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DNA damage, repair, and tanning acceleration

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Synopsis

Exposure of the skin to solar ultraviolet radiation (UV) leads to various adverse effects, such as the induction of cellular damage and mutations, suppression of the skin's immune system, and the induction of skin cancer. These effects are the consequence of various molecular alterations in the skin resulting from absorption of photons. Formation of DNA damage is one of these. In the present study the effect of tanning accelerators was investigated on the level of DNA damage after exposure to UV. With the use of a specific monoclonal antibody, DNA damage was quantified in human skin. Epicutaneous application of a complex of tanning enhancers (Unipertan® VEG-2002) before exposure to UV was found to reduce the level of DNA damage at 6 and/or 24 h thereafter. The results of *in vitro* studies (human skin organ cultures) as well as of a preliminary clinical trial are presented. The tanning enhancer was shown to increase removal of UV-DNA damage over normal repair measured at 6 and 24 h after UV exposure.

INTRODUCTION

Exposure of man to ultraviolet radiation (UV) is increasing due to changes in life style: sun holidays and the use of artificial tanning equipment have gained popularity, and a well-tanned skin is often considered a sign of good health. In addition, the depletion of stratospheric ozone may result in an increase in short-wavelength UV (UVB) reaching the earth's surface. The most serious harmful effect of UV for humans is the formation of malignant cells in the skin: mutated cells resulting from UV-inflicted DNA damage may be given the opportunity, during a state of UV-induced immunosuppression, to grow unimpeded and progress into a tumor (1–3).

Solar ultraviolet light is divided into two subregions: a short-wavelength region, UVB (290–315 nm), and a long-wavelength region, UVA (315–400 nm). Nucleic acids like DNA strongly absorb the radiant energy from UVB especially. The ensuing excitations of electrons result in chemical modification of DNA. Among the different types of DNA damage formed upon UVB irradiation, cyclobutane pyrimidine dimers (CPD) are predominant (Figure 1). The possible biological consequences of DNA damage are manifold. During cell replication a faulty base may be built into the new DNA strand opposite a modified base. This mutation may lead to altered expression of genes that play a central role in growth, differentiation, and carcinogenesis. In addition, CPD have been shown to play a role in the suppression of immune responses after UVB exposure (4).

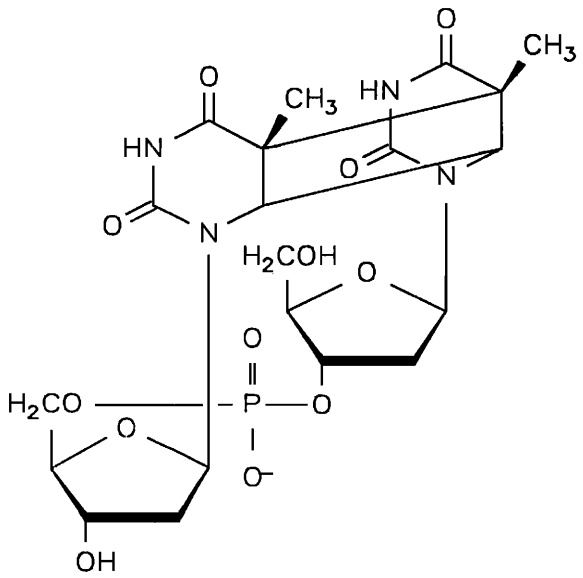

Figure 1. Schematic representation of a cyclobutane pyrimidine dimer.

UV-induced melanogenesis or tanning is a natural protective response of the skin: an increase in intracellular melanin reduces ultraviolet transmission (5). In addition, enzymatic repair systems are available to remove cellular damage inflicted by UV, whereas the skin's immune system destroys possible precancerous cells.

Tanning is generally considered a natural sunscreen, but its underlying mechanisms have not yet been fully elucidated. Melanin is biochemically synthesized from tyrosine. Epicutaneous application of a mixture of tyrosine/adenosinephosphates/riboflavin before UV exposure was shown to increase and accelerate the tanning response (6). Interestingly, UV-induced DNA damage and/or its repair have been linked to melanogenesis (7–9).

In the present study we have investigated the effect of the epicutaneous application of tanning accelerators (Unipertan® VEG-2002) on the level of DNA damage in epidermal cells at various time points after exposure to UV.

MATERIAL AND METHODS

TANNING ACCELERATION

Unipertan® VEG-2002 (international patents by Induchem, Switzerland) was used as a tanning accelerator, containing tyrosine (8%), adenosine phosphates (1%), riboflavin (0.3%), vegetable protein hydrolysate, and butylene glycol (20%). This complex of ingredients has been shown to be effective in tanning acceleration (6). Two $\mu l/cm^2$ of a 5% solution of Unipertan® VEG-2002 in water was applied to human skin, in line with recommendations by the FDA and COLIPA for the testing of sunscreens.

IN VITRO STUDY

Human abdominal skin was obtained directly after surgery from three female donors at

the academic Hospital of Utrecht (The Netherlands). Skin discs (8-mm diameter) were prepared and cultured on a microporous membrane as described by Van de Sandt and Rutten (10). A one-microliter mixture of tanning enhancers (5% Unipertan® VEG-2002 in sterile water) was brought onto the skin discs using a positive displacement pipette. The substance was evenly spread over the skin area with a disposable spatula (Poly-Pipets, Inc., Englewood Cliffs, NJ). Incubation with the substance was performed in a humidified incubator (37°C, 5% CO_2); the skin discs were exposed to UVB (0, 3000 J/m^2) from Westinghouse FS20 sunlamps (2 × 20 W). The UV-dose rate was 0.37 mW/cm^2 as determined with a UVX dosimeter (UV Products, San Gabriel, CA) equipped with a UVX-31 sensor for the measurement of UVB. At various time points after exposure to UV radiation (0, 6, 24 h) the skin was fixed with phosphate-buffered 4% formaldehyde and paraffin sections were prepared.

CLINICAL TRIAL

Besides the studies with human skin organ cultures, a clinical trial was performed in which three volunteers participated, two males and one female, with written informed consent. At first, each of the volunteer's personal minimal erythema dose (MED) was assessed using Westinghouse FS20T12 sunlamps (4×). The doses that resulted in a just perceptible redness were 1100 J/m^2 (volunteer 1), 1400 J/m^2 (volunteer 2), and 800 J/m^2 (volunteer 3). Two µl/cm^2 of a 5% solution of Unipertan® VEG-2002 was topically applied to one of the arms of each volunteer. After 1 h incubation the arm was irradiated with 3 MED, and punch biopsies were taken immediately after and at 24 h after irradiation; an additional unexposed skin biopsy was obtained from the same treated arm. The other arm was treated with UV only, and two skin biopsies (t = 0, 24 h) were taken. The skin samples were immediately transferred to a fixative comprised of phosphate-buffered 4% formaldehyde, and paraffin sections were prepared.

IMMUNOSTAINING OF UV-DNA DAMAGE

Paraffin sections were deparaffinized by subsequent 2-min incubations in xylene (2×), ethanol 100% (2×), ethanol 96%, ethanol 70%, and PBS. The slides were then boiled for 10 min in 10 mM citrate buffer (pH 6.0), rinsed with PBS (2×), and used for immunostaining. The skin sections were stained with monoclonal antibodies against cyclobutane pyrimidine dimers [hybridoma clone H3 (11)] and with goat-anti-mouse-IgG fluorescein-labeled secondary antibodies. The nuclei of the skin cells were counterstained with propidiumiodide. Nuclear green fluorescence in the epidermal cells proportional to the level of pyrimidine dimers was assessed with a scanning laser microscope (Zeiss LSM-41, Oberkochen, Germany) using image processing and image analysis. The procedure for the immunostaining and measurement of the fluorescence has been described (11,12).

RESULTS

IN VITRO STUDY

Skin organ cultures from three donors were used. Irradiation of skin discs with 3000

J/m^2 UVB resulted in significant induction of cyclobutane pyrimidine dimers, as indicated by the quantified fluorescence due to binding of the antibody (Figure 2). In Figure 3 the mean fluorescence is given for the skin samples (two skin samples per time point), of which approximately 400 cells were measured. Fluorescence in non-irradiated samples was around 26 arbitrary units and was not affected by application of Unipertan® VEG-2002 (data not shown). Background fluorescence is accounted for by non-specific binding of the antibody preparations, and also by the noise of the laserscan microscope. Application of Unipertan® VEG-2002 before exposure to UV did not result in a decrease of UV-induced CPD and, therefore, it does not act as a sunscreen creme.

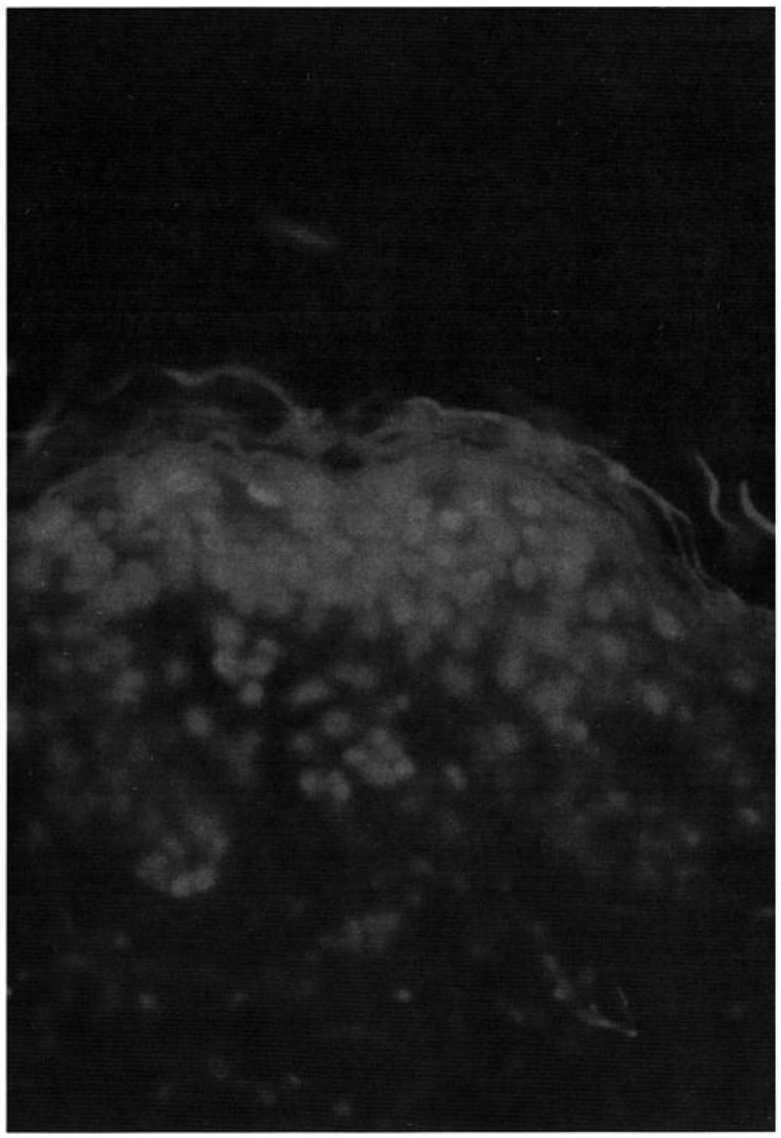

Figure 2. Section of human skin stained with the antibody against UV-DNA damage (cyclobutane pyrimidine dimers).

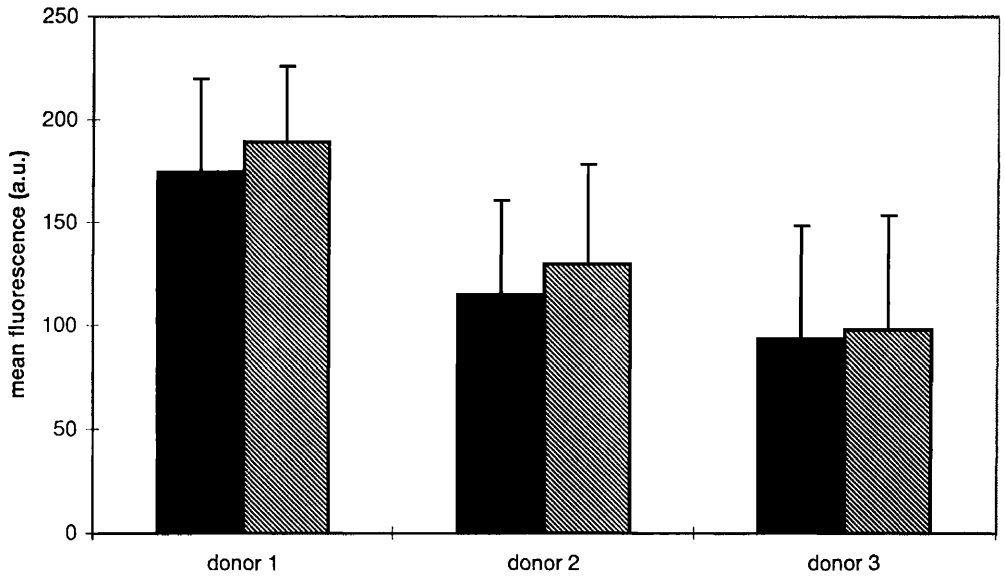

Figure 3. Mean fluorescence due to antibody binding, measured in nuclei from epidermal cells. Human skin organ cultures were used from three donors. Treatments: 3000 J/m^2 UVB (black bar) and Unipertan® VEG-2002 followed by 3000 J/m^2 UVB (hatched bar).

No reduction in the level of CPD at 24 h after exposure was observed in skin samples obtained from donor 1; in donors 2 and 3, however, reduction was 20% and 44%, respectively, at 24 h after exposure. When Unipertan® VEG-2002 was applied before irradiation, an additional reduction in the level of CPD was observed after 6 and 24 h. The reduction in the level of CPD increased to 12%, 41%, and 79% at 24 h after exposure in the three donors, respectively. Results are presented in Figure 4.

CLINICAL TRIAL

After the demonstration of enhancement of removal of UVB-DNA damage in *ex vivo* human skin samples, a preliminary clinical trial was performed with three volunteers. Unipertan® VEG-2002 was applied to the forearms at 1 h before exposure to 3 MED UVB. The punch biopsies taken at t = 0 and t = 24 h were processed for immunostaining, analogous to the *in vitro* experiments. No reduction in the level of CPD at 24 h was found in one volunteer; in another volunteer reduction was intermediate (43%), and almost complete (90%) in the third. Application of Unipertan® VEG-2002 reduced the 24-h level of DNA damage in the first two volunteers, resulting in a reduction of 34% and 77%, respectively. In the third volunteer the level of CPD after Unipertan® application was comparable to that of the control. This volunteer had already removed all of the initially induced DNA damage within 24 h, and no further reduction in CPD was expected. The results are summarized in Figure 5.

DISCUSSION

Two important facts are made clear by the present study: (a) the *in vitro* data are

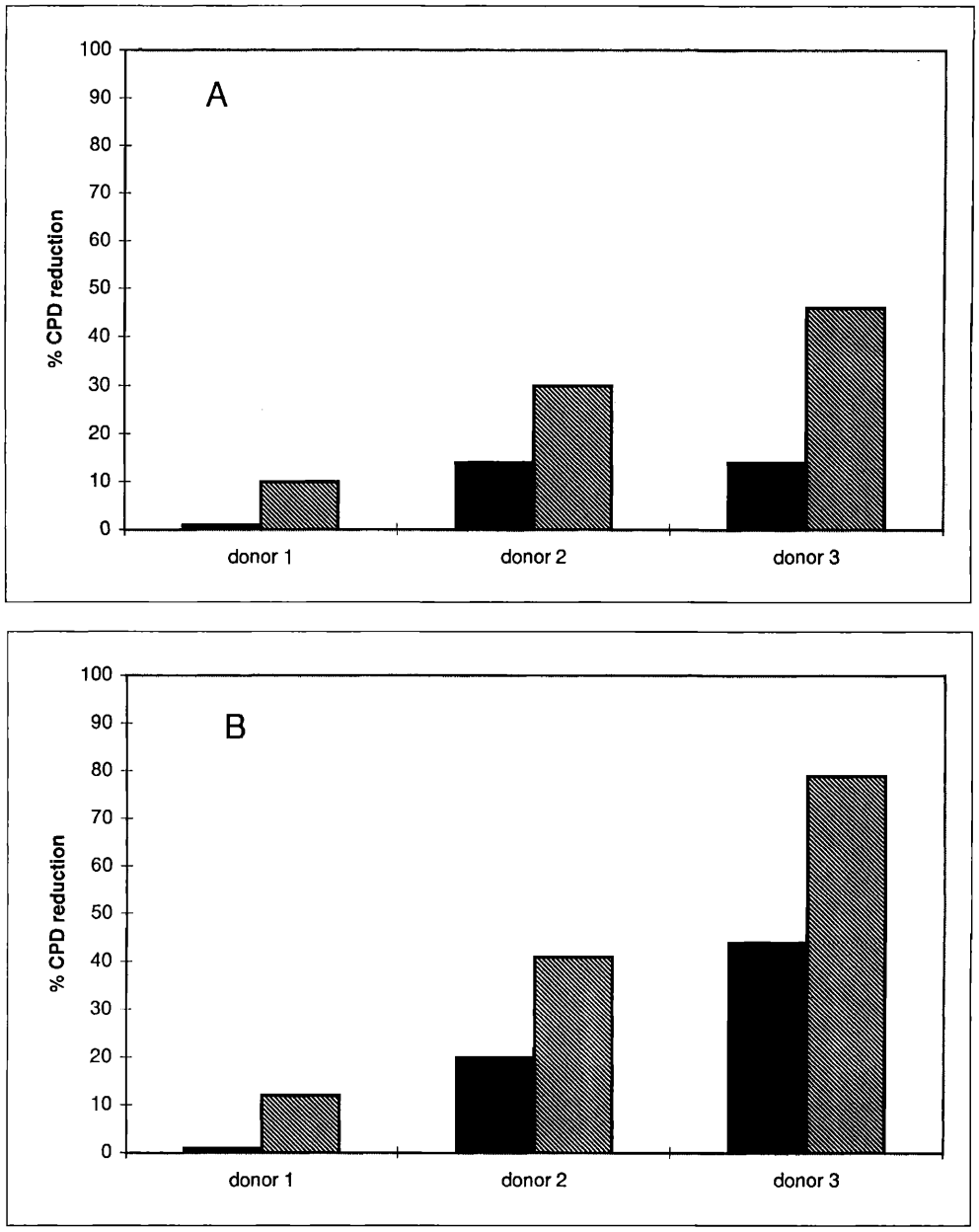

Figure 4. CPD reduction measured at 6 h (A) and 24 h (B) after irradiation of human skin organ cultures from three donors. Treatments: 3000 J/m^2 UVB (black bar) and Unipertan® VEG-2002 followed by 3000 J/m^2 UVB (hatched bar).

representative of the data obtained with human volunteers, and therefore the human skin organ culture appears to be a suitable model for photobiology studies; (b) variation exists in the reduction of DNA damage levels when comparing skin from different individuals, ranging from no to almost complete reduction of CPD within 24 h after exposure. When the level of DNA damage found at 24 h is reduced to what is measured immediately

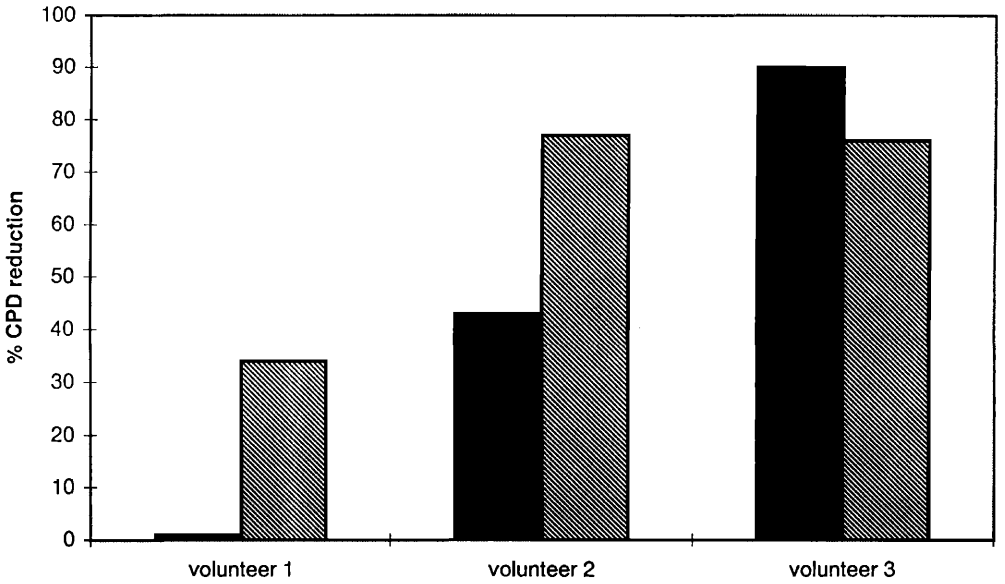

Figure 5. CPD reduction measured at 24 h after *in vivo* irradiation of skin from three volunteers. Treatments: 3 MED UVB (black bar) and Unipertan® VEG-2002 followed by 3 MED UVB (hatched bar).

after exposure, this reduction is very likely to be the result of the skin's repair system. Alternatively, one should take into account the theoretical possibility that within this time period the photoproduct has not actually been repaired but it merely no longer detectable. In the present discussion we prefer to refer to the term "repair" when dimer levels are reduced.

The observation of absence of repair in the skin of one of the donors used in this study is in line with other studies. Berg *et al.* (13) described a large inter-individual variation in dimer-repair capacities among the volunteers used. Stimulation of DNA repair may be especially important in individuals that display little to no repair. Young *et al.* (14) observed hardly any removal of dimers in human skin. The relationship between DNA repair and skin cancer is evident from the rare genetic disease xeroderma pigmentosum in which defective DNA repair results in a 1000-fold increase in skin cancer susceptibility (15).

The photoprotective role of tanning seems obvious when comparing the induction of erythema in fair-skinned individuals, who burn easily, with that in darker-skinned people, who are less susceptible to sunburn. More importantly, darker-skinned individuals are less at risk compared to fair-skinned individuals to develop skin cancer (16). The induction of DNA damage is one of the initiating molecular events underlying UV-induced mutagenesis, immunosuppression, and skin cancer (17,18). Melanin was recently shown to form supranuclear caps over nuclei, absorbing incoming light, and thereby reducing UV-induced DNA photoproducts in human epidermis (19). The reduction in the formation of DNA damage occurred in a melanin concentration-dependent manner.

Besides this obvious inverse relationship between melanin and photoproduct induction, a possible link between melanin synthesis and induction and/or repair of DNA damage

has been suggested (7–9). Enhancement of DNA repair, by delivery of repair enzymes to UV-damaged skin cells or cultured melanocytes, was shown to increase UV-induced melanin synthesis. Moreover, application of repair products (e.g., small DNA fragments), thereby mimicking DNA repair, was shown to induce tanning in unexposed skin via the upregulation of mRNA for tyrosinase. It was concluded that one of the signals initiating UV-induced melanogenesis is the excision of DNA photoproducts. It can be hypothesized that both pathways, melanogenesis and the DNA repair process, share one or more mediators.

The objective of the present study was to investigate whether this relationship between DNA repair and tanning is also true the other way around, whether stimulatoin of melanin synthesis results in enhancement of DNA repair. In order to activate melanogenesis and to induce DNA photoproducts, human skin was exposed to UVB that had been epicutaneously treated with tanning enhanceres (Unipertan® VEG-2002). This complex contains several precursors of melanin and, when applied to skin before UV exposure, has been shown to increase tanning significantly (6). As expected, 1-h incubation of the skin with Unipertan® VEG-2002 followed by a single dose of UV did not result in protection against formation of DNA damage. For tanning to be induced, providing a natural sunscreen, more time and preferably multiple exposures are required.

A stimulatory effect was found on DNA damage removal, both *in vitro* in cultured skin (three donors) and *in vivo* (two out of three volunteers). The effect observed on photoproduct repair may indicate that one or more, as yet unknown, mediators shared by the tanning response and the repair pathway have been activated by the tanning accelerators. Alternatively, one or more of the ingredients of Unipertan® VEG-2002 may have positively affected the repair process.

CONCLUSIONS

The present study indicates that tanning enhancers (Unipertan® VEG-2002) may provide photoprotection in two ways: (a) UV-induced melanogenesis is enhanced, providing a natural sunscreen, and melanin effectively absorbs UV, preventing further induction of cellular damage; (b) removal of DNA damage, which has been shown to contribute to UV-induced mutagenesis, immunosuppression, and skin cancer, is enhanced.

ACKNOWLEDGMENT

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