

Comparative Effects of Sunscreens Alone vs Sunscreens Plus DNA Repair Enzymes in Patients With Actinic Keratosis: Clinical and Molecular Findings from a 6-Month, Randomized, Clinical Study

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ABSTRACT

Recent experimental irradiation studies have shown that the addition of DNA repair enzymes (photolyase and endonuclease) to traditional sunscreens may reduce ultraviolet radiation (UVR)-induced molecular damage to the skin to a greater extent than sunscreens alone. In this 6-month, randomized, clinical study, we sought to compare the clinical and molecular effects of sunscreens plus DNA repair enzymes vs. those of traditional sunscreens alone in patients with actinic keratosis (AK). A total of 28 AK patients were randomized to topically apply sunscreens plus DNA repair enzymes (enzyme group; n = 14) or sunscreens alone (sunscreen group; n = 14) for 6 months. The main outcome measures included 1) hyperkeratosis, 2) field cancerization (as measured by fluorescence diagnostics using methylaminolaevulinate), and 3) levels of cyclobutane pyrimidine dimers (CPDs) in skin biopsies. Both regimens produced a significant reduction of hyperkeratosis at 6 months, with no difference between the two groups. Field cancerization was significantly reduced by both regimens, but the decrease observed in the enzyme group was significantly more pronounced than in the sunscreen group ($P < 0.001$). At 6 months, CPDs decreased by 61% in the enzyme group and by 35% in the sunscreen group compared with baseline values ($P < 0.001$). These findings indicate that, despite a similar effect on hyperkeratosis, the addition of DNA repair enzymes to sunscreens was more effective in reducing field cancerization and CPDs than sunscreens alone. Taken together, our findings indicate that sunscreens plus DNA repair enzymes may be superior to traditional sunscreens alone in reducing field cancerization and UVR-associated molecular signatures (CPDs) in AK patients, potentially preventing malignant transformation into invasive squamous cell carcinoma in a more efficient manner.

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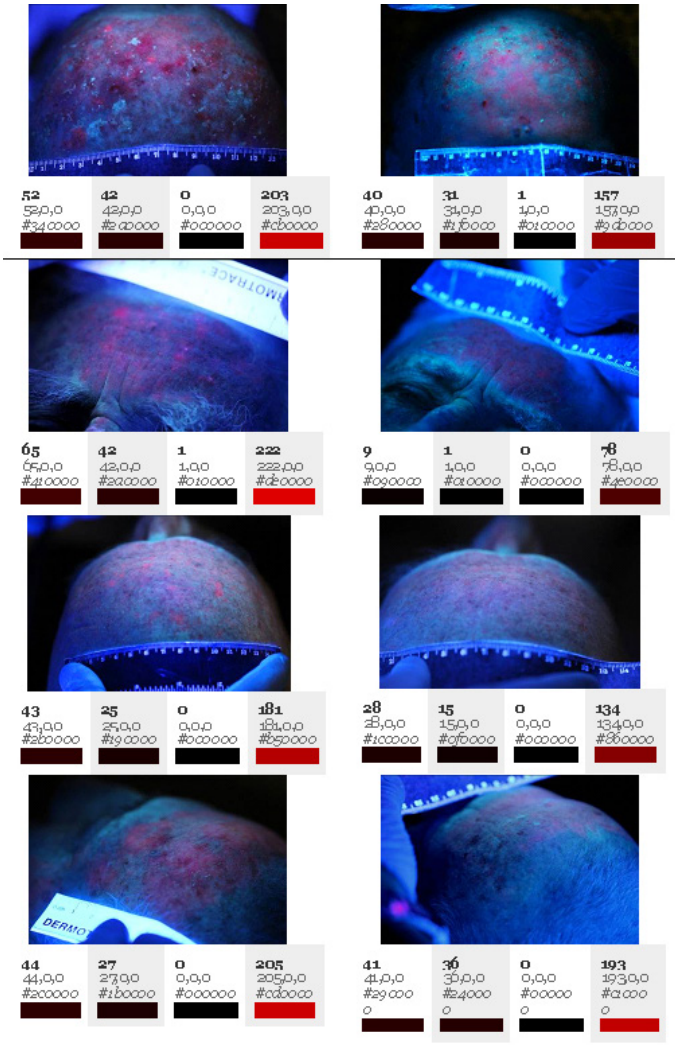
INTRODUCTION

Non-melanoma skin cancer is a complex multistep disease that arises from accumulating genetic alterations of critical cell regulatory genes associated with a high degree of genomic instability in skin keratinocytes.[1] Chronic exposure to ultraviolet radiation (UVR) in fair-skinned patients is universally recognized as the primary risk factor for the development of skin cancers, with these lesions occurring primarily on chronically sun-exposed skin areas (e.g., the head, face, and back of the hands).[2,3] DNA in keratinocytes readily absorbs UVR and is highly susceptible to forming photoproducts, mainly in the form of cyclobutane pyrimidine dimers (CPDs). CPDs have been shown to induce bulky lesions in the structure of DNA, ultimately representing the primary cause of mutations in skin tumorigenesis.[4,5]

Actinic keratoses (AKs) are common premalignant intraepidermal lesions that are generally considered as precursors of squamous cell carcinoma (SCC).[6,7] The presence of AKs may be regarded as a major warning sign that subclinical UVR-induced photodamage has accumulated over time.[8] The subclinical field changes suggest the widespread presence of DNA-damaged cells which are called field cancerization.[9] The term field cancerization is therefore used to indicate the presence of extensive skin areas with multiple precancerous lesions (clinically visible AKs and subclinical AKs characterized by histologic atypia) that have a potential of developing into SCC, indicating the presence of a spectrum of malignant progression.[10] Notably, it has been suggested that the number of subclinical AKs in chronically photodamaged areas can exceed the number of clinically visible AKs by ten-fold.[11]

The clinical usefulness of regular use of sunscreens for preventing the development of AKs and possibly reducing the risk of skin cancer in the long-term has been initially demonstrated in the seminal work by Thompson et al.[12] Subsequently, several independent investigators have confirmed that sunscreens

FIGURE 1. Fluorescence methyl aminolevulinate-based diagnostics of field cancerization before (left images) and after treatment (right images) in four patients treated with sunscreens plus DNA repair enzymes. Field cancerization was assessed quantitatively as the red-colored luminescent area. Using the Color Histogram module and the statistics of the red color distribution, the skin areas under examination were compared. The higher the mean intensity on a scale of 0 to 255, the greater field cancerization is.



offer protection against AKs both in the general population and in high-risk subjects.[13-15] Interestingly, recent experimental investigations have shown that the addition of xenogenic DNA repair enzymes (photolyase and endonuclease) to traditional sunscreens may reduce UVR-induced CPDs formation in the skin to a greater extent than do sunscreens alone.[16,17] However, no clinical studies have directly compared the clinical and molecular effect of regular use of sunscreens plus DNA repair enzymes vs those of traditional sunscreens in AK patients. In this 6-month, randomized, clinical study, we sought to address this issue by including both clinical and molecular endpoints, ie, 1) hyperkeratosis, 2) field cancerization (as measured by fluorescence diagnostics using methyl aminolevulinate [MAL]), and 3) levels of CPDs measured in skin biopsies.

PATIENTS AND METHODS

Study Participants

This 6-month, randomized, clinical study was conducted at two research sites (Centro Ortopedico di Quadrante, Omegna and Erba-Renaldi Hospital, Menaggio, Italy). Continuous enrollment took place between October 2012 and June 2013. The study population comprised 28 Caucasian patients aged >65 years (21 males and 7 females; age range: 65–82 years) with mild photodamage and clinical evidence of AKs (grade I and II) on the face and scalp. Exclusion criteria were as follows: lack of capacity to give informed consent; patient refusal; inability to communicate in Italian; history of known allergies, photosensitive disorder, or arsenic exposure; previous use of ablative laser procedures, dermabrasion, chemical peel, cryotherapy, curettage, surgical excision, or chemodestruction within six months prior to study initiation. Patients were randomly assigned to topically apply to the photodamaged areas sunscreens plus DNA repair enzymes (sun protection factor [SPF] 50 plus 1% photolyase from *Anacystis nidulans* and 1% endonuclease from *Micrococcus luteus*; enzyme group; n = 14) or sunscreens alone (SPF 50; sunscreen group; n = 14) for 6 months. The products were applied at an application density of 2 mg/cm^2, whereas the size of the treated areas ranged from 40 to 100 cm^2. Each contained between four and 10 clinically evident AKs. The study protocol was approved by the Institutional Review Boards and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent before participation in the study.

Procedures

All participants were asked to withdraw any topical product 14 days before the beginning of the study. In addition, they were not allowed to use any topical intervention throughout the entire study period. The study participants were randomly assigned a treatment using a web-based research randomizer (http://www.randomizer.org). Two sets of 14 unique numbers per set were created for the numbers 1–28, thus randomly allocating 14 patients to each of the 2 groups. Randomization was performed by allocation of the consecutive patients to the lowest available number from the randomization list. Consequently, half of the participants (n = 14) were assigned to the enzyme group, whereas the other half (n = 14) to the sunscreen group. After the randomization and the baseline assessment, patients were instructed to apply the study products over the photodamaged areas twice per day (once in the morning and once in the evening). A final visit was scheduled at the end of the 6-month product application period.

Outcome Measures

Primary outcomes of interest for this study were 1) hyperkeratosis, 2) field cancerization (as measured by fluorescence

diagnostics using methylaminolaevulinate), and 3) levels of CPDs measured in skin biopsies. Adverse events (including pain, erythema, crusting, and ulceration) and pigmentary changes that occurred during therapy were recorded.

Hyperkeratosis

Hyperkeratosis was graded on a 4-point scale, as follows: 0 (no visible keratosis), 1 (mild hyperkeratosis barely visible; affected area less than 5 mm^2), grade 2 (moderate hyperkeratosis with affected area between 5–10 mm^2) and grade 3 (severe hyperkeratosis with affected area bigger than 10 mm^2).

Field Cancerization

Field cancerization was assessed in a non-invasive manner by means of photodiagnosis.[18] A thin layer of the photosensitizing percursor (MAL, Metvix® Photocure ASA, Oslo, Norway) was accurately applied on each patient's AK-affected skin areas a 10-cm margin. The areas were occluded for 3 hours using a plastic film as the first layer, followed by aluminum thin foil as the top layer. A Wood's lamp was then used to illuminate the affected areas while images of the fluorescent areas were digitally recorded. Finally, fluorescence-sensitive images taken under Wood's lamp illumination were analyzed for colorimetric image segmentation using a red, green, and blue (RGB) color space software (freeware ImageJ v1.33 with the Colour Histogram plug-in, both downloaded from the NIH website (http://rsb.info.nih.gov/ij) which evaluated the luminescent area within the regions of interest. Field cancerization was assessed quantitatively as the red-colored luminescent area. Using the Color Histogram module and the statistics of the red color distribution, the skin areas under examination were compared. The higher the mean intensity on a scale of 0 to 255, the greater field cancerization is (Figure 1).[19]

CPDs measurement

A 4-mm punch biopsy was performed centrally in one clinically identified AK in the field cancerization area at the baseline visit. A second punch biopsy was performed one week after the end of the 6-month treatment course. The skin biopsy specimens were cleaved in half, and one piece was thawed at room temperature, minced, and lysed by three cycles of freezing (in an ethanol-dry-ice bath) and thawing (at 95°C). For the measurements of CPD, samples were digested for 12 h at 60°C with proteinase K in 100 mmol/liter Tris-HCl (pH 7.4), 150 mmol/L NaCl, and 10 mmol/L EDTA (pH 8.0). Proteinase K was then heat inactivated at 95°C for 10 min, and homogenates were extracted using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). The kit contains two main reagents: cell lysis and protein precipitation solutions. In brief, DNA was extracted from homogenates using a lysis buffer solution and then treated with RNase A. The kit removes proteins using a precipitation solution, followed by 2-propanol to pellet the DNA. CPDs were measured in duplicate by a commercial ELISA kit [OxiSelect Cellular UV-Induced DNA Damage ELISA Kit; Cell Biolabs, San Diego, CA, USA)] according to the manufacturer's protocol. The results of CPDs measurements were expressed in arbitrary units.[17] All laboratory analyses were made blinded to the treatment arm.

Statistical Analysis

The study variables are given as mean ± standard deviation or counts, as appropriate. Chi-square testing was used for categorical data. Unpaired t-test analysis was performed to compare the general characteristics between the two study groups at baseline. One-sample paired t-tests were performed for within-group comparisons between baseline and post-treatment values. Statistical analysis was performed using the Statistical Package for Social Sciences software version 17.0 (SPSS, Inc., Chicago, IL, USA). Two-tailed P values < 0.05 were considered statistically significant.

RESULTS

The baseline characteristics of the patients in the two treatment groups did not differ significantly with regard to age ($P = 0.74$) and sex ($P = 0.65$). Moreover, baseline hyperkeratosis, field cancerization, and levels of CPDs measured in skin biopsies did not differ significantly in the two study groups (Table 1). No significant adverse events and pigmentary changes were observed in both arms.

Hyperkeratosis

Both regimens produced a significant reduction of hyperkeratosis scores at 6 months, with no difference between the two groups (Table 1).

TABLE 1.

Baseline Characteristics and Parameter Changes at 6 Months in the Two Study Groups

	Enzyme group (n = 14)			Sunscreen group (*n* = 14)		
	Baseline	6 months	*P**	Baseline	6 months	*P**
HK	2.5 ± 0.7	1.4 ± 0.4	<0.001	2.3 ± 0.9	1.6 ± 0.5	<0.001
FC	165 ± 27	117 ± 11	<0.001,†	157 ± 21	141 ± 15	<0.05
CPDs	34 ± 11	13 ± 6	<0.001,†	29 ± 14	19 ± 7	< 0.001

HK, hyperkeratosis; FC: field cancerization; CPDs: cyclobutane pyrimidine dimers. Results are expressed in arbitrary units.
*Paired Student's *t* test. † $P < 0.001$ vs sunscreen group.

Field Cancerization

Field cancerization was significantly reduced by both regimens, but the decrease observed in the enzyme group (-29% compared with baseline values) was significantly more pronounced ($P < 0.001$) than in the sunscreen group (-10% compared with baseline values, Table 1).

CPDs Measurement

At 6 months, CPDs in skin biopsies decreased by 61% in the enzyme group and by 35% in the sunscreen group compared with baseline values. Again, the decrease observed in the enzyme group was significantly more pronounced than in the sunscreen group ($P < 0.001$; Table 1).

DISCUSSION

AKs are one of the most common skin condition caused by chronic solar exposure.[20] In patients with multiple and/or the suspicion of subclinical (non-visible) AKs, the main aim is to reduce field cancerization to prevent the emergence of novel clinical lesions and minimize the risk of malignant transformation to SCC.[9,10] To this aim, effective and safe topical field-directed therapies are eagerly awaited. This study compared the clinical and molecular effects of sunscreens plus DNA repair enzymes vs. those of traditional sunscreens alone in AK patients using hyperkeratosis, field cancerization, and levels of CPDs as the main outcome measures. To the best of our knowledge, no direct clinical comparison on the effect of sunscreens plus DNA repair enzymes vs. those of traditional sunscreens alone have been carried out in AK patients. The results of our randomized study showed that both approaches had a similar positive effect on hyperkeratosis after 6 months of treatment, whereas the addition of DNA repair enzymes to sunscreens was more effective in reducing field cancerization and CPDs than sunscreens alone.

In field cancerization, large areas of the skin have been preconditioned by long-term exposure to the carcinogenic effects of UVR.[9] In this preconditioned epithelium, multifocal premalignant lesions can develop as a result of an increased mutational burden and genomic instability. Traditionally, sunscreens have been used as a field-directed treatment to reduce and/or delay the progression of AKs into invasive SCC.[11-14] However, the use of specific and sensitive molecular markers of UVR-induced damage like CPDs has dramatically increased our understanding of the process of skin carcinogenesis.[21] Evidence for the involvement of CPDs in photocarcinogenesis is provided by the presence of p53 mutations foci (TC to TT or CC to TT transitions) detected at bipyrimidine sites in skin tumors.[22] Moreover, CPDs may cause immunosuppression in human skin, which can allow cancer growth while inducing a decrease of immunosurveillance.[23] In this scenario, CPDs are the main candidate biomarkers of the processes of field cancerization and multistep tumor development in subjects with photodamaged skin or AKs. Photolyase from *A. nidulans* and endonuclease from *M. luteus* are xenogenic DNA repair enzymes which can reverse the molecular events associated with skin carcinogenesis caused by chronic UVR exposure by directly repairing CPDs.[17,24] In recent years, several studies have suggested that a molecular approach to photoprotection involving the use of DNA repair enzymes that are extracted from bacteria or algae and encapsulated into liposomes may be useful in the prevention of UVR-induced skin lesions.[17,24-26] The results of our study showed that sunscreens plus DNA repair enzymes were more effective than traditional sunscreens alone in reducing field cancerization and CPDs, in line with what previously reported in experimental irradiation reports.[17,24] Taken together, our data suggest that sunscreens plus DNA repair enzymes represent an optimal option for the field-directed molecular treatment of AKs; besides improving hyperkeratosis, they also improved both field cancerization and CPDs better than sunscreens alone. However, our results should be interpreted within the context of some limitations. The present investigation was designed as an exploratory pilot project. The primary outcome measures in this study were hyperkeratosis, field cancerization, and levels of CPDs in skin biopsies. Thus, the study was not powered to detect changes in the risk of SCC transformation as a result of the application of sunscreens plus DNA repair enzymes vs. those of traditional sunscreens alone in AK patients. Greater numbers and longer treatment duration are required to evaluate this possibility fully. However, our findings provide a rationale for such a study.

"In patients with multiple and/or the suspicion of subclinical (non-visible) AKs, the main aim is to reduce field cancerization to prevent the emergence of novel clinical lesions and minimize the risk of malignant transformation to SCC."

In summary, our findings in AK patients indicate that sunscreens plus DNA repair enzymes may be superior to traditional sunscreens alone in reducing field cancerization and UVR-associated molecular signatures (CPDs). Further studies are necessary to clarify whether DNA repair enzymes added to traditional sunscreens may potentially prevent malignant transformation into invasive SCC in a more efficient manner than sunscreens alone.

DISCLOSURES

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