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Photoprotection by Topical DNA Repair Enzymes

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Many of the adverse effects of solar UV exposure appear to be directly attributable to damage to epidermal DNA. In particular, cyclobutane pyrimidine dimers (CPD) may initiate mutagenic changes as well as induce signal transduction responses that lead to a loss of skin immune surveillance and micro-destruction of skin structure. Our approach is to reverse the DNA damage using prokaryotic DNA repair enzymes delivered into skin using specially engineered liposomes. T4 endonuclease V encapsulated in liposomes (T4N5 liposome lotion) enhanced DNA repair by shifting repair of CPD from the nucleotide excision to the base excision repair pathway. Following topical application to humans, increased repair limited UV-induction of cytokines, many of which are immunosuppressive. In a recent clinical study, topical treatment of UV-irradiated human skin with T4N5 liposome lotion reduced the suppression of the nickel sulfate contact hypersensitivity response. Similarly, the photoreactivating enzyme enhances repair by directly reversing CPDs after absorbing activating light. Here also treatment of UV-irradiated human skin with photoreactivating enzyme in liposomes and photoreactivating light restored the response to the contact allergen nickel sulfate. These findings confirm in humans the observation in mice that UV induced suppression of contact hypersensitivity is caused in part by CPDs. We have tested the ability of T4N5 liposome lotion to prevent UV-induced skin cancer in patients with xeroderma pigmentosum (XP), who have an elevated incidence of skin cancer resulting from a genetic defect in DNA repair. Daily use of the lotion for one year in a group of 20 XP patients reduced the average number of actinic keratoses by 68% and basal cell cancers by 30% compared to 9 patients in the placebo control group. Delivery of DNA repair enzymes to skin is a promising new approach to photoprotection.

Key words: DNA repair, T4 endonuclease V, cyclobutane pyrimidine dimer, liposomes, xeroderma pigmentosum

INTRODUCTION

Xeroderma pigmentosum is an autosomal recessive genetic disease in which patients develop progressive actinic damage, pigmentation abnormalities and malignancies in sun-exposed skin [1]. They share a biochemical defect in processing DNA damage, particularly the types of damage induced in DNA by the ultraviolet light in sunlight (UV-B, 290 – 320 nm).

In 1975, Tanaka, Sekiguchi and Okada [2] demonstrated that the bacteriophage T4 DNA repair enzyme, T4 endonuclease V, could initiate excision repair of UV-induced DNA damage in XP cells. T4 endonuclease V specifically recognizes cyclobutane pyrimidine dimers (CPD) in DNA, the most common lesion caused by sunlight in DNA. Although this method of initiating excision repair is not the same as the pathway followed by the endogenous human DNA repair enzymes, once the cleavage at CPD has been made by T4 endonuclease V,

the endogenous human DNA repair enzymes, once the cleavage at CPD has been made by T4 endonuclease V, enzymes of the host cell then follow to remove the hanging CPD lesion by exonuclease activity and then resynthesize undamaged single-strand DNA using the opposite strand as a template. The *den*V gene for T4 endonuclease V has been cloned and sequenced [3]. The three dimensional structure has been deduced by X-ray diffraction [4].

In the late 1980's, our laboratory discovered that this enzyme could be delivered into cells using liposomes as a vehicle. Liposomes are microscopic spheres composed of lipid bilayers that spontaneously organize in water from lipids, and under the right conditions T4 endonuclease V can be entrapped between the membranes. Such liposomes are called T4N5 liposomes, and they have been shown to efficiently delivery T4 endonuclease V into cells in culture [5]. T4N5 liposomes in a hydrogel lotion and applied to either mouse or human skin penetrate and deliver the T4 endonuclease into cells in less than one hour [5,6]. The enzyme localized in the epidermis, with little delivery beyond into the dermis.

ENHANCED DNA REPAIR

DNA repair was studied in UV-B irradiated human skin explants treated with T4N5 liposome lotion. Epidermis was collected from explants 6 hours after irradiation with UV-B and treatment with increasing doses of T4N5 liposome lotion [6]. The results showed a steep reduction in the fraction of CPD remaining with increasing dose of T4N5 liposome lotion up to a plateau at approximately 0.5 μg/ml. At higher doses of liposomal enzyme there was no further increase in CPD repair, suggesting that increasing T4 endonuclease accelerates the repair process, until we reach a level of incision in which the excision machinery is working as fast as possible, and incision is no longer the rate limiting step.

PROTECTION FROM UV IMMUNOSUPPRESSION

UV exposure inhibits the contact allergic response. Mice treated with UV-B and then sensitized at the site to dinitrofluorobenzene (DNFB) or fluoroisothiocyanate (FITC) antigen fail to develop a contact allergic response and the Langerhans cells at the site have reduced size and number and loss of dendricity [7, 8]. However, treatment of the UV-B irradiated site with T4N5 liposomes prior to sensitization to DNFB or FITC restores the immune response, and microscopic examination of the skin reveals a preservation of the Langerhans cell density and morphology.

These findings have been replicated in human studies, but for ethical reasons only the elicitation of the immune response is readily examined. In 2000 Dr. Jean Krutmann and colleagues reported a study of UV suppression of the allergic response to nickel [9] using exogenous delivery of photoreactivating enzyme in liposomes. They first delivered an erythemal dose to the forearm of volunteers. Then the topical application of the DNA repair liposomes and exposure to photoreactivating light not only reduced the erythema but prevented the UV-induced immunosuppression of the allergic response to a nickel challenge in these subjects. A similar result was recently observed by Drs. Gary Halliday and Johanna Kuchel of the University Sydney using T4N5 liposome (personal communication). In their protocol they irradiated 15 nickel allergic volunteers with 4 sub-erythemal solar-simulating light doses each day, followed by T4N5 liposome lotion or control lotion treatment, for 4 consecutive days. They then challenged with nickel and observed the allergic responses. With the control

liposomes, the protocol produced statistically significant immunosuppression. At the T4N5 liposome treated sites, the immunosuppression was measurable but was not statistically significantly different than the control/no UV site.

CYTOKINE GENE EXPRESSION

TNFα and IL-10 are primary cytokines that are induced by UV and that contribute to suppression of the contact hypersensitivity response. The ability of T4N5 liposome lotion to suppress expression of TNFα has been confirmed in human studies [10]. UV-irradiated volunteers were treated with T4N5 liposomes and after 6 hours skin biopsies from treated sites were analyzed for TNFα mRNA by in situ hybridization. TNFα gene transcription was dramatically reduced after T4N5 liposome lotion treatment compared to controls. IL-10 induction, measured both by IL-10 gene transcription and IL-10 protein expression, was dramatically reduced after T4N5 liposome lotion treatment compared to controls. These results suggest that DNA damage is responsible for induction of cytokines, and these cytokines participate in the suppression of the immune response.

SKIN CANCER PREVENTION

The effect of enhancing DNA repair on the development of new skin cancers has been examined for the first time in XP. Thirty patients with a history of skin cancer or AK were recruited to a prospective, multi-center, placebo-controlled, randomized and blinded study on the incidence of new basal cell carcinoma (BCC) and actinic keratosis (AK) in XP patients applying T4N5 liposome lotion daily for one year [11]. The annual rate of new BCCs was reduced 30% by drug treatment, from 5.4 in the placebo group to 3.8 in the T4N5 Liposome Lotion group. The annual rate of AKs was also reduced 68%, from 25.9 in the placebo group to 8.2 in the T4N5 Liposome Lotion group. No serious adverse affects were observed, and the difference in the rates of AK between the groups was observed as soon as 3 months after beginning treatment.

CONCLUSION

DNA damage following UV exposure is important because it induces mutations that activate oncogenes and silence tumor suppressor genes. It is now becoming clear, however, that the immunosuppressive effects of UV are also important in determining the incidence of skin cancer. Increasing DNA repair has the promise both of removing the initiating lesion before mutations can arise, and of reducing the signals that allow nascent tumors to escape immune surveillance. This also suggests that even in skin that has sustained many years of damage, ongoing processes determine the appearance of new skin lesions, and that DNA repair now can improve the outcome of damage sustained in the past.

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