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Contrasting effects of ultraviolet A1 and ultraviolet B exposure on the induction of tumour necrosis factor-α in human skin

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Summary Ultraviolet B (UVB) irradiation of the skin causes immunosuppression which is relevant to the induction of skin cancer. The mechanism of this immunomodulation is unclear but various regulatory molecules have been implicated, including *cis*-urocanic acid (*cis*-UCA) and the cytokines tumour necrosis factor-α (TNF-α) and interleukin 10 (IL-10). Whether ultraviolet A (UVA) induces similar changes has not been investigated fully. We studied the effect of *in vivo* UVB and long-wave UVA (UVA1) exposure on the induction of TNF-α, IL-10 and *cis*-UCA in human skin. Volunteers were irradiated with three minimal erythema doses (MED) of UVB or UVA1. At different times after irradiation, suction blisters were raised from irradiated and from non-irradiated (control) skin. The TNF-α and IL-10 protein concentration, and the percentage of *cis*-UCA in the blister fluid, were then determined. UVB irradiation of human skin led to a rapid and significant increase in TNF-α concentration in suction-blister fluid, with maximal values 6 h after irradiation ($n = 6$, $P < 0{\cdot}05$). In contrast, UVA1 irradiation led to a decrease in TNF-α concentration in the suction-blister fluid compared with non-irradiated skin, with the lowest values 6 h after irradiation ($n = 6$, $P < 0{\cdot}05$). Both UVB and UVA1 exposure of the skin induced a slight increase in IL-10 concentration. However, the increase in IL-10 was only significant after UVB irradiation (UVB, $n = 6$, $P < 0{\cdot}05$; UVA, $n = 7$, $P < 0{\cdot}1$). As previously shown, both UVB and UVA1 result in the photo-isomerization of *trans*-UCA and an increased percentage of *cis*-UCA was found in the suction-blister fluid. Thus the results show differential effects of UVB and UVA1 irradiation on the induction of immunoregulatory molecules, which may help to explain the variation in immune responses after UVB and UVA1 exposure of human skin.

Exposure to sunlight includes both ultraviolet B (UVB, 280–320 nm), and short- and long-wave ultraviolet A (UVA2 320–340 nm and UVA1 340–400 nm) wavelengths. Irradiation of the skin by UVB causes immunosuppression which is thought to be a contributing factor to the induction of skin cancer. Several mechanisms have been implicated in UVB-mediated immunomodulation, including the synthesis and release of immunoregulatory molecules, such as *cis*-urocanic acid (*cis*-UCA)[1,2] and the cytokines tumour necrosis factor-α (TNF-α)[3,4] and interleukin-10 (IL-10).[5,6] Whether UVA irradiation of human skin also induces similar changes has not been fully investigated.

UVB irradiation of keratinocytes *in vitro*[7] and of human skin *in vivo*[8] leads to the synthesis and release of TNF-α. In mice TNF-α plays an important part in UVB-mediated suppression of contact hypersensitivity.[3,4] In normal epidermis UCA is found in high concentration as *trans*-UCA, which on UV exposure is photoisomerized to *cis*-UCA. The latter has been proposed as a photoreceptor for UV irradiation and suppresses immune responses in a variety of experimental systems, including delayed hypersensitivity responses to herpes simplex virus.[9] It has been suggested that the action of *cis*-UCA may be mediated through TNF-α, although the data are conflicting.[10,11] In addition to *cis*-UCA and TNF-α, IL-10 is an important cytokine for UVB-induced immunosuppression in mice.[5] UVB and UVA1 irradiation of human keratinocytes *in vitro* induces the release of IL-10[12] and one study has shown upregulation of IL-10 m-RNA after UVB irradiation of human skin *in vivo.*[13]

Taken together, only a few studies have investigated the effect of *in vivo* UVB irradiation on the induction of TNF-α and IL-10 in human skin and none have assessed UVA irradiation. The aim of the present study was to examine the concentration of TNF-α, IL-10 and *cis*-UCA in suction-blister fluid after *in vivo* exposure of human skin to UVB and long-wave UVA (UVA1) radiation.

Materials and methods

Subjects

Twenty-five volunteers were recruited after approval had been obtained from the local ethics committee (KA95222). All subjects were of skin types II–III, aged between 19 and 29 (mean 24) years and with no history of chronic disease. All the volunteers were recruited at one centre, in Copenhagen.

Ultraviolet exposure

The light source for UVB exposure was a Philips model TL12 2×20 W, the UVB lamp emitting a spectrum of 280–365 nm with a peak emission at about 310 nm (the main part of the energy had wavelengths between 280 and 320 nm). The light source for UVA1 exposure was an UVAsun 5000 (Mutzhas, Munich, Germany) delivering only energy at wavelengths above 340 nm, with the main part of the energy between 340 and 400 nm and a peak emission about 350 nm. Minimal erythema doses (MED) were determined for the volunteers receiving UVB and UVA1 radiation, and equivalent amounts of UVB and UVA1, judged by erythema, were subsequently administered. The total UV radiance of the lamps was determined with a IL 1700/760D/782 A spectroradiometer system (International Light, Newburyport, MA, U.S.A.). The area of the irradiated spots was $2{\cdot}8\ cm^2$ and the mean MED dose for UVB was $117\ mJ/cm^2$ and for UVA1 $81 \times 10^3\ mJ/cm^2$.

Suction blisters

Suction blisters were raised on normal non-irradiated skin and skin irradiated with 3 MED immediately before and after 6, 24 (UVB, $n = 6$ and UVA1, $n = 9$) and 72 h (UVB, $n = 6$). In four volunteers the blisters were produced 6 h after irradiation with 0, 1, 2 and 3 MED UVB to determine the UVB dose–response for the induction of TNF-α. All the blisters were raised under a negative pressure of 100–250 mmHg from skin on the medial aspect of the forearm. The difference in pressure used to raise the blisters was caused by interindividual variation and because the blisters were usually raised at a lower pressure on irradiated skin. The time stated represents the point at which suction was applied; it requires 2–3 h to raise the blisters. The blister fluids were collected and stored at −70 °C. TNF-α and IL-10 protein levels were determined by ELISA (Human HS TNF-α, Quantikine R&D System, Minneapolis, MN, U.S.A. and human IL-10, Endogen, Cambridge, MA, U.S.A.). The concentration of total UCA and percentage as the *cis*-isomer were determined by HPLC, as previously outlined.[14]

Statistical analysis

The Wilcoxon matched-pairs signed-rank sum test was used to compare the cytokine and *cis*-UCA concentrations in blister fluid from normal and irradiated skin of each subject. The statistical analysis was performed using SYSTAT for Windows (Systat Inc., Evanston, IL, U.S.A.).

Results

Tumour necrosis factor-α concentrations in suction-blister fluid

UVB irradiation of human skin led to a rapid and significant increase in TNF-α concentration in suction-blister fluid (Fig. 1). The increase in TNF-α concentration was already detectable in fluid from blisters created immediately after 3 MED UVB irradiation, and maximal values were found 6 h after irradiation (Fig. 1; $P < 0{\cdot}05$). The increase was transitory because the TNF-α concentration in fluid from blisters created 24 h after irradiation was equal to non-irradiated skin (Fig. 1, $P = 0{\cdot}75$). Furthermore, 72 h after 3 MED UVB irradiation the mean (± SD) TNF-α concentration in the blister fluid was lower (7 ± 5 pg/mL) than in fluid from non-irradiated skin (44 ± 16 pg/mL; $n = 6$). In all four volunteers, the TNF-α concentration in blister fluid increased with increasing UVB dose, with the highest values 6 h after 3 MED UVB (non-irradiated skin 57 ± 38 pg/mL; after 1 MED UVB 133 ± 71 pg/mL; 2 MED UVB, 196 ± 112 pg/mL; and 3 MED UVB, 347 ± 127 pg/mL; $n = 4$). In contrast to UVB irradiation, 3 MED UVA1 irradiation led to a decreased TNF-α concentration in the suction-blister fluid. The lowest TNF-α values were found immediately and 6 h after UVA1 irradiation (Fig. 1). In suction-blister fluid from blisters produced 6 h after UVA1 irradiation, the concentration of TNF-α was significantly lower than in fluid from non-irradiated skin (Fig. 1; $P < 0{\cdot}05$).

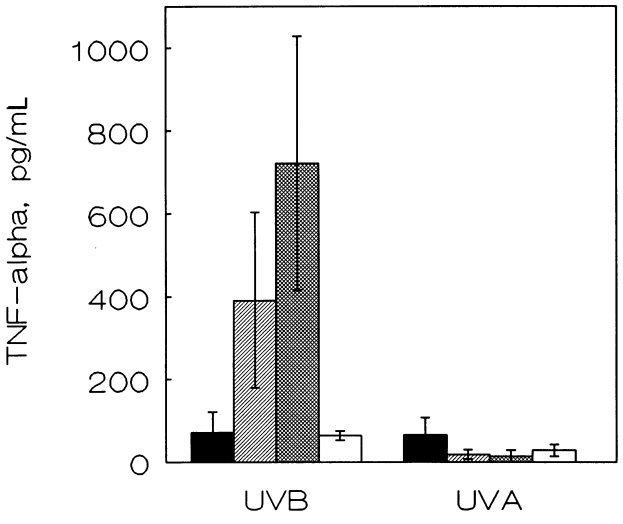

Figure 1. Tumour necrosis factor (TNF)-α concentrations in suction-blister fluid; normal volunteers were irradiated with 3 MED UVB ($n=6$) or UVA1 ($n=6$) at three spots immediately (0 h; hatched bars), 6 h (double hatched bars) and 24 h (white bars) and before suction blisters were raised at the irradiated sites, and at a control site (solid bars). The TNF-α protein level in the blister fluid was determined using a commercial ELISA kit and expressed as the mean (±SD).

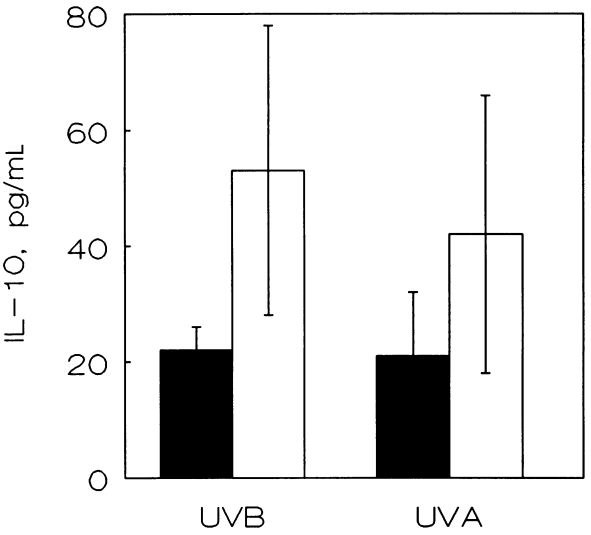

Figure 2. IL-10 concentration in suction blister fluid; normal volunteers were irradiated with 3 MED UVB ($n=6$) or UVA1 ($n=7$) at 24 h after irradiation, suction blisters were raised at the irradiated site (white bars) and at a control site (solid bars). The IL-10 protein level in the blister fluid was determined using a commercial ELISA kit and expressed as the mean (±SD).

Interleukin 10 and cis*-UCA concentrations in suction-blister fluid*

Three MED UVB irradiation of human skin resulted in a slight but significant increase in IL-10 concentration in suction-blister fluid, with maximal values at 24 h after irradiation (Fig. 2; $P<0{\cdot}05$). Like UVB irradiation, 3 MED UVA1 irradiation of human skin led to a slight increase in IL-10 concentration in blister fluid, but this was not significant (Fig. 2; $P<0{\cdot}1$). The percentage of *cis*-UCA in the suction-blister fluid 24 h after 3 MED of both UVB and UVA1 irradiation of the skin was significantly higher than that in fluid from non-irradiated skin (Table 1).

Discussion

UVB irradiation of human skin but not UVA1 irradiation resulted in an increased concentration of TNF-α in suction-blister fluid. In mice, TNF-α is involved in UVB-induced suppression of contact hypersensitivity.[3,4] TNF-α injected into the dermis of mice mimics the effect of UVB irradiation and furthermore, neutralizing TNF-α antibody abrogates the immunosuppressive effect of UVB irradiation. Also, UVB irradiation of human skin suppresses the development of contact hypersensitivity, although similar biological doses of UVA1 (judged by erythema) did not do so.[15] One of the mechanisms by which UVB irradiation of human subjects mediates suppression of contact hypersensitivity may be through the induction of TNF-α. This is supported by the present finding that UVB, but not UVA1, irradiation induced the release of TNF-α.

Small amounts of TNF-α were also present in suction-blister fluid from normal skin, indicating that the blistering procedure can induce the release of TNF-α. The source of TNF-α in human skin remains unknown. Keratinocytes may be the main candidate; UVB irradiation of keratinocytes *in vitro* leads to release of TNF-α and one recent study has shown that UVB exposure of human skin induces up-regulation of TNF-α mRNA in keratinocytes.[8] However, other cells may be important; Langerhans cells can secrete TNF-α, at least under pathological conditions.[16] Dermal mast cells contain TNF-α[17,18] and both *in vivo* and *in vitro* experiments have shown that UVB irradiation can trigger the release of TNF-α from them.[19]

The present results show that UVA1 irradiation of human skin significantly decreased the TNF-α concentration in suction-blister fluid, possibly because there are fewer dermal mast cells in skin after high-dose UVA1 treatment.[20] These results, together with the observation that high-dose UVA1 is an effective therapy for patients with urticaria pigmentosa,[21] support the hypothesis that dermal mast cells are one of the target cells in high-dose UVA1 therapy.[22]

Table 1. The mean urocanic acid (UCA) content and percentage of *cis*-UCA in suction blister fluid 24 h after UVB ($n = 6$) and UVA1 ($n = 7$) exposure of human skin

	Control	UVB irradiated	Control	UVA1 irradiated
Mean (±SD)				
Total UCA (nmol/mL)	0·11 (0·04)	0·16 (0·04)	0·15 (0·03)	0·16 (0·02)
% *cis*-UCA	10·7 (14·7)	38·4 (20·4)*	7·6 (7·2)	19·3 (9·5)*

* $P < 0·05$

UVB irradiation of human skin also induced a significant increase in IL-10 concentration in suction-blister fluid, while UVA1 irradiation led to a slight but insignificant increase. The main source of IL-10 in the skin is thought to be keratinocytes; both UVB and UVA1 irradiation of keratinocytes *in vitro* has been shown to induce IL-10 mRNA production and protein release.[12] In addition, macrophages, found in the epidermis after UVB[23] but not after UVA1 irradiation,[24] synthesize large quantities of IL-10.[25] In mice, IL-10 is a potent inhibitor of delayed and contact hypersensitivity,[5,26] probably by inhibition of Th1 cells and activation of Th2 cells, resulting in tolerance.[26,27] Also, UVB-induced IL-10-producing macrophages are able to down-regulate immune responses in human subjects.[28]

Previous studies have shown that UVB and long- and short-wave UVA (UVA1 and UVA2) irradiation lead to photoisomerization of *trans*-UCA.[29] In agreement with these findings, an increase in the percentage of *cis*-UCA in suction blister fluid was detected after both UVB and UVA1 exposure of human skin. It has been suggested that the immunosuppression caused by *cis*-UCA is mediated, at least partly, by TNF-α induction.[10] However, the present study indicates that UVA1 can increase the concentration of *cis*-UCA, but not of TNF-α, in the blister fluid. Therefore, the present results support another study showing that the effect of *cis*-UCA is not mediated by increased TNF-α production,[11] finding instead evidence for the involvement of IL-10.[30]

As a part of natural sunbathing and when using tanning salons, the skin is exposed to both UVB and UVA radiation. In addition, the increasing use of sunbeds, which filter out UVB but allow more exposure to UVA without burning, is a cause for concern. It is therefore important to determine the effect of both UVB and UVA irradiation on the skin immune system. The present results show differential effects of UVB and UVA1 irradiation on the induction of TNF-α. Furthermore, the results agree with an important role suggested for TNF-α in UVB-mediated suppression of contact hypersensitivity.

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