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Systemic Effect of Ultraviolet Irradiation on Non-immunologic Immediate Contact Reactions to Benzoic Acid and Methyl Nicotinate

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Systemic effects of ultraviolet irradiation B (UVB) and ultraviolet irradiation A (UVA) on non-immunologic immediate contact reactions (NIICRs) induced by benzoic acid (BA) and methyl nicotinate (MN) were studied in healthy volunteers. NIICR tests with four concentrations of BA and MN in white petrolatum were performed on the skin of the upper back on exposed and non-exposed areas, before and at intervals 1–14 days after exposure to 1) 0.20 J/cm^2 of UVB, 2) after the first of daily doses on five consecutive days of 0.04 J/cm^2 of UVB, 3) after the first of daily doses on five consecutive days of 20 J/cm^2 of UVA, and 4) 1–8 weeks after the first of twelve consecutive doses of 20 J/cm^2 of UVA given three times a week over a period of four weeks. After the last of twelve exposures of UVA, stratum corneum was stripped off from both the exposed and the non-exposed skin. Forty minutes after application of the test substances, erythema and edema reactions were observed visually, and changes in the blood flow were monitored using a laser-Doppler flowmeter. All dosages of UV-light inhibited the NIICRs on exposed areas. UVB as given repeatedly inhibited NIICRs to 125 mM BA on non-exposed areas. The twelve doses of UVA also had a systemic inhibitory effect on NIICRs both on stripped and non-stripped test areas. The results indicate a systemic inhibitory effect of UV light on NIICRs. *Key words: Contact urticaria: Laser-Doppler flowmetry.*

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In our previous study, both ultraviolet B (UVB) and ultraviolet A (UVA) irradiation was found to diminish skin reactivity to substances able to produce non-immunologic immediate contact reactions (NIICRs) in man (1, 2). UV irradiation alters the reactivity of the skin obviously in many ways, e.g. by increasing the number of suppressor T-cells and depleting the expressivity of DR-antigens of keratinocytes and Langerhans' cells (3–7).

It was not possible to determine whether the inhibitory effect of UV irradiation on NIICRs is only local on the basis of the previous results. We therefore investigated the systemic effects of UVB and UVA on NIICRs induced by benzoic acid (BA) and methyl nicotinate (MN) in man.

MATERIAL AND METHODS

Test subjects and methods

Four groups of healthy voluntary medical students (age 20–30) participated in the study (Table I). They received no anti-inflammatory analgesic or antihistaminic drugs for three days before the tests, and none during the tests. UVB irradiation was given in a microprocessor-controlled Waldmann UV 6002 device (Herbert Waldmann GmbH & Co, Villingen-Schwenningen, FRG, output 290–350 nm, peak 315 nm). The UVA light source was an Airam PUVA 22 (Airam Ltd., Helsinki, Finland) device equipped with Philips lamps TL-05-80 W/09 (output 320–400 nm, peak 355). One side of the upper back was covered with 4-fold green cloth, sealed with acrylic tape, and the rest of the body was irradiated. In the fourth group, immediately after the last of twelve exposures of UVA, on the exposed and non-exposed sides, 2×15 cm skin areas were stripped five times with strips of cellophane tape (Scotch brand 'magic' transparent tape, 810; 3M Co., Beauchamp, France).

Test substances

Ten microlitre doses of BA 250, 125, 62, and 31 mM (groups I–IV) and MN 10, 2.5, 0.4 and 0.08 mM (groups I–III) and MN 10, 5, 2.5 and 0.5 mM (group IV) (Sigma Chemical Co., St. Louis, MO, USA) both in white petrolatum, and white petrolatum as a reference, were applied without occlusion to 1×1 cm areas of the exposed and unexposed upper back skin, 2 cm apart from each other. In the fourth group, 250, 62 mM BA and 10 and 2.5 mM MN and petrolatum were applied on the stripped areas. The test substances were wiped away with blotting paper 20 min after application. Erythema and edema were graded visually 40 min after application of the test substances as follows: −, no reaction; +, faint; ++, moderate; +++, intensive, and the cutaneous

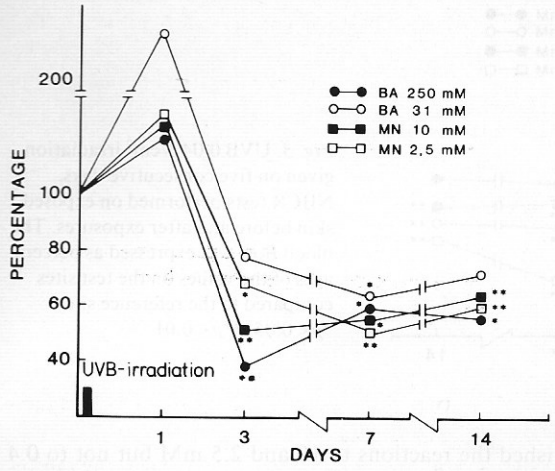

Fig. 1. The effect of 0.20 J/cm^2 of UVB on reactions induced by benzoic acid (BA) and methyl nicotinate (MN). BA was applied to irradiated skin before 1, 3, 7, and 14 days after exposure. The blood flow was measured 40 min after application and expressed as percentages of the values on the test sites compared to values before exposure. $*p<0.05$, $**p<0.01$.

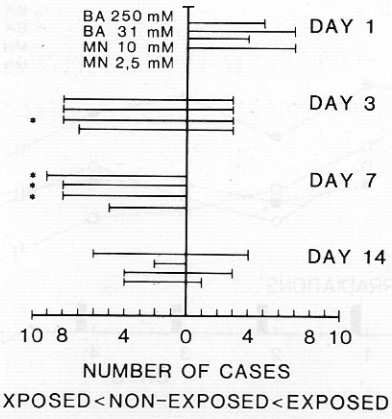

Fig. 2. Visual erythema of non-immunologic immediate contact reactions induced by benzoic acid (BA) and methyl nicotinate (MN) after exposure to 0.20 J/cm^2 of UVB. The results are expressed as numbers of test subjects showing stronger or weaker erythema on the irradiated skin than the erythema reaction before exposure. $*p<0.05$ in Wilcoxon signed rank test.

blood flow was measured using a laser-Doppler flowmetry (LDF) device (Periflux® PF1, Perimed KB, Stockholm, Sweden) according to the manufacturer's recommendations (8). The device was equipped with a special multifibre probe. The test subjects were sitting in a room at a temperature of 23±2°C. The results of the LDF measurements were expressed as percentages of the blood flow values on the test sites compared with values on the reference site.

Student's *t*-test for paired observations was used for the statistical analysis of LDF results, and the Wilcoxon signed rank test for paired observations for the visual observations.

RESULTS

UVB 0.20 J/cm^2

This single dose of UVB produced visual erythema in all twelve cases. NIICRs to all concentrations of BA and to two highest concentrations of MN were weaker than initial reactions on the 7–14 days after exposure on the exposed test areas (Fig. 1), but the blood flow in the tests performed on the non-exposed areas did not differ from the initial value. On the 3rd and the 7th days after exposure, visual erythema and edema from the three highest concentrations of BA and from 10 mM MN were weakened on the exposed areas ($p<0.05$) (Fig. 2), but no change was seen on the non-exposed areas. 0.08 mM MN did not produce NIICRs at all.

UVB 5×0.04 J/cm^2

This dosage of UVB itself caused neither erythema nor changes in the skin blood flow. Blood flow in NIICRs from all concentrations of BA and from 10 and 2.5 mM MN was found to be decreased on the exposed skin of the upper back on the 7th day after exposure, and on the 14th day blood flow in NIICRs from 125, 62 and 31 mM BA and 10 and 2.5 mM

Table I. *Composition of the series and the study schedule. Repeated UV irradiation in groups 2 and 3 were performed on 5 consecutive days. NIICR tests were performed before and after repeated daily UV irradiations.*

Skin types: II usually burn, slight tan; III sometimes burn, always tan; IV never burn, always tan

	Group 1 UVB single	Group 2 UVB repeated	Group 3 5×UVA	Group 4 12×UVA
No. of subjects (males/ females)	12 (2/10)	13 (3/10)	17 (5/12)	13 (1/12)
Skin type				
II	4	5	5	4
III	8	7	11	8
IV	–	1	1	1
Irradiation J/cm^2	0.20	5×0.04	5×20	12×20

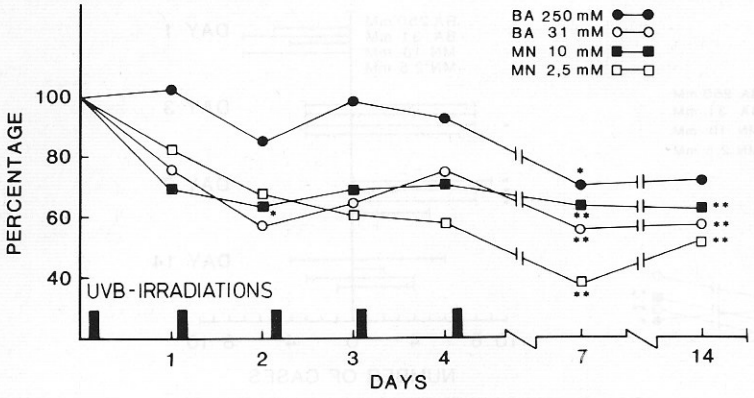

Fig. 3. UVB 0.04 J/cm² irradiation given on five consecutive days. NIICR tests performed on exposed skin before and after exposures. The blood flow was expressed as percentages of the values on the test sites compared to the reference sites. $*p<0.05$, $**p<0.01$.

MN was diminished on the exposed area (Fig. 3). Only one reaction on the non-exposed area, namely that to 125 mM BA on the 14th day, was weaker ($p<0.05$) than initially.

Visual erythema and edema from 62 and 31 mM BA and edema from 2 mM MN on the 7th day after the first exposure and edema from 63 and 31 mM BA on the 14th day after the first exposure were also significantly diminished on the exposed test area ($p<0.05$) (Fig. 4), but the strengths of the visual NIICRs on the non-exposed area did not alter during the test period.

UVA 5×20 J/cm²

UVA itself induced neither visual erythema nor change in the blood flow. UVA irradiation diminished the reactions to 10 and 2.5 mM but not to 0.4 mM MN on the UV exposed areas on the 4th, 7th and 14th days after exposure in both LDF and visual assessments (Figs. 5 and 6). The NIICRs induced by 62 and 31 mM BA but not those from higher concentrations were weaker on the exposed area on the 14th day in LDF measurements. No effect on NIICRs on the non-exposed area was found.

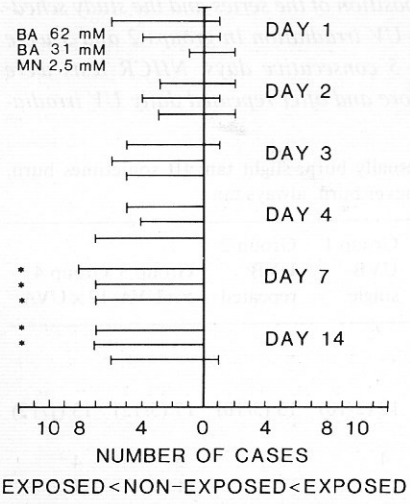

Fig. 4. Visual edema induced by BA and MN after exposure to five consecutive doses of 0.04 J/cm² of UVB. The results are expressed as in Fig. 2.

UVA 12×20 J/cm²

The local inhibitory effect of UVA on blood flow in NIICRs on the UV-exposed area was seen from week 2 through to week 8 (Fig. 7 *a*). The systemic inhibitory effect of UVA on non-exposed test areas was found not earlier than after last of the twelve exposures of UVA in LDF measurements (Fig. 7 *b*). Both local and systemic inhibition of NIICRs was still measurable for 4 weeks after the last exposure of UVA. Stripping of the skin did not influence the NIICRs either on the UV-exposed or the non-exposed skin (Figs. 7 *a*, *b*).

In visual assessment, the effect of UVA on the erythema reactions in NIICRs on UV-exposed skin could be seen from week 2 onwards, the inhibition being strongest in week 3. On the non-exposed area, the only decrease in erythema reactions could be seen visually in reactions from 10 mM MN on stripped skin after last exposure of UVA.

DISCUSSION

In this study, both single and repeated UVB and repeated UVA irradiation diminished NIICRs to BA and MN on exposed test sites, confirming our earlier results (2). Systemic effect of UVB on NIICRs was found in only one concentration of BA on the 14th day when UVB was given in repeated dosages. This

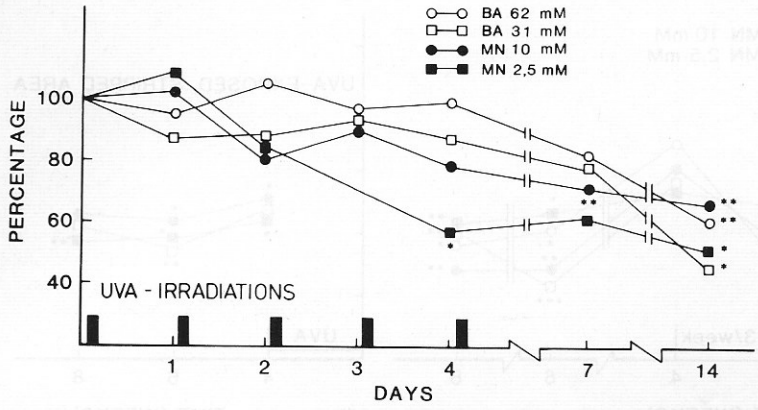

Fig. 5. The effect of 20 J/cm² UVA, given on days 0–4 on reactions induced by BA and MN applied on irradiated skin. The blood flow was expressed as percentages of the values on the test side compared with the values before exposure. $*p<0.05$, $**p<0.01$.

may only be a statistical coincidence because the evidence was only at 5% confidence.

When given three times weekly during a period of four weeks UVA had both local and systemic inhibitory effect on NIICRs from both substances investigated. It must be pointed out that the UVA lamps used in this study emit also UVB in some extent.

The inhibitory effect of UV-light on NIICRs could be caused either by an influence of UV on the immune system or by thickening of stratum corneum which, in turn, diminishes the amount of absorbed chemicals.

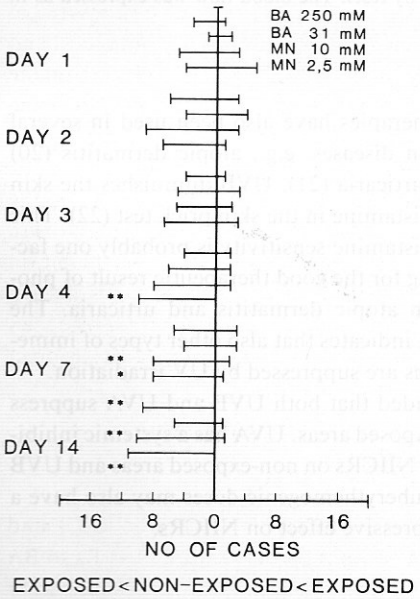

Fig. 6. Visual erythema induced by BA and MN after exposure to five consecutive doses of 20 J/cm² of UVA. The results are expressed as in Fig. 2. $*p<0.05$, $**p<0.01$.

To rule out the latter possibility, stripping of the keratin layer was done immediately after the last of the 12 UVA irradiations. Five strippings did not reach the living epidermis. Because no difference in the strength of the NIICRs between stripped and non-stripped skin was seen, the effect of UVA was probably the impairment of the inflammatory immune system.

Several studies have implicated the eicosanoids as mediator substances in different types of UVB-induced erythema (4, 9). The liberation of these mediators may not have any influence on the effect of UV irradiation on NIICRs, because the inhibitory effect of UV-exposure on NIICRs can be seen with non-erythemogenic doses of UV irradiation.

In recent years, studies of the effect of UV light on the skin has concentrated mainly on urocanic acid (UCA) (10, 11). UCA, a substance present in the stratum corneum, has been implicated as the photoreceptor for UV-induced suppression of the immune system. The mechanism of immunosuppression after UV radiation has been mainly investigated a) on antigen specific contact hypersensitivity, b) with the range of UVB irradiation, because the absorption spectrum of UCA, 240–310 nm, falls within the UVB range, c) in experimental animals or in vitro (12–15). The possible role of UCA as an immunomodulator was suggested in 1983, when DeFabo and Noonan (3) found that contact hypersensitivity could be suppressed, tumour rejection was delayed in mice as a result of UV irradiation and the immuno-suppressive effect of UV could be eliminated by the removal of the keratin layer. UCA or its photoproducts initiate a cascade of events resulting in degeneration of suppressor T lymphocytes. Later, Noonan et al. (16) were able to show that i.v. administration of UCA in mice

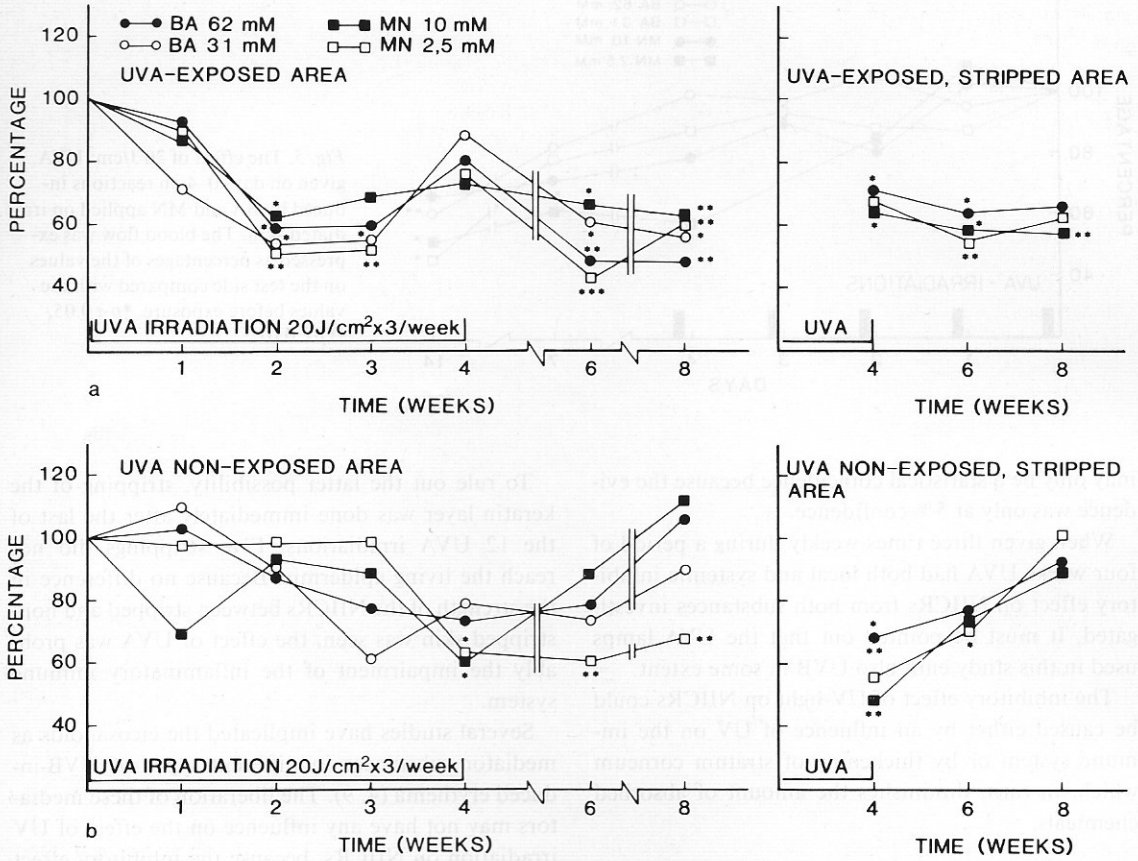

Fig. 7. The local a) and systemic b) effect of 20 J/cm² of UVA given for four weeks on reactions induced by BA and MN. After the last exposure of UVA, a skin area was stripped 5 times followed by tests. The blood flow was expressed as in Figs. 2 and 3.

initiates an antigen presentation defect of splenic dendritic cells in the same way as UV-light (250–300 nm).

Only a few studies have been carried out on the effects of UVA on immunosuppression. Schwarz et al. (17) showed with UVA (doses 10 and 20 J/cm²) a dose-dependent photoisomerization of trans-UCA to cis-UCA in vivo, but unexpectedly not in vitro, and Hersey et al. (18) found an increase in suppressor T cell activity after repeated exposure of solarium irradiation in man.

UVB therapy has been used in chronic hand eczema (19). In this particular study, therapy given only to the hands was found to be more effective than placebo light therapy but local and systemic UVB together was the most effective of the three regimens. Thus, systemic UVB may have therapeutic significance in inflammatory skin diseases affecting only small areas of the body.

UV-light therapies have also been used in several unrelated skin diseases, e.g., atopic dermatitis (20) and chronic urticaria (21). UVB diminishes the skin response to histamine in the skin prick test (22). This decrease of histamine sensitivity is probably one factor accounting for the good therapeutic result of phototherapies in atopic dermatitis and urticaria. The present result indicates that also other types of immediate reactions are suppressed by UV irradiation.

It is concluded that both UVB and UVA suppress NIICRs on exposed areas. UVA has a systemic inhibitory effect on NIICRs on non-exposed areas and UVB in repeated suberythemogenic doses may also have a systemic suppressive effect on NIICRs.

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