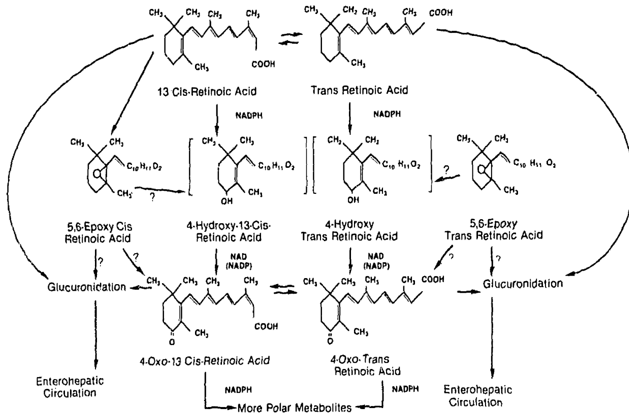

FIGURE 5. Proposed schema for the in vivo metabolism of 13cRA.

I. Pharmacokinetics

The pharmacokinetics of 13cRA in humans have been studied in two clinical settings: (1) normal subjects and patients with keratinizing skin disease or acne and (2) cancer patients. Pharmacokinetics of 13cRA have been examined after both acute single dose and after chronic dosing.

Colburn et al. studied the pharmacokinetics of a single dose of oral 13cRA in 15 normal male volunteers.[44] The subjects were fasting and received 80 mg of 13cRA as a liquid formulation. Oral intake was resumed 3 hr after drug administration. Blood and urine samples were collected for up to 72 hr. Blood samples were analyzed for 13cRA and 4-oxo-13cRA by the HPLC method of Vane et al.[9] A summary of the pharmacokinetic data obtained by these authors is outlined in Table 2. The mean ± SD of the T_{max}, C_{max}, and CxT for 13cRA were 1.47 ± 0.92 hr, 699 ± 148 ng/mℓ, and 6292 ± 1859 ng × hr/mℓ, respectively. The respective values for 4 oxo-cRA were 7.8 ± 1.6 hr, 371 ± 83.6 ng/mℓ, and 15,684 ± 4518 ng × hr/mℓ. The harmonic mean terminal phase elimination $T_{1/2}$ of 13cRA was 17.4 hr. Neither tRA nor cRA was found in the urine. These authors concluded:

1. 13cRA as an oral suspension is rapidly absorbed with biphasic elimination mean $t_{1/2}$s of 1.3 and 17.4 hr, respectively.
2. The maximum plasma concentrations of 13cRA occur between 1 and 4 hr.
3. Maximum concentrations of the major metabolite, 4-oxo-13cRA, are approximately one half those of 13cRA and occurred between 6 and 16 hr after dosing.
4. Due to the longer half-life of 4-oxo-13cRA, the area under the CxT curve for the metabolite was approximately 2.5 times that of 13cRA.

Another group of patients in which the retinoids have been studied are individuals with cancer and preneoplastic lesions. In general, these patients are a different group than "normal" subjects since many may have lost considerable body weight due to anorexia, have vitamin deficiencies, and some degree of malabsorption. For these rea-

Table 2
MODEL INDEPENDENT PARAMETERS ESTIMATED FROM ISOTRETINOIN AND 4-OXO-ISOTRETINOIN BLOOD CONCENTRATION-TIME DATA FOLLOWING AN 80-MG ORAL DOSE OF SUSPENSION

Parameters	Subject number: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean ± SD
Isotretinoin																
T_{max} (hr)	2	1	1	3	1	1	1	2	1	1	1	4	1	1	1	1.47 ± 0.92
C_{max} (ng/mℓ)	626	937	693	480	950	727	715	650	868	719	710	436	658	546	777	699 ± 148
AUC (ng hr/mℓ)	6531	9288	4969	6878	6954	5587	5359	5100	7181	8780	4579	4148	4056	5067	9899	6292 ± 1859
4-oxo-Isotretinoin																
T_{max} (hr)	6	8—16*	6	8	8	8	8	6	8	8	8	8	8—12*	6	6—8*	7.8 ± 1.6
C_{max} (ng/mℓ)	329	465	320	424	526	452	375	337	351	431	349	444	237	298	241	371 ± 83.6
AUC (ng hr/mℓ)	16697	22800	12414	20914	17281	21052	17039	12230	15413	22211	11317	13164	7809	12487	12437	15684 ± 4518
Ratio $\frac{AUC}{AUC}$	2.56	2.45	2.50	3.04	2.49	3.77	3.18	2.40	2.15	2.53	2.47	3.18	1.93	2.46	1.25	2.56 ± 0.59

* Average of the two values was used to calculate the mean ± SD values.

From Colburn, W. A., Vane, F. M., and Shorter, H. J., *Eur. J. Clin. Pharmacol.*, 24, 689, 1983. With permission.

sons, we considered it important to study the pharmacokinetics of 13cRA in cancer patients enrolled in a phase II trial at the University of Arizona.[8]

In contrast to the study of Colburn et al.,[45] patients in our study received 13cRA as oral gelatin capsules and not as a liquid formulation.[8] After an overnight fast, patients were administered the retinoid as an oral bolus and fasted for an additional 3 hr. Six patients received a dosage of 3 mg/kg, two patients 4 mg/kg, and five 5 mg/kg. At 30- to 60-min intervals, 7 to 10 mℓ of blood was collected in foil-wrapped vacutainers containing 200 units of sodium heparin. Samples were centrifuged, stored in the dark, and frozen at −25°C until analyzed. Fractional urines were collected for the first 8 hr and then up to 24 hr.

Plasma concentrations of 13cRA vs. time data were obtained for each patient and fitted to a multiexponential equation using a nonlinear regression computer program. The pharmacokinetic parameters of oral 13cRA obtained from nonlinear regression fitting of the plasma concentration vs. time data are summarized in Table 3. The mean time to peak plasma concentration of 13cRA of five patients receiving 3 mg/kg was 192.0 ± 65.7 min. The peak plasma concentration of 13cRA in these patients varied from 20 to 86 ng/mℓ with a mean of 50 ng/mℓ. The mean initial plasma $T_{1/2}$ for 13cRA was 12.0 ± 43.7 min. Because of its apparent prolongation and the requirement for daily dosing, the terminal phase half-life of 13cRA could not be determined accurately. Patients receiving 5 mg/kg had a mean time to peak plasma concentration of 270 ± 142 min and a mean peak 13cRA plasma concentration of 74 ng/mℓ. These peak plasma concentrations showed marked variability with a range from 19 to 150 ng/mℓ. The initial phase $T_{1/2}$ of 144 ± 97.3 min was not significantly different from that observed at 3 mg/kg. The elimination phase half-life was again prolonged and could not be accurately determined. Figure 6 shows a computer-plotted plasma concentration curve of 13cRA from two patients receiving 3 mg/kg. Both patients had 13cRa detectable in the plasma soon after administration of the initial dose with peak concentrations achieved between 2 and 3 hr. 13cRA was detected in the plasma of both patients at 24 hr. Although receiving the identical dose on a body weight basis, there was a considerable difference in the CxT between patients. This probably reflects the interpatient variability for gastrointestinal absorption of the drug.

tRA was detected in the plasma of most patients receiving 13cRA. Plasma concentrations of tRA varied and ranged from 0 to 30% of the simultaneous plasma concentration of 13cRA. 13cRA, tRA, and retinol were not detected in any urine samples collected. One patient studied showed two peaks in her plasma concentration curve of 13cRA. As other workers have described, this probably represents enterohepatic circulation of 13cRA and/or its conjugates.[12] Other than tRA, we did not detect any metabolites of 13cRA in the patient's plasma and were unable to identify any retinoids in the urine. However, it is important to note that the HPLC method did not detect the more polar metabolites of 13cRA, specifically 4-oxo-cRA.

These results in cancer patients are very similar to those in normal subjects described by Colburn et al.[44] Maximal blood concentrations of 13cRA and initial and terminal phase half-life appeared similar in both groups of subjects. Additionally, 13cRA appeared to have a variable bioavailability in both studies. Biliary secretion and enterohepatic circulation may account for some of the variation observed in plasma 13cRA clearance in some patients. With terminal phase half-lives ranging from approximately 10 to 40 hr, steady-state blood concentrations with daily dosing should be achieved in approximately 1 week.

The pharmacokinetics of 13cRA during repetitive dosing schedules in patients with either dermatologic or malignant disease have been reported. Brazell et al. studied a population of 11 patients with disorders of keratinization and 10 patients with cystic acne.[45] Each patient received an initial 13cRA dose of 80 mg which was followed by 40

Table 3
PHARMACOKINETIC PARAMETERS OF ORAL 13cRA IN CANCER PATIENTS

Patient	Dose (mg/kg)	Day of study	Time to peak plasma concentration (min)	Peak plasma concentration (μg/mℓ)	$T_{1/2\beta}$ (min)	C × T (μg × min/mℓ)	$T_{1/2\gamma}$ (min)
1	3	1	180	0.20	114.6	75.3	Indeterminate[b]
2	3	1	300	0.86	160.7	380.5	1225
3[a]	3	1	180	0.30	169.9	161.0	Indeterminate[b]
	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 ± 65[d]	0.50 ± 0.26	120 ± 43.7	221 ± 113	
7	4	1	180	0.15	82.5	20.0	Indeterminate[b]
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[b]
11	5	1	240	0.52	96.5	301.7	1889
12	5	1	480	1.15	290.1	760.8	Indeterminate[b]
13	5	1	180	0.93	86.0	271.1	Indeterminate[b]
Mean[f]	5		270 ± 142	0.74 ± 0.35	144 ± 97.3	368 ± 271	

[a] Patient 3 was studied on days 1 and 12 of daily drug administration.
[b] *Half-life appeared very prolonged and could not be accurately determined.*
[c] Mean values are obtained from the 5 patients who were studied on day 1 only.
[d] Mean ± SD.
[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.

From Goodman, G. E., Einsphar, J. G., Alberts, D. S., Davis, T. P., Leigh, S. A., Chen, H. S. G., and Meyskens, F. L., *Cancer Res.*, 42, 2087, 1982. With permission.

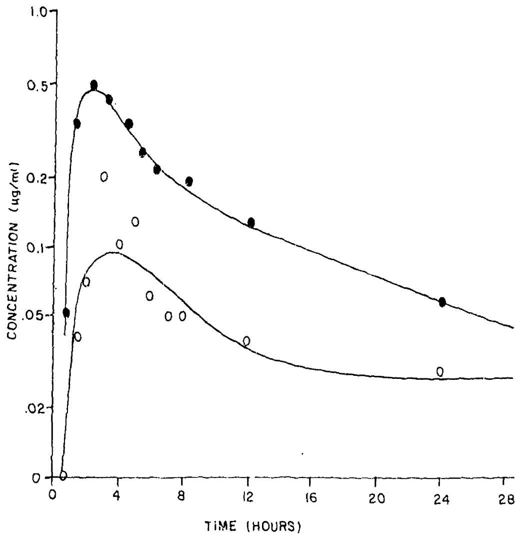

FIGURE 6. Computer-plotted plasma disappearance curve for two patients receiving 3 mg/kg 13cRA at time 0. (From Goodman, G. E., Einspahr, J. R., Alberts, D. S., Davis, T. P., Leigh, S. A., Chen, H. S. G., and Meyskens, F. L., *Cancer Res.*, 42, 2087, 1982. With permission.)

mg twice daily for 25 days. The patient then fasted for 8 hr and received a single dosage of 80 mg. Blood samples were obtained at appropriate intervals following the first 80-mg dosage, during the 25 interval days of twice-daily dosing and following the final 80-mg dosage. Figure 7 shows the mean plasma concentrations of both 13cRA and 4-oxo-13cRA during the study period. Although there was a tendency for a slight accumulation of the drug, there was no significant difference between the first and last dose in terms of: (1) time to maximum plasma 13cRA concentration; (2) maximum plasma 13cRA concentration; and (3) the characteristics of the profile for 13cRA. The areas under the plasma concentration time curves ($AUC_{0-\infty}$ vs. $AUC_{0-\tau}$) and the elimination half-lives were similar for the first and the last 80-mg dosage, suggesting that the drug does not induce its own metabolism and does not accumulate in the plasma. The average 13cRA terminal phase half-life was approximately 10 hr. Trough concentrations of 13cRA also remained relatively stable during the 25-day interval period. Although there was no apparent change in the disposition of 13cRA during multiple dosing, there was a significant difference in the pharmacokinetic parameters of its metabolite, 4-oxo-13cRA. The area under the 4-oxo-13cRA plasma concentration-time curve was greater after the last 80-mg dose than the first 80-mg dose at steady state. Because of these high plasma concentrations, it is important to consider that 4-oxo-13cRA may play a role in the in vivo activity of 13cRA.

Kerr et al. reported the pharmacokinetics of 13cRA in ten cancer patients receiving daily oral therapy.[46] They found 13cRA to be irregularly absorbed with large interindividual variations. Because each of their study patients received multiple daily drug

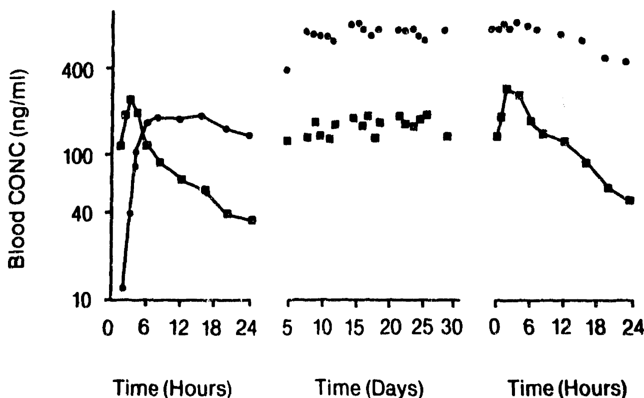

FIGURE 7. (■) 13cRA and (●) 4-oxo-13cRA mean blood concentrations (ng/mℓ) following multiple-dose administration to ten acne patients. The first graph represents the mean concentration time profiles after the first 80-mg dose of 13cRA; the second graph is the mean trough concentrations during the 25 days of 40 mg b.i.d. dosing; and the third graph represents the mean concentration time profiles following an 80-mg single dose administered 12 hr after the last 40-mg b.i.d. dose. (From Brazzell, R. F., Vane, F. M., Ehmann, C. W., and Colburn, W. A., *Eur. J. Clin. Pharmacol.*, 24, 695, 1983. With permission.)

doses, it was not possible to compute the terminal elimination $T_{1/2}$s. These authors did suggest that the drug should be administered every 4 hr to assure the maintenance of adequate plasma concentration.

Because many clinical investigators, including ourselves, plan to use lower doses (i.e., 0.05 to 0.15 mg/kg/day) of 13cRA in trials to evaluate its ability to prevent the development or recurrence of high-risk epithelial cancers, we have initiated pharmacokinetic studies at the University of Arizona of 0.15 mg/kg/day in eight normal subjects. Full plasma disappearance curves of parent compound and 4-oxo-13cRA were obtained after the first dose and then at 3-month intervals during the 12-month study. In addition, monthly 8 a.m. and 12 noon blood samples were obtained to determine trough (i.e., 24 hr) and peak (i.e., approximately 4 hr after daily dosing) plasma concentrations of 13cRA. Although this study has been initiated only recently, the preliminary results from the first 2 months of plasma sampling are of considerable interest. The peak plasma concentrations of 13cRA on days 1, 30, and 60 have, respectively, averaged 37 (range 21 to 152), 41 (range 25 to 60), and 60 (range 5 to 120) ng/mℓ. Trough plasma concentrations on the same days averaged 10 (range 5 to 25 with two patients showing no evidence of 13cRA), 27 (range 5 to 55 with all patients having measurable concentrations), and 40 ng/mℓ, respectively. There appeared to be an upward trend in both peak and trough 13cRA plasma concentrations over a 60-day period in these eight normal subjects. The composite terminal phase $T_{1/2}$ of the parent compound after the first dose was 28 hr (although accurate assessment is difficult because of the very low plasma concentrations observed at 24 hr). On the basis of these preliminary data, it appears that even at the relatively low dose of 0.15 mg/kg/day, plasma concentrations of 13cRA can be used to monitor dosing.

During this pharmacokinetic trial we have also monitored the plasma concentrations of the 4-oxo-13cRA metabolite. These peak concentrations averaged 64 (range 29 to 101), 48 (range 0 to 104), and 66 ng/mℓ, respectively, on days 1, 30, and 60. Trough

plasma concentrations in these eight patients averaged 43 (range 29 to 58), 56 (range 0 to 72), and 64 (range 32 to 88) ng/mℓ, respectively, on these same days. Thus, the 4-oxo-metabolite data may prove very useful as an additional marker for monitoring compliance during low dose 13cRA chemoprevention studies. Of course, these are only preliminary data and final conclusions can only be made on the basis of full results over the 12-month study period.

J. Summary

To summarize:

1. The pharmacokinetics of 13cRA appear to be similar in normal subjects, patients with dermatologic disorders, and patients with cancer.
2. 13cRA undergoes variable gastrointestinal absorption and has a low absolute bioavailability. Significant amounts of the drug are degraded in the gut and absorbed or excreted in the stool either as metabolites or parent drug or in the urine as metabolites.
3. 13cRA and probably its oxidized metabolites undergo significant enterohepatic circulation via the glucuronic acid conjugates. This can result in intra- and interpatient variation in plasma concentrations.
4. 13cRA undergoes rapid in vivo oxidative metabolism to 4-oxo-13cRA. This is the major plasma metabolite and appears rapidly after the administration of 13cRA.
5. The pharmacokinetics of 13cRA are predictable from single-dose studies.
6. The elimination phase $T_{1/2}$ shows great interpatient variability. The terminal phase $T_{1/2}$ has been reported to range from 10 to 77 hr.
7. With chronic dosing (of approximately 1 mg/kg/day), 4-oxo-13cRA accumulates in plasma and has an area under the plasma concentration-time curves 5.1 times that of 13cRA. 4-oxo-13cRA may play a significant role in the in vivo therapeutic effectiveness of 13cRA.
8. With chronic dosing (1 to 5 mg/kg/day), plasma concentrations of 13cRA are likely to reach steady state in around 7 days.
9. With chronic dosing of 13-cRA at only 0.15 mg/kg/day both parent compound and the 4-oxo metabolite are detectable in plasma 24 hr after dosing and may be useful as markers of dosing compliance in patients entered into chemoprevention studies.

III. TOXICOLOGY

A. Short-Term Effects

The acute side effects of vitamin A overdosage have been well described and consist largely of central nervous system alterations, while chronic intoxication results in skin and mucous membrane dryness and liver impairment, which eventually become irreversible.[47,48] In large part, derivatives of vitamin A were synthesized with the intent to improve the therapeutic/toxicity ratio for the parent compound. Bollag developed a simple papilloma model which allowed rapid screening and identification of compounds with improved ratios.[49] 13cRA was selected on this basis and early clinical experience suggested an affinity for skin and subcutaneous tissues and absence of central nervous system and hepatic side effects. In the past decade, a considerable experience with 13cRA (generally <2 mg/kg) against dermatological conditions has been acquired and acute and subacute (<6 months) side effects have been well characterized.[50-52] These are listed in Table 4. Our experience with 13cRA at higher dosages (2 to 5 mg/kg) has been similar except that side effects are more severe and more headaches have been seen.[16]

Table 4
CLINICAL AND LABORATORY TOXIC FINDINGS IN 523 PATIENTS TREATED WITH ISOTRETINOIN IN THE U.S.

Findings	Incidence (%)
Clinical	
Cheilitis	90
Dry mouth	30
Xerosis; desquamation, especially face pruritus, itching	25
Palmoplantar desquamation	5
Hair thinning	10
Epistaxis, petechiae	25
Conjunctivitis, eye irritation	50
Bone/joint/muscle symptoms	15
Lethargy, fatigue	10
Headache	10
Nausea, vomiting, other gastrointestinal	20
Laboratory	
Elevated sedimentation rates	50
Elevated triglycerides	25
Decreased red cell parameters	10
Decreased white cell counts	10
Increased platelet counts	10
White cells in urine	10
Increases in liver function tests	10

From Windhorst, D. B. and Nigra, T., *J. Am. Acad. Dermatol.*, 6, 675, 1982. With permission.

B. Long-Term Effects

The major use of 13cRA is likely to be as a chemopreventive agent administered for long periods of time at relatively low doses (<0.25 mg/kg). Dermatological experience and the known disposition of 13cRA suggest that careful monitoring of several organ systems should be performed. These include reproductive and embryological function, serum lipid changes, and skeletal abnormalities.

Adequate vitamin A levels are required for normal spermatogenesis and embryonic development. Low or high levels can result in abnormalities. Retinoids such as Tretinoin and Etretinate have clearly been shown to be teratogenic in animals.[53-55] To date, comparative teratogenic studies of retinoids suggest that 13cRA was nearly devoid of adverse effects in several animal models.[54] Nevertheless, concern about possible 13cRA-associated terata has been raised since abnormal offspring have been reported in a few women taking 13cRA for acne.[56] Whether these reports indicate an increased risk or simply reflect background teratogenic events is unknown. However, patients entering prevention trials using 13cRA must be fully cognizant of this possible risk and contraceptive protection must be employed for both partners.

Lipid changes have been noted in animals taking retinoids.[57] They also occur in some patients after 13cRA administration.[58-62] In general, triglycerides have increased, but cholesterol has remained normal. Unfortunately, neither HDL cholesterol nor VLDL has been reported in these studies although the package insert for Accutane states that 15% of patients in clinical trials from whom serum lipid profiles were obtained developed decreases in HDL. Documentation of unfavorable changes in lipoprotein profiles would impair the use of this retinoid in prevention trials as decreased HDL cholesterol and increased VLDL have been associated with adverse cardiovascular changes. Care-

ful monitoring of lipid profiles over the duration of prevention trials would seem reasonable.

Vitamin A is necessary for normal bone development. In animals, excesses of either vitamin A or 13cRA have been associated with hyperostosis and bone fractures,[63,64] and bone and joint pain has been noted in 10 to 20% of patients receiving 13cRA.[16,50] Recently, diffuse idiopathic skeletal hyperostosis was noted in four patients with refractory ichthyosis who had received high doses (3 mg/kg) of 13cRA for greater than 18 months.[65] Although the incidence of this entity is unknown and the doses high, this observation suggests that careful monitoring for skeletal toxicity will also be important in long-term chemoprevention trials.

C. Monitoring of Side Effects and Toxicity

We have developed a comprehensive four-level clinical and laboratory scale which we use to monitor acute and chronic side effects of 13cRA (Tables 5 and 6). The level of toxicity which is acceptable to the patient and the physician for any category of use will vary considerably. For example, a patient who has advanced cancer and is receiving 13cRA will tolerate considerable toxicity (level 3 or 4) before wishing to discontinue therapy. In contrast, an individual receiving 13cRA to prevent actinic keratoses may have a considerably lower threshold for side effects. Additionally, investigators will need to consider the ethical implications of giving 13cRA long term to individuals who are nearly normal.

IV. CLINICAL USES OF 13cRA FOR HUMAN CANCER

A. Rationale

In the past decade a large number of laboratory investigations have documented that the retinoids can block phenotypic expression of cancer, whether initiated by chemical, viral, physical, or biological carcinogens.[5,66-68] Several important general principles have emerged from these studies, which are discussed extensively elsewhere.[66-69] Briefly, the effect of the retinoids is dependent on the type and dose of the carcinogen, the time of administration of the retinoid with respect to the carcinogen, and the type of retinoid. 13cRA has been a particularly effective antipromoter in the majority of these laboratory investigations.

In the past 5 years, a large number of studies have also demonstrated that retinoids have antiproliferative activity against a large number of cultured animal and human cancers of diverse histologies including cultured cells from long-term cell lines[6,70-80] and cells obtained from biopsies of human cancers and grown in semisolid agar.[81-83] The responses were dependent on the type of malignancy, the retinoid, and were in general dose dependent. In a few cases, stimulation was evident at low concentrations. Again, 13cRA was among the most effective of the retinoids.

Extensive clinical experience with 13cRA as an antiacne and antikeratinizing agent has been obtained and therefore its introduction for oncologic uses has not been prolonged. The retinoid has been used for both preventive and therapeutic indications. The approach to these two areas of clinical intervention are considerably different — with different premises, therapeutic/toxicity ratios, and endpoints — and therefore will be discussed separately.

B. Prevention

We have identified several levels of cancer prevention. These are summarized in Table 7 and include primary, secondary, and tertiary forms as discussed extensively elsewhere.[84] Primary prevention represents simple avoidance; the best and most important example is cessation of smoking. Intervention in the form of behavioral modification is important at this level, but active chemoprevention has no role.

Table 5
CLINICAL TOXICITY SCALE FOR PATIENTS TREATED WITH VITAMIN A AND ITS DERIVATIVES

Level I toxicity
- Dry skin, mucous membranes, mild, controlled with emollients
- Cheilitis, mild, controlled with emollients
- Epistaxis, mild, <2 episodes per week
- Peeling of palms and soles, mild, controlled with emollients
- Headache, mild, ≤2/week over pretreatment incidence
- Increased sunburn susceptibility
- Conjunctivitis, mild, controlled with artificial tears
- Menstrual changes, mild
- Nausea/vomiting, mild, <1/day
- Exanthema, fleeting or mild

Level II toxicity
- Dry skin, mucous membranes, moderate, partially controlled with emollients
- Chelitis, moderate, partially controlled with emollients
- Epistaxis, mild <2/week or significant epistaxis (>10 cc); >1 episode per week
- Peeling of palms and soles, moderate, partially controlled with emollients
- Headache, mild >2/week over pretreatment incidence
- Conjunctivitis, moderate, partially controlled with artificial tears
- Alopecia of scalp, mild
- Musculoskeletal symptoms (pain, stiffness), mild
- Chronic fatigue, mild (able to perform normal functions)
- Dysuria (urethral irritation), <3 episodes per week
- Skin infections, ≤2 episodes per month
- Menstrual changes, hypomenorrhea
- Nausea/vomiting, moderate or >1/day
- Exanthema, sustained (>1/day)

Level III toxicity
- Dry skin, mucous membranes, severe, poorly controlled with emollients
- Cheilitis, severe, poorly controlled with emollients
- Epistaxis with significant blood loss (>10 cc) >3 times per week
- Peeling of palms and soles severe, causing functional disability
- Headache, moderate, poorly controlled with analgesics, >5/week over pretreatment incidence
- Alopecia of scalp (clinically obvious) with moderate hair loss
- Musculoskeletal symptoms (pain, stiffness), moderate, requiring analgesics for relief of symptoms
- Chronic fatigue, moderate, difficulty in performing normal functions (working, driving)
- Menstrual changes, amenorrhea
- Nausea/vomiting, severe, >1/day
- Exanthema, severe, sustained, >1/day

Level IV toxicity
- Epistaxis with significant blood loss (>10 cc) >6 times per week, interferes with normal function
- Headaches, severe, poorly controlled with analgesics, >6 times per week, interferes with normal function
- Musculoskeletal symptoms (pain, stiffness), severe, poorly controlled with analgesics
- Chronic fatigue, severe, unable to perform normal functions (working, driving)
- Corneal opacities

Secondary prevention involves active intervention with pharmacological agents. The individual has been exposed to initiators and/or promoters and may be continually exposed to them, but no identifiable preneoplasia or neoplasia has been identified. Examples of groups at risk would include smokers (lung), asbestos workers (lung), aniline dye workers (bladder), and patients with prior skin cancers who have not yet developed identifiable preneoplasias. Active intervention trials could include dietary changes (increase vegetables, decrease fats) or pharmacological modifications. At the University of Arizona, we have initiated two large secondary prevention trials. These include stratified randomized trials of placebo vs. retinol in patients with eight or more prior resected actinic keratoses and of placebo vs. vitamin A vs. 13cRA in patients with

Table 6
LABORATORY TOXICITY FOR PATIENTS TREATED WITH VITAMIN A AND ITS DERIVATIVES

Enter laboratory results

Serum triglyceride__________mg/dl

Serum cholesterol__________mg/dl

HDL cholesterol__________mg/dl

RBC count__________
WBC count__________
Liver function tests__________
SGOT__________ IU/l SGPT__________ IU/l

Alkaline phosphatase__________ IU/l
Hepatomegaly__________ % increase on ^{99}Tc scan

Other

Toxicity Scale

Level I toxicity	
Serum triglyceride	25% above upper limit of normal
Serum cholesterol	25% above upper limit of normal
HDL cholesterol	25% below pretreatment baseline value
RBC count	10% below lower limit of normal
WBC count	25% below lower limit of normal
Liver function parameters — (alkaline phosphatase, SGOT, SGPT)	25% above upper limit of normal
Hepatomegaly	25% increase on ^{99}Tc scan
Level II toxicity	
Serum triglycerides	26 to 100% above upper limit of normal
Serum cholesterol	26 to 75% above upper limit of normal
HDL cholesterol	26 to 75% below pretreatment baseline level
RBC count	11 to 25% below lower limit of normal
WBC count	26 to 75% below lower limit of normal
Liver function parameters	26 to 200% above upper limit of normal
Hepatomegaly	25 to 50% increase on ^{99}Tc scan
Level III toxicity	
Serum triglyceride	>100% above upper limit of normal
Serum cholesterol	>75% above upper limit of normal
HDL cholesterol	>75% below pretreatment baseline level
RBC count	>25% below lower limit of normal
WBC count	>75% below lower limit of normal
Liver function parameters	>100% above upper limit of normal
Hepatomegaly	>50% increase in ^{99}Tc scan
X-ray evidence of bony hyperiostosis or calcification of spinal ligaments	Unknown

greater than eight prior resected squamous or basal cell carcinomas. In both studies the appearance of skin cancer lesions will be quantitated. Patients will remain on treatment until differences between groups are evident. In the latter study, differences between placebo and treatment arms should be rapidly evident as the recurrence rate in this high risk group of patients is over 40% per year. These and similar studies directed to other organ systems should provide answers within 5 years as to whether 13cRA is an effective suppressor of initiated and/or promoted cells in humans.

Table 7
PREVENTION AND TREATMENT OF CANCER WITH 13-*cis*-RETINOIC ACID

Therapeutic mode	Presence of precancer or cancer	Strategy	Examples
Prevention			
Primary	No	Decrease exposure	Smoking, heavy metals
Secondary	No, cells may change and thereby predispose them to carcinogenic damage	Supplement with natural inhibitors	Vitamin A- or C- or Se-deficient individuals
	No, damage at cellular level	Provide pharmacologic inhibitors	Smokers, ultraviolet light
Tertiary benign	Yes, phenotypically identifiable preneoplasia or benign proliferation	Pharmacological or natural inhibitors	Cervical dysplasia, leukoplakia, bladder metaplasia, lung metaplasia (abnormal sputum cytology)
Treatment			
Adjuvant	Yes, gross cancer lesion(s) excised, micrometastatic disease present	Prevent recurrence	Cancers
Advanced	Yes, cancer present	Treat to toxicity	Cancers

Tertiary prevention deals with histologically identifiable hyperproliferative or preneoplastic lesions. In these cases, initiation has occurred and the cells have been promoted to varying degrees. Abnormal cells in the form of hyperproliferative or preneoplastic changes can be identified. Examples include leukoplakia, laryngeal papillomatosis, abnormal sputum cytology, cervical dysplasia, bladder abnormalities (unifocal, multifocal, papillomas), several skin conditions including actinic keratosis, keratoacanthomas, and epidermal dysplasias, and disturbed hemopoiesis such as preleukemia, other dysplastic marrow states, and chronic myelogenous leukemia.

Many of these entities are amenable to locoregional delivery of the chemopreventive agent, thereby minimizing attendant systemic effects. Perhaps the best human model for this approach is cervical dysplasia. Several distinct phases have been delineated, including mild, moderate, and severe dysplasia (cervical intraepithelial neoplasia I, II, III). Some authors also divide the severe phase into severe dysplasia and carcinoma *in situ*. We have studied the effect of vitamin A acid delivered by a collagen sponge/cervical cap device on mild and moderate dysplasia[85] and have recently completed a phase I dosage escalation study.[86] The drug and device were well tolerated, but retinoid-related side effects did occur. Since in general 13cRA has produced fewer side effects than tRA, a trial with this or another retinoid would be of considerable interest.

To date, no locoregional trial of retinoids for preneoplastic lesions of the lung, bladder, or oral cavity has been reported. However, one uncontrolled study with oral 13cRA presented preliminary evidence that sputum cytology can be improved.[87] Additionally, preliminary results from two randomized, placebo-controlled studies suggest that Etretinate (25 to 50 mg per dose daily) reduces the recurrence frequency of superficial bladder cancers.[88,89] Koch has also reported the treatment of 75 cases of leukoplakia with several different types of retinoids.[90] 13cRA and aRA were most effective with both retinoids producing greater than a 50% response rate (complete response rates for 13cRA and aRA were 25 and 29%, respectively, with durations greater than 1 year). In another study, Shah et al. used an oral troche to deliver 13cRA and good responses were seen in 11 patients with leukoplakia.[91]

C. Hyperproliferative Conditions

We and others have shown that 13cRA produces clinical responses in patients with keratoacanthomas and epidermal dysplasia verruciformis,[16,92-94] and Moriarty et al. have demonstrated impressive activity of Etretinate against actinic keratosis.[95] Both keratoacanthomas and epidermal dysplasia verruciformis are associated with human papilloma viruses and we therefore explored the activity of 13cRA against the severe hyperproliferative disease laryngeal papillomatosis.[96] Five patients were studied and one complete and two partial responses were obtained. All three responses have been of long duration. In a preliminary report, Bichler has also reported the effect of oral Etretinate (1 mg/kg) in 42 patients with laryngeal papillomatosis with moderate to severe dysplasias.[97] Complete response was obtained in 28 patients and partial responses in 11 patients. The long-term outcome of these patients will be of considerable importance.

A large number of investigations will need to be done with 13cRA and other retinoids to determine whether frank preneoplasias can be reversed. As these entities are even more variable in their clinical course than the established malignancies, careful design will be critical to developing studies which will give interpretable and meaningful results 5, 10, and 15 years later.

D. Advanced Cancer

Preliminary evidence exists for anticancer activity of 13cRA against both epithelial and nonepithelial malignancies. The results from our initial phase II study are summarized in Table 8.[16] One hundred patients with advanced malignancies were treated. Of these, 71 were of epithelial, 13 of neuroepithelial, and 16 of nonepithelial origin. Epithelial cancers were further subclassified as to squamous and nonsquamous in origin. In the 45 nonsquamous epithelial malignancies, only 1 response was seen, a partial response of a pelvic mass lasting 3 months in a patient with ovarian cancer. Cassidy et al. have also found no activity of 13cRA in a phase II trial of metastatic breast cancer.[98] Of 26 patients with squamous epithelial malignancies, there were 6 responses all occurring in patients with head and neck or lung cancer, but limited to subcutaneous or skin sites. Of 13 patients with metastatic melanoma, 1 also exhibited a partial response of lung lesions.

Several large trials have been initiated by us and other institutions to confirm these findings. These include a randomized trial of 13cRA vs. methotrexate for advanced or metastatic head and neck cancer, a large phase II trial for lung cancer, stratified by subtype, and a randomized adjuvant trial of placebo vs. 13cRA for deep (>1.5 mm) stage I malignant melanoma. These and other studies will help to define the role of 13cRA for advanced epithelial and melanoma cancers.

In addition to its action as an antiproliferative agent against epithelial tissues, evidence has accumulated that 13cRA affects hematopoietic cells.[71,99-104] The retinoid stimulates normal CFU_C and BFU_E,[99,100] but inhibits leukemic CFU_C.[101] Additionally, 13cRA produces terminal differentiation of cells from a promyelocytic HL-60 cell line.[71,102] Douer and Koeffler followed up this finding and reported the effect of 13cRA on clonal formation of bone marrow cells in semisolid medium from 25 patients with myelogenous leukemia.[103] Only in those three patients with the promyelocytic variant was colony formation suppressed. Although no reports are yet available in patients with myeloproliferative leukemias, we (unpublished) and others[104] have tested the effect of 13cRA on patients with preleukemia, and favorable responses have been noted in some of the cases. Confirmation of these findings will be quite important inasmuch as there is no known treatment for preleukemia. Delineation of the cellular and/or clinical features of those patients who do respond may well lead to advances in our understanding of retinoid action and preleukemia as well.

Table 8
PATIENT DISTRIBUTION AND RESPONSE RATE OF ELIGIBLE AND EVALUABLE PATIENTS TREATED WITH ISOTRETINOIN

Histologic class	No. of patients	No. (%) of responses[a] Including mixed responses	Excluding mixed responses
Epithelial[b]			
Squamous cell			
Advanced	24	6 (25)	2 (8)
Low grade (preneoplastic)	5	3 (60)	3 (60)
Nonsquamous cell	45	1 (2)	1 (2)
Malignant melanoma	13	1 (7)	1 (7)
Nonepithelial	21	0	0

[a] Responses include only mixed, partial, and complete; improvements are not included.

[b] Tumor types included epithelial squamous cell, high grade (8 lung, 10 head and neck, and 6 other); epithelial squamous cell, low grade (3 multiple basal cell, 1 keratoacanthoma, and 1 epidermal dysplasia verruciformis); epithelial nonsquamous cell (8 breast, 12 ovarian, 12 colon, 4 bladder, and 9 miscellaneous); and nonepithelial (3 choriocarcinoma, 5 lymphoma, 4 leukemia, and 9 miscellaneous).

From Meyskens, F. L., Gilmartin, E., Alberts, D. S., Levine, N., Brooks, R., Salmon, S., and Surwit, E., *Cancer Treat. Rep.*, 66, 1315, 1982. With permission.

Another cellular site of action of 13cRA is the immune system. Vitamin A has been known for some time to be necessary for normal immune functions.[105] A series of studies have provided evidence that 13cRA enhances recruitment of cytotoxic T cells into a functional compartment as well as enhancing cytotoxic T-cell killing.[105-109] We therefore have treated patients with refractory mycosis fungoides with 13cRA.[110] The response of some patients was dramatic, with marked clearing of extensive lesions. These observations have been confirmed[111] and suggest that exploration of activity in other T-cell lymphomas and related diseases (AIDS?) should be tried. Recently, Claudy et al. have also reported clinical activity in a patient with cutaneous T-cell lymphomas (mycosis fungoides) treated with Etretinate.[112] These clinical results indicate that 13cRA may affect the T-cell system (or its regulators) by unsuspected means. Laboratory investigation of the interaction of mycosis fungoides and 13cRA should be informative.

A large number of hyperproliferative cutaneous conditions have been treated with the retinoids, including 13cRA. As discussed extensively by Peck[51] and others,[113] the range of effectiveness of retinoids for dermatological conditions varies considerably. For example, 13cRA is very potent against conglobate and cystic acne, but relatively ineffective and variable in slowing psoriasis. The aromatic derivative has the completely opposite spectrum. The range of activity for 13cRA or other retinoids for preneoplastic and neoplastic oncological conditions is presently unknown.

E. Summary

To summarize:

1. 13cRA is an effective chemopreventive agent in in vitro systems and in animals. Exploration of this activity in human conditions seems warranted. Secondary prevention of skin, lung, and perhaps bladder cancers is now an approachable issue. Carefully stratified and randomized studies will provide important information about feasibility and efficacy of this approach for these malignancies, as well as provide a foundation of experience for more difficult cancer sites.
2. 13cRA is effective against a number of cutaneous preneoplasias as well as laryngeal papillomatosis. The spectrum of activity should be further explored and other retinoids also tried.
3. 13cRA produces responses in patients with mycosis fungoides. Its activity should be explored against other T-cell lymphomas and the biological basis for these responses studied.
4. 13cRA has minor to moderate activity against squamous epithelial malignancies, which needs to be more widely confirmed and extended to combination studies with cytotoxic therapy. Studies with other retinoids would also seem worthwhile.

V. FUTURE PROSPECTS

The use of retinoids in the prevention and treatment of human cancer represents a new direction in oncologic therapeutics. The modest effects of 13cRA against epithelial malignancies indicate that the activity of other retinoids should be explored. The limited and specific spectrum for 13cRA against dermatologic conditions as compared to other retinoids also supports this approach. Since the side effects of 13cRA do not generally include bone marrow suppression, their inclusion in combination with conventional cytotoxic treatment would seem reasonable.

Two important and neglected areas in retinoid development also need to be considered. The major responses have been largely limited to cutaneous areas. Does this reflect the histologic origin of the tumor or its skin location of the tumor? Does the tumor respond because 13cRA concentrates in the skin? Consequently, we ask: Can retinoids with *different* tissue distributions be synthesized? Another important observation is that most proliferating tissues (and tumors) require vitamin A for growth. Many tumors also possess intracellular receptors for retinoic acid. We ask: Can antiretinoids be developed in a manner similar to the development of antiestrogens, which have been highly effective against breast cancer?

Our own estimate is that 13cRA will serve as a prototype molecule from which two new areas of therapeutics will develop: (1) the use of biological response modifiers to control frank malignant growth, and (2) chemoprevention of patients at high risk for malignancy.

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