

# Reversal of DNA Damage in the Skin with DNA Repair Liposomes

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## 15.1 Significance of Functional DNA Repair

Exposure of the skin to ultraviolet (UV) radiation leads to DNA damage and, if unrepaired, to subsequent tumor initiation and skin cancer formation (Ananthaswamy and Kanjilal 1996). The importance of effective DNA repair to restrain skin carcinogenesis is best highlighted by the genetic disease xeroderma pigmentosum (XP) in which a defective nucleotide excision repair leads to the development of multiple skin cancers starting early on in life at a rate more than a thousandfold greater than in the normal population (Kraemer et al. 1994). A molecular analysis of nonmelanoma skin cancers from XP patients and individuals from the normal population reveals typical UV fingerprints (i.e., C to T or CC to TT transitions) at dipyrimidine sites of tumor suppressor genes, such as p53, INK4a-ARF, and patched or oncogenes, such as RAS (Ananthaswamy and Kanjilal 1996), as a direct consequence of unrepaired DNA photoproducts, such as cyclobutane pyrimidine dimers (CPDs). However, UV-induced DNA damage is also one of the triggers of UV-induced immune suppression (Applegate et al. 1989; Kripke et al. 1992), which is crucial in skin carcinogenesis in rodents and, most likely, also humans. For instance, XP patients have been shown to exhibit severe immunologic alterations, which may accelerate tumor growth after tumor initiation (Norris et al. 1990). However, in individuals of the normal population, excessive sunlight-caused DNA damage that overloads the endogenous DNA repair capacity and/or a simultaneous presence of a slight weakness in DNA repair may result in skin cancer as well. Munch-Peterson et al. (1985) observed a decrease in the cellular tolerance to UV radiation in lymphocytes of normal individuals with multiple skin cancers, using unscheduled DNA synthesis. Lambert, Ringborg, and Swanbeck (1976) found reduced DNA repair after UV irradiation of lymphocytes *in vitro* in patients with solar keratoses,

which are potential precursors of squamous cell carcinoma (SCC). After irradiation with various doses of UV, the lymphocytes were incubated with [3H]thymidine in the presence of hydroxyurea. A dose-response relationship for the UV-induced DNA repair synthesis was established for each individual, and the average repair capacity in patients with actinic keratoses was approximately 30% below that of controls. Sbano et al. (1978) utilized autoradiographic counting to measure the UV-induced unscheduled DNA synthesis of skin fibroblasts from patients with chronic actinic keratosis and from healthy donors of similar age. In order to study a possible regional difference of DNA repair between the parts of the body ordinarily exposed and those parts unexposed to sunlight, two cell strains were used for each examined subject: one developed from the forehead skin and the other from the abdominal or axillary skin. Unscheduled DNA synthesis analysis confirmed a depressed DNA repair in actinic keratosis patients as compared with controls. Thielmann et al. (1987) demonstrated that fibroblast strains from patients with SCC and basal cell carcinoma (BCC) had diminished DNA repair capacity (as measured by the alkaline elution technique) upon exposure to UV radiation by up to 82% compared to controls. Alcalay et al. (1990) showed that the skin of subjects with BCC had decreased repair of solar simulated radiation-induced CPD (as measured by a dimer-specific endonuclease assay) compared to the epidermis of a normal control population. Grossman and Wei (1995) and Wei et al. (1994, 1995) reported molecular epidemiologic data indicating that impaired DNA repair is involved in the etiopathogenesis of BCC in the normal population. They found that a low DNA repair capacity correlated to the number of skin tumors in subjects with one or more BCC (Wei et al. 1994). Wang et al. (2005) assessed UVB-induced chromatid breaks in a hospital-based, case-control study and found a dose-response relationship between mutagen sensitivity and risk for both BCC and SCC.

## 15.2 Liposomes with DNA Repair Enzymes

### 15.2.1 Enzyme Technology

Despite the availability of powerful sunscreens with high sun protection factors, skin cancer remains a major health problem. For instance, BCC and SCC taken together as the most common forms of skin cancer affect more than a million people each year alone in the United States (<http://www.skincancer.org>). Therefore, besides sunscreens, secondary approaches to reduce sun damage are desirable.

Liposomes (consisting of multilamellar phospholipid layers) containing prokaryotic DNA repair enzymes, such as T4 endonuclease V (T4N5), have been introduced as a novel concept of secondary photoprotection (Wolf et al. 1995; Wolf, Yarosh, and Kripke 1993; reviewed in Cafardi and Elmetts 2008; Zahid and Brownell 2008). T4N5, a product of the denV gene of bacteriophage T4, initiates repair of CPD by cleaving the glycosylic bond of the 5' pyrimidine and then breaking the DNA at the apyrimidinic site, a process that is thought to be a rate-limiting step of excision repair (Yarosh et al. 1996 and cited herein). The denV gene has been cloned and the complete nucleotide and amino acid coding sequence determined (Radnay et al. 1984). The expressed T4N5 protein has been purified to homogeneity in commercial quantities (Table 15.1). T4N5 liposomes can penetrate the stratum corneum of murine skin and human skin explants and deliver T4N5 to epidermal cells (Ceccoli et al. 1989; Yarosh et al. 1994). The membrane of the liposomes employed is pH sensitive, which is what facilitates the intracellular release of the enzyme (Yarosh et al. 1994). However, liposomes are of importance not only to shuttle the enzyme into the skin but also to protect the enzyme from inactivation through bacteria and/or the acid environment of the skin surface. Moreover, liposomes have the capacity to assist in protein refolding by interaction

with a secondary protein structure (Zardeneta and Horowitz 1994). This may have happened in certain studies in which a heat-denatured enzyme was used as a vehicle control and found partially active (Yarosh et al. 1999). However, particularly under daily life conditions, liposome-assisted protein refolding may be of great help to preserve the activity of a preparation, for instance, on the beach when skin is transiently exposed to high temperatures that may damage the ingredients of such a preparation with DNA repair activity.

### 15.2.2 Preclinical Work with Topical DNA Repair Enzymes

T4N5 liposomes increased DNA repair in fibroblasts and keratinocytes cultured from XP patients and DNA repair-proficient subjects (Yarosh et al. 1991). In various other studies, DNA repair liposomes affected other UV-induced alterations. Gilchrest et al. (1993) reported that T4N5 treatment enhanced *in vitro* UV-induced melanogenesis as measured within 16 to 96 h after UV exposure by melanin content, tyrosinase activity, 14C-dopa incorporation, and visual assessment of both murine melanoma cells and human keratinocytes. The most significant increase in the T4N5-enhanced melanogenesis was seen at the late time points, consistent with the delayed tanning reaction induced by natural sunlight. Liposomes containing extracts from *Micrococcus luteus* (a widely dispersed organism found in the normal flora of the mammalian skin, unpasteurized milk, marine waters, and even soil) did also enhance DNA repair and melanogenesis in Cloudman S91 melanoma cells (Yarosh, Kibitel, and O'Connor 1997). However, the exact mechanisms of DNA repair enzyme-induced melanogenesis after UV exposure remain to be determined.

In repair-proficient mice, treatment with T4N5 liposomes after UV exposure had strong effects on a variety of biologic endpoints. T4N5 liposomes enhanced CPD repair (Yarosh et

TABLE 15.1 Summary of Clinical Studies on the Effect of Liposomes Containing DNA Repair Enzymes

Study	Enzyme(s)	Effect
Yarosh et al. 1996	T4N5	Improvement of DNA repair in XP patients
Wolf et al. 2000	T4N5	Abrogation of UV-induced IL-10 and TNF-alpha formation in skin cancer patients
Stege et al. 2000	Photolyase	Reduction of UV-induced CPDs and restoration of interferon-gamma-induced ICAM-1 expression in the skin
Yarosh et al. 2001	T4N5	Reduction of the number of actinic keratoses and BCCs in patients with XP
Halliday et al. 2004	T4N5	Prevention of UV-induced immune suppression in the nickel-allergy model
Kuchel, Barnetson, and Halliday 2005	T4N5	Reduction of UV-induced immune suppression in the nickel-allergy model and epidermal dendritic cell alteration and macrophage infiltration of the skin
Ke et al. 2008	<i>Micrococcus luteus</i> lysate	Reduction of local immune suppression in the DNCB contact allergy model
Lucas et al. 2008	<i>Micrococcus luteus</i> lysate	Prevention of immune suppression in the DNCB contact allergy model
DeBoyes et al. 2010	T4N5	Reduction in the number of actinic keratoses in normal individuals with moderate-to-severe photodamaged skin
Hofer et al. 2011	Photolyase and <i>Micrococcus luteus</i> lysate	Protection from PLE

T4N5, bacteriophage T4 endonuclease V; photolyase, extracted from *Anacystis nidulans*; *Micrococcus luteus* lysate, containing DNA-repair enzymes with UV-specific endoclease activity; DNCB, dinitrochlorobenzene.

al. 1992) and diminished UV-induced sunburn cell formation, altered Langerhans cells and Thy-1+ dendritic epidermal T cells (Wolf et al. 1995), impaired antigen-presenting cell function (Vink et al. 1996), produced the immunosuppressive cytokine Interleukin-10 (Nishigori et al. 1996), and induced the appearance of T suppressor (i.e., regulatory) cells (Kripke et al. 1992) as well as both local and systemic functional immune suppression (Kripke et al. 1992; Wolf et al. 1995; Wolf, Yarosh, and Kripke 1993). Moreover, T4N5 treatment delayed the abnormal rise of p53 protein expression in chronically UV-irradiated mice (Bito et al. 1995) and reduced both the incidence and yield of skin cancer in mice (Bito et al. 1995; Yarosh et al. 1992). Together, the data indicated that T4N5 liposomes can enhance DNA repair in UV-irradiated mice and may prevent skin cancer by reducing tumor-initiating DNA damage and simultaneously preserving the immune response essential for tumor rejection. Notably, T4N5 liposomes only marginally affected the sunburn reaction as measured by skin edema but clearly reduced DNA damage and other UV-induced biologic alterations, including immune suppression (Wolf et al. 1995). Vink et al. (1997) used liposomes containing photolyase, which, upon absorption of photoreactivating visible light, splits UVB-induced CPD to restore the function of antigen-presenting activity of dendritic cells in the skin of mice. They used isoporsalen plus UVA (PUVA) as a control to demonstrate that the photoreactivation repair process was specific for UVB damage because it was unable to restore PUVA photoproducts. In one of the studies in mice (Wolf, Yarosh, and Kripke 1993), the effect of sunscreens containing standard UV filters (2-ethylhexyl-p-methoxycinnamate, octyl-N-dimethyl-p-aminobenzoate, or benzophenone-3) was directly compared to that of T4N5 liposomes. Notably, the sunscreens much better protected against the UV-induced inflammatory response than the liposomes (protection up to 97% vs. 39%); in contrast, the liposomes much better protected against immune suppression (as measured by systemic suppression of delayed type hypersensitivity to *C. albicans*) than the sunscreens (82% vs. 42%).

### 15.2.3 Clinical Studies with Topical DNA Repair Enzyme Preparations

Yarosh et al. (1996) first reported on the acute and chronic safety testing of T4N5 liposomes, showing neither adverse reactions nor significant changes in serum chemistry or in skin histology of treated individuals. The skin of XP patients treated with the DNA repair liposomes had fewer CPD in DNA and showed less erythema than control sites.

Wolf et al. (2000) investigated the penetration of T4N5 liposomes and their effects on biologic endpoints in UV-exposed buttock skin of non-XP skin patients with a history of multiple skin cancers (Wolf et al. 2000). Transmission electron microscopic studies after immunogold labeling and anti-T4N5 staining indicated that T4N5 enzyme incorporated into the liposomes penetrated into skin. T4N5-labeled gold particles were found in the cytoplasm and nuclei of keratinocytes and Langerhans cells. The investigators observed no effect of the T4N5 liposomes on

UV-induced erythema as measured by skin reflectance spectroscopy and only weak improvement of removal of CPD as measured by immunohistochemical antibody staining. However, UV-induced upregulation of interleukin-10 and tumor necrosis factor-alpha was nearly entirely abrogated when the test fields exposed to two minimal erythema doses were treated after UV exposure with active T4N5 liposomes compared to heat-inactivated control liposomes. Together, this suggested that even a moderate increase in overall DNA repair may lead to large effects on subsequent UV-induced biologic alterations, such as immune function, presumably by selective repair of certain sites in the genome (Kripke et al. 1992; Yarosh et al. 1996).

Halliday et al. (2004) and Kuchel, Barnetson, and Halliday (2005) used T4N5 liposomes to determine whether CPD formation is involved in UV-induced suppression of efferent immunity. Nickel-allergic volunteers were irradiated with a range of doses of solar-simulated UV and T4N5, or empty liposomes were applied after irradiation. Nickel-induced recall immunity was assessed by reflectance spectrometry. T4N5 liposomes inhibited suppression of the nickel response, prevented solar-simulated UV radiation from reducing the number of epidermal dendritic cells, and reduced macrophage infiltration into irradiated skin. Consistent with the results of other studies (Wolf et al. 2000), T4N5 liposomes did not significantly affect the UV-induced erythema response.

Stege et al. (2000) employed the DNA-repair enzyme photolyase, derived from *Anacystis nidulans*, that specifically converts CPDs into their original DNA structure after exposure to photoreactivating light to repair UVB radiation-induced DNA damage. Topical application of photolyase-containing liposomes to UVB-irradiated skin and subsequent exposure to photoreactivating light decreased the number of UVB radiation-induced CPD by 40%–45%. Moreover, application of photolyase prevented UVB-induced suppression of intercellular adhesion molecule-1 (ICAM-1), a molecule required for immunity and inflammatory events in the epidermis. Photolyase treatment also prevented elicitation of hypersensitivity to nickel sulfate, sunburn cell formation, and erythema. No effect on all these responses was observed if empty liposomes (containing no photolyase) were used or photoreactivating light exposure was not given after administration of photolyase-filled liposomes.

Ke et al. (2008) investigated the effect of topical formulations containing either DNA-repair enzyme extracts from *Micrococcus luteus* or RNA fragments (UVC-irradiated rabbit globin mRNA to increase the resistance of human keratinocytes to UVB damage and enhance DNA repair) on UV-induced local contact hypersensitivity (CHS) suppression in eight human volunteers as measured *in vivo* using the contact allergen dinitrochlorobenzene. Exposure to a single 0.75 minimum erythema dose (MED) simulated solar radiation exposure resulted in 64% CHS suppression in unprotected subjects compared with unirradiated sensitized controls. In contrast, UV-induced CHS suppression was reduced to 19% with DNA-repair enzymes and 7% with RNA fragments. Biopsies from an additional nine

volunteers showed an 18% decrease in thymine dimers by both DNA-repair enzymes and RNA fragments, relative to unprotected UV-irradiated skin. The authors suggested that both topical RNA fragments as well as DNA-repair enzymes may be useful as photoprotective agents. Similar results on UV-induced suppression of the induction of CHS were reported by Lucas et al. (2008) in a study using a commercial moisturizer with DNA-repair enzymes.

Yarosh et al. (2001) reported the results of a prospective, multicenter, double-blind, placebo-controlled study in patients with XP in which the regular daily administration (as close to midday as possible) of an after-sun lotion containing T4N5 liposomes over a period of 12 months reduced the incidence of actinic keratoses and BCC by 68% and 30%, respectively. Interestingly, an effect on the number of actinic keratoses was observed as early as 3 months after the start of treatment, suggesting that a quickly acting process, such as DNA repair enzyme-induced immune protection (rather than prevention of mutation formation), might have been responsible for the therapeutic action of the after-sun lotion. However, there were discrepancies about the statistical methods used for analysis of the data (Lachenbruch et al. 2001; Yarosh 2001), and therefore, more work is necessary to substantiate the efficacy of T4N5 liposomes in XP.

More recently, DeBoyes et al. (2010) evaluated 17 normal individuals with moderate-to-severe photodamaged skin for differences in actinic keratoses following topical application of T4N5 liposome lotion over 48 weeks. Compared to baseline, a statistically significant reduction in the number of actinic keratoses was seen following the treatment period. Wolf et al. evaluated the effect of a proprietary SPF 30 sunscreen and after-sun lotion, both containing a combination of photolyase from *Anacystis nidulans* and *Micrococcus luteus* lysate in a pilot study of intensified photoprotection in repair-deficient and -proficient patients with a history of multiple skin cancers (<http://www.clinicaltrials.gov>, NCT00555633). Thirteen patients (including five XP, one XP variant, three basal cell nevus syndrome, and four normal patients) were instructed to apply the sunscreen regularly before sun exposure and the after-sun lotion to their face and arms daily as close to midday as possible for 24 months. There was a trend for less BCC occurring in the patients during the 24-month study period compared to a 24-month pre-study period. In addition, the results of this study indicated that the intensified photoprotection strategy may have helped in reducing skin aging. The analysis of patient self-reports revealed a statistically significant improvement for several endpoints, including smoothness, wrinkles, color spots, and telangiectasia of the skin, starting as early as at 3 months of treatment with a maximum effect seen at 12 months (Figure 15.1). No adverse effects were noted during the study.

Hofer et al. (2011) led the research with liposomal DNA-repair enzymes into the field of photodermatoses (Gruber-Wackernagel, Byrne, and Wolf 2009; Wolf, Byrne, and Gruber-Wackernagel 2009). They used a proprietary after-sun lotion, containing a combination of photolyase and *Micrococcus luteus* lysate in a

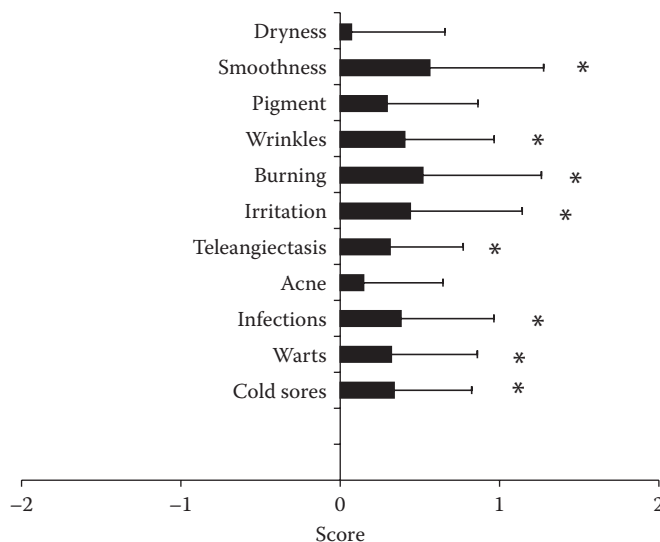


FIGURE 15.1 Effect of intensified photoprotection with a proprietary SPF 30 sunscreen and after-sun lotion, both containing a combination of photolyase from *Anacystis nidulans* and *Micrococcus luteus* lysate in a pilot study in repair-deficient (XP) or -proficient patients with a history of multiple skin cancers. Patients scored the skin status of treated areas (face, forearms, and hands) in 3-month intervals during a 24-month study period for various endpoints on a scale from -2 (maximum worsening) to +2 (maximum improvement). Data shown are from the 12-month observation time point.  $p < 0.05$  (Wilcoxon test).

randomized, double-blind, left-right body side comparison study in patients with polymorphic light eruption (PLE). Fourteen PLE patients were treated on four consecutive days with near-erythral solar-simulated UV exposures (followed by blue light treatment) in fields on symmetrically located, individual PLE predilection sites. As shown by a newly established specific PLE test score, PLE symptoms were significantly fewer on test sites treated with active DNA repair lotion than on untreated or placebo-treated test sites. At 144 h after first UV exposure (the time point of maximal PLE symptoms), the mean test score for the active enzyme-treated test fields were 61% lower compared to the untreated test fields (whereas the reduction by placebo treatment was only 27%). These results provided evidence that DNA damage may trigger PLE, and increased DNA repair may prevent the induction of PLE symptoms. However, the mechanism by which improved DNA repair may diminish PLE remains to be elucidated. Photoimmunoprotection is not a good mechanistic candidate because PLE patients have been shown to be more resistant to UV-induced immune suppression (as measured by induction of CHS) than normal controls (Koulu et al. 2010; Palmer and Friedmann 2004; van de Pas et al. 2004). Moreover, a lack of skin infiltration of neutrophilic granulocytes after UV exposure and subsequent failure of immune suppression to UV-induced neoantigens has been hypothesized to be responsible for PLE (Schornagel et al. 2004). Therefore, at least theoretically, immune protection through enhanced DNA repair should

deteriorate rather than prevent PLE. The authors speculated that enhanced DNA repair might reduce the formation of DNA photoproduct-related neoantigens as the potential starting point of the pathogenic chain in PLE. DNA-repair enzyme treatment had a highly significant effect on PLE symptoms but did not significantly alter UV-induced erythema. This is consistent with the results of previous studies in which topical DNA-repair enzymes affected the sunburn reaction only marginally or not at all but reduced DNA damage and other UV-induced biologic alterations, including immune suppression (Ke et al. 2008; Stege et al. 2000; Wolf et al. 1995, 2000).

### 15.3 Outlook

The topical application of liposomal-incorporated DNA repair enzymes is a novel strategy in photoprotection. Several companies have already started marketing cosmetic sun-care preparations (after-sun lotions and sunscreens) containing DNA-repair enzymes (photolyase from *Anacystis nidulans* and/or endonucleases from *Micrococcus luteus* lysate). Whereas conventional sunscreens containing chemical and/or physical UV filters must be applied before UV exposure to be protective, many liposomal preparations containing DNA-repair enzymes may be effective when applied after UV exposure and initiation of a sunburn reaction. However, they hardly protect against erythema (Kuchel, Barnetson, and Halliday 2005; Wolf et al. 2000). Therefore, they need to be combined in sunscreens with standard chemical and/or physical UV filters. The results of a controlled study have suggested that topical DNA-repair enzymes may reduce skin cancer, at least in DNA repair-deficient XP patients (Yarosh et al. 2001). However, so far, only cosmeceutical preparations with DNA-repair enzymes are marketed (Hofer et al. 2011; Lucas et al. 2008), and no pharmaceutical preparation is available for humans. More work and controlled clinical studies are necessary before a preparation with DNA-repair enzyme(s) may be able to receive drug approval, most likely first under orphan drug designation for patients with XP. Besides, comparative studies are necessary to determine which enzyme strategy (endonuclease-empowered excision repair vs. photoreactivating light repair) is most effective for secondary photoprotection.

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Q1: Table 15.1 was inserted here to be cited. Please check.

Q2: Please check if the edits made here are OK: “for instance, on the beach when skin is transiently exposed to high temperatures that may damage the ingredients of such a preparation with DNA repair activity.”

Q3: Please check if the edits made in the following are OK: “In various other studies, DNA repair liposomes affected other UV-induced alterations.”

Q4: Table 1 is not mentioned in the text of the chapter.

Q5: Please add the correct date to this reference.

