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Photolyase: Introduction and Clinical Data Review

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INTRODUCTION

ORIGINAL ARTICLE

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A Novel Technology: Sunscreen With Actinic Damage Repair

Leon H. Kircik MD

The incidence of actinic keratoses (AKs), a common dermatologic diagnosis, is likely to continue to grow in the United States in coming years. AKs develop as a response to cumulative ultraviolet (UV) damage, so as the American population continues to age, Baby Boomers and members of subsequent generations are likely to develop these pre-malignant lesions. As an additional cause for concern, anecdotal reports suggest that some dermatologists are diagnosing precancerous and cancerous skin lesions in growing numbers of patients in their thirties or younger.

Faced with growing incidence of skin cancers and precancerous lesions, American dermatologists tend to decry the public's apathy toward sunscreen use and UV avoidance. While it may be the case that younger patients aren't being UV savvy, we should reflect on the reality that many of our older patients lacked access to good quality, high sun protection factor (SPF) sunscreens with sophisticated technologies, for much of their lives. That means we have a population who have endured cumulative UV damage; thus, AKs have essentially become a hazard of aging.

Because AKs are a disease of chronic UV exposure, we recognize that individuals who develop these lesions have photodamaged skin. As such, they likely already have pre-clinical AKs and/or can be expected to go on to develop additional AKs with time. The notion of field-directed treatment using topical AK therapies or destructive modalities aims to address these pre-emergent lesions, and the approach is gaining acceptance. Still, many clinicians and their patients prefer to use destructive modalities to target individual lesions in certain cases. Regardless of the type of treatment used, physical modalities or topical treatments, the cost of AK treatment has become a huge burden to our medical system, especially to Medicare. Therefore, it would be wonderful to prevent AK formation. We, as dermatology providers, have been trying to do that by continuously emphasizing the importance of using sunscreen to our patients all day, every day. I tell my patients "your sunscreen should sit next to your toothpaste and you should put it on right after brushing your teeth in the morning". I also recommend that sunscreen use should not be reserved for special occasions such as hiking, biking, swimming, or only summer time use. I am happy to note that we have come a long way and become successful in our approach but apparently not one hundred percent since we still see a lot of patients with AKs every day.

Wouldn't it be a great idea if we had a product or an ingredient that would prevent AK formation before they became a problem? Ironically, the Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich, and Aziz Sancar "for mechanistic studies of DNA repair". Within the research of the Nobel team, a novel group of DNA repairing enzymes have emerged to do precisely that. Several products are now commercially available that contain the specific ingredient, photolyase, one of the DNA repairing enzymes shown to have clinical effect on human skin.[1,2]

The basic research and clinical findings, which will be further discussed in this supplement, are very promising for dermatologists who recognize the very real challenge of managing actinic damage. The potential of a scientifically formulated product that provides a high SPF along with DNA repair enzyme photolyase is a welcome development for all our patients with actinic damage.[2]

I hope you all find the information in the next couple of pages on this novel technology exciting and a beneficial tool that we will utilize in our clinics as a standard practice.

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 ORIGINAL ARTICLE Journal of Drugs in Dermatology

Understanding the Role of Photolyases: Photoprotection and Beyond

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ABSTRACT

The limitations of photoprotection modalities have been the inability to arrest the progression of photodamage. Chemoprevention strategies involving a sunscreen has been incomplete because of the need to induce sustained repair of mutations and slow carcinogenesis. Photolyases, or photoreactivation enzymes, serve the role of repairing mutations and damage to DNA induced by ultraviolet (UV) radiation and therefore influence the initiation phases of carcinogenesis. As these enzymes are absent in humans, exogenous forms have been manufactured and are now utilized in topical agents to supplement and augment the innate repair mechanisms that are mostly inefficient.

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INTRODUCTION

If there is a cookbook in Dermatology, the essential ingredient in every recipe is sunscreen. Patients presenting with acne, rhytides, melasma, actinic keratoses, or anything else within a dermatology office all need and should receive proper sunscreen and education. The concept of photoprotection is often the last but most resonating message that patients are given before they leave the clinic: "Don't forget to wear sunscreen" ... "wear it every 2 hours while outdoors" ... "protecting yourself from the sun will significantly reduce your skin cancer risk, prevent the pigmentation from getting worse, keep you looking younger..." and so on. A common analogy shared with patients and colleagues is that sunscreen is like toothpaste for the skin: we brush our teeth twice a day and after every meal, so the same should go for applying sunscreen routinely at breakfast and lunch and while outdoors. Excessive sun exposure could be the same as eating too many sweets. In addition, dermatologists not only treat but also provide methods to prevent consequences from cumulative UV exposure, like brushing our teeth every day is meant to prevent dental problems. We don't just brush one tooth, we brush them all, and just like cavities, where we see one, there is a good chance that more are on the way, one actinic keratosis (AK) or lentigo signals an entire field of photodamaged skin at risk, therefore necessitating full coverage of a daily sunscreen.

Until recently, our topical photoprotection modalities have had the limitations of being only for protection without any impact on or utility in reversing the process of what photodamage does to the skin. Cosmetic benefits from additives to commercial sunscreens such as retinol, antioxidants, or alpha-hydroxyacids and similar ingredients, have their role in the treatment of photoaging but are not proven to slow the progression to actinic keratosis and skin cancer simply because their effects are primarily on epidermal differentiation or improvement in collagen homeostasis. Having additional ingredients in our toolbox that could help address the progression of actinic damage would make daily application of sunscreen a more meaningful option for the high-risk patients, sun worshippers, transplant patients, and everyone in between. Photolyases, a type of photo reactivation enzyme, serves the role of repairing DNA mutations and damage induced by UV radiation and therefore has the potential to influence the development of carcinogenesis. As these enzymes have not been identified in humans, exogenous forms have been manufactured and are now utilized in topical agents to supplement and augment the innate repair mechanisms that are not completely efficient.[2]

In this supplement, there will be an introduction to the science of photolyases including a historic review, elucidation of their mechanisms of action, and effects on the process of

photocarcinogenesis. A second section will review the clinical implications and utilities of these compounds in daily clinical practice and relevance to the dermatology patient. The final chapter will focus on the evidence in the literature from the last 60 years and from recent clinical trials on how the understanding of photolyases has evolved into current applications and incorporation into preparations now available in the United States.

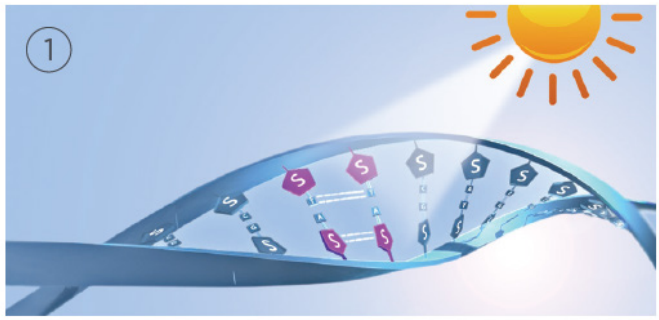
Just like toothpaste and dental care, sunscreens and photoprotection strategies are not a one stop solution for all patients and our approach and options continue to evolve. An understanding of the newest developments in photolyases and their position to supplement daily photo protection agents will help us expand our approach to photo protection for all patient types.

The Science of Photolyases: Historical Perspectives and Mechanisms of Action

Photolyases are naturally occurring enzymes whose purpose is to repair and reverse UV-induced DNA damage, such as thymidine dimers (also known as cyclobutane pyrimidine dimers, or CPDs). With the exception of placental mammals, they exist in most all species of plants and animals and are especially active in those organisms that have high cumulative exposure to UV radiation.[3,4] In simplest terms, the activity of photolyases is the reason plants and many outdoor animals do not develop the signs of photoaging. Ultraviolet B (290 nm – 320 nm) is primarily responsible for most epidermal changes linked to photoaging. In addition to the promotion of ROS activity, UVB is responsible for inducing cyclobutane pyrimidine dimers (CPDs, T-T abnormal nucleotide binding), and a less common defect known as 6-pyrimidine-4-pyrimidone (6-4 PP), both of which distort the helix, and alter replication.[5-7] The mutations that follow, such as in the p53 tumor suppressor gene, eventually interfere with normal keratinocyte differentiation and proliferation thus initiating carcinogenesis.[5-7] This is pronounced as we age due to immune senescence, natural and progressive cutaneous immunosuppression by loss of dendritic cell functioning related to aging and cumulative UV exposure in the skin, and decline in production of endogenous repair enzymes.[7] Humans depend on less efficient proteins that survey and repair the damage induced by ROS known as nucleotide excision repair (NER) proteins.[5-,7] The synopsis of this process is the extraction of the damaged DNA segment that created the break in the helix, which allows the undamaged single strand to re-connect from the repaired nucleotide sequence such that DNA polymerase can correctly transcribe the genome (Figure 1).[2]

The impact of photolyases was studied over nearly 70 years past with the discovery of endogenous process of enzymatic DNA repair known as DNA photoreactivation. This process results from the surge of ROS in photodamaged tissue. These ROS impact flavonoids, specifically flavin adenine dinucleotide (FAD), giving

FIGURE 1. Schematic of UV-induced cyclobutane pyrimidine dimers, characterized by new connection between thymidine nucleotide bases and break between uniform A-T binding.[1,2,8] (A=adenine, T=thymidine, S=sugar-phosphate base of helix)

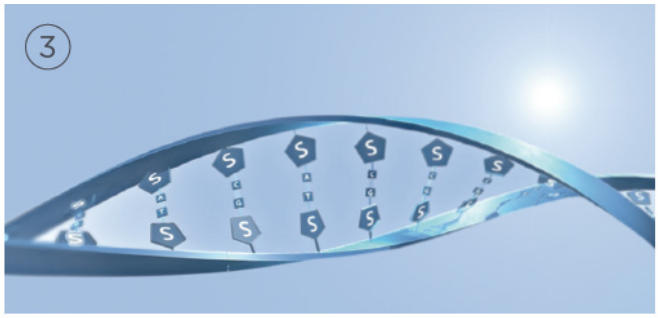


rise to activated forms of cofactors that absorb light within the UVA and blue spectra. As an integral component of photolyases, they exhibit chromophore-like properties by transferring their extra electrons (reduction) to the damaged DNA segments and therefore breaking them down to nucleotide monomers while restoring their own normal redox balance (Figure 2).[2] Nearly 45% of these UV-induced lesions can be repaired by the activity of DNA photo-reactivative enzymes, "photolyases".[8] Unfortunately, although humans do encode for enzymes similar in nature to photolyases that work in the blue light spectrum affecting our circadian rhythms, we do not have endogenous DNA photo-reactivative enzymes similar to non-mammals (Figure 3).[2]

There are two classes of photolyases found in nature: class I found in bacteria and some viruses, and class II in animals and plants. There are variations between the two classes of enzymes as to which utilize other cofactors, such as MTHF and 8-HDF, which augment the absorption capacity of the chromophores (Figure 4). Other classes have been described but the other cofactors have not been clearly elucidated and named, but their activation by reactive oxygen species (ROS) make them heavily involved in the process of DNA repair.[2] They exhibit non-covalent binding to

FIGURE 2. After photoactivation, photolyase identifies the defective DNA where the helix breaks by converting the dimers into monomeric bases. Normal transcription mechanisms by DNA polymerase then resume.[1,2,8]

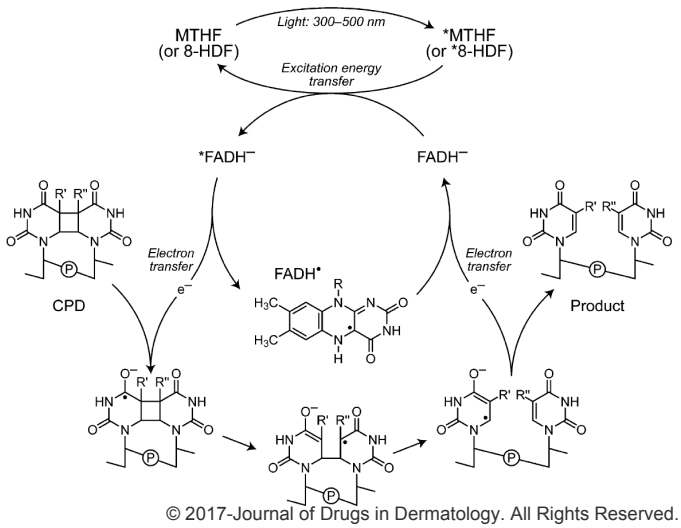
FIGURE 3. Almost half of the thymidine dimers can be repaired. Humans have other endogenous repair mechanisms such as T4N5 endonuclease, which are less efficient, therefore demonstrating the potential benefit of photolyases when exogenously applied.[1,2,8]



segments of DNA using the Flavin cofactor, one side being hydrophobic and the other with a polar charge, similar to the structures of thymidine dimers. The structure of the enzyme involves the cofactor being placed deep in the center, lined with the electrical charges in alignment with those of the DNA helix, and a central "cavity" that essentially swallows the faulty DNA and allows for the single strand to be repaired.[2,9] Similar activities are observed with DNA endonucleases and glycosylases despite the structural differences, but it is the efficiency of photolyases to bind to damaged DNA that make them unique especially in comparison to studies showing inferior binding to intact DNA strands.[2,10,11]

One of the most important class II photolyases that has been isolated and studied for potential utility as an exogenous agent comes from a cyanobacterium known as *Anacystis nidulans,* which is common in marine plants. The enzymes harvest energy from activation in the blue light spectrum, using the conversion of FAD and MTHF to excited forms to transfer energy through the enzymatic pathway. This reaction converts and repairs both CPD and 6-4 PP defects, resulting in an overall improvement in immune surveillance mechanisms, reduction in MMPs, and consequently reduction in damage via ROS and other cytokines.[2,7,12,13]

FIGURE 4. Proposed mechanism of CPD repair by photolyases, including the role of cofactor chromophores FAD in reduced form, and photoconversion of cofactors MTHF/8-HDF to augment light absorption. In the diagram, "R" represents Thymidine in DNA and Uracil in RNA. (Reprinted from: Weber, S., "Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase," *Biochimica et Biophysica Acta*, 2005, 1707:8.)



The primary goal of exogenous delivery of photolyases is to help improve the endogenous DNA repair capacity. Photolyase's mechanism of action help counter the progression of CPDs within photo damaged keratinocytes into actinic keratoses.[17] The exogenous delivery of photolyases has been studied and improved upon over many years, primarily in Europe, for use

This observation was examined by several in vitro assays for IL-6 concentration before and after exposure to photolyases. IL-6 has been demonstrated to impact apoptosis and dermal integrity aside from the effect on the Th-1 profile. The application of photolyases reduced IL-6 levels in vitro and it was hypothesized that there could be effects on the photoaging process.[7,14] This was followed by studies on keratinocyte adhesion molecule expression focusing on ICAM-1. As a result of photolyase application, expression of ICAM-1 in treated photodamaged skin was reduced indicative of improvement of overall immunocompetence and restoration of collagen stability.[7,15] A small study examined the histological impact of exogenous *A. nidulans* photolyases encapsulated in liposomes on UVB-irradiated human skin, measuring the efficiency of CPD repair in treated vs untreated skin. Measuring at 22.5 hours after application, there was 45% reduction in the formation of CPDs in the treated group compared to the untreated group.[8]

From these assays and observations, the potential application of photolyases was given consideration in strategies of photoprotection, photoaging, and photocarcinogenesis.

Clinical Applications of Photolyases in Dermatology

Photoprotection using daily sunscreen can reduce the progression of actinic keratosis to NMSC. Moreover, as demonstrated in several clinical trials, regular sunscreen use with high SPF also induced remission of existing lesions.[19,20] Although the FDA guides consumers to use a sunscreen with SPF>15 and for it to be applied every two hours, there is more evidence suggesting these may provide insufficient protection, given that the true sun protection factor is diluted by inconsistent user application.[20,21,22,24] These observations served as the template for the development of more advanced products that incorporate UV filters and liposomes containing photolyases for photoprotection as well as the potential role as chemoprevention of actinic keratoses.[22]

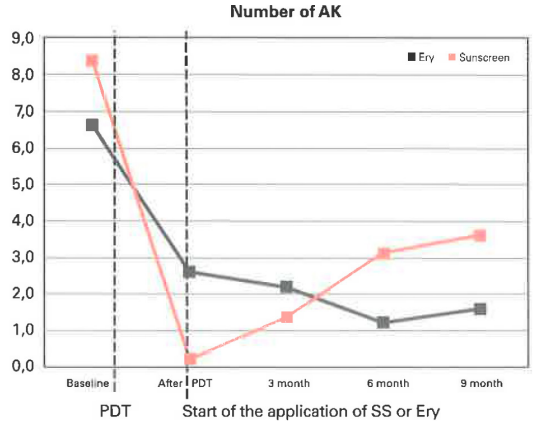
in cosmeceuticals and in sunscreens. As with any ingredient, the epidermal penetration and delivery are dependent on the ingredient's compatible molecular weight and configuration of the liposome to vehiculize the active ingredient. As important, the electron balance has to match to facilitate repair of the damaged helix.[7, 13] The delivery from liposomes into the epidermis with evidence of repair of targeted DNA in damaged keratinocytes has been demonstrated by Yarosh et al measurable within 60 minutes.[7,12] The same group also demonstrated the impact of the pH gradient that occurs over all layers of the epidermis, as the liposomes delivered from the topical application vehicle eventually rupture with subsequent intracellular enzyme release.[7,18]

The combination of photoprotective agents, such as physical sunblock and photolyase enzymes allow for agents to repair and protect patients from past and future damage. Sustained clinical observations in photodamaged skin are supported by a 9 month long study involving 30 patients after treatment with photodynamic therapy on the face or scalp.[26] The 15 subjects treated with sunscreen containing liposome encapsulated photolyase daily after one PDT treatment all had a longer remission time of AKs and did not need an additional PDT treatment in comparison to 10 of the 15 subjects in the group that was treated with sunscreen without photolyase that required a second treatment (Figure 5).[26] The findings suggest that the impact of PDT on restoration of epidermal differentiation markers, enhanced antigen presentation, and overall improvement of antitumor immune responses, can be maintained by the similar responses to skin treated with photolyases. [27] The concept of maintenance of the gains of destructive therapies in combination has, to date, been more associated with other topical modalities such as imiquimod, ingenol mebutate, and 5-FU, but appears to have a similar approach with photolyases in a sunscreen.

Another study involving 35 patients with AKs on the scalp was conducted in Spain where 12 subjects with actinic keratoses underwent treatment with photolyases in sunscreen or a SPF 50 sunscreen. Both groups were evaluated using dermoscopy as well as for lesion counts, and at the end of the study, the photolyases-treated group demonstrated nearly 70% resolution of clinically visible AKs but significant changes using the dermatoscope indicative of photodamage.[28]

The clinical utility of photolyases aside from their emergence in sunscreens is also evident in cosmeceuticals and topical photoaging strategies, often in combination or separate from retinoids, antioxidants, and other reparative ingredients such as T4N5 endonucleases.[7] However, the missing link in dermatology continues to be an effective topical modality for prevention of skin cancer; what has bypassed the conventional approaches in dermatology is the issue that anywhere between 0.025 and 16% of AKs can progress to invasive SCC

FIGURE 5. Evidence of sustained remission of previously treated AKs and preventative effects of photolyases compared to conventional sunscreens in patients treated once with photodynamic therapy. All patients in the photolyases group (ery) avoided a second PDT treatment given the outcomes while 10 of 15 subjects in the sunscreen group required a second treatment to maintain clearance.[26]



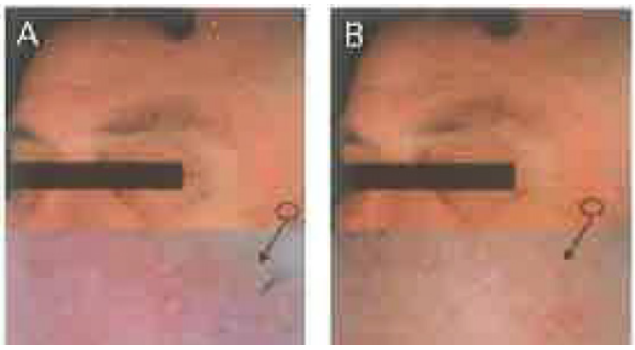
with risks varying with age, gender, chronic UV exposure, and location of AKs.[29,30] Using the toothpaste analogy again, just like the dental patient with a sweet tooth needing more than just prevention of cavities, the high-risk photodamaged patient is in need of an approach that changes the process instead of just protecting from further UV-induced damage. Research involving *Polypodium leucotomos* extract is another such ingredient that extends the possibilities of a topically applied product used for more than photoprotection to cancer prevention. As demonstrated in UV-treated mice models, after treatment with *Polypodium leucotomos* extract there were increases in epidermal p53 expression, delays in tumorigenesis, and restoration of apoptosis given the anti-oxidant impact.[31,32] Given the reported impact on DNA repair and on similar epidermal markers, photolyases may eventually play a similar role as a topical agent in the approach to photodamage as more long-term studies in humans are conducted.

Evidence and Data of Photolyases in Dermatology

One of the most complete studies published on the repair mechanisms of sunscreens containing photolyases was conducted in Barcelona by Puig et al. A study group of 20 patients was examined in the clinic, by biopsy, dermatoscope, and confocal microscope (honeycomb cells as a measure of dyskeratosis) after a two months treatment twice a day (at breakfast and lunch like dermatologists instruct patients), assessing the change in the field of photodamage and impact on AKs. The key to the study was the use of this sunscreen with photolyases as monotherapy, not just as an adjunct to other therapies with appropriate washout from previous AK treatment.[33]

At the conclusion of the 8 week treatment period the findings were as follows: 1) Overall clinical improvement in erythema,

FIGURE 6. Clinical and Dermatoscopic improvement in treatment area after 6 months of using sunscreens containing liposome encapsulated photolyases.[28]

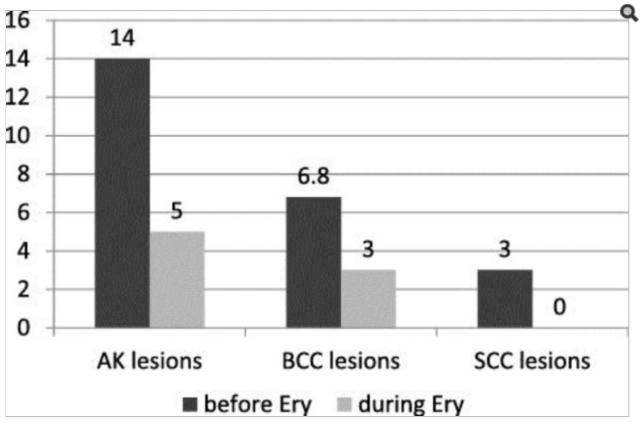


scaling, and dyschromia. 2) Confocal microscopic imaging revealing improvement in the atypical honeycomb appearance and detachment of keratinocytes. 3) Histological reduction of epidermal hyperplasia and presence of atypical keratinocytes. And 4) Immunofluorescence exams with decreased staining of CPDs and expression of Ki67, p21, and PCNA, all important markers of atypical epidermal proliferation in progression to actinic keratosis.[33] As a reminder, Ki67 and proliferating cell nuclear antigen (PCNA) are markers of epidermal proliferation, p53 and p21 are pro-apoptotic, and bcl-2 are anti-apoptotic markers.[32,33] Elevated expression of these markers was noted, suggesting a high level of actinic damages in the skin selected for the study. More specifically, over-expression of p21, which occurs from insult to the DNA helix and the subsequent cell cycle arrest in response to mutation to p53, may turn out to be the result of a decreased level of CPDs in the cells after the 8-week treatment course. These pivotal observations in this small study group identify this product as not just protective but therapeutic such that a patient treated for a longer period over a larger surface could experience improved protective effects from photocarcinogenesis.[33]

Another important study made comparisons of sunscreens with vs without photolyases on the important epidermal markers previously mentioned, specifically the population of CPDs, and the clinical correlation of progression of AKs in the treatment area.[34] A group of 35 patients treated with PDT for AKs were also randomized to receive the two different sunscreens. Biopsies were taken randomly from within the treatment field before and one month after the PDT treatments as well as one year later to measure long-term changes in expression of p53 and Ki67. Although at baseline both groups were comparable, after daily application for one year the expression of both markers was significantly reduced, even as early as one month for the expression of p53. These findings supported the observation of improvement in the entire field of photodamage, but also as a possible predictor for reduced carcinogenesis in these patients.[34]

Finally, a retrospective report was published on five women and three men with xeroderma pigmentosum and the potential benefits of sunscreen with exogenous liposome encapsulated photolyases. Using this population with the inherited defects in nucleotide exicion repair (NER) mechanisms and the ongoing formation of CPDs allows for demonstration of DNA repair on larger skin surfaces highly prone to new malignancies.[7,8,35] These eight subjects were treated with sunscreen either with or without photolyases for at least 12 consecutive months with documentation of the rates of new AK or NMSC during active treatment and for 12 months. New AK, BCC, and SCC mean lesion numbers during the yearlong treatment were 5, 3 and 0, respectively, in comparison with 14, 6.8, and 3 lesions, respectively, in the non-photolyase group. In summary, during the 1-year pre-treatment period with photolyases in sunscreen, there was a 65% reduction in appearance of new AK lesions and with 56 and 100% reductions in the incidence of new BCC and SCC lesions, respectively (Figure 7).[35]

FIGURE 7. Differences in the mean rate of production of AK and NMSC over a one year treatment time with sunscreen containing photolyases.[35]



Mean rate of skin lesions before and after Ery treatment (12-month time span).

CONCLUSION

Research, largely based in Europe, has expanded the clinical value of sunscreens containing liposome encapsulated photolyase DNA repair enzymes for patients undergoing treatment of actinic damage, high risk Fitzpatrick I-III patients, and patients requiring additional chemoprevention support due to chronic disease or medical treatments. These advances represent a significant opportunity to leverage photolyase containing products to dermatology patients to augment direct treatment modalities or within our cosmetic populations to enhance anti-aging regimens in place of traditional photoprotection options. The presented data demonstrates the distinction between conventional sunscreen options, compared to a novel and more complete approach combining photoprotection and photo repair with photolyase containing sunscreens.

DISCLOSURES

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