Chemoprevention of Human Actinic Keratoses by Topical DL-α-Tocopherol

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Abstract Prior research shows that topical application of free, nonfatty acid–conjugated vitamin E (DL-α-tocopherol) prevents skin cancer in mice, as well as immunosuppression induced by UVB radiation. This study investigated the chemopreventive potential of DL-α-tocopherol in humans through monitoring surrogate end point biomarkers in sun-damaged skin. Contralateral arms of healthy human volunteers with actinic keratoses (AK) were randomly assigned to receive either 12.5% DL-α-tocopherol or placebo in a crème base for 6 months. Changes in number of AKs, levels of p53 protein expression, proliferating cell nuclear antigen, and polyamines were assessed along with skin and systemic vitamin E levels. Following treatment, plasma concentration levels of DL-α-tocopherol were unchanged, but skin levels were highly elevated ($P < 0.001$). Levels of p53 and proliferating cell nuclear antigen did not change significantly, whereas number of AKs declined insignificantly in both placebo and treatment arms. Regression models showed significant decreases in putrescine, spermidine, spermine, and total polyamine concentrations following treatment. Topically applied DL-α-tocopherol was substantially absorbed in skin, but the 6-month application did not significantly reduce numbers of preexisting AKs on moderately to severely sun-damaged forearms. Increases in polyamine synthesis are expected during tumor initiation and promotion; conversely, the significant reductions in polyamine levels resulting from the topical DL-α-tocopherol application are consistent with reductions in tumorigenesis potential. Topical tocopherol did not normalize established sun-induced lesions, but DL-α-tocopherol–induced reductions in polyamine metabolism are consistent with the inhibition of skin squamous cell carcinogenesis as seen in previous human trials and animal models.

Incidence of both melanoma and nonmelanoma skin cancers is increasing in the United States at an alarming rate, reflecting trends both in increasing levels of solar UV radiation reaching the surface of the Earth and aging of the U.S. population (1, 2). Additionally, recent data suggest that skin cancer prevalence is increasing among younger adults (3, 4). The likelihood of continuance of these trends increases the urgency for the development of primary and secondary skin cancer prevention methods.

Vitamin E, a primary epidermal antioxidant rapidly depleted by oxidizing UV radiation, is a compound of interest in skin cancer prevention (5, 6). Research indicates that topical application of free, nonfatty acid–conjugated vitamin E (free DL-α-tocopherol) prevents skin cancer in mice, as well as immunosuppression induced by UVB radiation (7, 8). These findings suggest that free DL-α-tocopherol may be a promising chemopreventive agent among humans as well. However, clinical trials using the development of skin cancer as an end point are logistically difficult and costly. To evaluate the potential activity of topically administered DL-α-tocopherol in participants at high risk for developing nonmelanoma skin cancers, we pursued a two-pronged strategy of monitoring dorsal forearm actinic keratoses (AK) as well as levels of potentially relevant surrogate end point biomarkers [tumor suppressor protein p53, proliferating cell nuclear antigen (PCNA), and polyamine concentrations]. AKs are considered precursors or intermediate end points of squamous cell carcinoma development (9, 10) and are thus an obvious candidate for study. Levels of expression of p53 and PCNA are biomarkers that have been implicated in nonmelanoma skin cancer

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Table 1. Demographic characteristics of participants completing the 6-mo topical vitamin E study

	n	Weight, pounds (SE)	Age, y (SE)	Skin cancer history, no. (%)*
Males	36	182.4 (4.44)	68.0 (1.40)	16 (44.4)
Females	6	139.7 (8.27)	67.3 (1.40)	1 (16.7)
All	42	176.3 (4.60)	68.0 (1.22)	17 (40.5)

*Participants who answered "uncertain" to a history of skin cancer were considered as having a negative history.

carcinogenic pathways (11–15). The tumor promotion phase of UVR exposure is characterized by production of oxygenated free radicals and increased levels of polyamines. Polyamines play an important role in cell growth and proliferation with intracellular concentrations generally high during states of rapid cell turnover as in AKs (16, 17). Studying such biomarker end points can not only reduce the duration and size of clinical trials but also provide information about possible mechanisms of action for chemopreventive agents.

The primary hypothesis for this study was that topical administration of 12.5% DL-α-tocopherol in a crème base to sun-exposed skin in humans would result in significant reductions in polyamine levels, p53 protein expression, and PCNA labeling indices in AK lesions and perhaps induce clinical reversal of those lesions and/or prevention of new ones. The secondary hypothesis was that reduction in or disappearance of AK lesions would be correlated with changes in the assessed biomarkers (p53 and/or PCNA levels and/or putrescine, spermidine, and spermine polyamine levels).

Materials and Methods

Recruitment and visits

Fifty healthy volunteers 30 y of age or older who had at least 10 discrete and clinically diagnosable AKs on each dorsal forearm were recruited to this phase IIb, randomized, double-blind, placebo-controlled cancer prevention study. Exclusion criteria included current cancer or lateral forearm treatment for cancer or AKs within the past 30 d or initial clinical chemistry levels (SMA20) outside of normal limits.

Recruited participants completed extensive baseline questionnaires, including demographic characterization, dietary, medical, residential, and sun exposure histories. A 1-mo placebo run-in period ensured that the forearm skin was not damaged by recent sun exposure or dryness before the initial biopsies and also provided an estimate of compliance to the twice-daily topical application regimen. The placebo was a nonactive crème base, Vanicreme (Pharmaceutical Specialties, Inc.).

Clinical staff described and showed application of the study cream and the use of the provided calendar to record times of the twice-daily applications. Two tubes of cream were provided to the individual, bearing neon green or orange labels identifying the arm (right or left) to which the cream should be applied. The participant was asked to squeeze out 1 in of cream onto the appropriate forearm and apply the cream to the dorsal forearm (knuckles to the crease of the elbow) until the white color was no longer visible. Participants were also asked to record any adverse effects and call the clinic staff or doctor for any severe reactions or adverse effects lasting more than 24 h. Participants were asked to use a provided sunscreen (SolBar, Person and Covey) as necessary. The SolBar sunscreen was selected for its high sun protection factor of 50 and absence of tocopherol or other compounds known to interact with tocopherols.

After the 1-mo placebo run-in, participants returned to the clinic for toxicity and adherence assessments, forearm dermatologic examination including outlining AKs for baseline lateral forearm photographs, and shave biopsies. Participants with estimated topical application compliance of <75% during the placebo run-in period were excluded.

The dermatologist selected three AKs on each forearm and removed the six biopsies (three per arm), each ~4 mm in diameter, using the shave technique. Following the biopsy procedure, participants were provided two new tubes of study cream along with new adherence calendar pages.

Forearms of each participant were randomized such that one arm received free DL-α-tocopherol (Henkel Fine Chemicals) cream at a concentration of 12.5 g per 100 g of Vanicreme (12.5%) and the other arm received placebo (Vanicreme base with no additions). A progressive randomization program was used to ensure that the allocation of right arm versus left arm DL-α-tocopherol cream did not vary by gender, age, or initial AK count. This design guarded against the possibility that systematic differences in sun exposures for different arms could confound study results.

For the next 6 mo, participants returned to the clinic monthly for scheduled visits at which clinical staff inquired about possible complications. If adverse events had occurred, staff inquired about severity, date of onset, duration, and date of resolution. A predetermined protocol reduced or discontinued cream application if any side effects were rated as severe. At each visit, study staff also weighed the returned tubes of cream and provided a new set of study cream for the ensuing month.

At the end of 6 mo, participants returned to the clinic for physician-administered brief physical exams, forearm dermatologic exams including circling of AKs, photographs, and final biopsies. In addition to AK biopsies, three normal-looking sites per forearm were removed for assessment of dermal DL-α-tocopherol levels. Blood was drawn for a final clinical chemistry assessment with an additional 10 cm^3 sample in a heparinized container for systemic DL-α-tocopherol analysis.

Biochemical and immunohistologic assessments

To determine levels of free DL-α-tocopherol in plasma and skin, 250 μL ethanol (containing 0.1% butylated hydroxytoluene antioxidant) was added to a 250-μL aliquot of plasma to precipitate proteins. After vortexing, the sample was extracted with hexane, dried under nitrogen, and redissolved in the high-performance liquid chromatography mobile phase. For the skin biopsies, α-tocopherol was extracted as previously described, with minor modification (18). Briefly, skin was incubated with 0.5% collagenase and 0.2% protease (Sigma Chemical) at 37°C for 1 h. After skin homogenization, an equal volume of 1% SDS in ethanol was added and kept at the same temperature for 30 min. The sample was also extracted with hexane, dried under nitrogen, and redissolved in the mobile phase. The α-tocopherol was separated using a 5-μm ultrasphere octadecyl silane column (octadecylsilica 25 cm × 4.6 mm; Beckman Instruments, Inc.) and detected at a wavelength of 290 nm. The solvent system was acetonitrile-methanol-acetic acid (60:40:0.2, v/v/v) delivered at a flow rate of 2.4 mL/min (8, 19).

Table 2. Plasma and skin levels of free DL-α-tocopherol (n = 42)

	α-Tocopherol		P value of difference
	$\bar{X}$	SE	
Skin			
Treated arms	545.5	73.2	
Placebo arms	17.2	1.5	($P < 0.001$)
Plasma			
Baseline levels	12.5	0.8	
End of study	12.5	0.7	($P = 0.978$)

Skin polyamine concentrations were measured using a previously described reverse-phase high-performance liquid chromatography assay (20) with modification (21). The high-performance liquid chromatography effluent was monitored with a Kratos model Spectroflow 980 fluorescence detector (ABI Analytical). The excitation wavelength was set at 340 nm, and a 550-nm cutoff filter was used on the emission side. The limit of detection of dansylated polyamines in this system was <1 pmol. Levels of putrescine, spermidine, and spermine are reported in nmol/g wet skin.

To assess levels of PCNA and p53, skin biopsy specimens were paraffin embedded and cut into 3-μm sections. After deparaffinization, PCNA and p53 protein expression were measured by immunohistochemistry as previously described (11, 22, 23). For PCNA, immunostaining proceeded at room temperature using an avidin-biotin–based Vectastain avidin-biotin complex kit (Vector Laboratories). The complex was visualized using 0.25 mg/mL 3,3′-diaminobenzidine in PBS with 0.06% H_2O_2 (Sigma) added just before use. Light staining with H&E stain was followed by dehydration and coverslipping. For p53, immunostaining relied on a standardized streptavidin-biotin peroxidase system with a 3,3′-diaminobenzidine tetrahydrochloride chromagen and a hematoxylin counterstain (Ventana Medical Systems) on an automated VMS 320 immunostainer (Ventana Medical Systems). PCNA and p53 labeling indices were calculated by dividing the number of labeled cells by the number of total cells (labeled and unlabeled) in the counting area and multiplying by 100.

Analytic methods

Descriptive statistics included means, medians, SDs, and SEs. Initial assessment of differences in outcomes between placebo and DL-α-tocopherol arms relied on the Wilcoxon signed rank test because outcomes were not normally distributed. Additionally, a conditional change model that adjusts for the change on the untreated arm was used as previously described to assess the effects of α-tocopherol on number for all outcomes (19). Analyses were done using Stata, version 10 (24). Selected secondary analyses were completed using Statistical Analysis System 9.1 (25) or Statistical Package for the Social Sciences 15.0 (26).

Results

Demographics

Table 1 summarizes gender, weight, age, and previous cancer history. As a consequence of requiring at least 10 assessable AKs for study entry, most participants were male, and the average age for both genders was just under 70. Nearly half the males had a prior history of skin cancer compared with only one of the six female participants.

Participant attrition

Five of the original 50 participants did not complete the 6-month topical application regimen. Of these, two participants discontinued the study before being randomized: one after experiencing an aversion to the venipuncture procedure and the other due to a diagnosis of lymphocytic leukemia. After randomization, two participants discontinued the study due to ill health unrelated to the topical application regimen (recurrent problems following gall bladder surgery and death from cardiovascular disease). The third individual, who discontinued study medication after randomization, was unable to achieve the required 75% compliance to the topical application regimen, although compliance had been achieved during the run-in period.

Of the 45 participants completing the application regimen, 3 were nevertheless excluded from analysis of number of AKs. Of these, one participant had continued an oral treatment regimen (25,000 IU vitamin A daily) shown to be effective in reducing squamous cell skin cancer development in a previous chemoprevention trial (27, 28). For the other two, examination of the remaining contents of returned study cream tubes indicated that mislabeled tubes had been distributed during the study, so that each forearm had received both placebo and DL-α-tocopherol creams rather than a single cream throughout the study period. Thus, data from 42 individuals were used in the analysis of number of AKs. The excluded participants, 7 men and 1 woman, did not vary in age or initial characteristics from the 42 individuals who were fully evaluable.

The left arm biopsy on 1 individual was lost, so data from only 41 individuals could be used in the analysis of p53 and PCNA. Due to the limited availability of biopsy material, complete polyamine profiles were assessed for both arms of only 37 of the 41 study participants. For all analyses, we considered the use of an intent-to-treat approach. However, due to the study design, all subjects received both placebo and treatment (albeit on opposite arms), so that loss of a subject affected treatment and control groups identically. Thus, all analyses were conducted only on individuals for whom all data were available.

Left versus right arm

Baseline values of clinical outcome measures (AKs, p53, and PCNA) did not differ by left versus right arm or treatment

Table 3. Number of AKs by treatment group (n = 42)

	Baseline*		End of study*		Change†	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Placebo	34.3	3.8	26.3	3.8	–8.0	2.2
Treated	34.0	3.8	28.0	3.7	–6.0	2.2

*Only the initial number of AKs on the placebo arm was normally distributed.
†Change variables are normally distributed.

Table 4. Levels of p53 protein, PCNA expression (n = 41), and polyamine concentrations (n = 37) in forearm biopsy samples from topical vitamin E study participants

	Baseline		End of study		Change		P*
	Mean % expression	SE	Mean % expression	SE	Mean % expression	SE	
p53							
Placebo	20.3	2.4	25.4	2.9	5.1	3.1	0.1215
Treated	21.1	2.9	20.1	2.1	–1.0	3.3	
PCNA							
Placebo	8.4	1.0	9.0	1.1	0.6	1.5	0.4800
Treated	6.6	1.0	8.6	1.2	2.0	1.5	
Polyamines	**Mean nmol/g**	**SE**	**Mean nmol/g**	**SE**	**Mean nmol/g**	**% change SE**	**P***
Putrescine							
Placebo	120.83	7.73	131.59	10.14	10.76	12.2	0.0004
Treated	139.12	10.68	89.50	5.50	–49.62	9.3	
Spermidine							
Placebo	292.17	14.80	309.94	17.77	17.77	14.8	0.0074
Treated	302.10	15.17	259.6	11.40	–42.72	13.2	
Spermine							
Placebo	205.51	9.78	213.59	12.22	8.08	13.1	0.0955
Treated	246.77	10.26	226.61	8.69	–20.16	8.9	
Total polyamines							
Placebo	618.51	28.55	655.12	34.57	36.61	33.5	0.0014
Treated	687.98	29.13	575.49	21.05	–112.49	24.8	
Spermidine to spermine ratio							
Placebo	1.43	0.03	1.47	0.04	0.04	0.05	0.1048
Treated	1.26	0.06	1.15	0.04	–0.11	0.05	

NOTE: Information is included for only those participants for whom complete analytic specimen sets were available.
*Wilcoxon signed rank test on change variables.

versus control group (results not shown here). Moreover, in no analysis of those outcomes did arm status ever achieve statistical significance. Therefore, for simplicity, arm status was not included in subsequent tables or discussion.

Skin and plasma levels of DL-α-tocopherol

Levels of DL-α-tocopherol are presented in Table 2. Concentration of free DL-α-tocopherol was much higher in treated than in placebo arms, indicating that absorption into the skin occurred. However, as expected, there was no change in plasma levels of free DL-α-tocopherol from baseline to end of study.

Number of AKs

Table 3 presents the number of AKs at the time of randomization and after 6 months of treatment with placebo or DL-α-tocopherol. There were eight individuals at baseline and six individuals at the end of treatment, where AKs on one or both arms were so dense and confluent that the dermatologist had to classify them as "too numerous to count." For the purpose of analysis, including descriptive statistics, these cases have been treated as equal to 78 AKs, the highest countable number recorded in the data. These cases were distributed nearly identically across treatment status due to the randomization design. Sensitivity analyses were conducted (results not shown) excluding these individuals, and the pattern of results reported in Table 3 did not change in terms of sign or significance. Thus, the findings are robust to the transformation of too numerous to count status to a count of 78.

The third column in Table 3 indicates the difference in number of AKs from baseline to end of treatment. The number of AKs declined significantly on arms treated with DL-α-tocopherol (P = 0.004). However, they also declined significantly, and to a greater extent, on arms treated with placebo (P = 0.0002). The greater decrease in AKs among placebo arms was not statistically significant at P = 0.29. These changes amount to an approximately 20% to 25% decline in the number of AKs overall.

Skin p53 and PCNA expression and polyamine levels

The expression of p53 and PCNA over time is shown in Table 4 along with polyamine levels. No statistically significant differences for p53 and PCNA existed between placebo and treatment arms at baseline. Expression of p53 and PCNA increased in the placebo arms from baseline to end of study. However, neither increase in the placebo arm proved statistically significant (for p53, P = 0.18; for PCNA, P = 0.85). p53 expression declined in treated arms and PCNA expression increased, but these changes also were not statistically significant (for p53, P = 0.98; for PCNA, P = 0.10). Changes in

Table 5. Regression models, including coefficients, for effect of topical application of free DL-α-tocopherol on number of AKs and expression of p53, PCNA, or polyamine levels

	Overall effect of free DL-α-tocopherol		Effect of baseline level		R^2
	95% CI	*P*	95% CI	*P*	
AK*	2.0 (–1.3 to 5.3)	0.226	0.3 (–0.6 to –0.1)	0.007	0.17
p53†	–6.1 (–12.9 to 0.7)	0.078	–1.0 (–1.3 to –0.6)	>0.001	0.44
PCNA†	1.3 (–1.3 to 4.0)	0.308	–0.5 (–0.8 to –0.3)	0.001	0.27
Putrescine‡	–60.4 (–79.6 to –41.2)	<0.0001	–1.04 (–1.3 to –0.8)	<0.0001	0.65
Spermidine‡	–60.5 (–86.2 to –34.8)	<0.0001	–1.29 (–1.6 to –1.0)	<0.0001	0.70
Spermine‡	–28.2 (–45.2 to –11.3)	0.002	–1.25 (–1.5 to –1.0)	<0.0001	0.80
Total polyamines‡	149.1 (–214.3 to –83.9)	<0.0001	–2.18 (–3.6 to –2.0)	<0.0001	0.69
Spermidine to spermine ratio‡	0.005 (–.10 to 0.11)	0.11	1.04 (0.7 to 1.3)	<0.0001	0.60

*n = 42.
†n = 41.
‡n = 37.

biomarker expression did not differ significantly across placebo versus treatment arms (for p53, $P = 0.12$; for PCNA, $P = 0.48$). The overall levels of putrescine, spermidine, spermine, and total polyamine concentrations significantly decreased following treatment with free DL-α-tocopherol ($P < 0.0001$, $P < 0.0001$, $P = 0.002$, and $P < 0.0001$, respectively) compared with placebo. No significant difference in the ratio of spermidine to spermine was evident between the treatment arms, indicating similar decrements in the individual polyamines in response to the topical treatment.

Effects of free DL-α-tocopherol on number of AKs

Table 5 shows results of conditional change models for each of the three main outcomes. The first row captures the effects of free DL-α-tocopherol on number of AKs after controlling for baseline number of AKs. The findings are consistent with those presented in Table 3, confirming that free DL-α-tocopherol was not associated with any change in number of AKs beyond that associated with placebo.

Effects of free DL-α-tocopherol on levels of p53 and PCNA

The next two rows in Table 5 show similar results for the two biomarker outcomes. In each case, 95% confidence intervals (95% CI) for the intercept term indicate no statistically significant effect of free DL-α-tocopherol at the $\alpha = 0.05$ level. Note that for p53, there was a marginally significant effect ($P < 0.10$).

Safety monitoring and toxicity

Each monthly visit included the completion of a Safety Monitoring and Toxicity Report to ensure that all potential signs or adverse symptoms to the treatment were assessed. Initially and at each subsequent visit, participants were asked whether they had experienced any of the following: redness, itchiness, burning, dryness, or any other unexpected sensation on each arm. For each potential reaction, the severity was categorized as mild, moderate, or severe. The same form was completed if a participant called between clinic visits to report problems or when participants who had previously reported problems were called for follow-up. The study protocol required follow-up on any moderate or severe symptom. Nineteen of the 42 participants reported an absence of all signs or symptoms throughout the entire period of follow-up. Only 5% of the Safety Monitoring and Toxicity reports indicated any moderate or severe symptom. Table 6 provides the distribution of the most commonly reported signs and symptoms by treatment status. Redness and itchiness were the most frequently reported symptoms but were reported with equal frequency among arms receiving free DL-α-tocopherol or placebo treatment. These data do not suggest that the free DL-α-tocopherol treatment had a systematic effect on toxicity. Presumably, reactions also occurred in response to the delivery vehicle, the Vanicreme base.

Discussion

The current study shows that topically applied DL-α-tocopherol reduced polyamine content in the skin of patients

Table 6. Distribution of moderate and severe symptoms by treatment status

Symptom	Total no. reports	Arm(s) affected		
		Treated	Placebo	Both arms
Redness	5	2	2	1
Itchiness	5	2	2	1
Burning*	3	1	0	0
Dryness	1	1	0	0

*Information on which arm(s) experienced burning was not properly recorded in two cases.

with large numbers of established AKs on the forearms. Elevated polyamine levels are characteristically seen in many types of neoplastic cells and tissues. O'Brien and colleagues (29) and Peralta Soler and colleagues (30) have shown that in transgenic mice, overexpression of ornithine decarboxylase in skin leads to changes in tissue polyamine levels, particularly putrescine, and can modulate the development and maintenance of the neoplastic phenotype. Joshi (31) further hypothesized that polyamine synthesis is expected to promote endothelial cell proliferation and therefore angiogenesis. This reinforces the known role of polyamine biosynthesis in mediating skin tumor initiation and promotion (29–33).

The main findings of the present study can be summarized by the following statements. Daily topical application resulted in substantial uptake of free DL-α-tocopherol into the forearm skin. The significantly increased concentrations of tocopherol in skin were not associated with reductions in the number of established AK lesions (normalization of skin) or in the expression of skin biomarkers expected to mediate such changes, p53 and PCNA. Skin putrescine, spermidine, spermine, and the total polyamine concentrations were appreciably reduced relative to initial and placebo concentration levels. This finding is similar to observations of Khettab et al. (34) who showed that topical vitamin E inhibited the production of free radicals by ~60% and caused a significant reduction in polyamine biosynthesis ($P < 0.001$) in the epidermis of UV-irradiated hairless mice. In the present study, vitamin E significantly reduced skin polyamine concentrations, modifications consistent with the inhibition of squamous cell skin carcinogenesis as seen in previous human trials and animal models.

Although the number of AKs declined significantly over the course of this study, they did not seem to do so in response to treatment with free DL-α-tocopherol. Declines occurred in both the placebo control and the experimental intervention groups, suggesting that perhaps the SolBar 50 sunscreen daily applications were responsible for the change. Thompson et al. (35) reported significant regression effects of sunscreens administered daily over a period of a year to a group of Australians randomized to sunscreen application or placebo cream. Alternatively, the decline in AKs may have been due to an emollient effect masking the scale and roughness associated with AK rather than a physiologic change that would be likely to affect the development of subsequent skin cancer. The lack of a true free DL-α-tocopherol effect on AKs is also reinforced by the lack of a statistically significant effect of the intervention on p53 or PCNA skin biopsy labeling indices.

In the conditional change model for the overall effect of free DL-α-tocopherol, the 95% CI (–1.3 to 5.3) suggested that the study was powered to detect an effect of ±3.3 AKs as statistically significant. Referring back to Table 3, this would represent a 10% change in AK count from the baseline levels of ~34 AKs per arm. Thus, the study was sufficiently powered to detect a clinically significant change in numbers of AKs due to treatment. In a similarly designed previous study of topical difluoromethylornithine (DFMO) application, polyamine level decrements resulted in a 23% reduction in AK count (36). Two primary differences distinguish the topical DFMO from the current free DL-α-tocopherol study: (*a*) mean initial AK count of 28 among DFMO study subjects indicating a less sun-damaged population in comparison with the current study and (*b*) no sunscreen was provided during the DFMO study. Although the previous DFMO study also randomly assigned the arm (right or left) receiving the topical intervention cream, DFMO effectiveness was substantially greater in the right arm. Differential UVR likely due to left arm exposure during automobile driving was believed to affect study results. However, we currently cannot rule out the possibility that the addition of the highly protective (sun protection factor of 50) sunscreen to prevent differential sun exposure altered study results through an emollient effect or by adding UVR protection that altered the intervention time required for polyamine changes to significantly affect AK count. This topical study was not designed to accurately map size and location of specific AK lesions and capture potential subtle changes in AK appearance. The negative findings of the study therefore suggest that 6 months of daily applications of topical free DL-α-tocopherol has little or no normalizing effect on moderately to severely sun-damaged skin.

The vast majority of adult sunscreen preparations contain α-tocopherol acetate or another esterified fatty acid as an emollient. Previously, our group reported that α-tocopherol acetate (or succinate) could potentiate UVB skin carcinogenesis in a mouse model (8). Furthermore, in a companion human study, we showed that the long-term application of α-tocopherol acetate to sun-damaged skin resulted in a buildup of high concentrations of the esterified vitamin E compound in epidermal skin, a potentially adverse effect for skin cancer prevention (19).

In contrast, Gensler and Magdaleno (7) had shown that free DL-α-tocopherol applied topically to a UVB-induced, mouse skin carcinogenesis model proved significantly protective. Although the results of our present study indicated that free DL-α-tocopherol applied topically for 6 months to participant's arms containing multiple AKs did not significantly reduce the number of AK lesions, there was no evidence of a promotional effect on AK proliferation. Nevertheless, the continued incorporation of esterified forms of DL-α-tocopherol in commercially available sunscreens as an "inactive ingredient" emollient may pose a public health problem.

Topical application of free DL-α-tocopherol was well tolerated and resulted in substantial increases in skin vitamin E levels. The regimen was not associated with reduction in the number of established AK lesions nor biomarkers expected to mediate skin normalization. An inherent disadvantage to ensuring a clinical assessable end point, such as the reduction in the number of AK lesions, is the selection of moderately to severely sun-damaged skin indicative of substantial prior tumor promotion. Although unsuccessful at reducing the numbers of AK lesions, the application of free DL-α-tocopherol significantly reduced skin polyamine concentrations, modulations characteristically associated with the inhibition of neoplastic phenotypes and AK development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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