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T4 endonuclease V: review and application to dermatology

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Background: T4 endonuclease V was originally isolated from *Escherichia coli* infected with T4 bacteriophage. It has been shown to repair ultraviolet (UV)-induced cyclobutane pyrimidine dimers in DNA, which, when unrepaired, contribute to mutations that result in actinic keratoses and non-melanoma skin cancers (NMSC). This is a particular concern in patients with genetic defects in their DNA repair systems, especially those with xeroderma pigmentosum (XP). When packaged in liposomes and applied topically, T4 endonuclease V can traverse the stratum corneum and become incorporated within the cytoplasm and nucleus of epidermal keratinocytes and Langerhans cells. *Objective*: To review all major studies evaluating the efficacy of T4 endonuclease V in animals and humans, the toxicity and safety profile of the topical medication and its potential clinical uses. *Methods*: A literature search was performed through PubMed/Medline, using the keywords 'T4N5', 'T4 endonuclease V' and 'dimericine'. Papers found in the bibliographies of those identified in the initial search and deemed relevant were also included. *Conclusion*: This enzyme increases the repair of UV-damaged DNA and produces other beneficial effects on UV-damaged cells. In clinical trials in XP patients, topical application of liposome-encapsulated T4 endonuclease V reduced the incidence of basal cell carcinomas by 30% and of actinic keratoses by > 68%. Adverse effects were minimal, and there was no evidence of allergic or irritant contact dermatitis. Although the photoprotective effect of T4N5 has been investigated only in XP patients, the possibility exists that it may benefit others likely to develop premalignant keratoses and NMSC, such as organ transplant recipients receiving immunosuppressive therapy and individuals who have had numerous psoralen plus UVA photochemotherapy treatments. It may be also be effective for normal individuals.

Keywords: cyclobutane pyrimidine dimers, dimericine, DNA repair, excision repair, liposomes, skin cancer, T4 endonuclease V, T4N5, ultraviolet, xeroderma pigmentosum

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1. Introduction

1.1 Disease description and pathogenesis of NMSC

Cutaneous squamous cell and basal cell carcinomas, classified together as non-melanoma skin cancer (NMSC), are the most common malignancies in Caucasians [1], and there is indisputable evidence that ultraviolet (UV) irradiation plays a major role in their pathogenesis [2]. When the skin is exposed to solar ultraviolet B (290 – 320 nm) radiation, damage to DNA incurs in the form of cyclobutane pyrimidine dimers (CPD) and pyrimidine (6 – 4) photoproducts [3-5]. If these photoproducts reside within the ras oncogenes or the p53 and patched homolog (PTCH) tumor suppressor genes, they can initiate changes in keratinocytes, the cell of origin for NMSC, that ultimately result in the development of NMSC [6-11]. Because CPD and 6 – 4 photoproducts occur when the skin is exposed to even small amounts of UV irradiation, it is fortunate that all mammalian cells are

equipped with several DNA repair mechanisms which are able to protect the cell by removing the damaged DNA. [5,12] Without such repair processes in the skin, large numbers of UV-induced malignancies would result. Evidence of the importance of these DNA repair mechanisms for the prevention of UV-induced skin cancers is derived primarily from observations in patients with xeroderma pigmentosum (XP). XP is a genetically heterogeneous group of rare autosomal recessive diseases which are classified into seven complementation groups (XPA–XPG) plus a variant form. This group of diseases is caused by enzymatic defects in the initial stages of DNA repair [13]. These individuals have a predisposition to develop large numbers of UV-induced basal cell and squamous cell carcinomas and melanomas at an unusually early age [14]. The XP variant type is caused by defects in the post replication repair machinery in which nucleotide excision repair (NER) is not impaired [15].

It should be noted that CPDs are produced three times as frequently as (6 – 4) photoproducts [16] and (6 – 4) photoproducts are repaired much more quickly than CPDs in mammalian cells [17]. Although both are potentially mutagenic, CPDs are the major contributor to UV-induced mutations in skin cancer in mammals [16]. In a recent study, researchers generated transgenic mice that ubiquitously express CPD-photolyase, (6 – 4) photoproduct-photolyase, or both, which allows rapid light-dependent repair of CPDs or (6 – 4) photoproducts in the skin. Their studies revealed that the vast majority of semiacute responses in the UV-exposed skin (i.e., sunburn, apoptosis, hyperplasia, and mutation induction) can be ascribed to CPDs. Moreover, CPD-photolyase mice, in contrast to (6 – 4) photoproduct-photolyase mice, exhibited superior resistance to sunlight-induced tumorigenesis. This data identifies CPDs as the principal cause of NMSC [18]. Another recent study suggested that both CPDs and (6 – 4) photoproducts contribute to UV-induced apoptosis in NER-deficient cells, while in NER-proficient cells, CPDs are the only lesions responsible for UV-induced apoptosis [19]. The fast repair of (6 – 4) photoproducts in normal cells may be responsible for their inability to induce deleterious effects.

Depending on the type of DNA damage that occurs, one or two major repair pathways is utilized, either NER or base excision repair [5]. CPD and (6 – 4) photoproducts are repaired primarily by NER [20]. This process involves the use of several different enzymes. Under special circumstances, CPDs can be repaired by base excision repair, using the enzyme T4 endonuclease.

Non-melanoma skin cancers take many years to develop and evolve through a characteristic sequence of events. Basal cell carcinomas result from mutations in the PTCH gene, an element of the sonic hedgehog signal transduction pathway, and a disproportionate number of these tumors have UV-specific mutations in the DNA of this gene [9-11]. Basal cell carcinomas arise *de novo* and are not associated with a precursor lesion. In contrast to basal cell carcinomas, at least 90% of cutaneous squamous cell carcinomas contain UV signature mutations in the p53 gene [21-23]. Approximately 80% of squamous cell carcinomas of the skin evolve from actinic keratoses [24]. Actinic keratoses (AKs) are clinically apparent proliferations of mutant keratinocytes that are confined to the epidermis. AKs are one of the strongest determinants of skin cancer [25]. These lesions can progress into squamous cell carcinomas [26,27]. Estimates of the likelihood that an individual actinic keratosis will eventually become an invasive squamous cell carcinoma, are in the range of 1 – 10% [28,29].

1.2 Few proven approaches to the chemoprevention of non-melanoma skin cancer

The need for chemoprevention of AKs and NMSC is great, especially for high-risk patients such as those with a genetic predisposition for DNA damage (XP, basal cell nevus syndrome, albinism), organ transplant recipients on immunosuppressive therapy, patients who have received large numbers of psoralen plus UVA (PUVA) photochemotherapy treatments, and those with numerous arsenical keratoses [30]. Although NMSC are not a significant cause of mortality in the general population, they do cause significant morbidity and mortality in these patients. XP patients, for example, have at least a 1000-fold increase in skin cancer, with the first skin cancer typically occurring in children younger than the age of 10 [14,31,32]. The mainstay of treatment is surgery, and this results in numerous surgical procedures resulting in significant morbidity (disfigurement, pain, recovery time and financial strain). Ideally, a chemopreventative agent will inhibit skin cancer with minimal toxicity to the patient and would preferentially affect premalignant or malignant cells leaving normal cells unaffected.

While sunscreens are routinely recommended by dermatologists for the prevention of skin cancer for these conditions, studies have shown that they are only modestly effective against cutaneous squamous cell carcinomas and have no effect on basal cell carcinomas [33,34]. One of the limiting factors of sunscreen use has been that individuals apply these agents inconsistently and in amounts substantially less than that required to achieve the full sunburn protection factor (SPF) printed on the product label [35]. Also, application of a sunscreen with a high SPF seems to produce a false sense of protection. Since until recently, most sunscreens that were commercially available were much more efficient at protecting against UVB than UVA radiation, this has resulted in an increase in the amount of UVA radiation that individuals received [36,37]. UVA does not damage DNA by deleterious oxidative effects but rather produces CPDs [38]. Finally, while sunscreens can prevent further UV damage, they have no effect on pre-existing UV damage.

Due to the limitations of sunscreens, there is a great deal of interest in identifying alternative agents that will complement sunscreens as chemopreventative agents for UV-induced skin cancers. Oral retinoids were some of the first agents evaluated and were examined in XP patients.

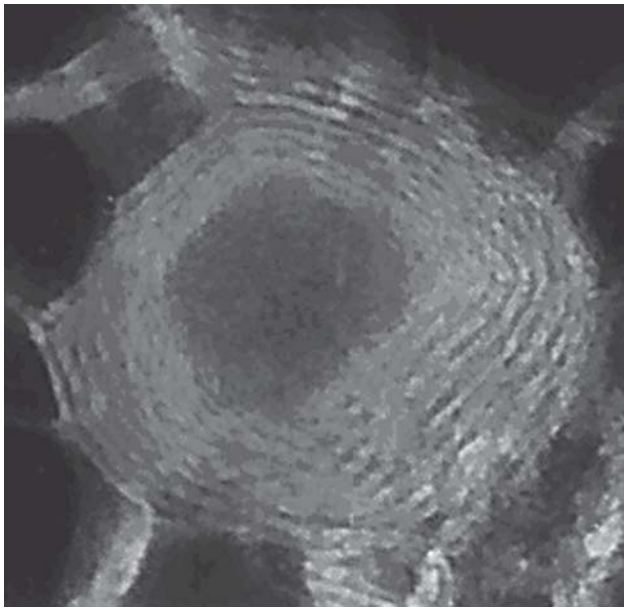

Figure 1. Electron microscopic photo of T4N5 liposome.
Courtesy of Cora Bucana and Daniel Yarosh.

While highly effective, large doses were required and the beneficial effects were no longer apparent when the drug was discontinued [39]. At these doses, known side effects including teratogenicity, hyperlipidemia, bony resorption, spinal hyperostosis, skin fragility and mucocutaneous dryness precluded long-term use of the agents [39]. Beta-carotene supplementation has also been evaluated as a potential chemopreventative therapy but no beneficial effect on the rates of AKs or NMSC was found [25,34] Dietary modification has been examined as a potential way to prevent the formation of AKs and NMSC, a concept which is based on positive results from animal studies [40,41]. Over a 24 month period, a diet that limited the fat intake to 20% of total caloric intake reduced the incidence of AKs by 70% in the group randomized to the low-fat diet [42]. This finding was confirmed in later studies with more subjects, which also showed statistically significant decreases in NMSC by decreasing the amount of fat intake in the diet [43,44]. Another chemopreventative approach has been the use of COX-2 inhibitors. COX-2 is overexpressed in AKs, squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) as well as in human epidermal cancer cell lines [45,46]. Specific inhibition of COX-2 with topical celecoxib has been shown to reduce UVB-mediated inflammation, edema, dermal neutrophil infiltration and activation, prostaglandin E2 levels and the formation of sunburn cells [47]. Epidemiological studies have demonstrated that regular users of NSAIDs have lower risks of SCC and fewer AKs than non-users [48]. Controlled, randomized clinical studies to evaluate the effect of oral COX-2 inhibitors on NMSC are currently in progress.

2. Liposome-encapsulated T4 endonuclease V

2.1 Overview of the market

Because DNA damage plays a fundamental role in photocarcinogenesis and T4 endonuclease V efficiently removes UV-damaged DNA, there has been interest in using the liposomal form of T4 endonuclease V (also known as T4N5) as a chemopreventative agent in UV-induced skin cancer and AKs. There are currently no topical DNA repair enzymes approved by the FDA in the US for the prevention of skin cancer.

2.2 Introduction to the compound

In 1975, Tanaka and colleagues demonstrated that the bacteriophage T4 endonuclease V, a 16,500-Da polypeptide isolated from *Escherichia coli* infected with bacteriophage T4, was able to augment nucleotide excision repair in human cells and initiate removal of CPDs [49]. In the late 1980s, Yarosh and colleagues discovered that this same enzyme could be delivered to cells using liposomes as a vehicle. Liposomes are microscopic spheres composed of lipid bilayers that spontaneously organize in water from lipids. Under the right conditions, T4N5 can be entrapped between the membranes [50]. Such liposomes are called T4N5 liposomes and have been shown to effectively deliver repair enzymes to cells in culture [51]. When applied topically to the skin *in vivo*, the liposomes can penetrate the stratum corneum and reach the deeper layers of the epidermis. The T4N5 single polypeptide can substitute for the multi-enzyme complex found in humans to initiate excision repair, and in so doing, can repair cyclobutane pyrimidine dimers [49]. In clinical trials, topical application of T4N5 liposomes was found to be successful in preventing the development of new AKs and basal cell carcinomas in patients with XP [52], a group of patients at high risk for AKs and NMSC.

2.3 Chemistry and mechanism of action

The T4N5 DNA repair enzyme has been encapsulated in pH-sensitive liposomes, and the combination is termed 'T4N5 liposomes'. These spherical objects are approximately 200 nm in diameter when viewed by electron microscopy (**Figure 1**) [53]. They have the ability to penetrate the stratum corneum and thus interact with living tissue [53]. Liposomes have many purposes and are useful in: i) stabilizing compounds; ii) retarding transit through the skin; iii) solubilizing, in water, compounds that are otherwise insoluble; iv) formulating a compound made up of two drugs that are otherwise incompatible with each other; and v) allowing a compound to permeate the epidermis that is otherwise impermeable [53].

Liposomes have been engineered to topically deliver active T4N5 into the lysozymal sacs of epidermal cells and to enhance DNA repair of UV-irradiated skin [54]. These liposomes bind to the cell surface within 1 min, and begin to coalesce by 10 min. Between 30 and 60 min, the labeled liposome membranes are found to be internally localized. Once inside

Box 1. Effect of T4N5 on cells.

- Interacts with mouse melanoma cells and human melanocytes
- Enhances repair in melanocytes and associated with increase in melanogenesis
- Increases DNA repair synthesis by removal of cyclobutane pyrimidine dimers
- Increases cell resistance to UV irradiation and to cell death
- Anti-mutagenic
- Reduces the number of apoptotic sunburn cells in the epidermis of UV-irradiated skin
- Suppresses UV-induced upregulation of TNFα expression in cells
- Prevents UV-induced upregulation of IL-10
- Blocks the UVB-induced inhibition of delayed-type hypersensitivity reactions
- Prevents the UVB-induced suppression of contact hypersensitivity
- Protects against morphological alteration of Langerhans cells
- Reduces activation of HIV transcription following UV irradiation

the cell, the DNA-repair liposome membranes are destabilized in the acidic lysozymal environment [51]. When applied topically, T4N5 liposomes are able to traverse stratum corneum of skin and localize in the epidermis. In murine studies, fluorescently labeled DNA-repair liposomes were found within the epidermis one hour after application, particularly in the cells surrounding the root sheath in the hair follicle [51].

In vitro and *in vivo* studies have been conducted to determine the effects of T4N5 liposomes on cells (**Box 1**). T4N5 increases DNA repair synthesis by removing CPDs (**Figure 2**) [49,55-58]. The enzyme catalyzes two reactions [59]. The first reaction utilizes the pyrimidine dimer glycosylase activity that hydrolyses the *N*-glycosyl bond of the 5′-thymidine in the thymine dimer site. In the second step, the apurinic/apyrimidinic endonuclease activity cleaves the phosphodiester bond at the abasic site, which occurs via β-elimination [60]. This abasic site is recognized by the much faster base excision repair (BER) system in human cells. An exonuclease removes bases around this site, and then a polymerase fills in the gaps.

It is important to note that not all CPD must be removed before dramatic biological effects are observed. In most experiments, T4N5 liposome lotion reduced the CPD frequency by less than 50% yet significant benefit was noted [53]. T4N5 increases resistance of cells to UV irradiation and cell death [58] and is antimutagenic [53]. It also suppresses UV-induced upregulation of TNF-α expression in cells [61]. TNF-α is a primary cytokine responsible for inflammation and the immunosuppressive activities of UVB radiation. UVB is a well-known inducer of TNF-α expression in mice and man [53,62]. Much lower doses of UV irradiation are required for induction of TNF-α in cells derived from XP patients than for repair-proficient cells, demonstrating the importance of DNA damage in TNF-α gene expression [53]. T4N5 also prevents UV-induced upregulation of the immunosuppressive cytokine IL-10 *in vitro* and *in vivo* [61,63,64]. When T4N5 is used to pretreat skin and then subsequently irradiated with UV light, there are fewer apoptotic sunburn cells in the epidermis compared with controls [65]. *In vivo* studies have also shown that it blocks UVB-induced inhibition of delayed-type hypersensitivity (DTH) reactions [66]. UV exposure of mice before immunization inhibits the induction of the DTH response to *Candida albicans* antigen injected subcutaneously at the unirradiated site [67]. Moreover, treatment of UVB-irradiated skin with T4N5 liposome lotion, prior to immunization, blocks suppression of the DTH response over a wide range of UVB doses [68]. T4N5 prevents suppression of contact hypersensitivity [66,69] and protects against morphological alteration of Langerhans cells [70]. When incubated with mouse melanoma cells and human melanocytes, the liposomes enhance repair in melanocytes and this is associated with an increase in melanogenesis [71]. Finally, DNA repair in cultured cells enhanced by T4N5 liposomes reduces activation of human immunodeficiency virus (HIV) transcription following UV irradiation [72]. It is known that the signals for HIV activation are more potent in DNA-repair-deficient cells [72,73].

2.4 Pharmacokinetics and metabolism

Laboratory testing has shown T4N5 to be somewhat durable and heat-resistant [74]. Quantitative image analysis has determined the half-life of T4N5 in mouse skin to be about 12 h, as determined by antibody staining [75]. The dose-response curve for T4N5 liposome lotion was similar in UVB-irradiated human skin explants to that found for cells in culture: increasing the dose of T4N5 liposome lotion between 0 and 0.5 μg/ml produced an increase in the rate of removal of CPD, which then reached a plateau with no further increase in repair at up to 2 μg/ml [57]. It is thought that this plateau means that increasing T4N5 accelerates the repair process to the point at which incision of damaged DNA is no longer the rate-limiting step. The kinetics of T4N5 liposome lotion has been examined by comparing half-lives of CPD. In previous studies there was a consistent increase in repair in normal cells and in normal subjects. In one particular study, there was an increase in repair after 6 h from ~ 10 to ~ 18%. This means a reduction in CPD half-life from ~ 27 h in normal skin to ~ 16 h in T4N5-treated normal skin [61]. Of note, very few (< 0.1%) of the topically applied liposomes appeared in the systemic circulation or peripheral organs at 6 – 12 h [57].

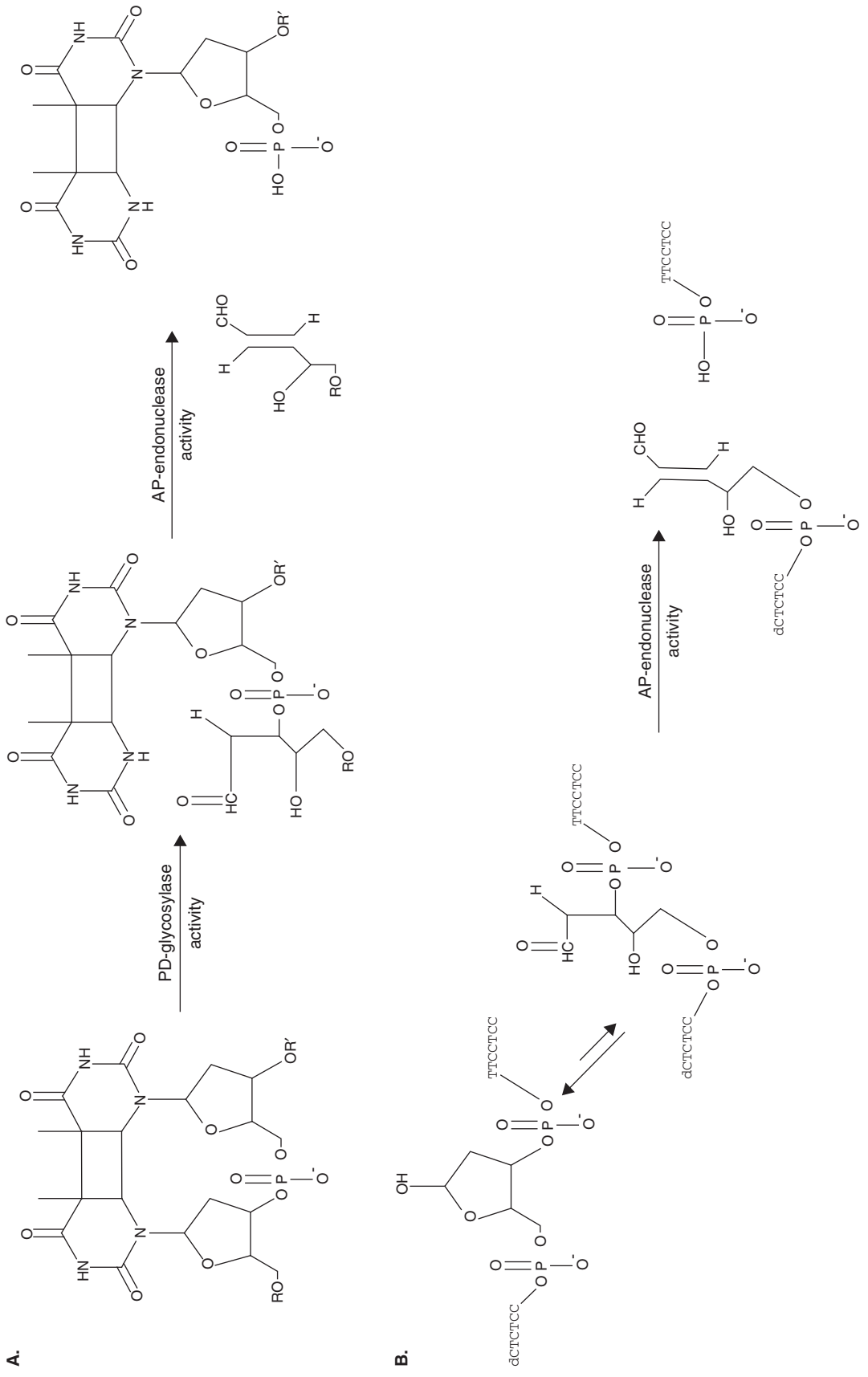

Figure 2. Reactions of T4 endonuclease V with a substrate containing a thymine dimer (A) or an abasic site (B).
Reprinted with permission from Oxford University Press. Original figure printed in Nucleic Acids Res 1992;20(18):4761–4 [81].

2.5 Toxicology

In preclinical toxicology testing, a single oral dose of 5 mg/kg of T4N5 liposome lotion (containing 0.5 μg/ml T4N5 in carbopol or hypan) given to rats produced no behavioral or clinical abnormalities, no deaths over 14 days and no gross abnormalities at necropsy. The medication was found not to be an ocular irritant to rabbits and SKH-1 hairless albino mice and showed no allergic reaction to repeated applications of the topical lotion. No changes were seen in histopathology after repeated applications nor after weekly applications over a prolonged period. Serum chemistries also remained stable; although minor changes were observed in some values, the changes were not found on a consistent basis [76]. T4N5 liposomes were neither carcinogenic nor photocarcinogenic in mice exposed to UV radiation three times a week for 30 weeks. In fact, T4N5 actually reduced the incidence of cancers in UV-irradiated mice over that period [57].

3. Clinical efficacy of liposome-encapsulated T4 endonuclease V

3.1 Phase I studies

Phase I studies were performed in 12 normal healthy volunteers of Fitzpatrick skin type III, with no history of abnormal sun response or skin disease. The volunteers consisted of seven men and five women, aged 22 – 46. Blood was drawn before and 5 days after application of T4N5 liposome lotion. Volunteers were UVB-irradiated with either 1.5 times the minimal erythema dose (MED) in Group 1 (five volunteers, 100 – 120 mJ/cm^2) or 3 times the MED in Group 2 (seven volunteers, 200 mJ/cm^2) on two 3 × 3 cm squares just above the buttocks. Immediately afterward, 4 mm punch biopsies were taken from each irradiated and one unirradiated skin site. One ml (approximately 100 μl/cm^2) from one of the two coded lotions (consisting of one active T4N5 liposome lotion and one control lotion) was applied to one irradiated site and the other lotion to the other site. Biopsies were taken from Group 1 at 4 h and 5 days from each irradiated site and from Group 2 at 24 h and 10 days. Erythema and histological assessment was performed. No consistent or meaningful differences were detected in histopathology when the active-treated site and placebo-treated skin were compared at 4 h, 24 h, and 5 days for Group 1, and at 4 h, 24 h and 10 days for Group 2. There were no adverse reactions to the lotion, and no significant changes were seen in serum chemistries [76].

In another study, the potential for the liposomal lotion to cause allergic contact dermatitis was examined using repeated-insult patch testing. The study involved 100 normal men and women with Fitzpatrick skin types I-IV. T4N5 liposome lotion was applied on Monday, Wednesday and Friday for 10 consecutive applications. After a 12-day rest, a final challenge patch was applied. All induction patches were scored as negative at 48 h and all challenge patches were negative at 48, 72 and 96 h after application [76].

3.2 Phase II studies

Studies were conducted to determine whether topical application of liposome-encapsulated T4N5 was capable of protecting against acute UV-induced erythema (i.e., sunburn). This was accomplished by performing MED testing on six Japanese XP patients. MED is an objective measurement of sensitivity of an individual to developing sunburn in response to UV radiation exposure. MED is generally determined by exposing an area of skin to increasing doses of ultraviolet radiation required to produce a uniform erythema over the irradiated site 48 h after UV exposure [77]. It is important to note that the MED is typically reduced in XP patients. In order to assess the effects of T4N5 liposome lotion on the sunburn reaction in these patients, the MED was determined at skin sites in six XP patients treated with both active and control lotion 48 h after UVB exposure. In five of the six patients, the site treated with active T4N5 liposome lotion had greater resistance to UV irradiation than the site treated with the control liposome lotion ($p = 0.02$, paired *t*-test) [76]. Previous studies with liposomal T4N5 have shown no change in MED in normal skin [61].

The unscheduled DNA synthesis (UDS) assay is widely used to assess DNA repair capacity because it measures the step of resynthesis of excised DNA using radiolabeled DNA precursors. XP patients show reduced UDS compared with normal controls [78,79]. UDS testing was performed on six XP patients 24 h after UV irradiation. Three sites, each of 4 mm in diameter, were exposed to 2 MED of solar simulated radiation (SSR). Immediately after exposure, one site was treated with T4N5 liposome lotion, a second with control lotion and the third with no lotion at all. In biopsies processed for unscheduled DNA synthesis 1 h after application of the lotions, three of the six patients showed an increase in UDS at the active-T4N5-liposome-treated site compared with the control site while two patients had little or no change. Overall, active T4N5 liposome treatment increased UDS in keratinocytes in upper regions of epidermis but had little or no effect on basal keratinocytes [76]. The doses of UV irradiation used in these XP patients were intentionally small, and it is likely that most of the damage was in the upper layer and not uniformly distributed throughout the skin. Additionally, the technology was new as this was among the first experiments performed. This may explain why the UDS repair was greatest in the upper layers. In later studies with DNA-repair liposomes in normal subjects given a measured MED, repair has in fact been greater in the basal layer when compared with that in the upper layer [80].

Another study involving 12 XP patients measured DNA repair by comparing the amount of CPDs in epidermal skin cells in biopsies taken from an untreated site immediately after irradiation with the amount of CPDs in the T4N5-treated sites 6 h after irradiation [76]. No differences were noted in

histopathology. CPD levels did not significantly decrease in irradiated, untreated skin during the 6 h post exposure. At the treated site, however, CPDs did decline in 9 of 11 patients during the 6 h after irradiation. CPDs decreased by 30 – 40% in four of the patients, and the average reduction was 20% compared with the untreated placebo site ($p = 0.024$, Student's *t*-test) [76]. The changes in serum chemistry noted 6 h after treatment were minor. A slight increase in triglyceride levels to a level only 10% above the normal range was noted, which may be related to the fact that patients were not required to fast. There was a slight increase in alkaline phosphatase (less than 10% overall and less than 25% for any one patient), which never exceeded the normal range.

Two XP patients, one male and one female, volunteered to use T4N5 liposome lotion daily for six months to survey problems and benefits that XP patients might encounter during long-term use. Serum chemistry and hematology tests were monitored monthly with no significant changes (none of 10% above or below accepted normal levels). Visual impressions of changes in skin were recorded monthly by a dermatologist. The female patient had a decrease in telangiectasias and solar lentigines in the second month and a decrease in AKs in the fourth month. The male patient had decrease in telangiectasias in the third month and a decrease in solar lentigines and AKs in the fourth month. Overall, most changes occurred in the fourth and fifth months of treatment [76].

A prospective, multi-center, placebo-controlled, randomized and double-blinded study involving 30 XP patients was conducted to assess the safety and efficacy of T4N5 for AKs and NMSC [52]. All of these XP patients had a prior history of skin cancer or AKs. The primary end point of the trial was the incidence of new basal cell carcinomas and AKs in XP patients applying T4N5 liposome lotion daily for 1 year. The annual rate of new basal cell carcinomas declined by 1.6 cancers per year, a 30% reduction ($p = 0.006$) by drug treatment. The annual rate of AKs also reduced by 17.7 lesions per year, a 68% decline ($p = 0.04$) after treatment. The diminution in the rate of AKs was observed within the first 3 months of treatment. No adverse events and no antibodies against the enzyme were detected in the patients' serum. The absence of toxicity confirms earlier studies [76]. Interestingly, during the 6 months after discontinuation of treatment, rates of new AKs and basal cell carcinomas did not increase, contrary to the experience with retinoids [39]. T4N5 lotion may repair CPDs, a fundamental and common source of these neoplasms, a phenomenon that is known to occur in mice and human skin [56,57]. As mentioned previously, (6 – 4) photoproducts in NER-deficient XP cells can clearly lead to apoptosis in cells. Since T4N5 solely repairs CPDs, this may account for the limited action of T4N5 in XP patients [19].

Currently, there is a prospective, multi-center, placebo-controlled, randomized and double-blinded study in progress that is evaluating the efficacy of T4N5 liposome lotion in renal transplant patients with a history of NMSC and AKs. The results of this study are not yet available.

4. Conclusion

The data from both laboratory and clinical studies suggest that T4N5 liposome lotion is very useful in DNA repair and reduces the number of AKs and basal cell carcinomas in XP patients. It remains to be determined whether T4N5 will be effective in other populations at risk for NMSC, such as organ transplant patients and those with genodermatoses predisposing to cutaneous carcinomas Additionally, patients with XP variant, who do not have a defect in NER, may have a different response to T4N5 liposomal lotion compared with other types of XP. It remains to be determined whether T4N5 is an effective preventative agent in normal individuals as well.

5. Expert opinion

T4N5 is a promising topical medication that shows great promise as a photoprotective agent for the prevention of AKs and NMSC in patients with XP. Clinical trials to date have shown that the medication is very safe and highly effective compared with other accepted therapeutic modalities for the prevention of skin cancer in this population. Its mechanism of action differs from that of traditional sunscreens, which block harmful ultraviolet radiation from entering the skin. T4N5 acts to repair and thus remove mutations produced by UV-induced cyclobutane pyrimidine dimers in DNA. Thus, in contrast to sunscreens, T4N5 is able to repair UV damage to DNA that has already occurred. The skin contains DNA repair enzymes but in XP, those enzymes are defective. T4N5 is able to restore the DNA repair capacity in XP.

While not to diminish the importance of T4N5 as a therapy for XP, it should be noted that XP is a very rare disease occurring in 1 in 250,000 in the United States. An equally exciting possibility is its use in other at risk populations that do not have a defect in their DNA repair capacity. For example, organ transplant recipients have an increased incidence of non-melanoma skin cancer because the immunosuppressive medications required to prevent rejection of their organs facilitate their growth and development. T4N5 has been shown to reverse the immunosuppressive effects of UVB radiation on the skin. The fact that even widespread application of T4N5 does not result in systemic absorption is an added advantage for this group of patients. Phase II studies are currently in progress to address the safety and efficacy of T4N5 in the transplant population.

It is reasonable to speculate that T4N5 may also benefit normal individuals who have developed UV-induced

malignancies and premalignancies because of excessive sun exposure. In normal subjects, it is theorized that AKs and NMSC develop because the amount of solar ultraviolet radiation that they receive overwhelms the normal DNA repair capabilities. T4N5 is a bacterial enzyme that repairs UV-damaged DNA via mechanisms that are distinct from those which are present in human skin, suggesting that T4N5 may have added benefit for the population at large.

Finally, it should be noted that ultraviolet radiation has pathological effects on the skin other than skin cancers and AKs. These include sunburn, photoaging and a variety of photosensitivity diseases. These are diseases in which T4N5 may have applicability but its efficacy in those conditions remains totally unexplored at this time.

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Declaration of interest

Dr Elmets is the lead investigator on an NIH-sponsored clinical trial, evaluating the photoprotective effects of T4N5 in renal transplant patients.

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