

## Differential modulation of inflammatory markers in plasma and skin after single exposures to UVA or UVB radiation *in vivo*

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**Background.** Solar light generates inflammatory responses in exposed skin. These effects are generally attributed to UVB light. However, skin is exposed to a huge quantum of UVA photons as UVA is a predominant part of sunlight and the radiation used in tanning beds. We examined the effects of a single exposure to UVA and UVB wavebands on cytokine levels in skin and plasma, myeloperoxidase (MPO) activity, expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) in skin.

**Methods.** Hairless mice were irradiated with either UVA (10 or 20 J/cm<sup>2</sup>) or UVB (200 or 800 mJ/cm<sup>2</sup>). The effects were assessed after 4/24 h. Plasma cytokine levels were evaluated using a Bio-Plex cytokine assay. Cytokine, iNOS and COX-2 levels in skin were determined by Western blot. Skin MPO activity was monitored spectrophotometrically.

**Results.** UVB induced up-regulation of interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6) and decrease in interleukin-10 (IL-10) mainly after 4 h. In contrast, UVA caused increase in levels of tumor necrosis factor-alpha (TNF- $\alpha$ ) and IL-6 after 4 h and up-regulated IL-10 and interleukin-12 (IL-12) after 24 h. The increase in MPO activity from infiltrated leucocytes was observed only in UVB irradiated animals. iNOS was up-regulated 4 h after UVA and UVB treatment. No significant effect on COX-2 expression was detected.

**Conclusions.** UVA and UVB light affected several inflammatory markers. For individual waveband, changes in plasma parameters did not correlate with those in skin. Thus evaluation of plasma samples cannot simply be replaced by determination in skin specimens and vice versa.

**Key words:** solar radiation, hairless mice, cytokine, myeloperoxidase, nitric oxide synthase, cyclooxygenase-2

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### INTRODUCTION

Human skin is normally exposed exclusively to wavelengths > 294 nm, as ozone in the stratosphere completely absorbs shorter wavelengths<sup>1</sup>. UVB wavelengths (295-315 nm) represent a minor (5-10%) but biologically very active part of incoming UV light. UVB is predominantly absorbed within epidermal layers and only a small fraction (~10%) reaches the dermis. In contrast, UVA (315-400 nm) is very abundant in incoming UV radiation (90-95%). It penetrates deep into the dermis (~50-70%) and even hypodermis (~10%) (ref.<sup>2</sup>).

Biological effects of UV radiation on skin include the inflammatory response. Characteristic findings after overexposure to sunlight are erythema, swelling and pain. Histopathologic changes include keratinocyte damage, Langerhans' cell depletion, dermal edema, mast cell degranulation, inflammatory cell infiltration and alteration to vascular functions<sup>3</sup>. Biochemical changes include increase in myeloperoxidase (MPO) activity and cyclooxygenase-2 (COX-2) expression, generation of arachidonic acid products and cytokines<sup>4,5</sup>. All these substances very likely assist in mediating the inflammatory reaction. Cytokines affect other outlying cells, mediating cellular

growth and differentiation as well as inflammatory and immune reactions. Thus, cytokines contribute to maintaining/disruption of cellular and intercellular homeostasis according to the actual concentration<sup>6</sup>.

Over decades, investigation has focused on specific and non-specific immune responses to UVB light, although sunlight as well as tanning lamps used in tanning beds are primarily UVA sources<sup>3</sup>. UVB exposure has been shown to augment production of various cytokines including tumor necrosis factor-alpha (TNF- $\alpha$ ), IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10 in human and/or mice skin<sup>7</sup>. Recent studies have provided evidence that UVA light can also affect the immune system. Similar cytokines have been identified as secreted on UVA exposure in keratinocytes *in vitro*<sup>1,8</sup>. Studies further suggest that UVA influences the immune system probably in a different way than UVB and that the consequences of UVA and UVB may interact with each other<sup>9</sup>. Apropos UVA and UVB penetration of the skin, UVB probably has more effect on the epidermis and UVA on the dermis. However, immunoregulatory effects of UVA have not been fully investigated and explained. The aim of this study was to examine and compare short-time (4 and 24 h) effects of single UVA or UVB exposure on selected inflammatory markers in hairless mice.

## MATERIALS AND METHODS

**Material.** Bio-Plex Pro™ Mouse Cytokines Group I kit was purchased from BioRad (USA). Rabbit polyclonal primary antibody anti-TNF- $\alpha$ , -IL-1 $\alpha$ , -IL-1 $\beta$ , -IL-6, -IL-10(p40), -IL-12, -COX-2, -iNOS, goat polyclonal primary antibody anti-actin and goat anti-rabbit and rabbit anti-goat horseradish peroxidase (HRP) conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (USA). 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. They 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 (295-3000 nm) corresponding to natural sunlight, and environmentally relevant UVA (10 or 20 J/cm<sup>2</sup>) and UVB (200 or 800 mJ/cm<sup>2</sup>) doses were employed. The simulator was equipped with a H1 or H2 filter transmitting wavelengths of 315-380 nm or 295-315 nm, respectively. The mice were randomly divided into 6 groups of 8 animals and dorsal skin was exposed to a single dose of UVB or UVA.

**Sample collection.** Blood samples were collected into Na<sub>2</sub>EDTA under ether anaesthesia at 4 or 24 h after exposure. The blood was centrifuged (2500 rpm, 10 min, 4 °C) to obtain the plasma, which was stored at -80 °C. 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.

**Plasma cytokine determination.** Levels of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, IL-12 (p40), IL-12 (p70), IFN- $\gamma$ , TNF- $\alpha$  in plasma were determined using Bio-Plex Mouse Cytokines Group I ELISA kit and Bio-Plex 200 System (BioRad, USA) according to the manufacturer's manual at the Laboratory of Diagnostics of Autoimmune Diseases, Academy of Sciences (Prague, Czech Republic).

**Skin cytokines determination.** Skin samples were homogenized in ice-cold cell lysis buffer (50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 mM EGTA; 20 mM NaF; 100 mM sodium orthovanadate; 0.5% NP-40; 1% Triton X-100; 1 mM phenylmethanesulfonyl fluoride; and protease inhibitor cocktail tablet). The homogenate was incubated 30 min on ice and centrifuged (14 000 rpm, 20 min, 4 °C). The supernatants

were collected for western blot analysis and stored at -80 °C. Samples were pooled and the protein content was determined by Bradford assay. Proteins were separated by 8% or 12.5% SDS-polyacrylamide gel electrophoresis, and blotted onto polyvinylidene difluoride membrane. Residual binding sites on the membrane were blocked using blocking buffer (100 mM Tris buffered saline (pH 7.5); Tween 20 (0.05 %; v/v); non-fat dry milk (5%; w/v)) for 1 h at room temperature. The membrane was then incubated with a primary antibody (rabbit anti-TNF- $\alpha$ , -IL-1 $\alpha$ , -IL-1 $\beta$ , -IL-6, -IL-10(p40), -IL-12, -iNOS, -COX-2 or goat anti-actin; in blocking buffer) overnight at 4 °C and then with a secondary HRP conjugated antibody (goat anti-rabbit antibody/rabbit anti-goat antibody; in blocking buffer) for 2 h at room temperature. The immunocomplex of individual proteins was detected by chemiluminescence using Western Blotting Luminol Reagent and autoradiography with XAR-5 film. The quantification of protein was performed by dosimetric analysis using ElfoMan 2.6 software.

**MPO activity determination.** The UV-induced leukocyte migration to the skin of hairless mice was evaluated using the MPO kinetic-colorimetric assay as previously described<sup>10</sup>. Samples of the total skin of hairless mice (1:20 dilution) were homogenized in 50 mM phosphate buffer (pH 6.0) and centrifuged (14 000 rpm, 10 min, 4 °C). The supernatant was discarded and the proteinaceous pellet was solubilised in 0.5 mL of ice-cold in 50 mM phosphate buffer (pH 6.0) with 0.5% hexadecyltrimethylammonium bromide (HTAB). Solubilisation was accomplished using a probe-type sonicator (10 bursts at an instrument power setting of 50 %) followed by freeze-thaw (3 cycles). Then homogenates were centrifuged (14 000 rpm, 10 min, 4 °C) and the supernatants were collected. The MPO activity of the skin tissue extracts was determined spectrophotometrically by continually monitoring the change in absorbance at 460 nm for a period of 3 min. Briefly, 1.76 mL of reaction mixture (50 mM phosphate buffer (pH 6.0) containing 0.5 mM o-dianisidine dihydrochloride and 0.25 mM HTAB) was mixed with 40  $\mu$ L of sample. The reaction was initiated by addition of the substrate, hydrogen peroxide (3 mM; 200  $\mu$ L). The results are presented as units/g protein.

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

## RESULTS

**Effects on plasma cytokine levels.** Four hours after exposure, the lower UVB dose significantly decreased IL-10 levels only ( $P<0.001$ ). The higher UVB dose reduced secretion of IL-10 and markedly increased levels of IL-1 $\beta$  and IL-6 to 1.25-fold and 24.7-fold, respectively (Table 1). At 24 h following UVB treatment, the IL-6 level was significantly enhanced in both irradiated groups. TNF- $\alpha$  concentration remained elevated in the group exposed to 200 mJ/cm<sup>2</sup> compared to controls (Table 1).

An increase in IL-6 and TNF- $\alpha$  secretion was detect-

**Table 1.** The plasma cytokine concentrations after UVB exposure.

Data represent results from 4 mice per group. Values are given in mean±SD. The statistical significance of difference between control and UVB treated group was determined by Student *t*-test; (\*) *P*<0.001 and (#) *P*<0.05.

Hours	Parameter (pg/mL)	Group of animals		
		Unirradiated	200 mJ/cm <sup>2</sup> UVB	800 mJ/cm <sup>2</sup> UVB
4 h	IL-1α	8.1±2.4	14.2±5.9	7.7±1.2
	IL-1β	55.4±7.4	53.4±6.4	<b>69.2±6.0<sup>#</sup></b>
	IL-6	2.4±1.9	5.0±2.8	<b>59.3±28.9<sup>#</sup></b>
	TNF-α	13.0±6.4	13.6±3.7	22.1±7.8
	IL-10	21.6±4.6	<b>5.0±1.2<sup>*</sup></b>	<b>6.7±4.2<sup>#</sup></b>
	IL-12(p40)	279.3±58.6	277.1±8.5	352.8±38.2
24 h	IL-1α	10.7±5.6	9.4±3.7	9.9±3.6
	IL-1β	55.2±6.6	61.4±18.4	47.4±13.9
	IL-6	1.9±0.4	<b>10.3±6.2<sup>#</sup></b>	<b>47.5±23.3<sup>#</sup></b>
	TNF-α	14.1±1.5	<b>23.3±3.8<sup>#</sup></b>	17.5±14.5
	IL-10	15.1±9.2	22.8±7.8	12.8±6.5
	IL-12(p40)	258.7±6.4	250.5±26.8	205.2±57.2

**Table 2.** The plasma cytokine concentrations after UVA exposure.

Data represent results from 4 mice per group. Values are given in mean±SD. The statistical significance of difference between control and UVA treated group was determined by Student *t*-test; (\*) *P*<0.005 and (#) *P*<0.05.

Hours	Parameter (pg/mL)	Group of animals		
		Unirradiated	10 J/cm <sup>2</sup> UVA	20 J/cm <sup>2</sup> UVA
4 h	IL-1α	6.9±2.7	5.9±1.1	4.0±2.2
	IL-1β	51.8±6.3	62.7±12.6	60.2±15.4
	IL-6	2.9±1.4	<b>46.2±15.6<sup>*</sup></b>	<b>198.5±36.4<sup>*</sup></b>
	TNF-α	8.0±1.5	<b>23.5±2.1<sup>*</sup></b>	<b>28.1±6.6<sup>*</sup></b>
	IL-10	26.0±6.5	25.0±4.6	26.5±15.4
	IL-12(p40)	266.7±71.2	285.7±96.4	361.4±88.4
24 h	IL-1α	4.6±2.1	4.5±2.1	6.9±3.2
	IL-1β	55.5±8.4	44.3±6.7	65.4±6.5
	IL-6	1.5±0.5	<b>5.4±1.8<sup>#</sup></b>	<b>11.5±6.7<sup>#</sup></b>
	TNF-α	8.8±0.1	11.0±3.9	8.9±3.2
	IL-10	21.8±6.6	<b>42.0±4.8<sup>#</sup></b>	<b>42.2±7.4<sup>#</sup></b>
	IL-12(p40)	221.3±63.2	214.1±45.8	<b>340.9±53.8<sup>#</sup></b>

ed 4 h after UVA treatment. The higher UVA dose also non-significantly enhanced the IL-12(p40) level at this time-point. At 24 h after irradiation, IL-6 concentration decreased compared to that at 4 h but the higher level persisted significant compared to control mice. The TNF-α concentration normalized in both irradiated groups. Significant increase in IL-10 level was found in both irradiated groups. The higher dose also induced a significant enhancement of IL-12(p40) level (Table 2).

Under our experimental conditions, the level of IL-12(p70) and INF-γ in both UVA and UVB irradiated animals was under the declared assay quantification limit (1.53 pg/mL and 1.84 pg/mL, respectively).

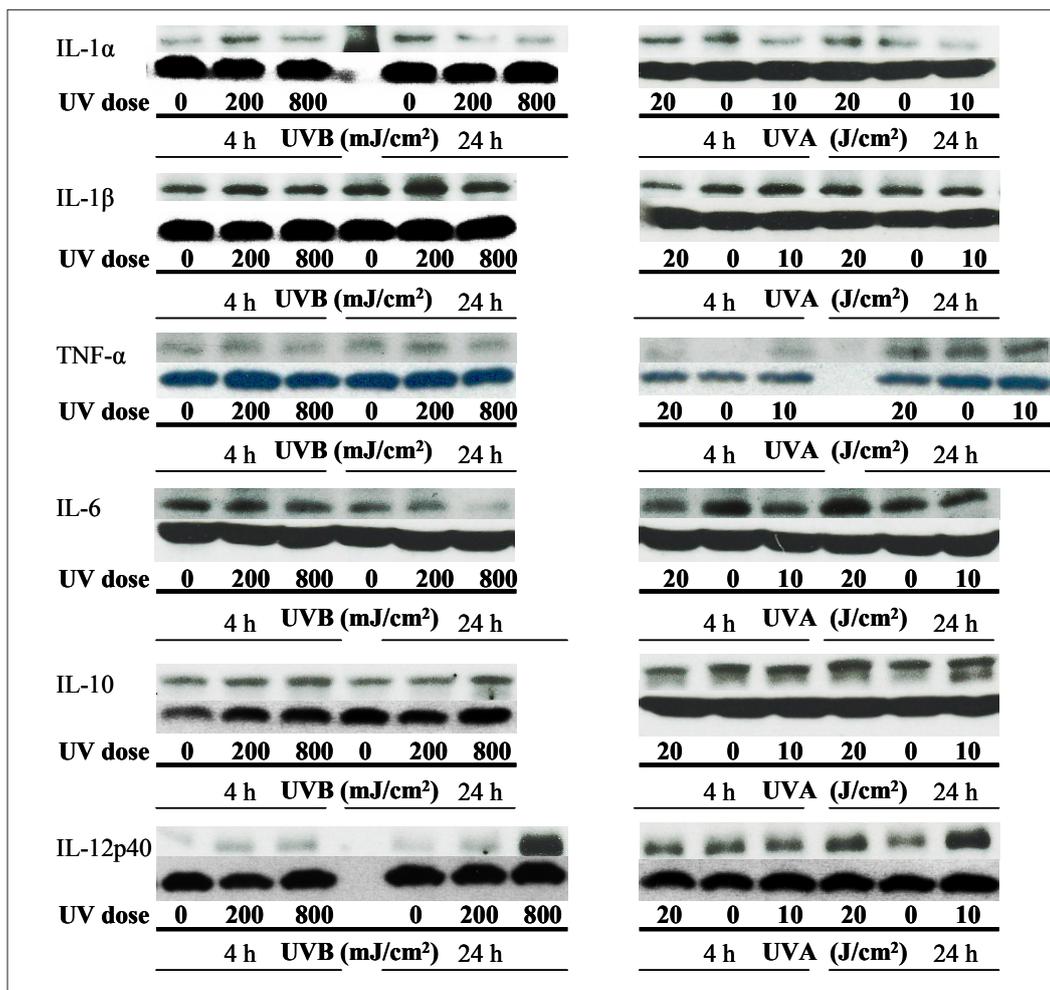
**Effects on skin cytokine expression.** Skin cytokine levels (Fig. 1, Table 3 and 4) detected by Western blot do not correlate highly with plasma levels analysed by the ELISA assay. The IL-1α level was increased in the group treated with lower UVB dose after 4 h and decreased in both groups after 24 h. IL-1α expression was reduced in mice exposed to the lower UVA dose at both time points and to 20 J/cm<sup>2</sup> after 4 h. We found an increased IL-1β level in the group exposed to 200 mJ/cm<sup>2</sup> of UVB after 4 and 24 h. The effect of UVA radiation on IL-1β level contrasted after 4 h. A dose of 10 J/cm<sup>2</sup> increased and the dose decreased levels. Normalization was found 24 h after UVA exposure. TNF-α level was nearly unaffected in skin irradiated with UVB waveband. In UVA exposed

**Table 3.** The skin cytokine concentrations after UVB exposure. Data represent a quantification of Western blot analysis results obtained from pooled skin homogenates of 4 mice per group. The quantification of protein was performed by dosimetric analysis using ElfoMan 2.6 software.

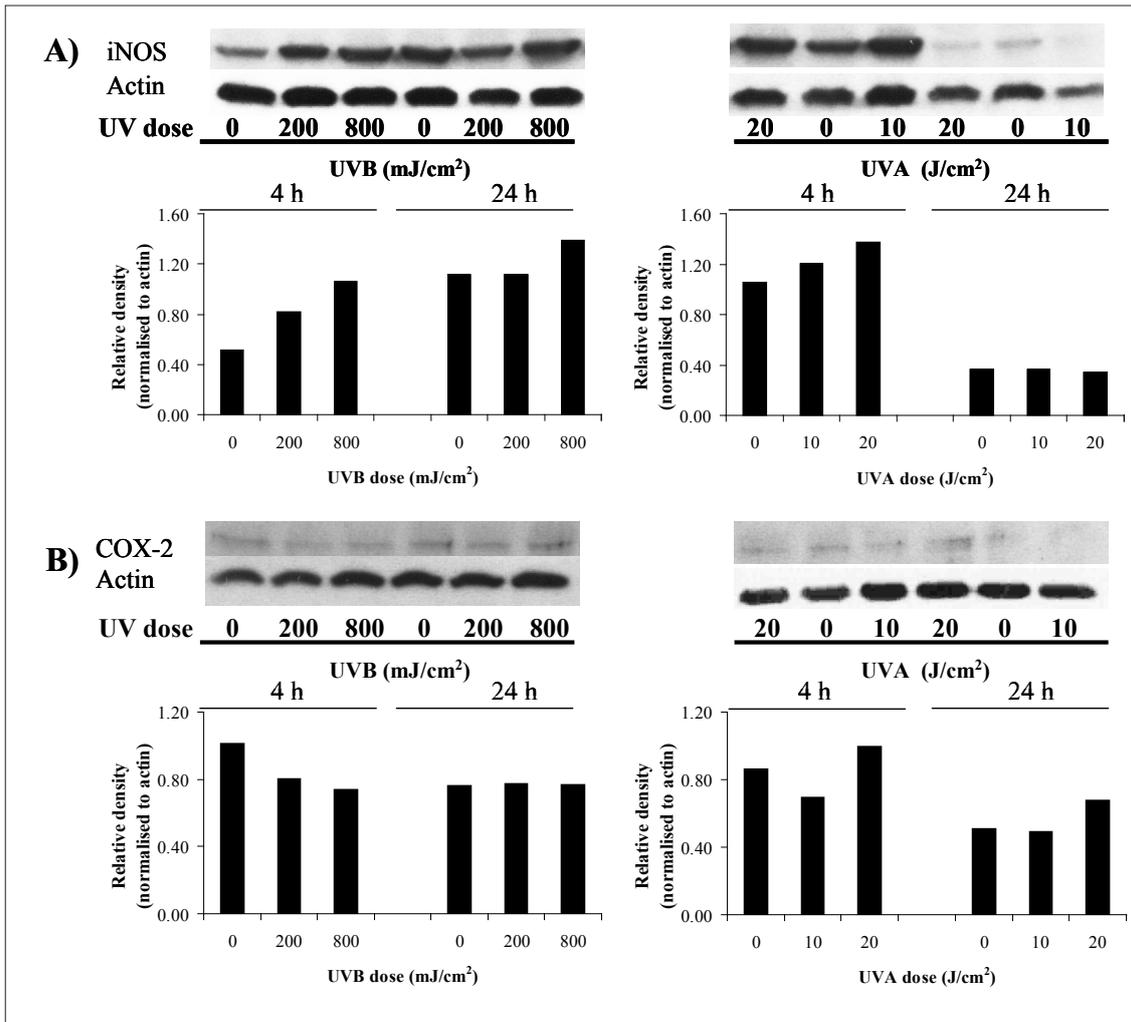
Relative density protein//actin	UVB (mJ/cm <sup>2</sup> )			
	0	200	800	
4 h	IL-1 $\alpha$	0.246	0.359	0.289
	IL-1 $\beta$	0.441	0.550	0.429
	IL-6	0.761	0.853	0.746
	TNF- $\alpha$	0.572	0.523	0.589
	IL-10	0.539	0.504	0.514
	IL-12(p40)	0.169	0.281	0.247
24 h	IL-1 $\alpha$	0.353	0.234	0.224
	IL-1 $\beta$	0.576	0.650	0.521
	IL-6	0.676	0.603	0.387
	TNF- $\alpha$	0.565	0.609	0.549
	IL-10	0.383	0.469	0.497
	IL-12(p40)	0.178	0.278	0.618

**Table 4.** The skin cytokine concentrations after UVA exposure. Data represent a quantification of Western blot analysis results obtained from pooled skin homogenates of 4 mice per group. The quantification of protein was performed by dosimetric analysis using ElfoMan 2.6 software.

Relative density protein//actin	UVA (J/cm <sup>2</sup> )			
	0	10	20	
4 h	IL-1 $\alpha$	0.487	0.343	0.289
	IL-1 $\beta$	0.613	0.657	0.429
	IL-6	0.996	0.863	0.746
	TNF- $\alpha$	0.691	0.809	0.589
	IL-10	0.566	0.582	0.514
	IL-12(p40)	0.459	0.382	0.247
24 h	IL-1 $\alpha$	0.507	0.302	0.224
	IL-1 $\beta$	0.662	0.553	0.521
	IL-6	0.835	0.934	0.387
	TNF- $\alpha$	0.810	0.866	0.549
	IL-10	0.567	0.788	0.497
	IL-12(p40)	0.377	0.586	0.618



**Fig. 1.** Effects of UVA and UVB irradiation on cytokine expression in hairless mice skin. Immunoblot analysis of selected proteins in pooled skin homogenates of hairless mice exposed to a single dose of UVA and UVB sacrificed 4 and 24 h after irradiation was performed as described in Materials and Methods. The quantification of protein is shown in Table 3 (UVB) and 4 (UVA).



**Fig. 2.** Effects of UVA and UVB irradiation on iNOS and COX-2 expression in hairless mice skin. Immunoblot analysis of the proteins in pooled skin homogenates of hairless mice exposed to a single dose of UVA and UVB sacrificed 4 and 24 h after irradiation was performed as described in Materials and Methods. The quantification of protein was performed by dosimetric analysis using ElfoMan 2.6 software.

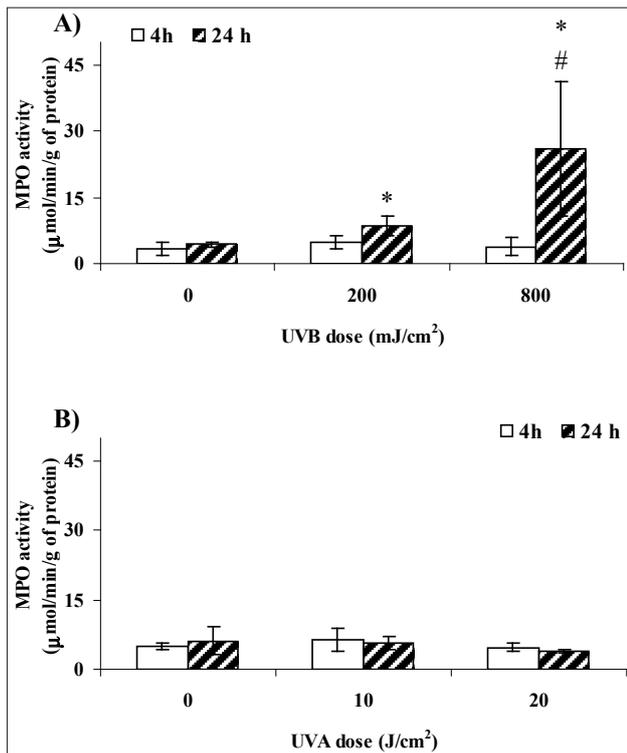
mice TNF- $\alpha$  was markedly elevated after 4 h in agreement with plasma results. 24 h following UVA irradiation the increase in TNF- $\alpha$  level was found only in the skin. IL-6 expression was not significantly affected following UVB irradiation except for a reduction caused by the higher dose after 24 h. However these results do not correlate with plasma cytokine level that was significantly augmented. UVA treatment induced a dose-dependent decrease in IL-6 after 4 h that contrasts with plasma level. However, an obvious increase was found in animals exposed to UVA after 24 h. IL-10 expression in UVB treated mice was nearly unchanged after 4 h and increased 24 h after exposure. UVA caused up-regulation of IL-10 in both irradiated groups 24 h following irradiation that corresponded to plasma levels. IL-12(p40) was augmented in both UVB treated groups at both time-points, especially in mice exposed to the higher UVB dose after 24 h. However, plasma IL-12(p40) level was moderately enhanced only in animals treated with the higher UVB dose 4 h after exposure. UVA waveband induced IL-12(p40) increase

in both irradiated groups after 4 h that only partially correlates with plasma levels (Fig. 1, Table 3 and 4).

#### Effects on iNOS and COX-2 expression in skin.

Expression of iNOS was significantly increased 4 h following UVB treatment. After 24 h the level was reduced in the group treated with the lower UVB dose. In UVA irradiated mice, iNOS was increased 4 h after exposure and normalized within 24 h (Fig. 2A). No significant effect of either UVA or UVB light was found on COX-2 expression (Fig. 2B).

**Effects on skin MPO activity.** In UVB exposed animals, no changes in skin MPO activity were observed after 4 h, but a significant dose-dependent increase was found after 24 h (Fig. 3A). UVA caused no significant alteration to MPO activity in exposed mice (Fig. 3B).



**Fig. 3.** Effects of UVA and UVB irradiation on MPO activity in hairless mice skin.

Female hairless mice were exposed to a single dose of UVB (200 and 800 mJ/cm<sup>2</sup>) (A) and UVA (10 and 20 J/cm<sup>2</sup>) (B) and sacrificed 4 and 24 h after irradiation. Measurements were performed in skin as described in Materials and Methods. The values are expressed as mean±S.D. of 4 animals per group. The value (\*) and (#) is significantly different ( $P<0.05$ ) from that of non-irradiated group of animals and from that of the group irradiated with the UVB dose of 200 mJ/cm<sup>2</sup>, respectively. Statistical significance was determined by t-test.

## DISCUSSION

Altered dermal and epidermal cytokine profiles have been implicated in the pathophysiology of various skin disorders and the sunburn reaction<sup>11</sup>. IL-1 and TNF- $\alpha$  have traditionally been understood to be the main inducer cytokines in the acute phase of inflammatory reaction<sup>12</sup>. In consensus we found increased plasma levels of TNF- $\alpha$  4 h after UVA exposure while after 24 h the levels were normalized. Only the lower UVB dose showed increased TNF- $\alpha$  level after 24 h. Similar changes in TNF- $\alpha$  level were found in UVB exposed skin. Levels of IL-1 $\alpha$  and IL-1 $\beta$  in plasma were unaffected following UVA and UVB treatment except for an increased IL-1 $\beta$  level in the group exposed to the higher UVB dose 4 h after irradiation (Table 1 and 2). On other hand, in skin we found UVA- and UVB-caused modulation of IL-1 and IL-1 $\beta$  but it was not dose- either time-dependent. The lower UVA and UVB dose was more effective and induced increase/decrease in those cytokines. The changes found in skin did not correlate with those in plasma. Comparing our results to published ones, Brink et al. demonstrated that sectional exposure of human volunteers to 1 and

3 MED (1.8–3.6 J/cm<sup>2</sup>) of solar simulated light (SSL, 290–400 nm) strongly elevated IL-1 $\beta$  and TNF- $\alpha$  mRNA levels in the skin 6 h after irradiation and then the levels decreased at 24 h (ref.<sup>13</sup>). A single exposure of human subjects to 3 MED of SSL (290–400 nm; 1.4–5.6 J/m<sup>2</sup>) also caused a rapid increase in TNF- $\alpha$  level in blister fluid that started at 4 h, peaked at 15 h and was normalized 48 h after exposure. On the other hand, IL-1 $\alpha$  and IL-1 $\beta$  level in blister fluid was significantly increased only at 15 h and remained elevated to 72 h after SSL exposure<sup>14</sup>. Similarly Hiromi et al. showed a rapid up-regulation of IL-1 $\alpha$  expression induced by UVB (275–305 nm; 200 mJ/cm<sup>2</sup>) in the skin of the Hirosaki hairless rats. IL-1 $\alpha$  protein increased after 3 h, peaked at 6 h and gradually decreased during 12–24 h after exposure<sup>15</sup>. In another trial, non-significant changes, decrease in TNF- $\alpha$  and increase in IL-1 $\beta$ , were found in blister fluid after sectional exposure of skin to SSL (1 and 2 MED) after 24 h (ref.<sup>16</sup>). Narbutt et al. found increased mRNA levels of IL-1 $\beta$  and TNF- $\alpha$  in human skin 24 h after a single local UVB (280–315 nm) exposure of 3 MED (ref.<sup>17</sup>). In contrast, no significant changes in TNF- $\alpha$  and IL-1 $\beta$  level were found in plasma of human subjects at 24 h following a single exposure to 3 MED of UVB (280–315 nm). Repeated UVB irradiation (10 x 0.7 MED) induced a statistically significant increase only in plasma TNF- $\alpha$  level; no effect was found on IL-1 $\beta$  levels<sup>18</sup>. Repeated SSL (280–400 nm; 96% UVA) exposures (10 x 120 J/m<sup>2</sup>) caused a significant increase in mRNA levels of IL-1 $\beta$  and TNF- $\alpha$  in human skin<sup>19</sup>, but no changes in plasma level of these cytokines 24 h after the last exposure<sup>18</sup>. Three whole-body exposures of human subjects to UVA (340–400 nm) or UVB (311–313 nm) did not affect TNF- $\alpha$  and IL-1 $\beta$  serum level at several time points (0–48 h) (ref.<sup>20</sup>).

IL-6 acts in both pro-inflammatory and anti-inflammatory ways and is critical for healthy wound healing. Both foregoing cytokines, IL-1 and TNF- $\alpha$  stimulate production of pleiotropic IL-6. Petit-Frère et al. also showed that release of IL-6 following UV radiation is wavelength-dependent and mediated by DNA damage, particularly by cyclobutane-pyrimidine dimers<sup>21</sup>. Using a solar simulator, we found a different time-course of plasma IL-6 production after UVA and UVB treatment. While IL-6 levels were more pronounced after 4 h (16- and 68- fold) and also more normalized after 24 h (4- and 8- fold) in UVA treated groups (Table 2), in UVB exposed animals, enhanced IL-6 levels at 4 h (2- and 25-fold) were nearly unchanged at 24 h (5- and 25- fold) following irradiation, see Table 1. The IL-6 profile that we found in mice skin following UVA and UVB irradiation (Table 3,4), contrasted with that in plasma as well as with results by Hiromi et al. Their immunohistochemical analysis of skin of the Hirosaki hairless rats exposed to UVB (275–305 nm; 200 mJ/cm<sup>2</sup>) demonstrated that IL-6 increased after 6 h, peaked at 12–24 h and gradually decreased at 48–96 h after the treatment<sup>15</sup>. In human subjects, Urbanski et al. found the peak IL-6 concentration in plasma at 12 h after following the treatment with a UV source emitting light of 250–600 nm (emission peaks 300 and 360 nm) (ref.<sup>22</sup>). Narbutt et al. found no significant changes in IL-6

level in plasma of human volunteers at 24 h following a single (3 MED) and repeated (10 x 0.7 MED) UVB (280–315 nm) exposure<sup>18</sup>. In contrast, massive increase in IL-6 mRNA level was found in human skin 24 h after a single exposure to 3 MED of UVB (280–315 nm) (ref.<sup>17</sup>). Similarly, repeated SSL (280–400 nm; 96% UVA) exposures (10 x 120 J/m<sup>2</sup>) caused a significant increase in mRNA levels of IL-6 in human skin<sup>19</sup>. Similarly, irradiation of human volunteers with SSL caused a significant increase in IL-6 level in blister fluid 24 h after exposure to 1 MED (6-fold) and 2 MED (46-fold). But after repeated SSL treatment (4 x 0.3 MED), IL-6 secretion was normalized within 24 h after the last exposure<sup>16</sup>.

UVB-induced IL-6 generation as well as DNA damage has been reported to up-regulate IL-10 secretion<sup>23</sup>. IL-10 is produced by activated immune cells, in particular monocytes/macrophages and T cell subsets, infiltrating skin following UV irradiation<sup>24</sup>. Under our conditions, IL-10 plasma level following UVB exposure was reduced 4 h after the treatment and then normalized. In contrast, skin IL-10 level was minimally affected 4 h after UVB treatment but increased in the both groups after 24 h. Shen et al. showed a significant up-regulation of IL-10 in UVB (3 MED, 0.554 mJ/cm<sup>2</sup>) treated hairless mice skin 24 h after irradiation that peaked at 72 h (12-fold increase) and decreased to control levels at 120 h (ref.<sup>25</sup>). A little different IL-10 profile was found in serum of Balb/c mice exposed to the dose of 15 kJ/m<sup>2</sup> of UV light (65% UVB and 35% UVA). IL-10 was increased 24 h after exposure, peaked at 36 h and was cleared at 72 h after irradiation<sup>26</sup>. In human subjects exposed to 3 MED of SSL (290–400 nm), IL-10 increase in skin was detected at 15 and 24 h after irradiation<sup>14</sup>. Local exposure of human volunteers to SSL (1 and 3 MED) induced a strong increase in IL-10 mRNA level 24 h after exposure<sup>13</sup>. Narbutt et al. found enhanced IL-10 mRNA level in skin<sup>17</sup> but unchanged protein level in plasma<sup>18</sup> at 24 h after a single exposure to 3 MED of UVB (280–315 nm) exposure. In UVA irradiated mice, we found a significant IL-10 increase in both plasma and skin samples after 24 h. Shen et al. reported that UVA (37.8 J/cm<sup>2</sup>) irradiation caused non-significant but detectable increase in IL-10 level in mice skin during 0–120 h after exposure<sup>25</sup>. On the other hand, repeated SSL (280–400 nm; 96% UVA) exposures (10 x 120 J/m<sup>2</sup>) of human volunteers caused a significant increase in mRNA levels of IL-10 in skin<sup>19</sup>, but no changes in plasma protein level of the cytokine 24 h after the last exposure<sup>18</sup>. The results suggest that UVB-caused IL-10 increase is probably linked to enhanced leukocyte infiltration and DNA damage (thymine dimmer formation) (ref.<sup>27</sup>). In contrast, UVA-caused IL-10 accumulation is not linked to leukocyte infiltration (Fig. 3B) or DNA damage<sup>28</sup>, and may be connected to UVA-stimulated overproduction of reactive oxygen species and the following oxidant/antioxidant imbalance.

IL-12 is a pleiotropic cytokine that is a potent inducer of INF- $\gamma$  but it blocks IL-10 production and counteracts its activity<sup>29</sup>. IL-12 is composed of two chains p40 and p35. Only the 70 kDa dimer is biologically active<sup>30</sup>. IL-12 is important in cutaneous allergies and inflammation and

it has also been shown to block UV-induced immunosuppression. This effect includes stimulation of DNA repair but without increasing the level of the repair enzymes<sup>31</sup>. Using wild-type (C3H/HeN) and IL-12-knockout mice Meeran et al. demonstrated that IL-12-deficiency significantly enhanced levels of pro-inflammatory cytokines (IL-1  $\beta$ , TNF- $\alpha$  and IL-6) and COX-2 as well as leukocyte infiltration (MPO activity) following UV treatment (80% UVB; 180 mJ/cm<sup>2</sup>) (ref.<sup>32</sup>). As shown in Table 1, under our conditions UVB treatment had no significant effect on IL-12(p40) plasma expression. However, a significantly increased IL-12(p40) level was detected in skin of mice treated with the higher UVB dose after 24 h (Fig. 1, Table 3). Following UVA exposure (20 J/cm<sup>2</sup>), plasma amount of IL-12(p40) was significantly up-regulated after 24 h. In skin, both UVA doses increased the protein level after 24 h. Our results show that UVA is more potent in alteration of IL-12 production than UVB irradiation and modulates the UVB-induced immunosuppressive effect. In agreement with our results, Shen et al. described a significant IL-12 increase in the epidermis of UVA (37.8 J/cm<sup>2</sup>) treated mice after 24 h, which peaked at 72 h (6-fold increase) and then declined. In contrast, UVB irradiation (0.554 mJ/cm<sup>2</sup>) resulted in a significant decrease in IL-12 levels 24 h after exposure and remained reduced for 4 days<sup>25</sup>. In human subjects exposed to 3 MED of SSL (290–400 nm), no significant effect on IL-12 was found in skin tissue at 4–72 h after irradiation<sup>14</sup>. Although UV light has been reported to stimulate INF- $\gamma$  production<sup>25</sup>, under our condition we found no effect on the plasma cytokine in either UVB or UVA irradiated mice.

UV-induced inflammation is also mediated by iNOS and COX-2 whose levels are normally low. We found up-regulation of iNOS expression at 4 h following UVA and UVB treatment. This corresponds with the results by Chang et al. who found significantly increased iNOS level in mouse skin 6 h after UVB (0.5 J/cm<sup>2</sup>) irradiation<sup>33</sup>. Repeated UVB exposure (80% of UVB, 7x180 mJ/cm<sup>2</sup>) of SKH-1 mice induced iNOS protein increase 24 h after the last treatment<sup>34</sup>. Unfortunately, we found no study on the effect of UVA radiation on iNOS expression skin *in vivo*. Concerning COX-2 protein expression, we found a moderate increase in mice exposed to the higher UVA dose at both time-points. No significant effect was detected following a single UVB exposure. It is not so surprising because COX-2 is understood to be mainly a marker of pro-inflammatory conditions and cancerogenesis that is linked to repeated UVB exposures<sup>35</sup>. Nevertheless in human keratinocytes, Buckman et al. found a moderate COX-2 protein increase at 6 h and strong one at 24 h after UVB (290–320 nm, 30 mJ/cm<sup>2</sup>) exposure<sup>36</sup>. Narbutt et al. also found a massive increase of COX-2 expression in human skin 24 h after a single exposure to 3 MED as well as repeated (10 x 0.7 MED) of UVB (280–315 nm) (ref.<sup>17</sup>). Similarly, Rhodes et al. reported up-regulated COX-2 level in human skin 24 h after UV exposure (290–400 nm, peak 313 nm) but not at 4 and 72 h (ref.<sup>37</sup>). Stimulation of COX-2 expression via UVA radiation of has been most probably described only *in vitro*<sup>38,39</sup>. A time-dependent study showed a rapid response with a peak of COX-2

mRNA and protein expression at 1-2 h and 4 h, respectively, following UVA exposure (250 kJ/m<sup>2</sup>; the emission peak at 365 nm) (ref. 38). Mahns et al. further found that UVA-induced COX-2 up-regulation is wavelength-dependent. While 320-350 nm stimulated COX-2 expression, 350-400 nm was practically ineffective<sup>40</sup>.

MPO is commonly used as a marker of infiltrating leukocytes (macrophages and neutrophils) in the skin<sup>34</sup>. In agreement with the inflammatory character of the UVB irradiation, we found a dose-dependent increase in the MPO activity (2- and 6-fold) in skin 24 h after exposure (Fig. 3). These results correspond to a study by Casagrande et al. who found a dose-dependent increase in MPO activity in HRS/J hairless mice skin 6 h after single UV exposure (270-400 nm, 73% UVB, 1.23-3.69 J/cm<sup>2</sup>) (ref.<sup>41</sup>). Similarly acute UVB treatment (0.224 J/cm<sup>2</sup>) of female SKH-1 mice induced a significant increase in skin MPO activity 48 h after exposure. However, in male mice a minimal change in MPO activity was found<sup>42</sup> and suggests that the effect is gender-dependent. On the other hand following UVA irradiation we found no significant effect on MPO activity. We also found no *in vivo* study that describes stimulation of MPO activity after UVA radiation to compare our results.

## CONCLUSION

There are a number of reports on the effects of UVB radiation on inflammatory biomarkers in plasma and/or skin. However, there is a lack of *in vivo* studies involving UVA light. Moreover, due to the use of different UV radiation sources, various experimental designs, animal models or human volunteers with differences in epidermal thickness and pigmentation, and different biological samples (skin, plasma, blister fluid) the results are often conflicting and thus it is difficult to generalise from, compare and/or relate to human beings. Here we have shown that a single exposure to solar simulated UVA and UVB light affected several cytokines at different time-courses in skin and plasma of mice. UVB- or UVA-induced changes in plasma parameters did not often correlate with those found in skin. This indicates that inflammatory marker analysis in plasma cannot substitute for analysis of skin tissue and vice versa. The variable quality, quantity and time-course of inflammatory response of the organism to UVA and UVB irradiation are connected to different mechanisms of action. Recent studies by Krutmann et al. indicate that the UV sources spectral output may be critical for the biological effects of UV light because there is some wavelength-dependent crosstalk at a molecular level that may significantly influence skin response<sup>1</sup>. For this reason, detailed *in vivo* and clinical trials arranged in dose- and time-dependent manner that will use the complete solar spectrum and isolated UVA and UVB wavebands together with exact characterization of UV sources are needed to understand and explain the molecular mechanisms of skin inflammation.

## ABBREVIATIONS

COX-2, Cyclooxygenase 2; HRP, Horseradish peroxidase; iNOS, Inducible nitric oxide synthase; IL-1 $\alpha$ , Interleukin-1 $\alpha$ ; IL-1 $\beta$ , Interleukin-1 $\beta$ ; IL-6, Interleukin-6; IL-10, Interleukin-10; IL-12, Interleukin-12; IFN- $\gamma$ , Interferon gamma; MED, Minimal erythema dose; MPO, Myeloperoxidase; TNF- $\alpha$ , Tumor necrosis factor-alpha; SSL, Solar simulated light; HTAB, hexadecyltrimethylammonium bromide.

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## CONFLICT OF INTEREST STATEMENT

**Author's conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

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