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Permalink

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Journal

Preventive medicine, 18(5)

ISSN

0091-7435

Authors

Plezia, PM
Alberts, DS
Peng, YM
et al.

Publication Date

1989-09-01

DOI

10.1016/0091-7435(89)90039-x

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The Role of Serum and Tissue Pharmacology Studies in the Design and Interpretation of Chemoprevention Trials^{1,2}

PATRICIA M. PLEZIA, PHARM.D.,*†‡ DAVID S. ALBERTS, M.D.,*†§³
YEI-MEI PENG, PH.D.,*† MIN JIAN XU, M.D.,*† SUSAN SAYERS, B.S.,*
THOMAS P. DAVIS, PH.D.,†§ EARL A. SURWIT, M.D.,†|| AND
FRANK L. MEYSKENS, JR., M.D.*†

**Section of Hematology and Oncology, Department of Internal Medicine, College of Medicine, University of Arizona; †Arizona Cancer Center, University of Arizona; ‡Department of Pharmacy Practice, College of Pharmacy, University of Arizona; §Department of Pharmacology, College of Medicine, University of Arizona; and ||Departments of Obstetrics and Gynecology and Surgery, College of Medicine, University of Arizona, Tucson, Arizona 85724*

The design and interpretation of chemoprevention trials are challenging tasks. Innovative methodological approaches to these investigations are in initial stages of development. Important pharmacologic issues should be addressed as early as possible in these trials to facilitate the optimal design of large, Phase III, randomized trials. These include determining the optimal dose of the compound and the toxicity profile. Other key areas involve the use of serum concentrations to monitor subject compliance, the evaluation of concentration of the chemopreventive agent in the target tissue, adequate assessment of the drug delivery systems, and the evaluation of the relationship between the dose administered and the serum or tissue concentrations achieved. Whenever possible the investigation of the relationship between serum or tissue concentrations of a chemopreventive agent vs its biologic activity should be determined. Specific examples involving the retinoids and carotenoids are presented. © 1989 Academic Press, Inc.

INTRODUCTION

Clinical Pharmacology Objectives

Historically, the role of pharmacology in clinical trials has been limited by the development of analytical techniques to measure drugs in biological fluids and tissues. Only in the last several decades have methodologies been established which accurately and precisely quantitate many compounds and their metabolic products. Agents currently under study or proposed for use in chemoprevention trials often provide particularly difficult challenges in this regard. As a result, many agents including some retinoids and carotenoids have been available in pharmaceutical dosage form for years, but our knowledge of their disposition in the body is only now emerging.

The goal of this article is to highlight some of the objectives of clinical phar-

¹ Supported in part by grants CA27502 and CA23074 from the National Institutes of Health, Department of Health and Human Services.

² Presented at the Third International Conference on the Prevention of Human Cancer: Chemoprevention, Tucson, AZ, January 12-15, 1988.

³ To whom reprint requests should be addressed at Arizona Cancer Center, University of Arizona, 1515 North Campbell Avenue, Tucson, AZ 85724.

macology that are especially pertinent to the design and conduct of chemoprevention trials. Whenever analytically feasible, these objectives should be met as early in the clinical investigation of an agent as possible. As such, well-designed Phase II chemoprevention trials should be performed for all new agents prior to the initiation of large, randomized clinical trials. In our opinion, Phase II studies of chemopreventive agents should be multifaceted and designed to provide the maximum pharmacologic information concerning the agent in a relatively short period of time (i.e., within 12 months). Ideally, four main objectives should be addressed. The first is to determine the maximally tolerated safe (i.e., nontoxic) dose for the new agent. Because chemopreventive agents, by definition, will be used in generally healthy populations for relatively prolonged periods of time, it is critical that these studies be designed to investigate both acute and chronic dosing schedules. The second major objective is to establish organ system toxicity, including predictability, extent, duration, and reversibility of toxic effects. In addition, the pharmacokinetics of the compound, including the metabolic profile and tissue distribution, should be determined. Last, whenever possible as dictated by available techniques, these initial trials should observe for evidence of cancer chemoprevention activity.

CURRENT ISSUES IN RETINOID AND CAROTENOID PHARMACOLOGY

Unfortunately, because they are relatively old compounds which were marketed prior to the initiation of the stringent premarketing requirements for new agents today, very little of this information is known for many of the compounds currently undergoing extensive clinical investigation. It is critical that this void be filled to enable us to design efficient Phase III trials that utilize the optimal dose of the chemoprevention compound. The remainder of the discussion will focus on several specific pharmacologic issues, concerning the retinoids and carotenoids, which have been addressed in our program.

The Use of Serum Concentrations to Monitor Compliance

An important, practical concern of all chronic dosing trials is subject compliance. While numerous approaches have been used to estimate compliance, most rely on information provided to the investigators by the subject and are, therefore, biased. The use of serum plasma concentrations of an agent to corroborate compliance is an attractive alternative, but must be used with appropriate restraint. It may be impossible to differentiate noncompliance from intersubject, or even intrasubject, variability in pharmacokinetic parameters, or from unique pharmacokinetic characteristics of the agent. For example, one of our recently completed trials involved the evaluation of the pharmacokinetics and metabolism of 25,000 IU per day of retinol, administered for 9 months to 13 normal subjects between 50 and 67 years of age (D. S. Alberts, L. McDonald, L. Edwards, *et al.* *JNCI*, submitted for publication). Following the first day of dosing and at 3, 6, and 9 months, serial blood samples for plasma retinol and retinyl palmitate were obtained in all subjects for up to 72 hr after the previous dose of retinol. Shown in Table 1 are the mean (plus standard error) predose and maximum retinol concentration and maximum retinyl palmitate concentration determined in all 13 subjects

TABLE I
MEAN PLASMA PHARMACOKINETIC DATA FOR RETINOL (ROH) AND RETINYL PALMITATE (RP) IN
13 STUDY SUBJECTS OBTAINED ON THE FIRST DAY AND AT 3, 6, AND 9 MONTHS OF LOW DOSE
RETINOL ADMINISTRATION

	Time from first retinol dose (months)								<i>P</i> value
	0 ^a		3		6		9		
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	
ROH									
Baseline (ng/ml)	733.8	67.8	798.5	75.7	742.9	74.2	764.5	54.1	0.573
Maximum level over 72 hr (ng/ml)	859.1	106.6	889.3	88.1	818.2	73.7	788.1	54.6	0.041
RP									
Peak (ng/ml)	515.5	129.1	930.7	147.1	906.1	151.0	652.2	100.4	0.007
Half-life (hr)	16.1	2.3	14.8	1.5	16.5	1.2	22.5	7.4	0.863

^a First day of retinol dosing. The *P* value arises from a test of the hypothesis of no differences among the time periods. Friedman's test was used to perform the analysis.

at each of the four time intervals. There was no change in baseline plasma retinol concentration ($P = 0.573$) and there was actually some evidence for a decrease in the maximum plasma retinol concentration observed, although it was of marginal significance ($P = 0.041$). If, without any knowledge of retinol pharmacology, an investigator attempted to use plasma retinol concentrations as a measure of compliance, the results would be quite misleading. In contrast, the mean peak retinyl palmitate concentration increased significantly ($P = 0.007$) between Days 1 and 90 of the study period. Thus, this major retinol metabolite could prove useful for monitoring dosing compliance in study subjects entered into chemoprevention trials with 25,000 IU doses of retinol. The fact that retinyl palmitate has an intermediate half-life (14.8 to 22.5 hr) makes it a suitable candidate for the evaluation of compliance during the time period immediately prior (i.e., 1 week) to the plasma determination. It is important to recognize that while the knowledge that a blood sample will be obtained may serve to motivate a subject toward better compliance, this approach does not provide information concerning the long-term compliance history. As a result, serum concentration information must be used in conjunction with other tools including pill counts and subject diaries.

Evaluation of Target Tissue Concentration

A second important issue for investigation is the determination of whether the potential agent actually reaches the proposed site of action in the tissues. This was a second, very important objective of the previously described study of retinol pharmacokinetics (D. S. Alberts, L. McDonald, L. Edwards, *et al.* *JNCI*, submitted for publication). Skin and subcutaneous fat biopsies were obtained during or shortly after termination of daily retinol administration in 7 study subjects and 13 age-matched control subjects undergoing cosmetic surgery. The control subjects were not receiving retinol or other vitamin A supplements. Shown in Table 2 are the results of the skin distribution study. The mean retinol concentration was 131.7 ± 34.34 ng/g skin in the study subjects versus 118.9 ± 16.29 ng/g skin in the

TABLE 2
CONCENTRATIONS OF RETINOL (ROH) AND RETINYL PALMITATE (RP) IN SKIN BIOPSIES OBTAINED FROM 7 RETINOL-TREATED STUDY SUBJECTS AND 13 NORMAL CONTROLS^a

	Retinol study subjects (<i>n</i> = 7) Mean $\pm$ standard error	Control study subjects (<i>n</i> = 13) Mean $\pm$ standard error	<i>P</i> value
ROH skin (ng/g)	131.7 $\pm$ 34.34	118.9 $\pm$ 16.29	0.968
RP skin (ng/g)	15.9 $\pm$ 6.39	25.5 $\pm$ 6.65	0.393

Note. Study subjects received daily retinol doses of 25,000 IU for up to 9 months. Control subjects were not receiving retinol supplements at time of cosmetic procedures.

^a The comparisons were carried out using the Wilcoxon rank-sum test. The *P* values indicate that none of the comparisons are statistically significant (*P* > 0.05).

matched controls. This difference was not significant (*P* = 0.968). Additionally, there was no difference between the two groups with respect to mean retinyl palmitate concentrations in the skin. This observation raises the issue of whether the current clinical trials administering retinol in doses of 25,000 IU per day in the prevention of skin cancer may be using a dose that is too low to increase concentrations at the proposed site of biologic action.

Assessment of Drug Delivery Systems

A third pharmacologic issue which should be addressed in all clinical chemoprevention trials is an evaluation of the drug delivery system employed. Since many of the compounds that are being proposed for chemoprevention trials are considered food products or nutritional supplements, manufacturers are often not required to demonstrate absorption and bioavailability data prior to marketing the compound. Even prior to these investigations, however, the dosage form should be analyzed to determine the actual content of the agent as well as the within- and between-lot variability, which can be substantial. Such studies must be completed prior to initiation of efficacy trials to demonstrate that the compound is released from the dosage form and is consistently absorbed by the population under study. Equally important, if a change in dosage form becomes necessary during a trial, these studies should be repeated to determine whether the two products are bioequivalent. Along these lines, we have been involved with the evaluation of cervical tissue uptake of all-*trans*-retinoic acid (TRA) delivered via a collagen sponge-cervical cap. This delivery device is being used in a Phase III randomized, placebo-controlled trial in patients with moderate or severe intraepithelial cervical dysplasia. The content and uniformity of TRA in the cream vehicle was confirmed by high-performance liquid chromatography analysis prior to patient entry.

In an initial dose finding trial (1), 10 patients consented to participate in this pharmacologic study. The TRA delivery system consisted of a cervical cap (Sky Biopolymers, Princeton, NJ) within which a collagen sponge was inserted. The delivery system has been described in detail in a previous publication (2). The uptake of TRA into cervical tissues was studied using tritiated compound. Five hundred microCuries of [³H]TRA (0.0917 mg) plus 1 ml of cold TRA (0.05 and 0.372%) were applied to the collagen sponge and the sponge-cervical cap device

was inserted into the vaginal vault and examined for proper placement. The device remained in place for 24 hr.

Cervical biopsies, including endocervical curettage, deep stroma, and epithelial cervix were obtained from each patient at 4 and 24 hr after removal of the delivery system. Post-treatment blood samples were also obtained at various times for 24 hr after dosing in selected patients to determine systemic exposure to TRA by HPLC analysis. The mean ($\pm$ standard deviation) cervical tissue TRA uptake results are presented in Table 3.

Mean concentrations of [^3H]TRA equivalents at 4 hr were substantially higher in both epithelial cervix and deep stroma when the higher concentration of TRA was administered. The difference reached statistical significance for the deep stroma comparison. The tissue concentrations of TRA decreased rapidly over the 24-hr post-treatment period, suggesting a short half-life of drug in the cervical tissue. There was no difference between the two dosage strengths at 24 hr. Serum concentrations analyzed for TRA indicated that there was no systemic absorption of the compound. The results of this study provided valuable information for use in designing Phase III trials with this system. The release of drug from the dosage form and uptake into the target tissue was documented. In addition, we were able to demonstrate a dose-response relationship for the two concentrations studied. The lack of systemic concentrations of the compound corroborates the lack of toxicity reported in earlier clinical trials.

Recently, the Sky Biopolymer cap has become unavailable and as a result we have begun using a cap provided by Lamberts-Dalston (Luton, England). We have just completed evaluation of what had to be considered an entirely new drug delivery system. Table 4 shows the comparison of tissue uptake of TRA at an administered dose of 0.372%. Although not statistically significantly different in this small population, the Lamberts-Dalston system resulted in substantially higher mean concentrations in the epithelial cervix at 4 hr and in the epithelial cervix and deep stroma at 24 hr. These differences will be an important consideration when the results of the ongoing clinical trial are evaluated.

Relationship between Dose and Serum/Tissue Concentrations

Another important objective under study in our program is to determine if a

TABLE 3
UPTAKE OF TRA INTO CERVIX TISSUES IN PATIENTS WITH MILD TO MODERATE DYSPLASIA

Patients	Dose TRA (%)	Tissue uptake of TRA (ng/g wet wt) (mean values)			
		At 4 hrs		At 24 hrs	
		Epithelial cervix	Deep stroma	Epithelial cervix	Deep stroma
1-5	0.05	161	98	45	16
6-10	0.372	1,953	2,079	70	45
Mann-Whitney <i>P</i> value		0.076	0.009	0.724	0.059

TABLE 4
UPTAKE OF TRA^a INTO CERVIX TISSUES IN PATIENTS WITH MILD TO MODERATE DYSPLASIA

Patients	Type cervical cap	Tissue uptake of TRA (ng/g wet wt) (mean values)			
		At 4 hrs		At 24 hrs	
		Epithelial cervix	Deep stroma	Epithelial cervix	Deep stroma
6-10	Sky polymers	1,953	2,079	70	45
11-13	Lamberts-Dalston	5,666	2,084	395	702
Wilcoxon Score <i>P</i> value		0.136	0.371	0.052	0.702

^a TRA administered in a dose of 0.372%.

relationship exists between the dose of the chemopreventive agent administered and the plasma and/or target tissue concentration achieved. An ongoing trial which addresses these questions is a β -carotene dose-finding trial in nonsmoking, healthy volunteers between the ages of 50 and 65 years of age. After a 3-month placebo compliance evaluation period, subjects are randomized to receive either placebo, 15, 30, 45, or 60 mg of β -carotene daily for 9 months. Additional objectives of this trial are to determine what, if any, toxicities are present during chronic administration of these β -carotene doses and to evaluate the possible role of plasma β -carotene in the assessment of compliance.

Monthly plasma and buccal mucosal samples are obtained from each subject. Full thickness skin and subcutaneous fat biopsies are obtained at three specified time intervals: while on placebo, between 5 and 7 months, and again between 10 and 12 months. In addition, all subjects are monitored intensively for comprehensive dietary intake throughout the study period. An important distinction between this study and the previously described retinol trial is the use of a placebo arm. The addition of a control group is especially useful when studying compounds found in normal diets, which may be subject to seasonal or other systematic variations.

Forty-six subjects have been enrolled into this ongoing study. Preliminary data from six subjects for baseline β -carotene, retinol, and retinyl palmitate concentrations in plasma and skin are presented in Table 5. Despite dietary counseling designed to limit dietary intake of β -carotene to 7.5 mg per day, a very wide range of baseline concentrations were found. In this small sample size, there appears to be a trend toward higher skin concentrations of β -carotene in the three subjects who had the highest plasma values. In addition, skin retinol and retinyl palmitate concentrations appear to follow the same trend as plasma and skin β -carotene concentrations. While these data await verification, this preliminary analysis raises some interesting pharmacologic issues concerning the interrelationship of these compounds in various biologic fluids and tissues.

Subjects in this trial have been randomized to active capsules within the last month. Information concerning the effect of supplementation with different doses of β -carotene will be available within the next year. An integral part of this analysis will be the evaluation of whether there is a linear relationship between β -carotene dose and plasma concentration or area under the plasma concentration

TABLE 5
CONCENTRATIONS OF β -CAROTENE, RETINOL, AND RETINYL PALMITATE IN PLASMA AND SKIN OF OLDER SUBJECTS

Subject No.	β -Carotene		Retinol		Retinyl palmitate	
	Plasma (ng/ml)	Skin (ng/g)	Plasma (ng/ml)	Skin (ng/g)	Plasma (ng/ml)	Skin (ng/g)
1	412	73	614	103	35	23
2	339	103	405	137	20	14
3	385	78	434	119	22	5
4	54	36	513	68	8	ND ^a
5	44	51	810	70	52	ND ^a
6	46	ND ^a	727	32	8	ND ^a

^a ND, not detectable.

versus time curve. This pharmacokinetic information could aid the interpretation of the clinical results of various chemopreventive trials now underway which use a wide range of β -carotene doses.

Assessment of Chemopreventive Activity

Despite the fact that the compound is already marketed in the United States, this β -carotene trial closely parallels the Phase II study design described earlier. In addition to evaluating the effect of dose on toxicity and serum and tissue concentrations of β -carotene, an attempt is also being made to evaluate a biologic measure of chemopreventive activity. The methods currently available to determine short-term endpoints in this area are limited, but several promising approaches have been identified. TPA induction of ornithine decarboxylase (ODC) is a simple *in vitro* test system to evaluate the *in vivo* effects of biologic modifiers (3). TPA-induced ODC activity will be determined in skin biopsies obtained from the study subjects receiving various doses of the agent to evaluate whether any of the β -carotene doses are capable of inhibiting this measure of polyamine synthesis in the epidermis. This test, or others, may prove useful to help identify an optimal dose (based upon a measure of biologic activity) to be used in future trials. This particular test may be especially useful for skin cancer prevention trials.

SUMMARY

Chemoprevention research is in the very early stages of development. Little information is available concerning the clinical pharmacology/pharmacokinetics of many of the potentially active compounds. Continued research in this area will determine whether intrasubject variability in disposition of these compounds can, at least partially, explain the clinical response observed. In addition, this information will allow the design of the most efficient Phase III trials, thereby making the optimal use of the limited available resources.

ACKNOWLEDGMENTS

We acknowledge the important contributions of the following individuals: Alison Culling-Berglund, Vivan Graham, Dava Kendall, Linda McDonald, and Donald Slymen.

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