Acute Exposure to Solar Simulated Ultraviolet Radiation Affects Oxidative Stress-Related Biomarkers in Skin, Liver and Blood of Hairless Mice

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The ultraviolet (UV) region of solar radiation is a critical factor in the initiation and development of a number of skin diseases. However, it is not only skin which is directly exposed to solar light that is affected by UV radiation, through low molecular weight mediators, generated upon irradiation, "non-skin" tissues can also be affected. The aim of this study was to examine in detail, the acute effects of UVA and UVB wavebands on hairless mice. Female SKH-1 hairless mice were exposed to a single dose of UVB (200, 800 mJ/cm^2) or UVA (10, 20 J/cm^2) using a solar simulator. The effects on haematological parameters, activity and/or expression of antioxidant enzymes, level of glutathione (GSH), markers of oxidative damage (lipid peroxidation and carbonylated proteins) were analysed in erythrocytes, plasma, liver and whole skin homogenates. No macroscopic changes were observed either 4 or 24 h after UVA/UVB exposure. The blood count showed a significant increase in leukocyte number and reduction of platelets 4 h following UVA and UVB irradiation, which disappeared 24 h after irradiation except for the higher UVA dose. Changes in oxidative stress-related parameters, particularly activity of catalase (CAT) and superoxide dismutase (SOD) and level of GSH and lipid peroxidation products, were found in skin, erythrocytes and liver. The expression of several enzymes (CAT, SOD, glutathione transferase (GST), nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase (NQO1) and hem oxygenase-1 (HO-1)) in skin was affected following UVA and UVB radiation. Increase in carbonylated proteins was found in plasma and skin samples.

Key words UVB; UVA; acute toxicity; hairless mouse; oxidative stress

Solar light is the main environmental factor implicated in various skin disorders. Extensive evidence supports the notion that the whole solar spectrum (UV, visible and infrared wavelengths) participates in skin cells damage.[1)] However, UV wavelengths are regarded as the most hazardous and most toxic. The sun is primarily a UVA source with an approximate terrestrial UVB content of about 5—10%. UVA (315—400 nm) penetrates deep into the skin. Approximately 80% of UVA reaches the dermal-epidermal junction and around 10% of UVA even reaches the hypodermis. UVA photons are less energetic than UVB and cause mainly indirect damage *via* increased generation of reactive oxygen and nitrogen species (RONS). These reactive species attack biomolecules resulting in several types of DNA damage (*e.g.* DNA single strand breaks, DNA interstrand cross-links and nucleotide base modifications), formation of oxidized fragments and products of lipids (*e.g.* lipid alkoxyl radicals, aldehydes, alkanes, lipid (hydro)peroxides and epoxides) and oxidatively modified proteins and saccharides. In contrast, incoming UVB (295—315 nm) is mostly absorbed by the epidermis (90%). UVB is directly absorbed by the aromatic heterocyclic bases of DNA. As a result of UVB photons absorption cyclobutane-pyrimidine dimers and pyrimidine-(6-4)-pyrimidone photoproducts are formed.[2)] Aromatic amino acids such as tryptophan and tyrosine also act as potent UVB radiation absorbers and their interaction with high energetic UVB photons leads to the generation of several derivatives. Of these, 6-formylindolo[3,2-*b*]carbazole (FICZ) has been recognized to have fundamental importance.[3)] Amino acid modification alters protein function as well as affects cellular signalling. However, the division between UVA and UVB is arbitrary and UVB participates in RONS production as well.[4)]

Skin cells are equipped with several non-enzymic (ascorbic acid, tocopherol, ubiquinol, and glutathione (GSH)) and enzymic antioxidants (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX)) that maintain the prooxidant/antioxidant balance by rapid RONS elimination, resulting in cell and tissue stabilization. However, flooding of reactive species causes antioxidants depletion and further formation of reactive products that both result in oxidative stress. Production of modified biomolecules is also accompanied by alteration to various enzyme activity and regulation of gene expression in several pathways such as inflammatory cytokines, mitogen-activated protein kinases, matrix metalloproteinases, nuclear factor-κB, nuclear factor erythroid-2 related factor 2 (Nrf2) and phase 2 detoxifying enzymes such as nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase (NQO1), hem oxygenase-1 (HO-1), glutathione transferase (GST) and glutathione reductase (GSR).[4,5)]

Over 50 years of UV light research, a number of authors have examined the effects of chronic and/or repeated exposures. However, lacking of number reports has pursued the acute effects of UVA/UVB exposure.[6—12)] Further, studies on alterations caused by UVA/UVB light have involved predominantly skin tissue, as the skin is the first barrier between the body and the environment. Only a limited number of reports describe the effects of UV light on non-skin (internal) tissues. However, damaged biomolecules as well as signal molecules generated by UV exposure may reach the blood circulation and in this way affect blood cells and internal organs.

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Further, deep penetrating UVA photons themselves may affect blood vessels in the dermis and hypodermis and thus increase oxidative stress. The aim of this study was to determine the acute effects of single exposure to UVA and UVB radiation in hairless mice skin, blood and liver with special emphasize on parameters related to RONS-caused damage.

MATERIALS AND METHODS

Material ABC VET LMGE Reagent Pack was obtained from Horiba ABX (France). The Oxy-Blot protein oxidation detection kit was from Chemicon International (U.S.A.). Protease inhibitor cocktail tablete CompleteTM was purchased from Roche Diagnostic (Germany). Western blotting luminol reagent for chemiluminiscent horseradish peroxidase detection, primary antibodies (rabbit polyclonal anti-SOD1/2, -GPX, -GSR, -GST, and -HO-1, goat polyclonal anti-actin), and horseradish peroxidase (HRP) conjugated secondary antibodies (goat anti-rabbit and rabbit anti-goat) were purchased from Santa Cruz Biotechnology (U.S.A.). Primary antibodies (goat polyclonal anti-NQO1 and -CAT) and all other chemicals were purchased from Sigma-Aldrich (U.S.A.).

Animals Female albino SKH-1 hairless mice (5 weeks old), purchased from AnLab s.r.o. (Praha, Czech Republic), were used in this study. The animals were housed in plastic cages containing dust-free sawdust and maintained throughout under standard conditions: 24±2 °C temperature (checked daily), 50±10% relative humidity and 12 h light/12 h dark cycle. Mice were kept in groups of 8 per cage and fed a standard diet and water *ad libitum*. The animal protocol for this 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 After 4—5 weeks acclimatization mice were used for the experiment. For irradiation, a solar simulator SOL-500 (Dr. Hönle UV Technology, Germany), with a spectral range (295—3000 nm) corresponding to natural sunlight was used. The simulator was equipped with a H1 filter (Dr. Hönle UV Technology, Germany) transmitting wavelengths of 315—380 nm or H2 filter (Dr. Hönle UV Technology, Germany) transmitting wavelengths of 295—315 nm. The UVA and UVB output measured by an UVA- or UVB-meter (Dr. Hönle UV Technology, Germany) was 3.0 or 0.5 mW/cm^2, respectively.

Mice (26.7±1.9 g body weight) were randomly divided into 6 groups of 8 animals and dorsal skin was exposed to a single dose of UVB (200 or 800 mJ/cm^2) or UVA (10 or 20 J/cm^2). During UV irradiation, mice were held in plastic cages. The distance between the light source and target skin was *ca.* 45 cm for UVA/UVB irradiation. The used UVA (20 J/cm^2) and UVB (800 mJ/cm^2) dose was the equivalent of exposure to approximately 2 and 1.5 h, respectively, of natural May sunlight at our latitude (49°N).

Collection of Samples Four or 24 h after UV exposure, macroscopic examination of the skin was performed. The animals were weighed and under ether anaesthesia, blood samples were collected into ethylenediaminetetraacetic acid disodium salt (EDTA). An aliquot of the blood (0.025 ml) was immediately used for blood count. The rest of the blood was centrifuged (2500 rpm, 10 min, 4 °C) to obtain the plasma which was stored at −80 °C and used for oxidized proteins determination. Erythrocytes were washed with phosphate buffer saline (PBS) and stored at −80 °C for determination of antioxidant enzyme activities, GSH content and lipid peroxidation. After blood collection, mice were killed by cervical dislocation. Livers were removed, weighed, washed in cold PBS and stored at −80 °C for determination of antioxidant enzymes activities, GSH content and lipid peroxidation. Dorsal skin was removed, washed in cold PBS, the subcutis was eliminated and skin was stored at −80 °C for determination of antioxidant enzyme activities, GSH content, lipid peroxidation, amount of oxidized proteins and expression of selected proteins (HO-1, NQO1, GST, CAT, GSR, GPX, SOD1, SOD2).

Haematological Parameters White blood cells (WBC), red blood cells (RBC), haemoglobin (HBG), hematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were measured in blood using haematology analyzer Vet ABC animal blood counter and ABC VET LMGE Reagent Pack (Horiba ABX, France).

Lipid Peroxidation Assay Lipid peroxidation was assessed as thiobarbituric acid reactive substances (TBARS) in skin and liver homogenates and lysate of erythrocytes using thiobarbituric acid (TBA) reaction method.[13] Briefly, the mixture of TBA (0.375%, w/v) and TCA (15%, w/v) was combined with samples (9 : 1) and heated (30 min, 95 °C). The samples were then cooled and centrifuged (10 min; 13000 rpm; 4 °C). The amount of TBARS was determined spectrophotometrically at 535 nm (Sunrise, Tecan, Switzerland). TBARS amount was expressed as mmol/g of haemoglobin or protein.

GSH Assay The level of glutathione (GSH) in erythrocytes, skin and liver was determined according to Sedlak and Lindsay using Ellman's reagent.[14] Briefly, lysate of erythrocytes or tissue homogenate was precipitated (trichloroacetic acid (25% (v/v), thoroughly mixed and centrifuged (10000 rpm, 10 min, 4 °C). The supernatant (0.02 ml) was combined with assay buffer (0.8 M Tris, 0.02 M EDTA; pH 8.9) mixed with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) and the absorbance was read at 412 nm after 4 min. GSH amount was expressed as mmol/g haemoglobin or protein.

Superoxide Dismutase Activity Assay SOD activity in skin and liver homogenates and erythrocyte lysates was measured using the indirect spectrophotometric method based on the generation of $O_2^{\cdot -}$ by a mixture of reduced nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) in the presence of nitro blue tetrazolium (NBT).[15] Briefly, lysate of erythrocytes (0.01 ml) was directly mixed with assay buffer (0.1 mM EDTA, 50 mM KH_2PO_4; pH 7.4) that contained NBT (60 μM) and NADH (100 μM). The reaction was started with PMS (35 μM) and the absorbance was measured at 560 nm after 5 min. In the case of skin and liver tissues, the homogenate (0.05 ml) was combined with water (0.45 ml), chloroform (0.125 ml) and solid KH_2PO_4 (30 mg), thoroughly mixed and centrifuged (4000 rpm, 30 min, 4 °C). The supernatant (0.01 ml) was mixed with assay buffer, the reaction started with PMS and

the absorbance measured as described above. SOD activity was expressed as U/g haemoglobin or protein.

Glutathione Peroxidase Activity Assay GPX activity in skin and liver homogenates and erythrocyte lysates was evaluated spectrophotometrically at 340 nm by the modified method of Tappel.[16] The reaction mixture included GSH (0.39 mM), NADPH (0.19 mM), glutathione reductase (1.55 U/ml) in assay buffer (50 mM Tris, 0.1 mM EDTA; pH 7.6) and sample (10 μl). The enzyme reaction was initiated by cumen hydroperoxide (0.1%, v/v). Activity was expressed as mU/g haemoglobin or protein.

Glutathione *S*-Transferase Activity Assay GST activity in erythrocytes, skin and liver homogenates was assayed spectrophotometrically at 340 nm by the method of Habig *et al*.[17] The reaction mixture contained sample (10 μl), GSH (5 mM) in assay buffer (0.1 M KH_2PO_4; pH 7.4) and 1-chloro-2,4-dinitrobenzene (1.25 mM) which was used as a substrate. The enzyme activity was expressed as U/g haemoglobin or protein.

Glutathione Reductase Activity Assay GSR activity in skin and liver homogenates was determined spectrophotometrically at 340 nm as described by Carlberd and Mannervik.[18] GSR activity is proportional to NADPH decay. The sample (5 μl) was mixed with GSSG (0.05 mM) in assay buffer (0.1 M KH_2PO_4, 0.5 mM EDTA; pH 7.4) and started by NADPH (5 mM). The enzymatic activity was expressed as U/g haemoglobin or protein.

Catalase Activity Assay CAT activity in erythrocyte lysates and skin and liver homogenates was evaluated according to Beers and Sizer.[19] The assay mixture contained 50 mM phosphate buffer (pH 7.0) and 6 mM H_2O_2, in a final volume of 2.45 ml, with 50 μl of sample. Changes in optical density at 240 nm were spectrophotometrically monitored (UV–VIS spectrophotometer UV-2401PC; Shimadzu, Japan). CAT activity was expressed as mU/g haemoglobin or protein.

NQO1 Activity NQO1 activity in skin homogenates was determined spectrophotometrically at 600 nm.[20] After centrifugation (10000 rpm, 10 min, 4 °C), homogenate (50 μl) was added to reaction mixture (800 μl; 25 mM TRIS (pH 7.6), Tween 20 (0.2%, v/v), bovine serum albumin (BSA; 0.07%, w/v), 2 mM NADPH. The reaction was initiated by addition of 2,6-dichlorphenol indophenol (40 μM) as the substrate. NQO1 activity was expressed as U/g protein.

Haemoglobin Determination The haemoglobin concentration in erythrocyte lysates was measured spectrophotometrically (540 nm) using Drapkin's reagent.

Protein Determination The protein concentration in skin and liver homogenates was determined by the Bradford method.[21]

Carbonylated Protein Determination Amount of oxidatively modified proteins was detected in pooled plasma samples and skin homogenates by Western blot analysis using the OxyBlot™ Protein Oxidation Detection kit (Chemicon International, U.S.A.) according to the manufacturer's protocol. Briefly, samples of 20 mg proteins were subjected to 2,4-dinitrophenylhydrazine (DNPH) derivatization. Incubation of equal aliquots with a control solution (without DNPH) served as negative control. The DNPH-derivatized protein samples were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and blotted onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked (1 h; room temperature; shaking) using blocking buffer (5% BSA in 100 mM Tris–buffered saline (pH 7.5) with Tween 20 (0.05%; v/v)). Then the membranes were incubated with a rabbit anti-DNPH primary antibody in blocking buffer (1 : 150; 1 h, room temperature) and finally with a goat anti-rabbit immunoglobulin G (IgG) peroxidase-coupled secondary antibody in blocking buffer (1 : 300; 1 h; room temperature). The membranes were then treated with chemiluminescence substrate to visualize protein bands. Protein carbonylation was determined by autoradiography with XAR-5 films.

Expression of Antioxidant Enzymes and Oxidative Stress-Related Proteins Skin samples were homogenized in ice-cold lysis buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA); 20 mM NaF; 100 mM Na_3VO_4; NP-40 (0.5%, v/v); Triton X-100 (1%, v/v); 1 mM PMSF; and protease inhibitor cocktail tablet). The homogenate was incubated 30 min on ice and centrifuged (14000 rpm, 20 min, 4 °C) and the supernatants were collected for Western blot analysis. The samples were stored at −80 °C. The protein content was determined by Bradford assay. Expression of proteins was evaluated in pooled samples.

Proteins were separated by 8% or 15% SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membrane. Residual binding sites on the membrane were blocked using blocking buffer (5% non-fat dry milk (w/v) in 100 mM Tris-buffered saline (pH 7.5) with Tween 20 (0.05%; v/v)) for 1 h at room temperature. The membrane was then incubated with a specific primary antibody (diluted in blocking buffer) overnight at 4 °C and then with a secondary horseradish peroxidase conjugated antibody (goat anti-rabbit or rabbit anti-goat; in blocking buffer) for 2 h at room temperature. Individual proteins were detected by chemiluminiscence using Western Blotting Luminol Reagent and autoradiography with XAR-5 film.

Statistical Analysis The data were expressed as means ± S.D. and a *t*-test was used for testing the significance of differences ($p<0.05$).

RESULTS

Macroscopic Changes UVB and in lesser extent, UVA wavelengths have been described in the literature to induce erythema.[22] Thus we compared the visual aspect of the skin of non-irradiated and irradiated animals before killing them. Under our experimental conditions, there were no visible macroscopic changes (redness, swelling) in the skin of non-irradiated mice and animals exposed to a single dose of UVB (200 or 800 mJ/cm^2) or UVA (10 or 20 J/cm^2) after 4 and 24 h.

Possible effects on internal organs such as liver were then examined. We found no abnormalities in liver appearance or weight in UVA irradiated groups or control animals at 4 h (control − 1.6 ± 0.1 g, 10 J/cm^2 − 1.5 ± 0.2 g, 20 J/cm^2 − 1.4 ± 0.3 g) and 24 h (control − 1.5 ± 0.1 g, 10 J/cm^2 − 1.4 ± 0.1 g, 20 J/cm^2 − 1.5 ± 0.2 g). Similarly no effects were found in UVB treated mice after 4 h (control − 1.9 ± 0.1 g, 200 mJ/cm^2 − 1.8 ± 0.1 g, 800 mJ/cm^2 − 1.9 ± 0.2 g) and 24 h (control − 1.8 ± 0.1 g, 200 mJ/cm^2 − 1.8 ± 0.3 g, 800 mJ/cm^2 − 1.7 ±

0.2 g).

Effects of UV on Haematological Parameters It has been reported that 80% of UVA light reaches the dermo-epidermal junction and penetrates deeper to the dermis and hypodermis.[2] For this reason we assumed that blood vessels and blood cells might also be affected by UV exposure. Individual blood cells were counted and other related parameters were analyzed. The results showed a significant dose-dependent increase in WBC number in mice exposed to 200 mJ/cm^2 UVB (145.5%) and 800 mJ/cm^2 UVB (174.5%) at 4 h after irradiation compared to the control group (100%). UVB light also caused a significant decrease in PLT number in animals irradiated with 200 mJ/cm^2 (64.2%) and 800 mJ/cm^2 (69.0%). The observed effects on WBC and PLT disappeared within 24 h. Other haematological parameters were not significantly affected either 4 or 24 h after UVB exposure (Table 1).

A significant dose-dependent growth in WBC number was also observed in animals exposed to UVA, particularly 10 J/cm^2 (149.7%) and 20 J/cm^2 (166.1%). In the group treated with 20 J/cm^2, a significant WBC increase persisted 24 h after exposure (137.8%). UVA light further caused a significant decrease in PLT number in mice irradiated with 10 J/cm^2 (75.2%) and 20 J/cm^2 (76.8%) after 4 h. Within 24 h, the PLT amount increased to the control level in the group exposed to 10 J/cm^2. However, it remained significantly reduced in mice treated with a higher dose (20 J/cm^2; 84.0%). UVA-caused changes in other haematological markers were not significant at $p<0.05$ (Table 2).

Effects of UV on Antioxidant Parameters in Erythrocytes As shown in Table 3, UVB exposure resulted in increase in TBARS amount (112.5%) after 4 h. This phenomenon disappeared after 24 h. SOD activity was the other significantly affected parameter that was decreased in both UVB treated groups after 4 and 24 h. However a significant difference was found in the group exposed to 200 mJ/cm^2 after 4 h (88.9%) and to 800 mJ/cm^2 after 24 h (87.3%). There were no statistically significant variations in other measured parameters except for an increase in GSH level in the group exposed to 200 mJ/cm^2 after 24 h (112.6%).

UVA elicited changes were predominantly found in animals exposed to the higher UVA dose (Table 4). TBARS amount was surprisingly decreased in animals exposed to the higher dose (81.6%) after 4 h even though non-significant increase was found in mice treated with 10 J/cm^2. GSH was depleted after 4 h but increased after 24 h. CAT activity was increased after 4 h but reduced after 24 h (88.8%). The dose of 10 J/cm^2 caused only non-significant changes.

Effects of UV on Antioxidant Parameters in Liver Hepatocytes represent metabolically active cells that are essential for numerous biological functions and activities. Thus liver tissue was chosen to examine possible alterations of UVA and UVB light to internal organs. In UVB exposed animals (Table 5), significant changes were observed in SOD activity and GSH level. SOD activity was decreased in a dose-dependent manner (91.7, 85.7% of control) after 4 h and a non-significant reduction persisted in the group exposed to higher dose after 24 h. GSH depletion was obvious at 24 h (89.5, 73.0%). We found no statistically significant ($p<0.05$) changes for other parameters.

Table 1. Haematological Parameters in UVB Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	200 mJ/cm^2 UVB	800 mJ/cm^2 UVB
4 h	WBC	10^3/mm^3	6.55±1.80	9.53±1.10*	11.43±3.91*
	RBC	10^6/mm^3	8.93±0.37	8.90±0.15	9.02±0.11
	HGB	g/dl	15.23±0.30	14.88±0.66	15.23±0.34
	HCT	%	44.50±1.10	44.58±1.63	44.90±0.75
	PLT	10^3/mm^3	444.50±175.30	338.50±57.53	364.00±67.51
24 h	WBC	10^3/mm^3	8.03±3.04	8.13±1.76	9.30±2.05
	RBC	10^6/mm^3	8.72±0.27	8.93±0.23	8.77±0.18
	HGB	g/dl	14.83±0.70	14.45±0.17	14.78±0.29
	HCT	%	44.75±2.51	44.53±1.84	45.25±0.70
	PLT	10^3/mm^3	486.00±187.74	391.25±84.13	429.75±133.84

WBC, white blood cells; RBC, red blood cells; HGB, haemoglobin; HCT, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration. The values are expressed as mean±S.D. of 4 animals per group. The value (*) is significantly different from value of non-irradiated group of animals ($p<0.05$). Statistical significance was determined by *t*-test.

Table 2. Haematological Parameters in UVA Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	10 J/cm^2 UVA	20 J/cm^2 UVA
4 h	WBC	10^3/mm^3	5.93±1.24	8.88±1.57$^{\#}$	9.58±0.69$^{\#}$
	RBC	10^6/mm^3	9.52±0.25	9.63±0.15	9.61±0.06
	HGB	g/dl	14.98±0.38	14.88±0.40	14.73±0.65
	HCT	%	49.18±0.82	49.20±1.22	47.93±2.51
	PLT	10^3/mm^3	392.55±44.89	295.05±59.91*	301.65±49.25
24 h	WBC	10^3/mm^3	6.60±1.83	7.25±0.31	9.10±1.30*
	RBC	10^6/mm^3	9.38±0.30	9.36±0.36	9.44±0.20
	HGB	g/dl	14.93±0.41	14.40±0.44	14.78±0.21
	HCT	%	47.20±1.10	46.43±1.80	47.03±0.72
	PLT	10^3/mm^3	371.00±53.25	354.67±10.21	311.75±34.83*

WBC, white blood cells; RBC, red blood cells; HGB, haemoglobin; HCT, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration. The values are expressed as mean±S.D. of 4 animals per group. The value (*) and (#) is significantly different from that of non-irradiated group of animals ($p<0.05$) and ($p<0.001$), respectively. Statistical significance was determined by *t*-test.

Table 3. Oxidative Stress-Related Parameters in Erythrocytes of UVB Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	200 mJ/cm^2 UVB	800 mJ/cm^2 UVB
4 h	TBARS	mmol/g	0.32±0.02	0.36±0.02*	0.36±0.02*
	GSH	mmol/g	13.52±0.69	13.84±0.58	13.69±0.76
	SOD	U/g	3.52±0.24	3.13±0.20*	3.24±0.25
	GPX	mU/g	119.88±11.56	117.44±9.14	118.34±3.62
	GST	U/g	10.93±1.82	10.62±1.08	11.69±1.21
	CAT	mU/g	27.63±2.80	26.27±2.74	28.17±1.71
24 h	TBARS	mmol/g	0.37±0.03	0.38±0.05	0.35±0.02
	GSH	mmol/g	13.59±1.05	15.30±1.11*	13.22±1.10
	SOD	U/g	3.79±0.26	3.56±0.25	3.31±0.18$^{\#}$
	GPX	mU/g	143.25±7.34	146.28±5.28	141.67±12.68
	GST	U/g	10.98±2.34	11.36±1.48	10.35±1.38
	CAT	mU/g	39.98±4.00	38.76±1.82	39.95±3.11

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances. The values are expressed per 1 g of haemoglobin. The values are expressed as mean±S.D. of 4 animals per group. The value (*) and (#) is significantly different from value of non-irradiated group of animals ($p<0.05$) and ($p<0.01$), respectively. Statistical significance was determined by *t*-test.

Table 4. Oxidative Stress-Related Parameters in Erythrocytes of UVA Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	10 J/cm^2 UVA	20 J/cm^2 UVA
4 h	TBARS	mmol/g	0.49±0.06	0.56±0.07	0.40±0.02*
	GSH	mmol/g	13.88±0.76	13.58±1.48	12.40±1.09*
	SOD	U/g	3.24±0.28	3.34±0.21	3.44±0.14
	GPX	mU/g	125.43±3.69	131.58±5.62	125.18±13.52
	GST	U/g	10.41±0.48	10.32±0.90	9.55±0.81
	CAT	mU/g	30.11±2.44	31.96±2.83	33.42±2.44
24 h	TBARS	mmol/g	0.51±0.06	0.50±0.04	0.46±0.02
	GSH	mmol/g	12.15±2.48	12.78±1.79	15.22±1.20*
	SOD	U/g	3.46±0.20	3.54±0.10	3.41±0.28
	GPX	mU/g	133.98±9.12	127.98±12.84	134.33±15.26
	GST	U/g	9.71±1.04	9.55±0.31	10.31±1.76
	CAT	mU/g	26.54±2.92	28.91±1.26	23.58±1.33*

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances. The values are expressed per 1 g of haemoglobin. The values are expressed as mean±S.D. of 4 animals per group. The value (*) is significantly different from that of non-irradiated group of animals ($p<0.05$). Statistical significance was determined by *t*-test.

Table 5. Oxidative Stress-Related Parameters in Liver of UVB Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	200 mJ/cm^2 UVB	800 mJ/cm^2 UVB
4 h	TBARS	mmol/g	0.06±0.01	0.06±0.01	0.06±0.01
	GSH	mmol/g	17.84±1.48	16.82±1.56	16.84±1.86
	SOD	U/g	78.22±6.77	71.79±7.28	67.01±7.31*
	GPX	mU/g	335.40±45.69	342.36±33.60	329.89±20.73
	GST	U/g	0.52±0.05	0.56±0.11	0.52±0.08
	GSR	U/g	12.98±2.05	13.02±1.81	13.89±2.19
	CAT	mU/g	49.31±7.06	50.80±8.04	49.70±8.88
24 h	TBARS	mmol/g	0.08±0.01	0.08±0.02	0.08±0.01
	GSH	mmol/g	20.59±2.97	18.42±1.15	15.02±1.33$^{\#}$
	SOD	U/g	92.98±18.26	92.80±10.29	82.76±7.29
	GPX	mU/g	330.23±65.24	348.71±30.01	361.84±27.62
	GST	U/g	0.52±0.12	0.57±0.06	0.55±0.06
	GSR	U/g	16.18±3.81	17.40±2.13	15.49±1.81
	CAT	mU/g	46.57±10.01	53.71±7.17	50.34±6.13

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; GSR, glutathione reductase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances. The values are expressed per 1 g of protein. The values are expressed as mean±S.D. of 4 animals per group. The value (*) and (#) is significantly different from that of non-irradiated group of animals ($p<0.05$) and ($p<0.005$), respectively. Statistical significance was determined by *t*-test.

Minimal changes were observed in liver of UVA irradiated mice 4 h after exposure (Table 6). On the other hand, 24 h after UVA exposure CAT activity was significantly reduced ($p<0.01$) in both irradiated groups (70.6, 75.1% of control). Reduction in GSH level (74.6%) and activity of GSR (65.3%) was found in animals exposed to 10 J/cm^2, while in the group irradiated with 20 J/cm^2 both parameters were virtually unaffected. In mice treated with the higher UVA dose, a significant decrease in TBARS amount was found.

Effects of UV on Antioxidant Parameters in Skin Skin is the primary target of UV radiation and in our experiment skin was naturally the tissue most affected by irradiation. At 4 h after UVB treatment, a significant decrease in GST activity (76.9, 84.5%) and increase in SOD activity (123.2, 122.4%) was observed in both irradiated groups, whereas GSH level (89.0%) and CAT activity (70.3%) reduction and NQO1 activity increase were found only in the group exposed to the higher UVB dose. The activity of GST and CAT remained reduced while the activity of NQO1 increased in both irradiated groups 24 h after exposure. TBARS amount (75.0%) and SOD activity (76.0%) were decreased after 24 h only in mice treated with 800 mJ/cm^2. GPX and GSR activities were unaffected in both irradiated groups at both time

Table 6. Oxidative Stress-Related Parameters in Liver of UVA Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	10 J/cm^2 UVA	20 J/cm^2 UVA
4 h	TBARS	mmol/g	0.05±0.01	0.048±0.003	0.06±0.01
	GSH	mmol/g	15.34±2.21	16.34±3.42	15.46±1.37
	SOD	U/g	53.67±6.00	57.69±7.54	58.97±11.67
	GPX	mU/g	257.27±7.66	257.58±70.34	258.12±45.32
	GST	U/g	0.39±0.05	0.43±0.10	0.41±0.08
	GSR	U/g	9.43±1.36	8.09±1.64	9.08±2.35
	CAT	mU/g	34.10±5.06	39.29±12.10	40.01±6.99
24 h	TBARS	mmol/g	0.10±0.004	0.10±0.014	0.09±0.007*
	GSH	mmol/g	21.09±4.28	15.73±2.69*	20.37±1.59
	SOD	U/g	106.33±13.39	96.72±7.30	105.90±12.35
	GPX	mU/g	429.19±57.39	408.10±22.27	428.68±29.05
	GST	U/g	0.64±0.09	0.63±0.04	0.73±0.12
	GSR	U/g	17.47±5.20	11.41±2.70*	16.88±5.20
	CAT	mU/g	57.51±4.96	40.58±3.70$^{\#}$	39.00±3.71$^{\#}$

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; GSR, glutathione reductase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances. The values are expressed per 1 g of protein. The values are expressed as mean±S.D. of 4 animals per group. The value (*) and (#) is significantly different from that of non-irradiated group of animals ($p<0.05$) and ($p<0.001$), respectively. Statistical significance was determined by *t*-test.

Table 7. Oxidative Stress-Related Parameters in Whole Skin Homogenates of UVB Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	200 mJ/cm^2 UVB	800 mJ/cm^2 UVB
4 h	TBARS	mmol/g	0.25±0.04	0.24±0.05	0.23±0.02
	GSH	mmol/g	13.51±0.85	12.69±1.03	12.05±1.00*
	SOD	U/g	64.24±8.19	79.15±10.30*	78.62±10.00*
	GPX	mU/g	168.12±20.11	153.82±12.01	160.26±29.02
	GST	U/g	0.13±0.01	0.10±0.03*	0.11±0.01$^{\#}$
	GSR	U/g	18.94±3.67	18.71±2.46	18.84±5.94
	NQO1	U/g	53.15±5.91	57.69±13.15	60.45±2.27*
	CAT	mU/g	5.83±1.04	5.11±1.09	4.10±0.70*
24 h	TBARS	mmol/g	0.44±0.05	0.50±0.11	0.33±0.04$^{\#}$
	GSH	mmol/g	18.37±3.27	21.21±2.77	18.70±1.61
	SOD	U/g	84.72±7.72	92.78±12.44	60.18±18.33*
	GPX	mU/g	153.25±44.17	139.00±8.02	114.54±26.97
	GST	U/g	0.14±0.04	0.10±0.01*	0.09±0.01*
	GSR	U/g	18.36±9.39	19.27±6.84	23.98±5.94
	NQO1	U/g	56.49±9.95	69.75±7.43*	59.68±11.53
	CAT	mU/g	6.42±1.20	4.86±0.70*	3.24±0.40$^{\#}$

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; GSR, glutathione reductase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances; NQO1, nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase. The values are expressed per 1 g of protein. The values are expressed as mean±S.D. of 4 animal per group. The value (*) and (#) is significantly different from that of non-irradiated group of animals ($p<0.05$ and ($p<0.01$), respectively. Statistical significance was determined by *t*-test.

Table 8. Oxidative Stress-Related Parameters in Whole Skin Homogenates of UVA Irradiated Hairless Mice

	Parameter	Unit	Group of animals		
			Unirradiated	10 J/cm^2 UVA	20 J/cm^2 UVA
4 h	TBARS	mmol/g	0.31±0.05	0.32±0.06	0.61±0.12$^{\#}$
	GSH	mmol/g	9.74±0.53	10.10±2.08	11.35±26.27*
	SOD	U/g	100.13±14.22	108.61±11.61	107.04±26.27
	GPX	mU/g	166.47±32.64	179.25±26.59	209.62±19.95*
	GST	U/g	0.10±0.01	0.12±0.01*	0.12±0.012*
	GSR	U/g	36.25±8.81	40.76±22.46	28.27±7.43
	NQO1	U/g	61.46±9.82	78.34±7.99*	61.45±17.36
	CAT	mU/g	7.38±1.56	6.56±1.43	5.20±1.11*
24 h	TBARS	mmol/g	0.34±0.05	0.43±0.02$^{\#}$	0.44±0.04$^{\#}$
	GSH	mmol/g	17.30±3.07	17.12±1.56	15.15±5.35
	SOD	U/g	109.73±39.18	114.15±17.38	173.77±41.33*
	GPX	mU/g	170.66±38.09	211.86±46.37	204.17±69.73
	GST	U/g	0.15±0.02	0.16±0.01	0.15±0.02
	GSR	U/g	37.83±10.47	35.47±15.48	32.08±3.93
	NQO1	U/g	68.31±19.58	64.73±18.72	68.56±7.98
	CAT	mU/g	7.16±1.26	7.70±0.88	6.49±1.37

SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione transferase; GSR, glutathione reductase; CAT, catalase; GSH, glutathione; TBARS, thiobarbituric acid reactive substances; NQO1, nicotinamide adenine dinucleotide (phosphate) quinone oxidoreductase. The values are expressed per 1 g of protein. The values are expressed as mean±S.D. of 4 animals per group. The value (*) and (#) is significantly different from that of non-irradiated group of animals ($p<0.05$) and ($p<0.01$), respectively. Statistical significance was determined by *t*-test.

points. The results are summarized in Table 7.

In UVA treated mice, significant changes were observed mostly in the group exposed to the higher dose (Table 8). 20 J/cm^2 of UVA caused a significant ($p<0.01$) increase in TBARS amount after 4 and 24 h (187.5 and 129.4%), in GST activity after 4 h and in SOD activity after 24 h. CAT activity was reduced after 4 h (70.0% of control) and remained non-significantly decreased at 24 h after exposure. In mice treated with the dose of 10 J/cm^2 only GST activity and TBARS amount were increased after 4 and 24 h, respectively.

Effects of UV on Plasma and Skin Protein Oxidation Oxidation of amino acid residues (*e.g.* lysine, proline, arginine) results in the formation of carbonyl derivatives that may be detected by modified Western blot analysis. In skin homogenates of UVB irradiated mice, we found an obvious increase in carbonylated proteins especially after 24 h (Fig. 1A). In skin samples from UVA exposed animals (Fig. 1B) the increase was evident after 4 h (both doses) and 24 h (20 J/cm^2). Analysis of plasma samples revealed an accumulation of oxidatively modified proteins in both UVA and UVB irradiated mice particularly at higher doses after 4 and 24 h (Fig. 2).

Effects of UV on Expression of Antioxidant Enzymes and Oxidative Stress-Related Proteins in Skin Expression of several antioxidant enzymes and oxidative stress-related proteins was evaluated in whole skin homogenates (Fig. 3). Expression of CAT was significantly reduced in animals exposed to 800 mJ/cm^2 of UVB at both time intervals. Following UVA treatment, an obvious decrease in CAT amount was found in both irradiated groups after 4 h but the effect disappeared within 24 h. The protein amount of SOD1 (Cu-ZnSOD) was not or only moderately affected after UVB and UVA exposure. On the other hand, expression of SOD2 (MnSOD) was obviously increased by a lower dose at both

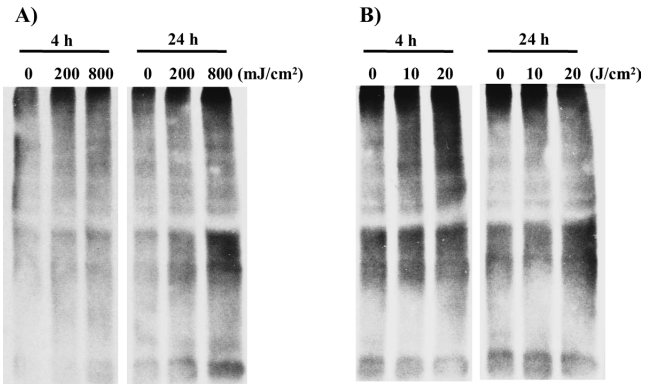

Fig. 1. Analysis of Carbonylated Proteins in Hairless Mice Skin after UVA and UVB Radiation

Protein samples (20 μg) from (A) UVB treated mice skin or (B) UVA treated mice skin were incubated with DNPH, separated by SDS-PAGE electrophoresis, blotted onto a nitrocellulose membrane and incubated with polyclonal rabbit anti-DNPH antibody.

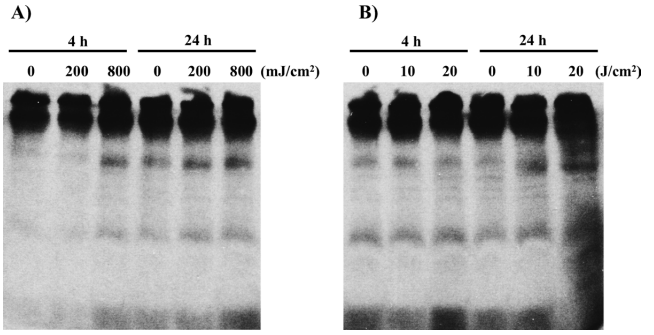

Fig. 2. Analysis of Carbonylated Proteins in Plasma of Hairless Mice Skin UVA and UVB Radiation

Protein samples (20 μg) from (A) plasma of UVB treated mice or (B) plasma of UVA treated mice were incubated with DNPH, separated by SDS-PAGE electrophoresis, blotted onto a nitrocellulose membrane and incubated with polyclonal rabbit anti-DNPH antibody.

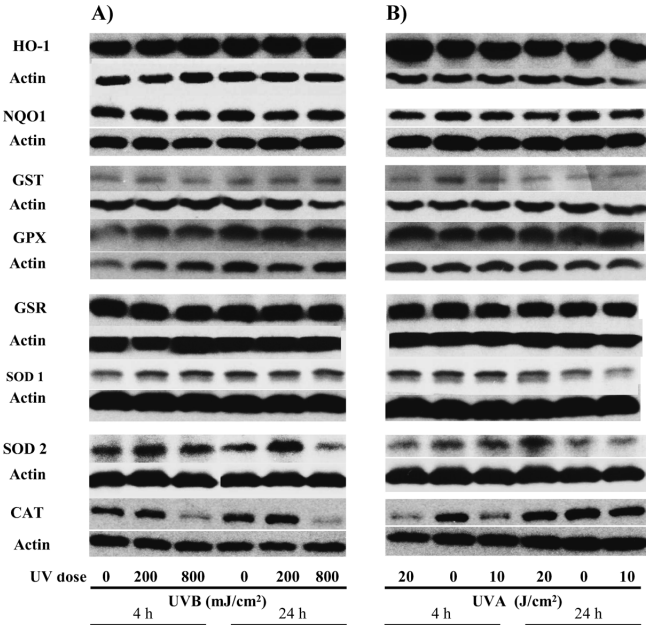

Fig. 3. Effects of UVA and UVB Irradiation on Expression of Antioxidant Enzymes and Oxidative Stress-Related Proteins in Hairless Mice Skin

Immunoblot analysis of selected proteins in skin homogenates of hairless exposed to a single dose of UVB (A) and UVA (B) as described in Materials and Methods.

time intervals and decreased by a higher UVB dose after 24 h. The effect of UVA on SOD2 appeared following exposure to a higher dose after 24 h. These findings correlate with observed changes in CAT and SOD activity in skin homogenates. Expression of GPX, GSR and GST was not markedly affected following UVA or UVB exposure. Protein level of HO-1 was enhanced in mice exposed to higher UVB (4, 24 h) and UVA dose (4 h). The effect was more pronounced in UVA treated animals. Skin expression of NQO1 was increased following exposure to the lower UVB dose in both time-intervals. NQO1 activity was increased in the group exposed to 800 mJ/cm^2 of UVB after 4 h and in the group irradiated with 200 mJ/cm^2 of UVB after 24 h. UVA treatment caused NQO1 protein decrease at both time points.

DISCUSSION

A number of publications have described the (photo)toxic effects of UVA and UVB light on skin cells *in vitro*. Several studies on different animal species have involved long-term effects of UVB radiation. However there are only a limited number of reports on the acute effects of UV light and a generally dearth on UVA toxicity *in vivo*. Further, only minimal information exists on changes in "non-skin" tissues following UVA and UVB radiation except for some that evaluated photoprotection. For these reasons we studied the effects of a single UVB and UVA exposure on oxidative stress-related parameters in the skin, blood and liver of hairless mice over a relatively short period (4, 24 h) after irradiation. To approximate natural sunlight, we used a solar simulator that produces radiation corresponding almost completely to natural solar light. The higher chosen dose of UVA (20 J/cm^2) and UVB (800 mJ/cm^2) was comparable with exposure to natural May sunlight at our latitude (49°N) for approximately 2 and 1.5 h, respectively.

UV photons, particularly UVA, penetrate deep into the dermis and can affect blood and lymphatic vessels. However, *via* excessive RONS generation in skin cells, both UVA and UVB light may influence the vessels as well. For all that effect of UV radiation to vascular system has been marginalized yet. Our results show that a single exposure to UVA and UVB radiation reduced the number of PLT in irradiated hairless mice. We were unable to find any literature for blood count reflecting acute effects of UVA/UVB light *in vivo* to confirm our results. The only reference described a UVB-induced decrease in PLT count in chronically irradiated sheep.[23] We further found some data on the effect of ionizing X-rays on blood cell parameters but the results of these studies are contrary; some authors observed a decrease[24,25] in PLT count and others an increase.[26,27] *In vitro* treatment of human PLT concentrates (used for transfusion) with psoralens plus UVA light[28] or UVB[29] alone has been tested to decontaminate the concentrate. No changes in PLT count were observed in the concentrate even after treatment with a very high UVB dose of 10000 mJ/cm^2.[29] The decrease in PLT number that we observed seems to be related to a process occurring after UV exposure *in vivo*. Several publications have suggested that PLT activity (*e.g.* PLT activation, adhesion to vascular endothelium, or aggregation) is modulated by RONS.[30] Both UVA and UVB light trigger generation of RONS that result in RONS excess cells together with antioxidant depletion. In this way UV radiation may alter the cells of the vessel walls (endothelial cells, vascular smooth muscle cells, and fibroblast) and then PLT function. The other affected parameter of blood count was the number of leucocytes that was significantly increased following UVA and UVB exposure. Available data involving UVB radiation *in vivo*, however, have yielded contradictory results. Lymphocyte proliferative responses increased,[31] decreased[32] or were unchanged[33] from those of un-exposed subjects. The reason for these conflicting results may be linked to the type of radiation source and/or other irradiation conditions, post-irradiation period and sample collection. Acute effects single of UVA exposure on lymphocyte number has probably not been described yet.

The number of erythrocytes was not affected in short-time period (4, 24 h) following UVA and UVB exposure (Tables 1, 2); however, some oxidative stress-related biomarkers were modified. We found (Table 3) an increase in TBARS amount and GSH level (200 mJ/cm^2, 24 h) and decrease in SOD activity following a single UVB treatment. In agreement with our data, Saral *et al.* found a significant rise in GSH level and SOD activity in the erythrocytes of guinea pigs at 24 h after treatment with a single UVB dose of 900 mJ/cm^2. However TBARS level was first moderately reduced (24 h) and then increased (48 h).[34] Mulero *et al.* found no changes in erythrocyte GSH amount in irradiated hairless rats. However GSH levels in plasma were significantly increased after application of markedly higher UV dose of 7000 mJ/cm^2 (ratio UVB/UVA was 0.9). They also found a significant decrease in erythrocyte CAT activity.[35] Another study found a rise in activities of GST, GPX and CAT in hairless rat erythrocytes after acute UVB irradiation but the doses used (1540—2410 mJ/cm^2) were quite high.[36] Only one paper showed the effects of UVA light on erythrocyte antioxidant parameters *in vivo*, but the changes related to repeated UVA exposure. The authors found a significant decrease in CAT activity and GSH level 48 h after the third exposure (3×18 J/cm^2). SOD activity was nearly unchanged.[37] Our results are quite different. We observed GSH depletion at a higher UVA dose after 4 h. However the level increased within 24 h. The dose of 20 J/cm^2 UVA also significantly reduced CAT activity after 24 h.

The stress of solar/UV light on the whole organism has not been target of phototoxic studies. There are only a small number of reports on effects of solar UV light on the internal organs. Skin cells exposed to UV produce several low-molecular weight signalling molecules and stress proteins that may influence non-skin tissues. In our study we focused on liver, an organ essential for a number of functions including harmful compounds detoxication. We observed a significant decrease in SOD activity and GSH level following UVB exposure and a reduction in GSH level and GSR and CAT activities after UVA exposure. Hasegawa *et al.* described a decrease in GPX activity and increase in lipid peroxidation product level but they found no alteration in SOD activity in the liver of IRC mice subjected to acute UVB irradiation (1400 mJ/cm^2) after 24 h.[38] Repeated UVA treatment (3×18 J/cm^2) of Spraque-Dawley rats resulted in a significant decrease in liver CAT activity, as in our experiment. These authors also describe a reduction in SOD activity but no effect on lipid peroxidation products or GSH level.[39] Overall our

results showed that liver was less affected by acute UVA or UVB exposure than in erythrocytes or skin. However, when one takes into account that the liver is not directly exposed to UV rays the observed alterations may be a serious problem especially after intensive or irregular exposures to high UV doses and should not be trivialized.

A number of reports have dealt with UV effects on ROS-related biomarkers in animal skin. However, due to the use of different UV light sources and various experimental designs the results are conflicting and difficult to generalise from or compare. For example, the effect of UV on SOD activity is unclear in earlier studies. Reports showed an increase[36] or no change[10,40] in SOD activity following UVB and a drop in the activity following UVA[41] or UVA+UVB[6,42] treatment. Under our conditions the activity first increased (4 h) but decreased following a higher UVB dose (800 mJ/cm^2) within 24 h. In contrast, UVA (20 J/cm^2) light induced a rise in SOD activity after 24 h. These changes in SOD activity are in an agreement with modification of Mn-SOD expression that we found by Western blot analysis. Shindo *et al.*[6,42] and Lopez-Torres *et al.*[43] further suggest that the reduction in SOD activity is linked to its drop in dermis rather than in epidermis. The activities of other important enzymes participating in RONS elimination, GPX and GSR were not significantly affected by a single UVA or UVB exposure except for GPX increase in mice irradiated with the higher UVA dose after 4 h. These findings only partially agree with published results. Ahn *et al.* found no changes in either GPX or GSR immediately after UVB (280—290 nm) treatment (4×100 mJ/cm^2).[40] Fuchs *et al.* described no change in GPX but decrease in GSR (to 82% of control) after 300 mJ/cm^2 of UVB.[10] Shindo *et al.* found a moderate reduction in GPX and GSR activities following solar light exposure.[6,42] Repeated UVA treatment caused GPX depletion in rat skin.[41] In hairless rats, activities of both GPX and GSR were increased following UVB exposure (1540—2410 mJ/cm^2).[36] Previous *in vitro*[44] and *in vivo*[41] studies showed that UVA radiation caused massive CAT activity depletion in skin cells. Similarly, several studies on hairless mice revealed CAT activity reduction following UVB exposure.[6,10,11] Our analysis confirmed a significant decrease in CAT activity in whole skin following UVB and UVA (20 J/cm^2; 24 h) exposure. Although human skin differs from animal models (thickness of individual layers), Rhie *et al.* showed a similar CAT activity response in human skin *in vivo*. They also found reduction in protein and mRNA level after a single UVB treatment (2 minimal erythemal dose (MED).[45] The essential non-enzymatic antioxidant GSH was depleted after 4 h but increased to control level within 24 h in UVB irradiated mice. Curiously a higher UVA dose (20 J/cm^2) induced a rise in GSH amount after 4 h that normalized within 24 h. Earlier studies mostly showed GSH decrease immediately[6,10,42] or after 24 h[46,47] following UVB or solar light exposure. On the other hand Mulero *et al.* found that lower doses (1.54 and 1.93 J/cm^2 UVB+UVA) reduced GSH level but 2.41 J/cm^2 has no effect at 72 h after exposure.[36] Repeated UVA irradiation resulted in non-significant GSH reduction in rat skin.[41] The temporary GSH depletion may be linked to its participation in GST reaction resulting in thioesters formation and their subsequent excretion.[48] Zhu and Bowden also found that UVB light induced a slight decrease in activity of γ-glutamate cysteine ligase, a rate-limiting enzyme in GSH synthesis.[49]

RONS overflow on UV exposure results in antioxidant depletion and increased susceptibility oxidative damage to biomolecules. As expected, UVA light induced an increase in lipid peroxidation measured as TBARS level. In UVB treated mice, the TBARS amount was unchanged after 4 h but decreased significantly at higher UVB dose (800 mJ/cm^2) after 24 h. Mulero *et al.* also found a decrease in lipid peroxidation in hairless rats 72 h after exposure to UVB/UVA (with ratio of 0.9; 1540 and 1930 mJ/cm^2).[36] On the other hand *Saral et al.* observed increase in lipid peroxidation 24 and 48 h after a single UVB exposure (900 mJ/cm^2) in guinea pig[34] and Erden Inal *et al.* after repeated UVA irradiation in rats.[41] During interaction of RONS and lipid peroxidation products with proteins, carbonyl derivatives are formed. Modifications affect protein functions and may leads to the proteins accumulation and further disruption of normal cell activities.[50] In agreement with previous results,[40,46] we found an increase in protein carbonyls in hairless mice skin following both UVB exposure and UVA treatment. Moreover we also detected a significant increase in oxidized protein level in plasma of UVA and UVB irradiated mice. The results showed that carbonylated proteins are more stable and thus represent a more sensitive marker of oxidative damage than TBARS.

Phase 2 enzymes such as GST, NQO1, HO-1 play a critical role in cell protection against xenobiotics and oxidative stress responses. In relation to sunlight exposure, they participate in elimination of toxic electrophilic and oxidative compounds including oxidized lipid, DNA and catechol products that are generated *via* interaction of RONS.[51] A single UVA exposure resulted in a significant increase in GST activity after 4 h that was normalized within 24 h. On the other hand, in UVB exposed mice a significant decrease in GST activity was found at both time intervals. No effect was found on GST expression. We found no reports on UVA effects on GST. After UVB irradiation (30, 50 mJ/cm^2) Seo *et al.* found a significant decrease in GST activities in normal human keratinocytes *in vitro* and in hairless mice within 24 h. They found no changes in protein expression or mRNA amount[52] and this is consistent with our results. A single UVB exposure enhanced activity of NQO1 in skin after 4 h (800 mJ/cm^2) and 24 h (200 mJ/cm^2). No effect was observed in UVA treated mice. In contrast, a recent study showed a dose dependent increase in NQO1 expression in human keratinocytes cell culture 24 h following UVA (320—400 nm) and solar light (300—400 nm). The effect was moderately greater after exposure to UVA than solar light.[53] A previous study showed an induction of HO-1 mRNA expression in hairless mice at several time points following UVA treatment (38.7 J/cm^2). No mRNA was observed in animals exposed to UVB (0.550 mJ/cm^2). Activity of HO-1 in skin of UVA irradiated mice was increased (after 72 h) as well.[54] Under our experimental conditions we found analogous results for the UVA waveband that increased HO-1 expression 4 h after exposure. Moreover, the higher UVB dose also increased HO-1 levels at both time points. Marrot *et al.* also showed that the effect of UVA was obviously higher than of solar light and induction of HO-1 gene was significantly higher in human skin melanocytes (13-fold increase) than in keratinocytes (4-fold increase) after UVA irradiation (44.5 J/cm^2; 5 h).[53] It has

been shown that in the regulation of phase 2 genes expression, a specific transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) is involved. Under oxidative stress Nrf2-dependent genes are strongly stimulated.[53] RONS generation is main result of UVA exposure and thus reactive species are more stimulated by UVA than UVB wavelengths. These may explain the difference between UVA and UVB modulation in phase 2 gene activity/expression. Moreover a recent study proposes that p53 pathway that is activated by UVB *via* pyrimidine dimers and photoproducts formation could compete with Nrf2 promoters, suppressing transcription of antioxidant response genes.[55]

We can conclude that a single exposure to both UVA and UVB radiation results in significant changes in antioxidant parameters of skin tissue. Moreover, UVA and UVB also induce alterations to blood cells count, oxidation of plasma proteins, and changes in liver and erythrocyte antioxidant characteristics. The experiments were performed on SKH-1 mouse model and this has some limitations in comparison to human skin; it mainly differs from human skin in epidermis thickness and pigmentation that may relevantly influence UV skin penetration. For this reason the results may not be completely comparable to humans. However, sunlight exposure may negatively influence not only unprotected skin but also internal organs especially during intensive and/or irregular exposure in days with high UV intensity.

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REFERENCES

1) Zastrow L., Groth N., Klein F., Kockott D., Lademann J., Renneberg R., Ferrero L., *Skin Pharmacol. Physiol.*, **22**, 31—44 (2009).
2) Verschooten L. S., Claerhout S., Van Laethem A., Agostinis P., Garmyn M., *Photochem. Photobiol.*, **82**, 1016—1023 (2006).
3) Sindhu R. K., Wagner F. E., Kikkawa Y., *Adv. Exp. Med. Biol.*, **527**, 297—306 (2003).
4) Svobodová A., Walterová D., Vostálová J., *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.*, **150**, 25—38 (2006).
5) Wondrak G. T., *Curr. Opin. Investig. Drugs*, **8**, 390—400 (2007).
6) Shindo Y., Witt E., Han D., Parker L., *J. Invest. Dermatol.*, **102**, 470—475 (1994).
7) Shindo Y., Witt E., Han D., Epstein W., Packer L., *J. Invest. Dermatol.*, **102**, 122—124 (1994).
8) Shindo Y., Witt E., Hand D., Tzeng B., Aziz T., Nguyen L., Packer L., *Photodermatol. Photoimmunol. Photomed.*, **10**, 183—191 (1994).
9) Aricioglu A., Bozkurt M., Balabanli B., Kilinç M., Nazaroglu N. K., Türközkan N., *Biol. Trace Elem. Res.*, **80**, 175—179 (2001).
10) Fuchs J., Huflejt M. E., Rothfuss L. M., Wilson D. S., Carcamo G., Packer L., *Photochem. Photobiol.*, **50**, 739—744 (1989).
11) Pence B. C., Naylor M. F., *J. Invest. Dermatol.*, **95**, 213—216 (1990).
12) Sasak H., Akamatsu H., Horio T., *Photochem. Photobiol.*, **65**, 707—713 (1997).
13) Buege J. A., Aust S. D., *Methods Enzymol.*, **52**, 302—310 (1978).
14) Sedlak J., Lindsay R. H., *Anal. Biochem.*, **25**, 192—205 (1968).
15) Ewing J. F., Janero D. R., *Anal. Biochem.*, **232**, 243—248 (1995).
16) Tappel A. L., *Methods Enzymol.*, **52**, 506—513 (1978).
17) Habig W. H., Pabst M. J., Jakoby W. B., *J. Biol. Chem.*, **249**, 7130—7139 (1974).
18) Carlberg I., Mannervik B., *J. Biol. Chem.*, **250**, 5475—5480 (1985).
19) Beers R. F., Sizer I. W., *J. Biol. Chem.*, **195**, 133—140 (1952).
20) Lin Y. L., Ho I. C., Su P. F., Lee T. C., *Toxicol. Appl. Pharmacol.*, **214**, 309—317 (2006).
21) Bradford M. M., *Anal. Biochem.*, **72**, 248—254 (1976).
22) Chew S., DeLeo V. A., Harber L. C., *J. Invest. Dermatol.*, **89**, 410—414 (1987).
23) Ivanov V. L., Ipatova A. G., Demichev V. V., Efimenko N. V., Kozlov V. A., Sukhanova N. N., Shevchenko T. S., Shevchenko A. S., *Kosm. Biol. Aviakosm. Med.*, **25**, 32—35 (1991).
24) Hsu H. Y., Lin C. C., *J. Ethnopharmacol.*, **55**, 43—48 (1996).
25) Hsu H. Y., Yang J. J., Lian S. L., Ho Y. H., Lin C. C., *J. Ethnopharmacol.*, **54**, 69—75 (1996).
26) Pecaut M. J., Gridley D. S., Smith A. L., Nelson G. A., *Immunol. Lett.*, **80**, 67—73 (2002).
27) Gridley D. S., Rizvi A., Luo-Owen X., Makinde A. Y., Coutrakon G. B., Koss P., Slater J. M., Pecaut M. J., *In Vivo*, **22**, 159—169 (2008).
28) Grass J. A., Hei D. J., Metchette K., Cimino G. D., Wiesehahn G. P., Corash L., Lin L., *Blood*, **91**, 2180—2188 (1998).
29) Snyder E. L., Beardsley D. S., Smith B. R., Horne W., Johnson R., Wooten T., Napychank P. A., Male P., Buchholz D. H., *Transfusion*, **31**, 491—496 (1991).
30) Krötz F., Sohn H. Y., Pohl U., *Arterioscler. Thromb. Vasc. Biol.*, **24**, 1988—1996 (2004).
31) Goettsch W., Garssen J., Hurks M. H., De Gruijl F. R., Van Loveren H., *Photochem. Photobiol.*, **60**, 373—379 (1994).
32) Morison W. L., Parrish J. A., Bloch K. J., Krugler J. I., *Br. J. Dermatol.*, **101**, 513—519 (1979).
33) Spellman C. W., Woodward J. G., Daynes R. A., *Transplantation*, **24**, 112—119 (1977).
34) Saral Y., Uyar B., Ayar A., Naziroglu M., *Physiol. Res.*, **51**, 285—290 (2002).
35) Mulero M., Rodríguez-Yanes E., Nogués M. R., Giralt M., Romeu M., González S., Mallol J., *Exp. Dermatol.*, **17**, 653—658 (2008).
36) Mulero M., Romeu M., Giralt M., Folch J., Nogués M. R., Fortuño A., Sureda F. X., Linares V., Cabré M., Paternáin J. L., Mallol J., *J. Toxicol. Environ. Health A*, **69**, 1371—1385 (2006).
37) Kahraman A., Inal M. E., *J. Appl. Toxicol.*, **22**, 303—309 (2002).
38) Hasegawa T., Kaneko F., Niwa Y., *Life Sci.*, **50**, 1893—1903 (1992).
39) Erden Inal M., Kahraman A., *Toxicology*, **154**, 21—29 (2000).
40) Ahn S. M., Hwang J. S., Lee S. H., *Biol. Pharm. Bull.*, **30**, 692—697 (2007).
41) Erden Inal M., Kahraman A., Köken T., *Clin. Exp. Dermatol.*, **26**, 536—539 (2001).
42) Shindo Y., Witt E., Packer L., *J. Invest. Dermatol.*, **100**, 260—265 (1993).
43) Lopez-Torres M., Thiele J. J., Shindo Y., Han D., Packer L., *Br. J. Dermatol.*, **138**, 207—215 (1998).
44) Shindo Y., Hashimoto T., *J. Dermatol. Sci.*, **14**, 225—232 (1997).
45) Rhie G. E., Seo J. Y., Chung J. H., *Mol. Cells*, **30**, 399—404 (2001).
46) Vayalil P. K., Elmets C. A., Katiyar S. K., *Carcinogenesis*, **24**, 927—936 (2003).
47) Sharma S. D., Meeran S. M., Katiyar S. K., *Mol. Cancer Ther.*, **6**, 995—1005 (2007).
48) Masella R., Di Benedetto R., Vari R., Filesi C., Giovannini C., *J. Nutr. Biochem.*, **16**, 577—586 (2005).
49) Zhu M., Bowden G. T., *Photochem. Photobiol.*, **80**, 191—196 (2004).
50) Levine R. L., *Free Radic. Biol. Med.*, **32**, 790—796 (2002).
51) Lee J. S., Surh Y. J., *Cancer Lett.*, **224**, 171—184 (2005).
52) Seo K. I., Cho K. H., Park K. C., Youn J. I., Eun H. C., Kim K. T., Park S. C., *J. Dermatol. Sci.*, **13**, 153—160 (1996).
53) Marrot L., Jones C., Perez P., Meunier J. R., *Pigment Cell Melanoma Res.*, **21**, 79—88 (2008).
54) Allanson M., Reeve V. E., *J. Invest. Dermatol.*, **122**, 1030—1036 (2004).
55) Faraonio R., Vergara P., Di Marzo D., Pierantoni M.G., Napolitano M., Russo T., Cimino F., *J. Biol. Chem.*, **281**, 39776—39784 (2006).