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ORIGINAL ARTICLE

d-limonene prevents ultraviolet irradiation: Induced cyclobutane pyrimidine dimers in Skh1 mouse skin

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Abstract

AIM: To establish whether *d*-limonene can protect against induction of cyclobutane pyrimidine dimers (CPDs) and sunburn in ultraviolet irradiation (UVR) irradiated mouse skin.

METHODS: The *d*-limonene was given in 4 daily oral 20 μL aliquots at different concentrations as follows: 100%, 10% or 1% in liponate and 100% liponate as control. One day after the final *d*-limonene treatment, the mice were anesthetized with *i.p.* sodium pentobarbital and placed in boxes to allow a rectangular (2 cm × 4 cm) region of dorsal skin to be irradiated with a single, ultraviolet radiation dose of 1.5 kJ/m^2. Skin samples from UVR irradiated area were obtained at 5 min after UVR exposure for CPD detection, at 6 d after UVR exposure, skin samples were obtained for in situ analysis for N-myc downstream regulating gene 1 (NDRG1) (a stress response gene), proliferating cell nuclear antigen (PCNA) (an S-phase marker) and filaggrin (a barrier integrity gene). Based on immunohistochemistry staining, the number of CPD, NDRG1 and PCNA positive cells, as well as unstained cells was counted in 3 different individually selected areas and percentage of positive cells was established.

RESULTS: CPD reduction occurred as follows: liponate only-none; 1% *d*-limonene-54.3% reduction of CPDs; 10% *d*-limonene-73.4% reduction of CPDs; 100% *d*-limonene-86.1% reduction of CPDs, the latter equivalent to a UV dose of only 0.21 kJ/m^2. Sunburn was also dose-dependently reduced by d-limonene. The NDRG1 protein was strongly induced by UVR (70.0% ± 10.4% positive cells), but 1% *d*-limonene reduced the response to 64.6% ± 9.2%, 10% *d*-limonene reduced the response to 16.2% ± 3.4% and 100% *d*-limonene reduced the response to 6.3% ± 1.7%. Similarly, PCNA was 52.4% ± 9.9% positive in UVR exposed skin, and 1% *d*-limonene reduced it to 42.9% ± 8.1%, 10% *d*-limonene reduced it to 36.2% ± 6.7% and 100% *d*-limonene reduce it to 13.8% ± 3.4%. NDRG1 and PCNA were increased by *d*-limonene or UVR separately, but combined they produced less than either agent separately owing to the protective effect of pre-exposure to *d*-limonene.

CONCLUSION: Overall *d*-limonene acted to protect against ultraviolet B-induced DNA photodamage and sunburn in UVR exposed skin.

Key words: Sunburn; Ultraviolet irradiation; *d*-limonene; Cyclobutane pyrimidine dimers; N-myc downstream regulating gene 1; Proliferating cell nuclear antigen

Core tip: Skh-1 hairless mice were given 4 daily 20 μL aliquots of different concentrations of *d*-limonene, and then irradiated to a single ultraviolet irradiation. Skin samples from the ultraviolet-exposed area of mice showed that

ultraviolet irradiation induced cyclobutane pyrimidine dimers formation, N-myc downstream regulating gene 1 and proliferating cell nuclear antigen expression, pretreatment of *d*-limonene significantly reduced these responses. Pure *d*-limonene also induced the expression of epidermal barrier protein filaggrin. In conclusion, *d*-limonene protected the mice skin from UV-induced DNA photodamage and sunburn in mice skin.

Uddin AN, Wu F, Labuda I, Tchou-Wong KM, Burns FJ. *d*-limonene prevents ultraviolet irradiation: Induced cyclobutane pyrimidine dimers in Skh1 mouse skin. *World J Dermatol* 2014; 3(3): 64-72 Available from: URL: http://www.wjgnet.com/2218-6190/full/v3/i3/64.htm DOI: http://dx.doi.org/10.5314/wjd.v3.i3.64

INTRODUCTION

Understanding how aroma terpenes prevent sunburn and/or skin cancer in mice could lead to more effective and safer ways of blocking sun damage to human skin. As one of the most common terpenes in nature, *d*-limonene is listed in the Code of Federal Regulations as generally recognized as safe (GRAS) as a flavoring agent (http://www.cfsan.fda.gov/-Ird/fcf182.html) and is found in common foods, such as, fruit juices, soft drinks, baked goods, ice cream, and pudding at low concentrations[1]. Limonene has well-established chemopreventive activity against many cancer types in animal models[2,3], including mammary[4], skin[5], liver[6], lung[7] and forestomach cancers[8].

Previously, we showed that β-damascenone, an aroma terpene, protected against sunburn by activating both keratinocyte and sebaceous gland pathways that fortified and thickened the cornified barrier layers of mouse epidermis[9]. Our immunohistological and gene expression results of keratin, caspase 14, filaggrin or loricrin were consistent with the idea that fortification of the cornified envelope by the activation of new sebum secretion was the key mechanism of β-damascenone-induced sunburn protection by absorbing ultraviolet irradiation (UVR) and reducing its penetration into the skin[9].

In the current study, the effect of *d*-limonene on the protection of mouse skin against UVR-induced sunburn and DNA photodamages was quantified in UVR-irradiated mice skin. The stress-related N-myc downstream regulating gene 1 (NDRG1) gene protein, proliferation [as assessed by proliferating cell nuclear antigen (PCNA)] and expression of skin barrier function gene, filaggrin, were detected by immunohistochemistry. Results indicated that *d*-limonene protected against DNA photodamages and sunburn of the mice skin. The protection was likely associated with activation by the *d*-limonene of keratinocyte and sebaceous gland proliferation and induction of endogenous UV absorbents in the outer cornified envelop of skin, thereby increasing UVR absorption and protecting underlying cutaneous tissues.

MATERIALS AND METHODS

Experimental animal

Albino, female Skh-1 mice of 6 wk old used in all experiments were purchased from *Charles River Laboratories (Wilmington, MA)*.

d-Limonene

The test terpene, *d*-limonene, was obtained as a 99.99% pure compound from Biokeys for Flavors(Norwood, NJ, United States). For 10% or 1.0% compounds, pure *d*-limonene was diluted with the triglyceride, liponate. *d*-Limonene was administered orally, sometimes topically.

Sunburn dose-response experiments

Thirty mice were grouped as follows: (1) control (3 mice); (2) UVB alone (3 mice); (3) 100% *d*-limonene (6 mice, 3 topical, 3 oral); (4) 100% *d*-limonene + UVR (6 mice = 3 topical *d*-limonene + 3 oral *d*-limonene); (5) 10% *d*-limonene + UVR (6 mice = 3 topical *d*-limonene + 3 oral *d*-limonene); and (6) 1.0% *d*-limonene + UVR (6 mice = 3 topical *d*-limonene + 3 oral *d*-limonene). *d*-Limonene was given topically or orally. For topical exposure, 20 μL *d*-limonene (100% or diluted) was applied on the dorsum of the mice in the same amounts as was administered by feeding tube.

Exposure of mice to UVR

One day after the final *d*-limonene application, mice were exposed to a single dose of 1.5 kJ/m^2 of UVR (7.5 min duration). For positional restraint during UVR exposures, the mice were anesthetized with *i.p.* sodium pentobarbital (Nembutal) at a dose of 35 mg/kg body weight and placed in boxes configured to allow a rectangular (2 cm × 4 cm) region of dorsal skin to be exposed to UVR. The UVR was generated by of a bank of four parallel Westinghouse fluorescent sun lamps (FS-20, Westinghouse, Bloomfield, NJ, United States). The lamps were positioned 24 cm above the skin surface. The dose rate of UV was 0.20 kJ/m^2 per minute as measured with a digital radiometer/ photometer (1400A, International Light Inc., Wilmington, MA, United States). Sunburn was evaluated and photographed at a peak response generally 4 to 6 d after UV exposure. A minimal erythema dose (MED) experiment was performed in the dose range of 0.25 kJ/m^2 to 2.0 kJ/m^2. All experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee of New York University School of Medicine.

DNA photodamage experiments

For detection of cyclobutane pyrimidine dimer (CPD) photoproducts, a separate group of 27 Skh1 mice were used as control, UVR alone, UVR + 100/10/1.0% *d*-limonene for oral applications. Four daily *d*-limonene doses and a single UVR exposure was applied as described in sunburn experiments. Skin samples from the dorsal area of mice exposed to UVR were obtained at 5 min after

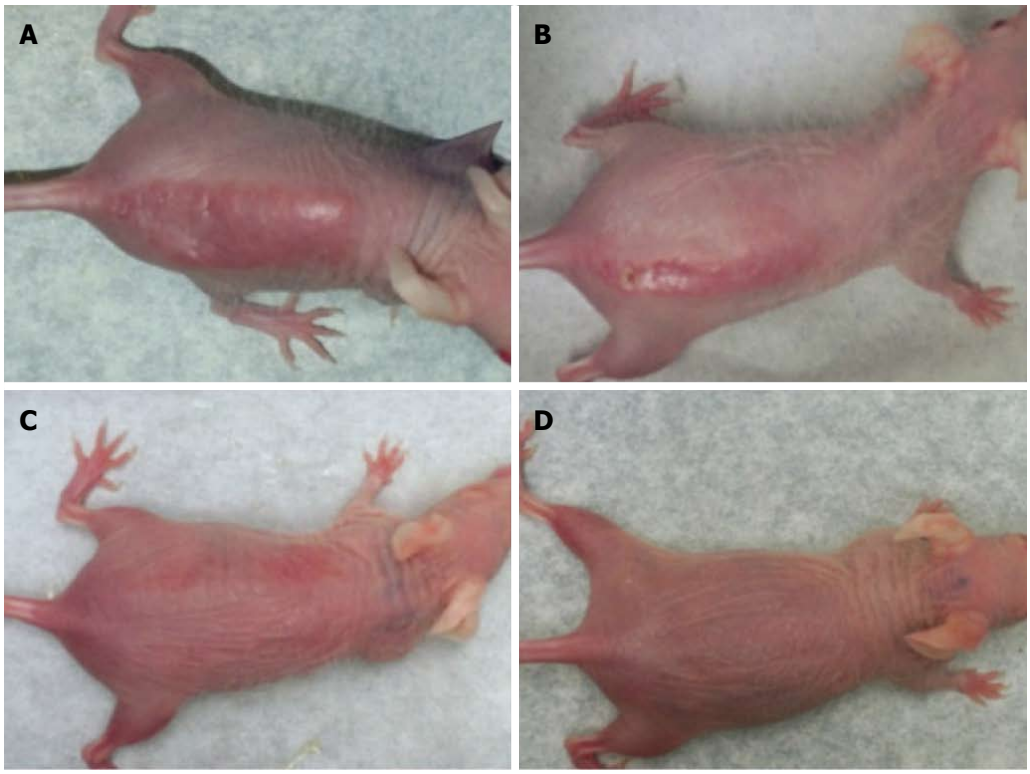

Figure 1 *d*-Limonene prevented sunburn formation. Mice were exposed orally to 20 μL of pure or diluted d-limonene daily for 4 consecutive days followed by 1.5 kJ/ m^2 of ultraviolet B exposure generated by a commercial sunlamp 1 d later. The maximum sunburn response occurred as shown by skin erythema on 6th day of UVR exposure was completely eliminated by pure *d*-limonene. A: UVR alone; B: UVR + 1.0% *d*-limonene in liponate; C: UVR + 10% *d*-limonene in liponate; D: UVR + 100% *d*-limonene. Topical application of d-limonene followed by UVR yielded similar results (data not shown). UVR: Ultraviolet irradiation.

each UVR exposure for CDP detection. For NDRG1, PCNA and filaggrin detection, skin samples were obtained at 6 d after UVR exposure.

CPD detection

CPDs were detected with conventional streptavidin-biotin methods. Formalin-fixed paraffin-embedded sections were deparaffinized in xylene and absolute ethyl alcohol. The DNA was denatured by incubating slides in 2N HCl for 10 min and then neutralizing by incubating in 50 mmol/L Tris-base for 5 min at room temperature. After blocking with non-immune serum, slides were incubated overnight at 4 ℃ with mouse monoclonal anti-CPD antibody (Cosmo Bio Co., Tokyo, Japan) at a dilution of 1:300 in PBS. Slides were then incubated for 15 min with biotinylated antibody and then with the streptavidin-biotin peroxidase system Ultra Streptavidin Detection System kit (Covance Research Products, Dedham, MA, United States).

Immunohistochemical analysis

Immunostaining of NDRG1, PCNA and filaggrin were performed on paraffin sections of skin samples. The sections were incubated overnight at 4 ℃ with different antibodies as follows: a rabbit polyclonal anti-NDRG1 antibody (Abcam, Cambridge, MA, United States) at a dilution of 1:300 in PBS, a mouse monoclonal anti-PCNA antibody (Covance Research Products) at a dilution of 1:100 in PBS, a purified polyclonal anti-filaggrin antibody (Covance Research Products) at a dilution of 1:300 in PBS. Then immunostaining was performed according to the protocol of with the streptavidin-biotin peroxidase system Ultra Streptavidin Detection System kit (Covance Research Products).

Quantitative analysis of CPD, NDRG1 and PCNA-stained cells

Stained cutaneous tissues were observed under a light microscope and images were created by using a Canon Powershot SD500 digital camera with a Scopetronix microscope adapter. The number of CPD, NDRG1 and PCNA positive cells as well as unstained cells was counted in 3 different randomly selected areas of equal length for each sample and the percentage of positive cells was calculated. Values are expressed as mean ± SE.

RESULTS

Sunburn protection

Administration of 100% *d*-limonene orally significantly prevented DNA photodamage as well as sunburn/erythema in Skh-1 mice exposed to 1.5 kJ/m^2 of UVR. Four doses of 20 μL each (0.95 μg/g body weight) of 100% *d*-limonene provided complete protection from UVR-induced sunburn. Topical *d*-limonene prevented UVR-induced sunburn slightly more effectively than oral *d*-limonene. Sunburn protection was gradually re-

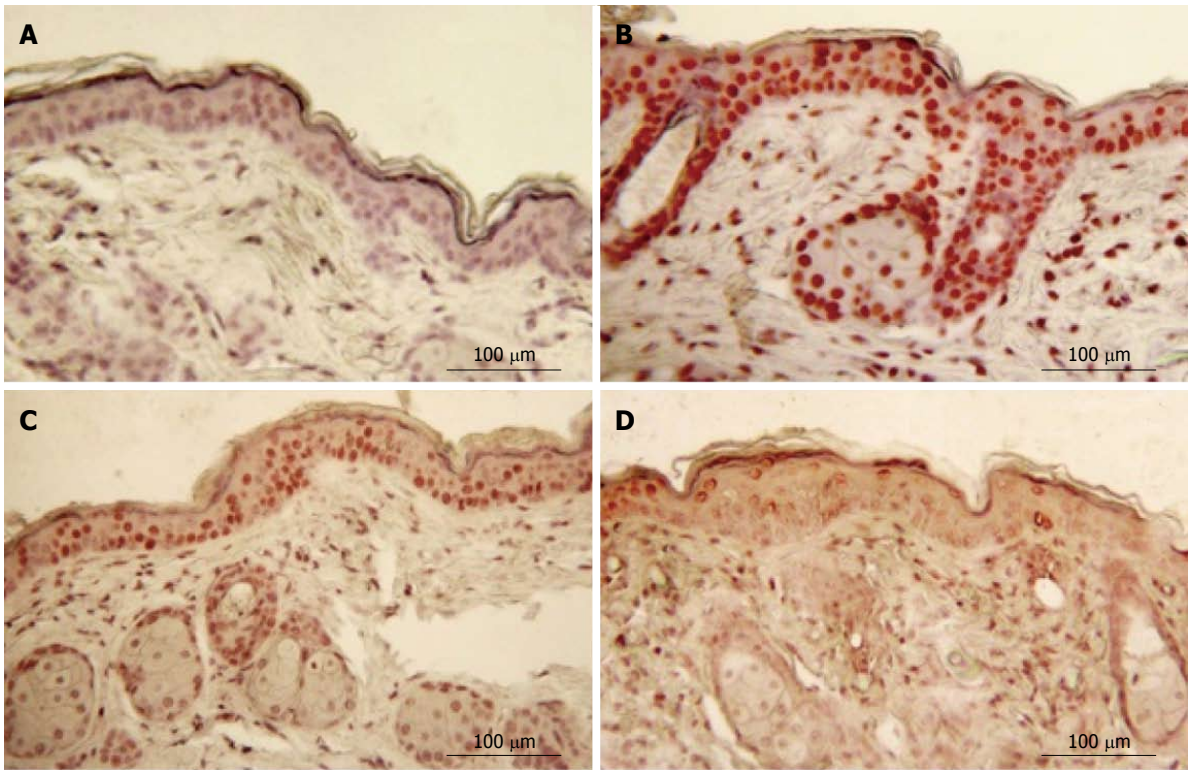

Figure 2 Immunostaining for cyclobutane pyrimidine dimers following oral exposure to *d*-limonene. Skin samples were collected 5 min after UVR exposure. (A) Only unstained, CPD-negative cells are seen in control epidermis (B) intense CPD-stained nuclei are seen in skin samples (C, D). Oral *d*-limonene prevented CPD photoproducts formation in dose-dependent manner. UVR: Ultraviolet irradiation; CPD: Cyclobutane pyrimidine dimer.

duced with lower concentrations such as 10%, 1% or 0% *d*-limonene in a dose-dependent fashion (Figure 1). The sunburn protection was less but still significant at 10% *d*-limonene but was absent at 1% *d*-limonene. A minimal erythemal dose (MED) for these mice was established at 0.75 kJ/m^2.

DNA photoproducts formation

No staining for CPDs was observed in epidermis that had not been exposed to UV (Figure 2). Five min after UVR exposure, CPD-stained cells were observed among the epithelial cells superficial dermal fibroblasts. UV irradiation significantly increased the CPD staining as shown in Figure 2B. Mice given oral 100% *d*-limonene before UVR exposure exhibited only 15% of the UVR-induced CPDs in compared to mice not treated with *d*-limonene (Figure 2D). The photoproducts were reduced for 10% or 1% *d*-limonene in a dose-dependent fashion as follows and as shown in Table 1. The percentage of CPD positive cells was 94.7% ± 11.1% in UV exposed, 45.7 ± 8.3% (52% reduction) in 1% *d*-limonene pretreated, 26.6 ± 5.8% (72% reduction) in 10% *d*-limonene pretreated, and 13.9 ± 4.5% (85% reduction) in 100% *d*-limonene pretreated mice skin epidermis.

NDRG1 protein analysis

Mice exposed to UVR strongly expressed NDRG1 (Figure 3C), however, mice exposed to UVR following treatment with *d*-limonene, showed reduced NDRG1 protein in epidermis (Figure 3D-F), even though *d*-limonene alone produced an increased level of NDRG1 (28.1% ± 5.2%, Figure 3B). In control skin, little or no NDRG1 was observed in epidermis but low levels were detected in sebaceous glands. The NDRG1 protein, a cytoplasmic protein involved in stress response, indicated that 70.0% ± 10.4% of keratinocytes were affected by the DNA damaging effect of the UVR. The UVR-induced NDRG1 index remained at 64.6% ± 9.2 % at 1.0% *d*-limonene, but was significantly reduced at 10% *d*-limonene to 16.2% ± 3.4% and at 100% *d*-limonene to less than 6.3% ± 1.7% (Table 1) ; the latter indicating a nearly complete elimination of cellular damage or response to damage; a finding that is consistent with sunburn prevention and CPD inhibition.

Epidermal proliferation as measured by PCNA-positive cells

As shown in Figure 4 both *d*-limonene and UVR induced proliferative stimulation of in the epidermis, the hair follicles (not shown) and the sebaceous glands (not shown). However, prior treatment of mice with *d*-limonene exposure significantly reduced the PCNA-positive cells in skin compared to that of UVR alone mice. The percentage of PCNA-positive nuclei (S-phase nuclei) in epidermis of *d*-limonene-treated mice was 74.6% ± 11.1%, *vs* 7.1% ± 1.7% in controls; a 10.5 folds increase (Table 1). The increased proliferation of keratinocytes was apparently balanced by an increased rate of differentiation, because

Table 1 Quantitative analysis of the effect of d-limonene on cyclobutane pyrimidine dimer, N-myc downstream regulating gene 1 and proliferating cell nuclear antigen induction

Groups	CPD-positive cells (%)	NDRG1-positive cells (%)	PCNA-positive cells (%)
Control	0.0	6.0 ± 1.4	7.1 ± 1.7
UVR only	94.7 ± 11.1	70.0 ± 10.4	52.4 ± 9.9
UVR + 1.0% *d*-limonene	45.7 ± 8.3	64.6 ± 9.2	42.9 ± 8.1
UVR + 10% *d*-limonene	26.6 ± 5.8	16.2 ± 3.4	36.2 ± 6.7
UVR + 100% *d*-limonene	13.9 ± 4.5	6.3 ± 1.7	13.8 ± 3.4
100% *d*-limonene alone	0.0	28.1 ± 5.2	74.6 ± 11.1

d-Limonene was administered orally daily for 4 consecutive days prior to 1.5 kJ/m^2 of UVB radiation. CPD samples were obtained on the 5th day 5 min after each UVR exposure. Skin samples for NDRG1 and PCNA were obtained 6 d after the UVR exposures. An approximate number of CPD, NDRG1 and PCNA positive cells as well as unstained cells were counted in 3 different areas of equal length for each sample and percentage of positive cells was calculated. UVR: Ultraviolet irradiation; PCNA: Proliferating cell nuclear antigen; NDRG1: N-myc downstream regulating gene 1; CPD: Cyclobutane pyrimidine dimer.

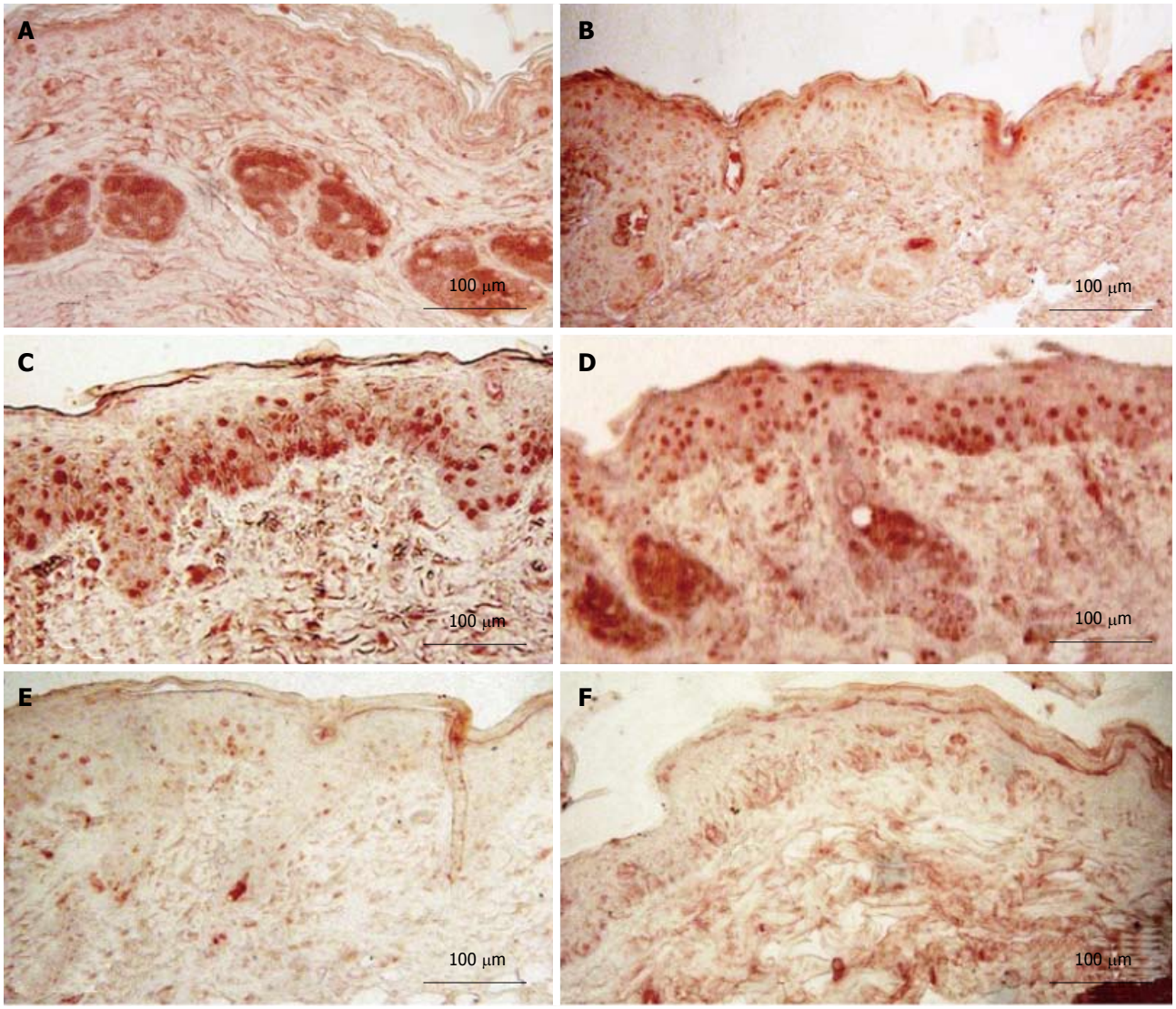

Figure 3 Immunostaining of N-myc downstream regulating gene 1. Skin samples were obtained 6 d after UVR exposure. A: In control skin little or no NDRG1 was seen in epidermis but low levels were detected in sebaceous glands, Control; B: 100% *d*-limonene alone, mouse skin exposed to UVR strongly expressed NDRG1 both in epidermis and sebaceous glands (C-E); C: UVR alone; D: UVR + 1.0% *d*-limonene; E: UVR + 10% *d*-limonene; F: UVR + 100% *d*-limonene. Skin exposed to UVR with prior treatment of d-limonene, show significantly reduced NDRG1protein in epidermis and hair follicles (F). UVR: Ultraviolet irradiation; NDRG1: N-myc downstream regulating gene 1.

the total nucleated keratinocyte count was unaffected. The percentage of PCNA positive cells was 52.4% ± 9.9% in UV exposed skin, but 1% *d*-limonene reduced the number to 42.9% ± 8.1%, 10% *d*-limonene reduced the

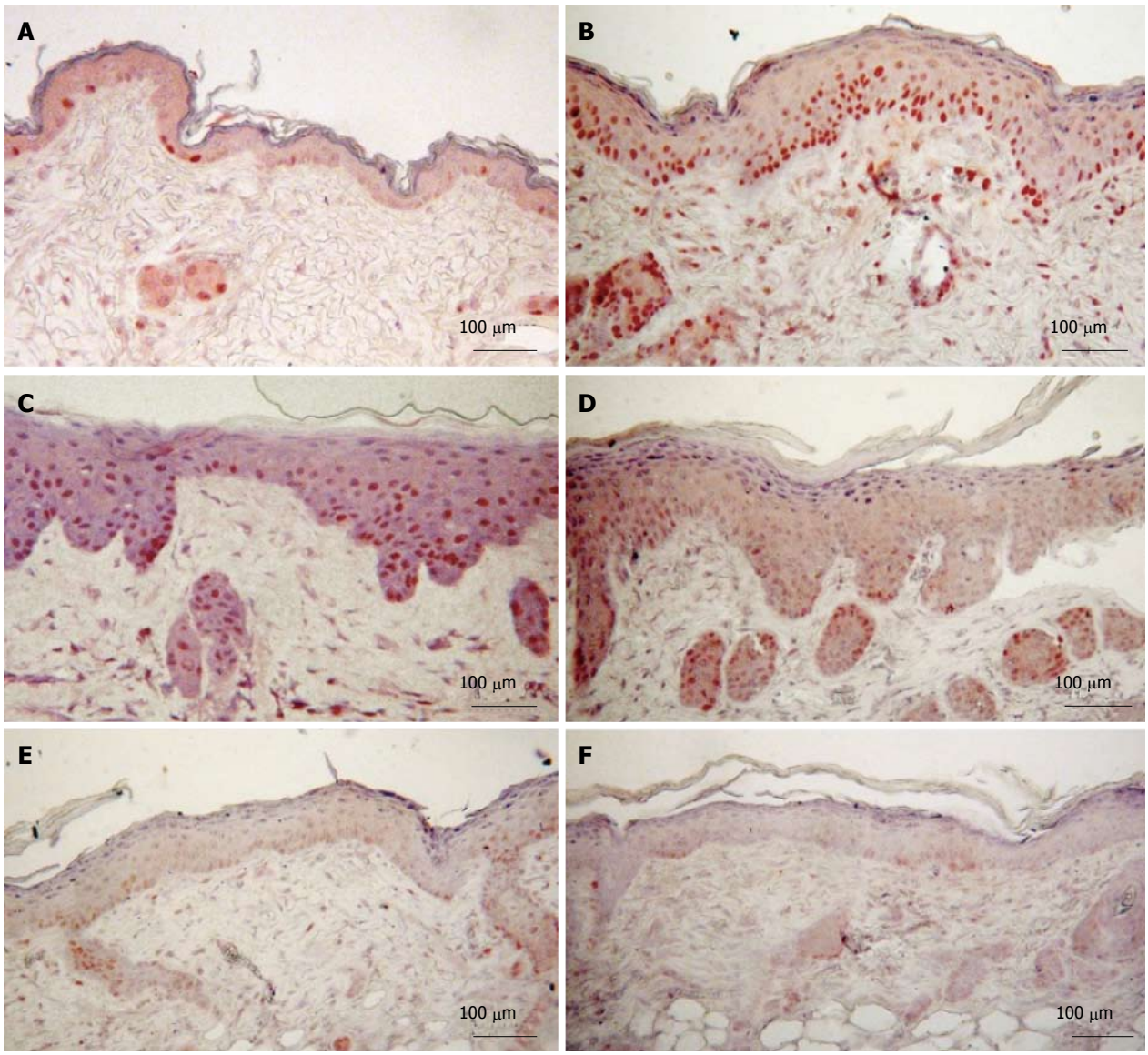

Figure 4 Immunostaining of proliferating cell nuclear antigen. Skin samples were obtained on 6 d after UVR exposure. A: Control; B: 100% *d*-limonene alone; C: UVR alone; D: UVR + 1.0% *d*-limonene; E: UVR + 10% *d*-limonene; F: UVR + 100% *d*-limonene. (A) In control skin few PCNA-positive cells are observed in epidermis. Both 100% d-limonene alone (B) and UVR alone (C) induced proliferative stimulation in epidermis and hair follicles. However, pre-treatment of mice with d-limonene significantly reduced the PCNA-positive cells in skin compared to UVR-only mice (D-F), which verifies the CPD finding that oral d-limonene was protective against UVR-induced tissue damage. UVR: Ultraviolet irradiation; PCNA: Proliferating cell nuclear antigen; CPD: Cyclobutane pyrimidine dimer.

number to 36.2% ± 6.7% and 100% *d*-limonene reduced the number to 13.8% ± 3.4%.

Induction of filaggrin by d-limonene

As shown in Figure 5, *d*-limonene elevated the filaggrin level in the epidermis and sebaceous glands in comparison to control, similar to what was observed in our β-damascenone study[3]. *d*-Limonene increased the thickness of the cornified envelope layer of epidermis for a prolonged period up to 12 d.

DISCUSSION

DNA damage induced by UVR is thought to play an important role in the pathogenesis of skin cancers[10,11]. The major types of DNA damage induced by UVR are cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs). Approximately 75 % of UVR-induced DNA damage is CPDs and the remaining is 6-4PPs and the Dewar isomer of 6-4PPs[12]. These types of DNA lesions are repaired by nucleotide excision repair system in normal cells[13,14]. The formation and repair of DNA photoproducts appears to be crucial for cancer induction as cells from xeroderma pigmentosum (XP) patients, who are highly susceptible to UVR-induced skin cancer as a result of a mutation, cannot remove these photoproducts[15-17].

The results that either oral or topical *d*-limonene blocked UVB-induced sunburn in Skh1 mouse skin (Figure 1) are consistent with our previous observations that

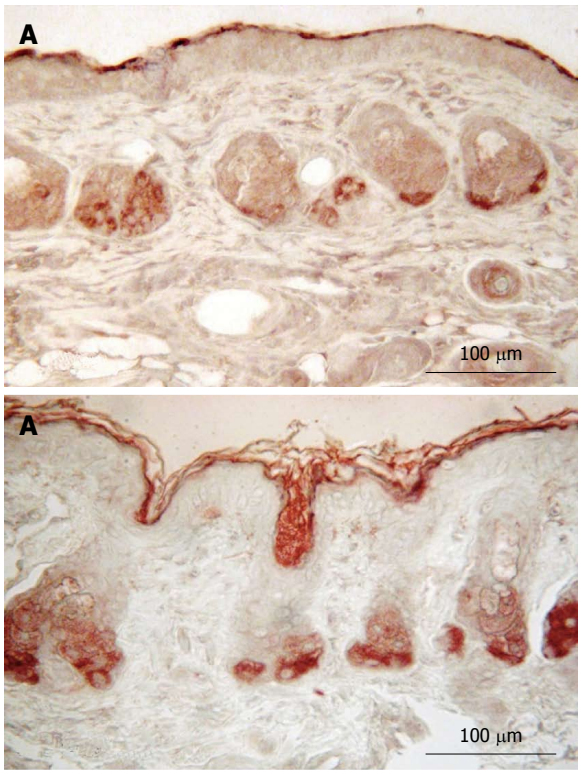

Figure 5 *d*-Limonene induced the expression of filaggrin. A: Control; B: 100% *d*-limonene alone. Sebaceous glands of d-limonene–treated mice show a higher filaggrin signal compared to control mice. Also shown is the typical increased thickness of filaggrin-negative materials distal to the dark, thin filaggrin-positive layer, in comparison to control skin.

β-damascenone, also an aroma terpene, acted as a sunburn protective agent similar to current observations[9]. The preventive mechanism of *d*-limonene exhibited two components, reducing the formation of DNA photodamages (Figure 2) and lowering of stress, proliferation genes, including NDRG1and PCNA (Figures 3, 4). It is noteworthy that while pure *d*-limonene induced NDRG1, PCNA, it eliminated the response of these same genes to UVR exposure. This can be explained by invoking *d*-limonene's stimulation of proliferation and differentiation leading to a thickened outer cornified barrier prior to UVR as a protective, absorptive effect. Pure *d*-limonene is not a cutaneous sensitizer, but it is reported to be irritating at high doses. Applications of 25% or 40% of *d*-limonene solutions failed to cause long-term irritation in the ears of rabbits, although application of undiluted *d*-limonene caused skin redness and irritation[18]. Present results are consistent with these earlier observations.

It is of utmost importance that mice given pure *d*-limonene followed by UVR exposure exhibited reduced UVR-induced CPD photoproducts in the epidermal DNA in a dose-dependent fashion. While the cellular response to *d*-limonene is still to be elucidated, the possibility of direct biochemical reaction between *d*-limonene and cellular DNA is unlikely as most of the oral *d*-limonene is rapidly excreted from the body after metabolized in liver to form perillyl alcohol and carveols which distributes in the blood to other parts of the body[19]. Possibly, *d*-limonene's ability to activate proliferation and differentiation of epidermis reflects an underlying, most likely vestigial, UVR protection system in mouse skin.

Another possibility is that *d*-limonene has triggered a gene cluster associated with the strengthening and thickening of UVR-absorbing cutaneous envelopes, possibly including the activation of NDRG1. *In situ* analysis showed an accumulation of NDRG1 in the suprabasal layers of the skin, as well as in the more differentiated areas of mouse skin papillomas[20]. Although NDRG1 protein is up-regulated during a variety of cell stresses[21,22], including DNA damage[23], nickel[24] and hypoxia[25], *etc*., its exact function remains unknown.

Prior treatment of mice with *d*-limonene followed by UVR exposure significantly reduced the NDRG1 expression in skin compared to that of UVR and *d*-limonene alone. UVR is known to initiate ROS and/or inflammatory stress responses[26,27], but *d*-limonene was ineffective when administered after the UVB (data not shown), which implies that the anti-oxidative or inflammatory activity of the *d*-limonene was not a likely basis for its UVB protective effect. *In vivo* studies with *d*-limonene have shown its efficacy against hepatocellular carcinoma, and inhibition of the overexpression of c-myc and c-jun proteins as one of the mechanisms by which *d*-limonene exhibits its anticarcinogenic effect[6]. NDRG1, as the downstream of target of N/c-myc may also involve the activity of the *d*-limonene.

Like β-damascenone, *d*-limonene increased the number and size of the sebaceous glands and also induced a higher level of the filaggrin protein in sebaceous glands and in follicular lumens near the skin surface. This result suggests that terpene-induced sebum formation may represent a secondary but inducible pathway whereby filaggrin is made available for strengthening and repairing the cornified envelop layer of epidermis, beyond the well-known filaggrin origin in keratinocytes. The latter filaggrin originates from the degradation of the large and insoluble polyprotein profilaggrin and is further proteolyzed to amino acids that serves as building blocks for epidermal structure, hydration and barrier function[28,29]. Filaggrin knockdown study in a human skin model showed increased skin sensitivity to UVR and significant decreased amount of urocanic acid, a product from fliaggrin degradation acts as an UV absorbent within the stratum corneum[30]. More study will be needed to resolve the question whether treatment with the *d*-limonene increased this endogenous UVB protective chromophore.

Taking together current *d*-limonene data and previously reported β-damascenone results, suggest that *d*-limonene protected against UVR-induced DNA damage, sunburn by activating stress response and proliferation and gene NDRG1, PCNA and filaggrin which strengthen the skin barrier against UV rays. Even though pure *d*-limonene alone seemingly had some adverse effect, it will protect the skin from UV damage by optimizing the dosage.

ACKNOWLEDGMENTS

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COMMENTS

Background

d-Limonene, an aromatic terpene, has been reported to protect against many cancer types, including mammary, skin, liver, lung and forestomach. Skin cancer caused by the ultraviolet component of sunlight is a major risk in human populations lacking the protection of melanin.

Research frontiers

The research explores the possibility of using very low concentrations of a safe, natural substance taken orally to provide effective and long-lasting protection against ultraviolet radiation-induced sunburn and, if DNA damage is prevented, skin cancer.

Innovations and breakthroughs

Whether taken orally or applied directly to the mouse skin surface, *d*-limonene prevented UVB-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and sunburn. Elevated ultraviolet irradiation (UVR) absorption was associated with a thicker and stronger skin barrier. *d*-Limonene also counteracted the UVR-induced expression of proliferating cell nuclear antigen and N-myc downstream regulating gene 1. These results show that aromatic terpenes prevent sunburn and DNA damage in mouse skin and are a new and innovative approach to protecting skin against ultraviolet radiation induced skin damage.

Applications

The ultraviolet radiation protection persisted for about 2 wk and might persist even longer in human skin because of its longer turnover time. The interesting discovery was that oral *d*-limonene was about equally effective as a topical application for activating what spears to be a previously unknown natural UVR protective system contained in mouse and human skin.

Terminology

Aromatic terpenes are non-nutritive substances found in citrus and other fruits and herbs. They contribute to the distinctive fragrance of plants, and are generally considered to be safe for consumption by humans and animals.

Peer review

This manuscript described an interesting finding that administration of *d*-Limonene is able to prevent UV-induced sunburn and CPD formation in mouse skin.

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