Featured Article

Safety and Efficacy of Dose-Intensive Oral Vitamin A in Subjects with Sun-Damaged Skin

David Alberts,[1,2,3,4] James Ranger-Moore,[2,4]
Janine Einspahr,[1,2] Kathylynn Saboda,[2]
Paul Bozzo,[2] Yun Liu,[2] Xiao-chun Xu,[8]
Reuben Lotan,[8] James Warneke,[2,5]
Stuart Salasche,[2,6] Suzanne Stratton,[1,2,3]
Norman Levine,[1,2,6] Rayna Goldman,[2]
Marcy Islas,[2] Laura Duckett,[2]
Deborah Thompson,[7] and Peter Bartels[2,7]

[1]Department of Medicine, [2]Arizona Cancer Center, [3]College of Medicine, [4]College of Public Health, [5]Department of Surgery, [6]Section of Dermatology, and [7]Optical Sciences Center, University of Arizona, Tucson, Arizona, and [8]University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

Purpose: Previously, we reported the results of a Phase III, placebo-controlled trial in 2,297 randomized participants with moderately severe actinic keratoses wherein 25,000 IU/day vitamin A caused a 32% risk reduction in squamous cell skin cancers. We hypothesized that dose escalation of vitamin A to 50,000 or 75,000 IU/day would be both safe and more efficacious in skin cancer chemoprevention.

Experimental Design: One hundred and twenty-nine participants with severely sun-damaged skin on their lateral forearms were randomized to receive placebo or 25,000, 50,000, or 75,000 IU/day vitamin A for 12 months. The primary study end points were the clinical and laboratory safety of vitamin A, and the secondary end points included quantitative, karyometric image analysis and assessment of retinoid and rexinoid receptors in sun-damaged skin.

Results: There were no significant differences in expected clinical and laboratory toxicities between the groups of participants randomized to placebo, 25,000 IU/day, 50,000 IU/day, and 75,000 IU/day. Karyometric features were computed from the basal cell layer of skin biopsies, and a total of 22,600 nuclei from 113 participants were examined, showing statistically significant, dose-response effects for vitamin A at the 25,000 and 50,000 IU/day doses. These karyometric changes correlated with increases in retinoic acid receptor α, retinoic acid receptor β, and retinoid X receptor α at the 50,000 IU/day vitamin A dose.

Conclusions: The vitamin A doses of 50,000 and 75,000 IU/day for 1 year proved safe and equally more efficacious than the 25,000 IU/day dose and can be recommended for future skin cancer chemoprevention studies.

Introduction

Vitamin A (*e.g.*, retinyl palmitate and retinol) and its analogs have shown activity as chemopreventive agents in a wide range of human intraepithelial neoplasias, including actinic keratosis and oral leukoplakia (1–3). In one large Phase III trial of orally administered retinol (25,000 IU/day for up to 5 years) in 2,297 randomized participants with evidence of moderate to severe actinic keratosis, the vitamin A intervention was associated with minimal toxicity (*i.e.*, mild elevation of serum lipids) and a 32% reduction in the risk of squamous cell carcinoma (1, 4). Having previously shown that vitamin A doses as high as 300,000 IU/day were well tolerated for up to 1 year in patients with advanced cancers, we were interested in evaluating the safety and efficacy of retinyl palmitate doses in the intermediate range of 25,000–75,000 IU/day in the treatment of patients with sun-damaged skin (5).

It is well documented that decades of UV exposure lead to the development of severely sun-damaged skin and, ultimately, over 1 million new cases of nonmelanoma skin cancers in the United States per year (6, 7). Chronic sun exposure causes cumulative DNA damage in nuclei from the basal and suprabasal cell layer (8, 9) in the skin. Previous research has shown that this DNA damage in human tissues can be expressed in nuclear chromatin patterns (*i.e.*, in the spatial and statistical distribution of chromatin) and that the distributional characteristics of chromatin can be quantified using a set of nuclear features (8). Based on 93 nuclear chromatin features, a progression curve for UV-induced skin DNA damage was derived wherein the deviation of nuclei from "normal" undergoes a monotonic change toward abnormal feature values found in actinic keratoses [AKs (8)]. In subsequent studies, we documented that chemopreventive interventions with topically administered α-difluoromethylornithine or oral vitamin A can halt UV-induced actinic damage and cause regression of lesions along a previously described progression curve (9, 10).

The primary objective of this study was to determine whether the effects of taking the chemopreventive agent, vitamin A, in intermediate to moderately high daily oral doses would be well tolerated and could be quantitatively measured by karyometric and retinoid receptor analyses in the skin of individuals with visually and histologically normal, sun-damaged skin.

Materials and Methods

Source of Clinical Material and Storage

Vitamin A (retinyl palmitate) and matched placebo were obtained from Knoll Pharmaceuticals (a subsidiary of BASF),

Whippany, New Jersey. Gelatin capsules containing 25,000 IU (and matched placebo containing pure vegetable oil) were encapsulated by Banner/Pharmacaps. Butylated hydroxytoluene, a preservative, was added to the drug vehicle to maintain potency and integrity of the retinyl palmitate for the duration of the study. Study drug and placebo were packaged in 1 month pill packs for each dose (25,000, 50,000, and 75,000 IU) and stored at room temperature.

Vitamin A Clinical Protocol

Participant Eligibility Criteria. All participants had moderate to severe sun damage with or without AKs on their posterior forearms at the time of enrollment into the study. Eligible participants could also have a history of two prior nonmelanoma skin cancers. Participants were required to be at least 50 years old and in general good health with no history of invasive cancer, cardiovascular events, strokes, or other serious diseases. Female participants had to be postmenopausal. Written informed consent, according to institutional and NIH guidelines, was obtained from all participants before any study-related assessments.

Participant Procedures. Participants were randomized to receive placebo or orally administered 25,000, 50,000, or 75,000 IU retinyl palmitate daily for at least 12 months. Participants were instructed to keep a daily log of medication intake and potential toxicities and to return all unused study drug. One-quarter-inch shave biopsies were obtained from buttock skin just below the belt line in an area that was not sun-damaged. Biopsies were also taken from sun-damaged skin on the widest surface of the posterior forearms both at baseline and at the end of 1 year of the study drug intervention. The skin biopsies were fixed in formalin, embedded in paraffin, stained with H&E, and stored at 25°C for subsequent histopathological and karyometric analysis (9–11).

Safety Evaluation. All randomized participants had baseline and 1 year follow-up physical examinations, including detailed skin evaluations by specially trained physicians (J. W. and N. L.). Complete blood counts with differentials and full blood chemistry panels including liver enzymes (aspartate aminotransferase and alanine aminotransferase) and serum lipid levels were obtained twice during the placebo run-in period and then at months 3, 6, 9, 12, and 15 with a follow-up 3 months later. Plasma retinyl palmitate level determinations were obtained every month and analyzed every 3 months using a previously described high-performance liquid chromatography method (12). As an additional safety procedure, all participants had a baseline nuclear liver scan. If the aspartate aminotransferase or alanine aminotransferase reached $3\times$ the upper limit of normal, the participant was to undergo a second nuclear scan to evaluate for evidence of fatty infiltration of the liver. Evidence of a significant nuclear liver scan change from baseline would require removal from study.

The rates of all toxicities or other adverse events were compared across placebo and treatment groups using Fisher's exact test. This statistical analysis was also performed on toxicity rates that were possibly related to drug administration.

Karyometric Analyses. Karyometric analysis methods have been described in detail in a recent publication by Bartels *et al.* (10) concerning the evaluation of chemopreventive agent activity in sun-exposed, histologically normal-appearing skin. An abbreviated description of those methods follows. Data recording was done with a 100:1 planapochromatic oil immersion objective, N.A. 1.40 (Zeiss, Oberkochen, Germany). The relay optics adjusted image sampling to 6 pixels/μm. Image recording was done with a Sony DXC9000 3CCD color camera (Melville, NY). For maximum contrast in the H&E-stained sections, only the red channel image was used for feature computation. Nuclei were chosen at random for segmentation from the basal cell and suprabasal cell layer.

After segmentation, a set of 93 karyometric features was computed for each nucleus. These provided numeric values for nuclear features that include nuclear area, total absorbance, variance of pixel absorbance values (3 features); histograms of pixel absorbance values at 0.10 absorbance intervals (18 features); the upper diagonal of the co-occurrence matrix of pixel absorbance values consisting of six absorbance intervals, each 0.30 absorbance unit wide (21 features); run length features consisting of six absorbance intervals, each 0.30 absorbance unit wide, *versus* six run length intervals from 1–2 pixels to 11–12 pixels (36 features); and a number of summarizing features of the chromatin texture, such as run length emphasis, pixel absorbance, and histogram shape [15 features (13, 14)].

Several discriminant functions (DFs) using karyometric features were derived (10). The first, DF I, was devised to separate nuclei from the buttock skin from the nuclei of sun-damaged forearm skin. On the basis of DF I, sun-exposed nuclei could be divided into categories of low and high levels of actinic damage, and a second DF (DF II) was developed (10). DF II was based on all nuclei, even though many were likely to be undamaged. For maximum sensitivity to the subtle changes expected in tissue that still appeared "normal" to the human eye, a third more sensitive DF was developed. Specifically, DF III was termed "second-order" because it was derived using DF II scores, rather than the original nuclear karyometric features. DF III focused on differences in the distributions of DF II scores before and after treatment.

Differences between treatment groups were assessed at the case level by comparing distributions of DF III scores with statistical significance determined on the basis of ANOVA (10). Also compared was the percentage of cases with decreased or increased actinic damage levels over time, measured as mean DF III scores. Statistical significance was determined using tests of proportions.

Retinoid Receptor Analyses. Specimens were obtained from the lateral forearm skin shave biopsies. The specimens were routinely fixed in 10% neutral formalin and embedded in paraffin. The paraffin blocks were then cut into 5-μm-thick sections, which were collected in a bath containing diethylpyrocarbonate-treated water to prevent RNase contamination (15, 16). For the same reason, the glass microscope slides used had been cleaned and baked at 180°C for 4 h and then coated with poly-L-lysine (Sigma Chemical Co., St. Louis, MO). A nonradioactive *in situ* hybridization method was used based on digoxygenin-labeled antisense riboprobes prepared for each of the six receptors, as described previously (16, 17). The quality and specificity of the digoxigenin-labeled probes were determined by Northern blot analysis (16). The specificity of the binding of antisense riboprobes was verified by negative control

Table 1 Baseline demographic and actinic keratosis characteristics in all 129 randomized participants

	Placebo	25,000 IU	50,000 IU	75,000 IU
Randomized	31	32	33	33
Completing trial	30	29	27	30
Males	18	20	22	22
Mean age (yrs)	63.6	62.9	64.0	64.5
Females	13	12	11	11
Mean age (yrs)	64.3	61.2	61.9	64.3
No. with AKs[a] (%)	16 (52)	24 (75)	18 (54)	22 (67)
Median no. of AKs per participant	9.0	7.5	7.5	11.5

[a] AK, actinic keratoses.

sections to which sense probes were hybridized or no probe was hybridized. These controls were found to be negative. The stained sections were reviewed with a Nikon microscope. Lesion or normal skin staining within each section was scored from 0–4 as follows: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining; and 4, very strong staining.

For each of the two time points (baseline and 1 year), 2 biopsies/person were assessed for staining intensity. Thus, the available data consisted of repeated ordinal measures by participant and study time. A mixed model was used for data analysis because such an approach allows for explicit modeling of the correlation structure inherent in repeated measures. An unstructured correlation matrix provided the best model fit. A Poisson link function was used to model the non-normally distributed receptor expression intensity levels. To initially assess treatment effects, the dose levels were treated as categorical to avoid assumptions about dose response. Treatment and time were considered to be fixed effects, whereas patient effects were considered to be random effects. For each treatment level, least square mean intensity levels and their 95% confidence intervals were estimated. Differences in least square means between treatment levels were assessed for statistical significance.

A second set of analyses looked for dose-response trends in each of the receptors. For those analyses, dose levels were coded as continuous, and Ps for the slope of receptor expression intensity levels over time were determined.

Results

Clinical Data

Demographics. Shown in Table 1 are the demographic data and actinic keratosis characteristics for the 129 randomized participants. Sixty-four percent of the participants were male, and 36% were female. The mean age for males was 63.8 years, and the mean age for females was 62.9 years. Mean age did not differ significantly across treatment groups. Because only about 60% of participants had clinically evaluable AKs on their forearms, it was not feasible to use actinic keratosis count as an efficacy end point in this study. Nevertheless, there was good balance between the four randomized intervention groups with respect to the mean number of forearm AKs in those participants with clinically evaluable AKs.

Participant Completion and Toxicities. As shown in Table 2, 116 of the 129 randomized participants (90%) com-

pleted the 1-year intervention. There were no statistically significant differences in completion rates between the four randomized groups, with completion rates ranging from 96.8% for the placebo group to 81.8% for the 50,000 IU/day treatment groups ($P = 0.277$, Fisher's exact test). Under the intent-to-treat design, all 129 participants were evaluated for toxicities. The percentage of subjects experiencing clinical or blood toxicities did not differ significantly across treatment groups ($P = 0.501$ and 0.228, respectively, by Fisher's exact test). Blood toxicities of any grade or type were uncommon. Overall, there was no evidence of dose-response-related toxicities to the vitamin A interventions.

Incidences of specific clinical and laboratory toxicities of any grade experienced by the 129 randomized participants are shown in Tables 2 and 3. There was no evidence for a dose response for any of these toxicities, including alopecia, cheilitis, conjunctivitis, dry skin, peeling, epistaxis, headache, muscle stiffness, dysuria, exanthema, serum liver function tests (*i.e.*, aspartate aminotransferase, alanine aminotransferase, and serum alkaline phosphatase), or serum triglycerides. Additionally, because liver scans were only to be repeated in participants who experienced severe clinical or laboratory-related toxicities, there were no participants who underwent repeat liver scans during this clinical trial.

Karyometry Results. An extensive discussion of the statistics underlying the karyometric analyses in this study can be found in a publication by Bozzo *et al.* (9). As the primary efficacy end point in this study, effects of vitamin A were assessed by comparing the percentage of participants at each dose level experiencing increases or decreases in overall levels of actinic damage over 1 year, measured as the mean DF III score across all nuclei within each case. Here, results are given only for subjects who completed the study. An intent-to-treat analysis, in which dropouts are assumed not to have changed in terms of their DF III scores, yielded qualitatively similar results.

In the placebo group, only 25.0% of cases (7 of 28) showed decreased damage. For the three vitamin A groups in ascending order of dose level, statistically significant decreases in actinic damage levels occurred in 64.5% of cases for the 25,000 IU/day dose (20 of 31; $P = 0.004$), 80.8% of cases for the 50,000 IU/day dose (21 of 26; $P < 0.001$), and 78.6% of cases for the 75,000 IU/day dose (22 of 28; $P < 0.001$), respectively, as seen in Fig. 1.

A vitamin A-related dose response is clearly shown from placebo to 50,000 IU/day vitamin A, after which the effect reached a plateau. A logistic regression model, based on the assumption that dose was a continuous measure, confirmed the statistical significance of this relationship ($P < 0.001$).

Up-Regulation of Retinoid Receptors. As a secondary biomarker end point, retinoid receptor expression was analyzed in the skin shave biopsies of vitamin A-treated participants at baseline and after 1 year of intervention as described earlier. Shown in Figs. 2–5 are least square mean estimates of the changes in receptor expression levels of retinoic acid receptor (RAR)-α, RAR-β, RAR-γ, and retinoid X receptor (RXR)-α, respectively, after 12 months of the four interventions. Treatment with oral vitamin A up-regulated vitamin A receptor levels significantly, especially at higher doses. Specifically, increases in RAR-α, RAR-β, and RXR-α in participants on 50,000 IU/day

Table 2 Participant study completion and toxicities experienced according to vitamin A treatment status

Laboratory test	Placebo $N = 31$ (%)	25,000 IU $N = 32$ (%)	50,000 IU $N = 33$ (%)	75,000 IU $N = 33$ (%)	Totals $(N = 129)$
Completed study	30 (96.8)	29 (87.8)	27 (81.8)	30 (90.9)	116
Clinical toxicities	7 (22.6)	13 (40.6)	11 (33.3)	10 (30.3)	41
Dose reductions	7 (22.6)	5 (15.6)	7 (21.2)	9 (27.3)	28
Blood toxicities	3 (9.7)	1 (3.1)	5 (15.2)	6 (18.2)	15
Serum triglycerides	3 (9.7)	1 (3.1)	4 (12.1)	5 (15.2)	
SGOT[a]	0	0	0	1 (3.0)	
SGPT	0	0	1 (3.0)	1 (3.0)	
Alkaline phosphatase	0	0	0	0	

[a] SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase.

Table 3 Number (percentage) frequency of highest grade clinical toxicity for the 129 randomized participants according to randomization group

Toxicity type	Placebo $(N = 31)$	25,000 IU $(N = 32)$	50,000 IU $(N = 33)$	75,000 IU $(N = 33)$
Alopecia				
Mild	2 (6.45)	4 (12.50)	2 (6.06)	2 (6.06)
Moderate	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.03)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Cheilitis				
Mild	0 (0.00)	2 (6.25)	3 (9.09)	2 (6.06)
Moderate	0 (0.00)	0 (0.00)	1 (3.03)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Conjunctivitis				
Mild	0 (0.00)	1 (3.13)	0 (0.00)	0 (0.00)
Moderate	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Dry skin				
Mild	2 (6.45)	1 (3.13)	2 (6.06)	1 (3.03)
Moderate	0 (0.00)	2 (6.25)	1 (3.03)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Dysuria				
Mild	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Moderate	0 (0.00)	0 (0.00)	1 (3.03)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Epistaxis				
Mild	0 (0.00)	3 (9.38)	0 (0.00)	0 (0.00)
Moderate	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Fatigue				
Mild	1 (3.23)	2 (6.25)	2 (6.06)	5 (15.15)
Moderate	1 (3.23)	1 (3.13)	0 (0.00)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Headache				
Mild	3 (9.68)	3 (9.38)	2 (6.06)	0 (0.00)
Moderate	1 (3.23)	1 (3.13)	0 (0.00)	0 (0.00)
Severe	0 (0.00)	1 (3.13)	0 (0.00)	0 (0.00)
Myalgias				
Mild	2 (6.45)	2 (6.25)	1 (3.03)	1 (3.03)
Moderate	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Severe	1 (3.23)	1 (3.13)	1 (3.03)	3 (9.09)
Nausea				
Mild	0 (0.00)	2 (6.25)	1 (3.03)	1 (3.03)
Moderate	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Severe	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

decline was statistically significantly different from zero for RXR-α and nearly so for RAR-α. Note that these comparisons are relative to no change in expression level. Figs. 2–5 show the 95% confidence intervals for changes in receptor levels, and these clearly demonstrate that the increases in receptor levels at the various treatment levels did not differ significantly from placebo, perhaps due to the limited amount of information provided by a five-point ordinal scale. However, additional analyses were conducted that treated dose as a continuous variable and tested for dose-response up-regulation. For two of the receptors, statistically significant dose-response effects were observed ($P = 0.0155$ for RAR-α and $P = 0.0171$ for RAR-γ).

Discussion

We have had a long-term interest in vitamin A as a skin cancer chemopreventive agent and have completed Phase I, II, and III clinical trials with varying vitamin A doses (1, 5). In our Phase III trial, we documented a 32% risk reduction for squamous cell skin cancers associated with a mean 3.5-year vitamin A intervention at 25,000 IU/day in 2,297 participants with evidence of multiple AKs on the face and/or forearms (1). Because there was virtually no vitamin A-related toxicity at the 25,000 IU/day dose level, we initiated the present study to evaluate the safety of 2- and 3-fold increases in vitamin A doses for a period of 1 year. As documented in this manuscript, vitamin A doses could be increased safely to 50,000 and 75,000
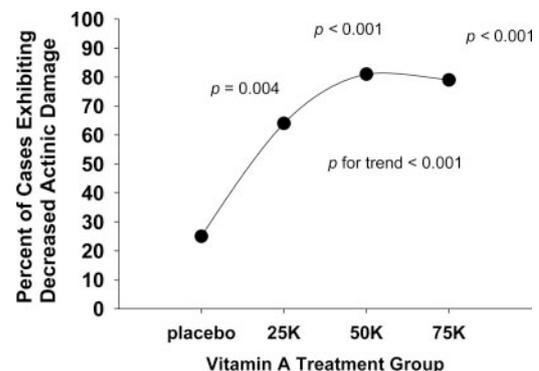

Fig. 1 Percentage of cases exhibiting decreased actinic damage as measured by change in the mean discriminant function III score from baseline to end of study by vitamin A dose level. *P*s associated with each dose group show comparisons with placebo.
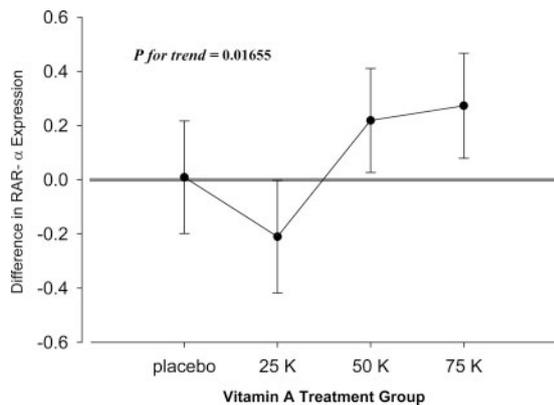
and increases in RAR-α and RAR-γ in participants on 75,000 IU/day were statistically significantly greater than zero. With the exception of RAR-γ, receptor levels appeared to be down-regulated in the group receiving 25,000 IU/day vitamin A. This

Fig. 2 Least square mean estimates and 95% confidence intervals for retinoic acid receptor α expression by vitamin A treatment group.
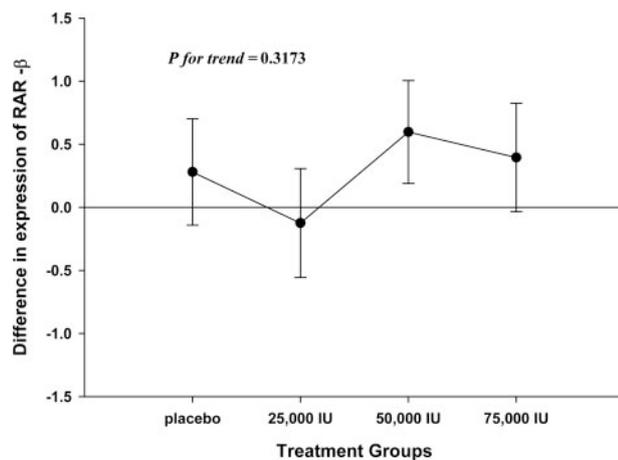

Fig. 3 Least square mean estimates and 95% confidence intervals for retinoic acid receptor β expression by vitamin A treatment group.

IU/day for the 1-year period with no evidence of differences in the rate of vitamin A-related toxicities in comparison with either placebo or 25,000 IU/day.

We previously published that long-term vitamin A dosing at 25,000 IU/day can raise cholesterol slightly and that this could increase coronary artery disease risk (4). More recently, it has been reported that long-term vitamin A dosing can result in loss in bone mineral density and increased risk for weight-bearing bone fractures (18). Obviously, these serious long-term toxicities must be considered in any clinical decision to use oral vitamin A for skin cancer chemoprevention.

As primary and secondary efficacy end points for this trial, we quantitated potential vitamin A-associated karyometric and retinoid receptor concentration changes in sun-damaged skin on the lateral forearms. There was a highly significant reduction of lateral forearm epidermal cell actinic damage associated with the 50,000 and 75,000 IU/day vitamin A doses. Furthermore, there was significant up-regulation of epidermal cell retinoid receptors at these dose levels, providing a mechanism-driven explanation for the positive karyometric results. It is thought that the anticancer effects of vitamin A and its analogs are

mediated through changes in gene expression by activating a signal transduction pathway in which nuclear RARs play a pivotal role (19, 20). These receptors can be divided into two types: RARs and RXRs. Each type includes three subtypes (α, β, and γ). RXR-RAR forms heterodimers that bind to a specific DNA sequence, called the RA response element, in the promoter regions of target genes (19, 20) to regulate expression of the target genes. Decreased expression of nuclear retinoid acid receptors and their induction by retinoids have been reported in skin cancers and premalignant lesions (see review in Ref. 21). The present study demonstrates that vitamin A increased expression of RARs and RXR-α and decreased karyometric scores in sun-damaged skins (skin premalignant lesions), especially at high doses (≥50,000 IU) of vitamin A, suggesting that the ability of retinoids to prevent skin carcinogenesis is mediated through nuclear retinoid receptors.

For two of the receptors, a dose-response up-regulation was observed. Taken together, these data strongly support both the safety and efficacy of the 50,000 IU/day vitamin A dose as a
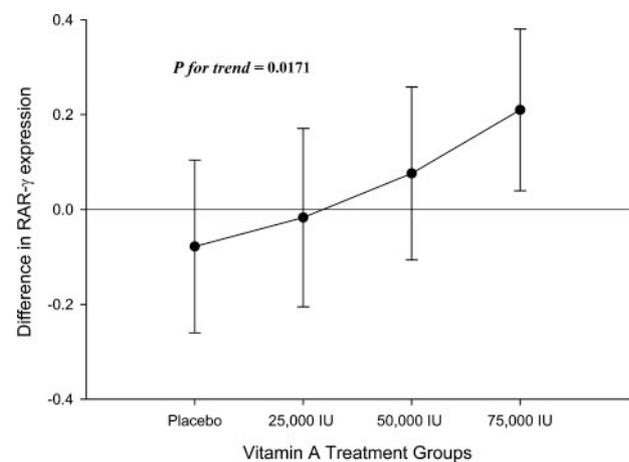

Fig. 4 Least square mean estimates and 95% confidence intervals for retinoic acid receptor γ expression by vitamin A treatment group.
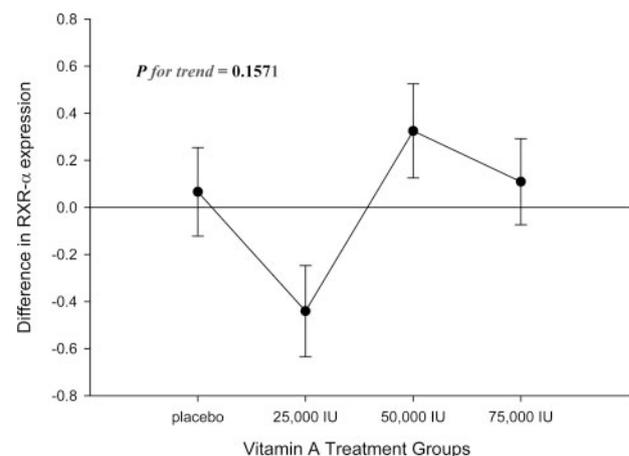

Fig. 5 Least square mean estimates and 95% confidence intervals for retinoid X receptor α expression by vitamin A treatment group.

replacement of the intermediate 25,000 IU/day dose for use in future skin cancer chemoprevention trials.

Our quantitative karyometric analysis data suggest that orally administered vitamin A is effective as a skin cancer chemopreventive agent by reducing the levels of actinic nuclear damage as measured by average nuclear abnormality levels and DF scores derived from appropriate karyometric features. DF analysis has proven useful in past analyses to discern progression to cancer in normal-appearing tissue (9, 10). The development of a second-order DF was necessary because the expected effects were small compared with natural epidermal cell DNA variability, a situation generally encountered in chemoprevention studies. This method demonstrated extreme sensitivity in detecting net changes among approximately 15% of epidermal cell nuclei, with average nuclear abnormality differences on the order of 0.15 SD.

Elsewhere we have documented a dose-response relationship between oral vitamin A doses of 25,000–75,000 IU/day and plasma concentrations of both 13-*cis*-retinoic acid and all-*trans*-retinoic acid (22). These findings underlie our evidence for a dose-response up-regulation of epidermal cell retinoid receptors and provide the mechanistic basis for the salutary oral vitamin A effects on sun-damaged skin and, ultimately, the previously documented reduction in risk of squamous cell carcinoma (1).

References

1. Moon TE, Levine N, Cartmel B, et al. Effect of retinol in preventing squamous cell skin cancer in moderate-risk subjects: a randomized, double-blind, controlled trial. Cancer Epidemiol Biomark Prev 1997;6: 949–56.

2. Hong WK, Endicott J, Itri LM, et al. 13-cis-Retinoic acid in the treatment of oral leukoplakia. N Engl J Med 1986;315:1501–5.

3. Lippman SM, Batsakis JG, Toth BB, et al. Comparison of low-dose isotretinoin with β-carotene to prevent oral carcinogenesis. N Engl J Med 1993;328:15–20.

4. Cartmel B, Moon TE, Levine N. Effects of long-term intake of retinol on selected clinical and laboratory indexes. Am J Clin Nutr 1999;69:937–43.

5. Goodman GE, Alberts DS, Earnest DL, Meyskens FL. Phase I trial of retinol in cancer patients. J Clin Oncol 1983;1:394–9.

6. Fry RJM. Ultraviolet radiation-induced skin cancer. In: Conti CJ, Slata TJ, Andres JP, Klein-Szanto JP, editors. Skin tumors: experimental and clinical aspects. New York: Raven Press; 1989,321–37.

7. Johnson TM, Dolan OM, Hamilton TA, et al. Clinical and histologic trends of melanoma. J Am Acad Dermatol 1998;38:681–6.

8. Bozzo PD, Vaught LC, Alberts DS, Thompson D, Bartels PH. Nuclear morphometry in solar keratosis. Anal Quant Cytol Histol 1998; 20:21–8.

9. Bozzo PD, Alberts DS, Vaught LC, et al. Measurement of chemopreventive efficacy in skin biopsies. Anal Quant Cytol Histol 2001;23: 300–12.

10. Bartels PH, Ranger-Moore J, Stratton MS, et al. Statistical analysis of chemopreventive efficacy of vitamin A in sun-exposed, normal skin. Anal Quant Cytol Histol 2002;24:185–97.

11. Bartels PH, daSilva VD, Montironi R, et al. Chromatin texture signatures in nuclei from prostate lesions. Anal Quant Cytol Histol 1998;20:407–16.

12. Peng YM, Alberts DS, Xu MJ, et al. Effects of high dietary retinyl palmitate and selenium on tissue distribution of retinoids in mice exposed to tumor initiation and promotion. J Nutr Growth Cancer 1986; 3:33–40.

13. Galloway M. Texture analysis using gray level run lengths. Comput Graph Image Process 1975;4:172–9.

14. Young T, Verbeek PW, Mayall B. Characterization of chromatin distributions in cell nuclei. Cytometry 1986;7:467–74.

15. Xu X-C, Wong WY, Goldberg L, et al. Progressive decreases in nuclear retinoid receptors during skin squamous carcinogenesis. Cancer Res 2001;61:4306–10.

16. Xu XC, Clifford JI, Hong WK, Lotan R. Detection of nuclear retinoic acid receptor mRNAs in histological tissue sections using non-radioactive in situ hybridization histochemistry. Diagn Mol Pathol 1994;3:122–131.

17. Lotan R, Xu C, Lippman SM, et al. Suppression of retinoic acid receptor β in oral premalignant lesions and its upregulation by isotretinoin. N Engl J Med 1995;332:1405–10.

18. Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. J Am Med Assoc 2003;287:47–54.

19. Chambon P. The retinoid signalling pathway: molecular and genetic analyses. Semin Cell Biol 1994;5:115–25.

20. Mangelsdorf DJ, Umesono K, Evans RM. The retinoid receptors. In: Sporn MB, Roberts AB, Goodman DS, editors. The retinoids. New York: Raven Press; 1994. p. 319–49.

21. Xu X-C, Lotan R. Aberrant expression and function of retinoic acid receptors in cancer. In: Nau H, Blaner WS, editors. Handbook of experimental pharmacology: retinoids. Berlin: Springer-Verlag; 1999. p. 323–43.

22. Alberts DS, Einspahr JG, Frank D, et al. Cost- and time-efficient evaluation of chemoprevention agent efficacy. Cancer Epidemiol Biomark Prev 2002;11(Suppl):1197s.