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SHORT COMMUNICATION

DNA damage after acute exposure of mice skin to physiological doses of UVB and UVA light

Alena Rajnochová Svobodová · Adéla Galandáková · Jarmila Šianská · Dalibor Doležal · Radka Lichnovská · Jitka Ulrichová · Jitka Vostálová

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Abstract Solar ultraviolet (UV) radiation is an important risk factor in skin carcinogenesis. This has been attributed mainly to the UVB waveband because the high-energetic photons are capable of interacting with DNA and inducing DNA damage. Recently, UVA light has also gained increasing interest in relation to DNA alteration. Although UVA photons are less energetic than UVB, they comprise a major fraction of sunlight UV radiation and penetrate deep into the skin. The study was carried out to compare the acute effects of UVA and UVB light on SKH-1 mice in relation to DNA damage and associated parameters. Mice were exposed to UVA (10 and 20 J/cm^2) or UVB (200 and 800 mJ/cm^2) radiation. The number of DNA single-strand breaks (SSB) in lymphocytes, amount of phosphorylated histone H2AX (gamma-H2AX) and apoptosis or DNA fragmentation (TUNEL-positive cells) in skin sections and level of gamma-H2AX, activated caspase-3 and phosphorylated p53 in skin were evaluated after 4 and 24 h. SSB analyzed by alkaline comet assay were found to be 4 and 24 h following UVB and UVA treatment, respectively. TUNEL and gamma-H2AX-positive cell were observed only in UVB exposed animals at both time intervals. The level of activated caspase-3 and phospho-p53 was increased 24 h after UVA and UVB radiation and was more apparent in UVB treated mice. The results indicate that the mechanism of DNA damage caused by acute UVA exposure includes formation of SSB (oxidative damage), but not double-strand breaks.

Keywords SKH-1 mice · Solar radiation · Single-strand breaks · DNA fragmentation · Gamma-H2AX · p53

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A. R. Svobodová (✉) · A. Galandáková · J. Šianská · J. Ulrichová · J. Vostálová
Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic
e-mail: alf.svoboda@seznam.cz

D. Doležal
Center for Laboratory Animals, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

R. Lichnovská
Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

Introduction

In equatorial countries, temperate areas in summer and during sunbed use, human skin can be exposed to huge quanta of solar ultraviolet (UV) radiation. Until recently, most of studies on UV-induced damage focused on UVB radiation (290–320 nm). The genotoxic potential of UVB is linked to its ability to provoke direct DNA damage, e.g., cyclobutane-pyrimidine dimers (CPD) and pyrimidine-(6-4)-pyrimidone photoproducts. However, recently there has been increasing interest in the contribution to DNA injury made by UVA (320–400 nm) light. A widely accepted action of UVA radiation is production of reactive oxygen and nitrogen species resulting in damage to DNA, lipids and proteins. These oxidatively modified reactive molecules adversely affect multiple molecular pathways in cells [15]. Recently, it was shown that CPD are also produced

after UVA irradiation, and surprisingly in larger yield than oxidative lesions (8-hydroxy-2-deoxyguanosine). The mechanism of UVA-stimulated CPD formation is different, probably via triplet energy transfer photosensitization [11].

Presently, there is a small number of reports on the effects of solar UVA radiation in vivo. Only a limited number of reports describe the effects of UV light on non-skin tissues [4, 5, 7, 12, 16]. However, more penetrating UVA photons cause damage to biomolecules in the dermis that may reach the blood circulation and affect blood cells. Via signal molecules, generation UVB light may also alter blood vessels and elements. Recently, we have shown that UVA and UVB radiation affect blood count and oxidative stress-related parameters in the erythrocytes and liver of hairless mice [16]. Here, we assessed the acute effects of a single exposure to solar simulated UVA light on DNA damage and related parameters in skin and lymphocytes of SKH-1 mice in comparison to those caused by UVB radiation.

Materials and methods

Materials

Primary (rabbit polyclonal anti-phospho-p53 (Ser 20) and anti-caspase-3, goat polyclonal anti-actin) antibodies were from Santa Cruz Biotechnology (USA). Primary mouse anti-γ-H2AX (phosphorylated at Ser 139) antibody was obtained from Cell Signalling (USA). In Situ Cell Death Detection Kit, AP (TdT-mediated dUTP-biotin nick end labeling—TUNEL) was obtained from Roche Applied Science (USA). Histopaque 1077, ethidium bromide and all other chemicals were purchased from Sigma-Aldrich (USA).

Animals

Female SKH-1 hairless mice were from a breeding facility Charles River Deutschland (supply equipment: AnLab s.r.o, Praha). Mice were housed in the animal facility and were maintained throughout under standard conditions: 22 ± 2°C, 50 ± 10% relative humidity and 12 h light/12 h dark cycle. Mice were fed a standard diet (St-1—complete mixture for rats and mice in the SPF breeding; supply equipment: VELAZ, s.r.o. Únětice) and water ad libitum. The animal protocol for the study was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine and Dentistry of Palacký University and Ethics Committee of Ministry of Education, Czech Republic and conducted in accordance with the Act No. 167/1993 on the protection of animals against cruelty.

UV irradiation

To approach the experimental conditions as close to natural conditions as possible, a solar simulator *SOL-500* (Dr. Hönle UV Technology), with a spectral range (295–3,000 nm) corresponding to natural sunlight, and environmentally relevant UVA (10 or 20 J/cm^2) and UVB (200 or 800 mJ/cm^2) doses were employed. The simulator was equipped with a *H1* or *H2* filter transmitting wavelengths of 315–380 or 295–315 nm, respectively. The mice were randomly divided into six groups of eight animals and dorsal skin was exposed to a single dose of UVB or UVA.

Sample collection

Blood samples for lymphocyte isolation were collected into Na_2EDTA under ether anesthesia at 4 or 24 h after exposure. The mice were then killed by cervical dislocation. The dorsal skin was removed, washed in cold phosphate buffered saline, the subcutis was discarded and the skin was stored at −80°C. Sections of dorsal skin were fixed in Baker solution for histological and immunohistochemical analysis.

Histopathological examination

Sets of histological sections were stained with hematoxylin–eosin and PAS. The histological evaluation was performed on an Olympus BX 40 light microscope.

TUNEL staining

Apoptotic cells were detected using the In Situ Cell Death Detection Kit following the manufacturer's protocol. Sections were analyzed under 400 magnification for TUNEL-positive cells.

γ-H2AX-positive cell staining

Tissue sections were de-paraffinized, rehydrated and treated with 0.1 mol/l sodium citrate buffer (pH 6.5) in HistosPro microwave histoprocessor (120°C, 5 min). The sections were quenched of endogenous peroxidase activity in 5% H_2O_2 [30 min, room temperature (RT)]. The sections were washed with Tris-buffered saline (TBS; 50 mmol/l Tris, 150 mmol/l NaCl; pH 7.6) and TBS with Tween 20 (0.05%; v/v) and then incubated with anti-γ-H2AX antibody (1 h, RT) and with HRP-conjugated goat-antimouse antibody (EnVision™ Detection Systems; Dako, Denmark; 1 h, RT). Finally, the slides were incubated with 3,3′-diaminobenzidine substrate solution (Dako, Denmark; 2 min, RT) and counterstained with hematoxylin (30 s).

The sections were observed under 400 magnification for γ-H2AX-positive cells.

DNA single-strand breaks

Peripheral lymphocytes were isolated on Histopaque 1077 gradient. The DNA breakage in cells was determined using an alkaline comet assay as described by Vodička et al. [18]. The number of single-strand breaks (SSB) was analyzed after staining by ethidium bromide on an Olympus IX 70 fluorescence microscope.

Protein level of p53, caspase-3 and γ-H2AX

Skin samples' preparation was performed as described previously [16]. The total protein samples were subjected to immunoblotting using standard procedures. The used monoclonal antibodies are specified in part "Materials".

Statistical analysis

The data were expressed as mean ± SD, and a t test was used for testing the significance of differences.

Results

Histopathological examination

Hematoxylin–eosin and PAS staining showed a formation of cells with pyknotic nucleus and shrunken eosinophilic cytoplasm, called sunburn cells, in the epidermis of UVB irradiated mice. The higher dose stimulated sunburn cells production and focal inflammation after 4 h and it gradually disappeared at 24 h. The lower UVB dose induced sunburn cells formation and focal inflammation after 24 h. In skin of UVA-treated animals, no or sporadic (only some sections) sunburn cells were detected (data not shown).

γ-H2AX-positive cell production

A significant dose-dependent increase in γ-H2AX-positive cells was found in the basal layer as well as the whole epidermis of UVB irradiated mice at both time intervals (Table 1; Fig. S1 in supplementary material). Although the percentage of γ-H2AX-positive cells was lower in the basal layer than in other layers, more intense staining for γ-H2AX was observed in proliferating cells of the basal layer. Separated positive cells were also in the dermis of UVB irradiated mice. In UVA exposed animals, there were no or sporadic γ-H2AX-positive cells (data not shown).

Table 1 Effect of UVB radiation on formation of γ-H2AX-positive cells in SKH-1 mouse skin

	γ-H2AX-positive cells (%)	
	Basal layer	Total
4 h		
Non-irradiated	1.0 ± 0.6	9.5 ± 1.1
200 mJ/cm^2	10.7 ± 6.1	34.0 ± 19.4
800 mJ/cm^2	18.9 ± 3.6*	63.2 ± 11.7*
24 h		
Non-irradiated	1.8 ± 0.7	10.9 ± 2.8
200 mJ/cm^2	10.2 ± 2.4#	56.7 ± 20.1#
800 mJ/cm^2	16.3 ± 1.1*	81.2 ± 10.7*

Quantification was assessed in immunohistochemically stained tissue sections. From each mouse, 5–10 representative fields were counted to calculate percent of γ-H2AX-positive cells over total cells in randomly selected fields (×400). Data represent results from four mice per group. The statistical significance of difference between control and UVB-treated group was determined by Student's t test; *$p < 0.001$ and #$p < 0.01$

Single-strand break formation

Exposure to both UVB doses significantly ($p = 0.001$) increased SSB number in lymphocytes 4 h after irradiation. The damage disappeared within 24 h (Fig. 1a). In UVA exposed mice, an increase in SSB number was apparent after 24 h. However, DNA damage was less frequent than in UVB treated animals after 4 h (Fig. 1b). In both UVA and UVB irradiated mice, lymphocyte DNA damage was mostly classified as group I (mild damage).

TUNEL positive cells

Number of TUNEL-positive cells increased in both UVB irradiated groups after 4 h and persisted at least for 24 h (Fig. 2; Table S2 in supplementary material). Positive cells were localized mainly in the follicle region and single cells were also in the basal layer. In the skin of UVA-treated mice, there were no or only sporadic apoptotic cells (data not shown).

Activation of H2AX, p53 and caspase-3

Western blot determination of γ-H2AX confirmed the formation of γ-H2AX after exposure to 800 mJ/cm^2 at 4 h and 200 mJ/cm^2 at 24 h and a significant increase in mice exposed to 800 mJ/cm^2 after 24 h (Fig. 3a). The used UVA doses did not stimulate γ-H2AX formation (Fig. 3b). The UVB treatment caused significant a dose-dependent increase in phospho-p53 (Ser 20) level 24 h (Fig. 3a). In UVA irradiated mice, a slight increase in phospho-p53

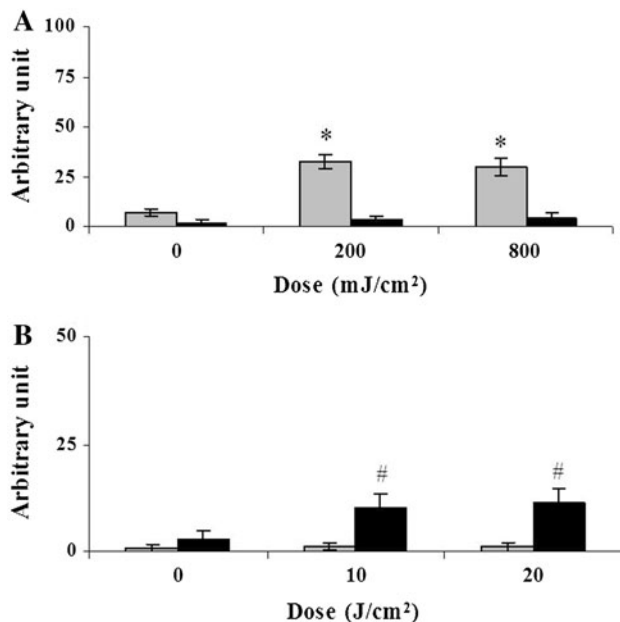

Fig. 1 DNA single-strand breaks formation in hairless mice lymphocytes after UVB (**a**) and UVA (**b**) exposure. The alkaline comet assay was used to analysis as described in "Materials and methods". Effect of irradiation was examined after 4 h (*grey bars*) and 24 h (*black bars*). To evaluate DNA damage, 100 cells (nuclei) per slide were analyzed. Total damage is expressed in arbitrary units, which range from 0 (undamaged DNA) to 400 (totally damaged DNA). Data are expressed as mean ± SD. The statistical significance of difference between control and UVA- or UVB-treated group was determined by Student's *t* test; $*p < 0.001$ and $^{\#}p < 0.01$

level was also found after 24 h (Fig. 3b). Significant caspase-3 activation was found in both groups of UVB exposed animals after 24 h (Fig. 3a). UVA caused augmentation of caspase-3 expression after 24 h (Fig. 3b).

Discussion

The sunburn cell is a morphological hallmark of skin over-exposed to sunlight. The functional role of the sunburn cell is rapid removal of cells with irreparable DNA damage to prevent their possible neoplastic transformation. We found that sunburn cell formation depends on time, UV dose and light character. Although in mice exposed to 800 mJ/cm^2 of UVB, the presence of sunburn cells was apparent 4 h after treatment and gradually decreased after 24 h; the lower UVB dose provoked the evident formation of the cells only after 24 h. Our results are consistent with a report by Lu et al. who described sunburn cell formation in UVB (180 mJ/cm^2) irradiated hairless mice between 8 and 24 h following exposure [10]. Another study using domestic and hairless pigs demonstrated a significant increase in sunburn cells after 24 h. However, the maximum was found 48 h after UVB exposure (250 mJ/cm^2) [8]. We further found that a single UVA exposure (10 and 20 J/cm^2) did not cause sunburn cell production at either 4 or 24 h. This is in agreement with the previous reports that sunburn cells are absent or rare after UVA [9, 17].

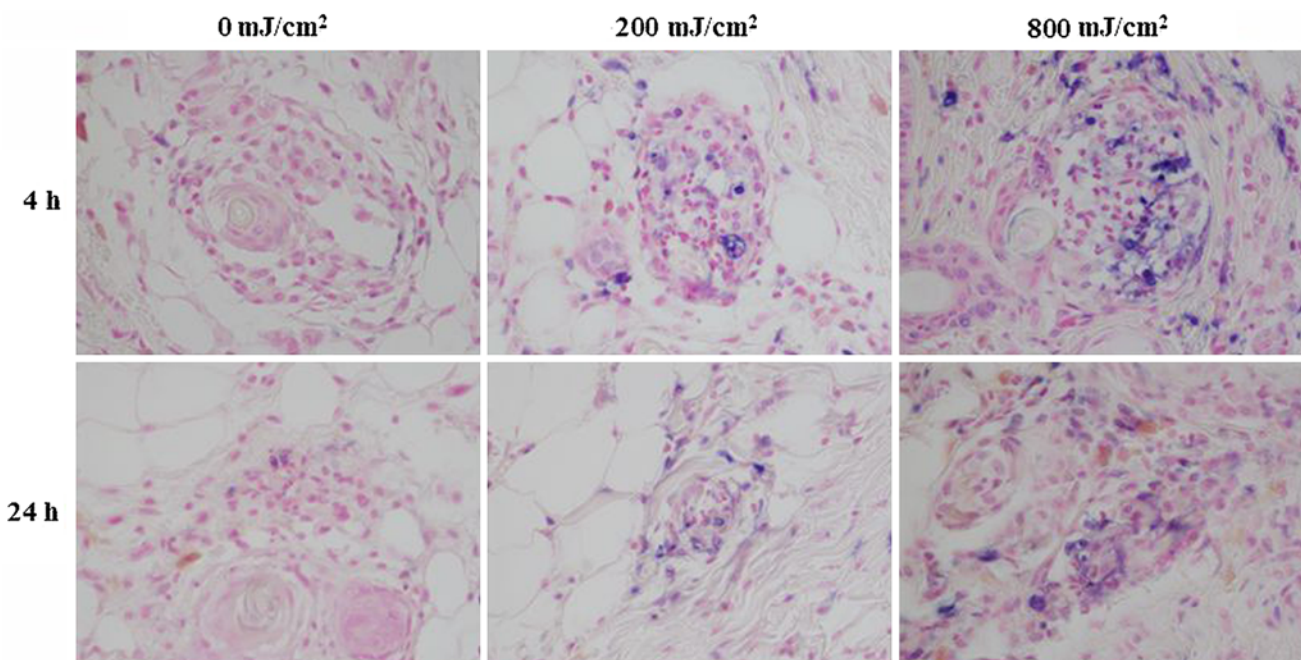

Fig. 2 Effect of UVB radiation on TUNEL-positive cells formation in SKH-1 mouse skin assessed by immunohistochemical analysis. Analysis of tissue sections was performed as described in "Materials and methods". In each case, *representative pictures* from unexposed (control) and UVB irradiated mice skin are shown. All *panels* represent a 400-fold magnification

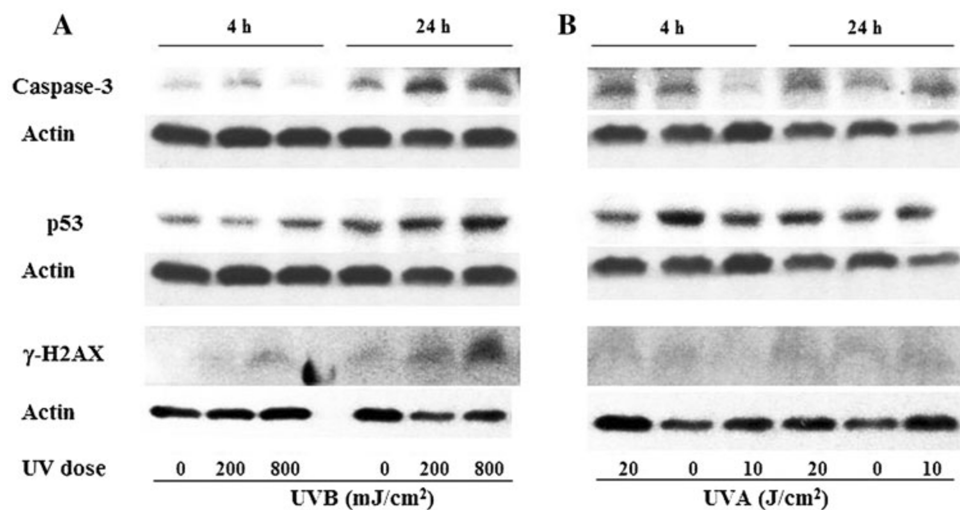

Fig. 3 Effects of UVA and UVB irradiation on expression of γ-H2AX, caspase-3 and p53 in hairless mice skin. Immunoblot analysis of selected proteins in skin homogenates obtained from hairless mice exposed to a single dose of UVB (**a**) and UVA (**b**) as detailed in "Materials and methods"

TUNEL assay is a popular method for determination of DNA fragmentation linked to apoptosis. Lu et al. [10] showed that the number of TUNEL-positive cells increased quickly after UVB (180 mJ/cm^2) exposure, stayed augmented for 12 h and then decreased. In UVB irradiated mice, we likewise observed an increase in apoptotic cells after 4 h that persisted for 24 h. Nevertheless, p53 phosphorylation and caspase-3 activation increased markedly only 24 h after the exposure. We failed to find a significant number of TUNEL-positive cells in UVA exposed mice. A recent study by Jiang et al. showed the formation of TUNEL-positive cells in chronically (12 $\times$ 5 J/cm^2) UVA irradiated C57BL/6J mice. However, the increase was not very dramatic (17 $\pm$ 4.1 per 100 cells) in comparison to control animals (8 $\pm$ 0.6 per 100 cells) [6]. Western blot analysis showed an increase in activated caspase-3 and phosphorylated p53 level in both groups of UVA irradiated mice after 24 h, but it was not as prominent as in UVB-treated animals. Immunohistochemical analysis in human skin exposed to approximately 40 J/cm^2 of UVA irradiation showed p53-positive cells only in the basal layer after 24 h [2].

Apoptosis is tightly bound with DNA damage. Comet assay is used to monitor DNA strand break formation. The alkaline version of the assay measures SSB and alkali-labile sites (apurinic/apyrimidinic sites or baseless sugars). These are not related only to oxidation. They might also represent intermediates in the repair process [3]. Here we showed probably for the first time that comet assay can use lymphocytes to detect UV caused DNA damage. Both UVA and UVB light induced detectable damage to lymphocytes in vivo. However, UVB radiation (200 and 800 mJ/cm^2) caused more apparent DNA injury than UVA (10 and 20 J/cm^2). The more aggressive UVB waveband induced the damage after 4 h and it was eliminated within 24 h, while UVA induced SSB after 24 h.

If SSB are not repaired during the G1 phase of the cell cycle, they can generate more hazardous double-strand breaks (DSB) during the S phase which then can lead to chromosomal aberrations [19]. The generation of the phosphorylated form of histone H2AX (γ-H2AX) has been identified as an early event after DSB formation and is considered the most sensitive assay for their detection [1]. A recent study demonstrated that γ-H2AX can be used to analyze DSB formation in lymphocytes and manifold tissues [14]. Following UVB treatment, we found time- and dose-dependent γ-H2AX formation in all epidermal layers of hairless mice skin. In contrast, we detected no γ-H2AX generation in the skin of UVA irradiated animals using western blot or the more sensitive immunohistochemical analysis. Recently Rizzo et al. demonstrated that UVA (20–50 J/cm^2) did not stimulate γ-H2AX expression in primary skin fibroblasts. They also observed no or only weak comet formation under neutral conditions (DSB) following UVA exposure [13].

The relative contribution of UVA and UVB wavelengths of solar light to DNA damage and related events is not completely understood. Here we have shown that a single exposure of UVA- and UVB-induced DNA damage at relatively low and physiologically relevant doses, which are quickly removed by repair machinery of skin cells. Our findings indicate that the mechanism of DNA damage caused by acute UVA exposure includes primarily the formation of SSB (oxidative damage). However, chronic UVA exposure may result in the accumulation of DNA lesions and DSB formation as described some in vitro experiments [19]. Moreover, repeated exposure to UVA light may also increase or multiply UVB-mediated DNA damage. Thus further experimental work is required to confirm the impact of different DNA damage responses to UVA and UVB and of interactions between UVA and UVB on mutagenesis.

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References

1. Barnes L, Dumas M, Juan M, Noblesse E, Tesniere A, Schnebert S, Guillot B, Molès JP (2010) GammaH2AX, an accurate marker that analyzes UV genotoxic effects on human keratinocytes and on human skin. Photochem Photobiol 86:933–941
2. Burren R, Scaletta C, Frenk E, Panizzon RG, Applegate LA (1998) Sunlight and carcinogenesis: expression of p53 and pyrimidine dimers in human skin following UVA I, UVA I + II and solar simulating radiations. Int J Cancer 76:201–206
3. Collins AR (2009) Investigating oxidative DNA damage and its repair using the comet assay. Mutat Res 681:24–32
4. Erden Inal M, Kahraman A (2000) The protective effect of flavonol quercetin against ultraviolet a induced oxidative stress in rats. Toxicology 154:21–29
5. Hasegawa T, Kaneko F, Niwa Y (1992) Changes in lipid peroxide levels and activity of reactive oxygen scavenging enzymes in skin, serum and liver following UVB irradiation in mice. Life Sci 50:1893–1903
6. Jiang S, Liu XM, Dai X, Zhou Q, Lei TC, Beermann F, Wakamatsu K, Xu SZ (2010) Regulation of DHICA-mediated antioxidation by dopachrome tautomerase: implication for skin photoprotection against UVA radiation. Free Radic Biol Med 48:1144–1151
7. Kahraman A, Inal ME (2002) Protective effects of quercetin on ultraviolet A light-induced oxidative stress in the blood of rat. J Appl Toxicol 22:303–309
8. Kawagishi N, Hashimoto Y, Takahashi H, Ishida-Yamamoto A, Iizuka H (1998) Epidermal cell kinetics of pig skin in vivo following UVB irradiation: apoptosis induced by UVB is enhanced in hyperproliferative skin condition. J Dermatol Sci 18:43–53
9. Lavker R, Kaidbey K (1997) The spectral dependence for UVA-induced cumulative damage in human skin. J Invest Dermatol 108:17–21
10. Lu YP, Lou YR, Yen P, Mitchell D, Huang MT, Conney AH (1999) Time course for early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice. Cancer Res 59:4591–4602
11. Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T (2006) Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci USA 103:13765–13770
12. Mulero M, Romeu M, Giralt M, Folch J, Nogués MR, Fortuño A, Sureda FX, Linares V, Cabré M, Paternáin JL, Mallol J (2006) Oxidative stress-related markers and langerhans cells in a hairless rat model exposed to UV radiation. J Toxicol Environ Health A 69:1371–1385
13. Rizzo JL, Dunn J, Rees A, Rünger TM (2011) No formation of DNA double-strand breaks and no activation of recombination repair with UVA. J Invest Dermatol 131:1139–1148
14. Rübe CE, Grudzenski S, Kühne M, Dong X, Rief N, Löbrich M, Rübe C (2008) DNA double-strand break repair of blood lymphocytes and normal tissues analysed in a preclinical mouse model: implications for radiosensitivity testing. Clin Cancer Res 14:6546–6555
15. Svobodová A, Vostálová J (2010) Solar radiation induced skin damage: review of protective and preventive options. Int J Radiat Biol 86:999–1030
16. Svobodová AR, Galandáková A, Šianská J, Doležal D, Ulrichová J, Vostálová J (2011) Acute exposure to solar simulated ultraviolet radiation affects oxidative stress-related biomarkers in skin, liver and blood of hairless mice. Biol Pharm Bull 34:471–479
17. Takeuchi S, Zhang W, Wakamatsu K, Ito S, Hearing VJ, Kraemer KH, Brash DE (2004) Melanin acts as a potent UVB photosensitizer to cause an atypical mode of cell death in murine skin. Proc Natl Acad Sci USA 101:15076–15081
18. Vodička P, Koskinen M, Vodičková L, Štětina R, Šmerák P, Bárta I, Hemminki K (2001) DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. Chem Biol Interact 137:213–227
19. Wischermann K, Popp S, Moshir S, Scharfetter-Kochanek K, Wlaschek M, de Gruijl F, Hartschuh W, Greinert R, Volkmer B, Faust A, Rapp A, Schmezer P, Boukamp P (2008) UVA radiation causes DNA strand breaks, chromosomal aberrations and tumorigenic transformation in HaCaT skin keratinocytes. Oncogene 27:4269–4280