Medium-wave Ultraviolet Radiation (UVB) is Important in Doxycycline Phototoxicity

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The phototoxic reaction to doxycycline is provoked by long-wave ultraviolet light (UVA). It was shown by the in vivo mouse tail technique, measuring phototoxic edema, that the addition of medium-wave ultraviolet light (UVB) immediately after, immediately before and especially 24 h before the phototoxic trauma, enhanced the reaction more than could be expected from simple addition, thus demonstrating the importance of photoaugmentation in this process. *Key words: Mouse tail technique; Photoaugmentation.* (Received March 24, 1986.)

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Doxycycline is a potent photosensitizer and is the tetracycline derivative which most frequently causes phototoxic reactions in Sweden as reported to the Drug Information Committee, Swedish National Board of Health and Welfare (1). The pronounced potency of doxycycline as well as demethylchlortetracycline (DMCT) and chlortetracycline compared with the other tetracycline derivatives has also been shown experimentally using red blood cells (2), lymphocytes (3) and fibroblasts (4) as well as in comparative studies in humans given tetracyline per os and exposed to natural sunlight (5, 6, 7). Doxycycline phototoxicity has also been demonstrated by in vivo experiments using the quantitative mouse tail technique although it should be pointed out that the required dose was very high compared with other well known photosensitizers (8).

In spite of this mass of evidence it has been difficult to reproduce the phototoxic reaction in man using an artificial UVA source and doxycycline (orally) (9, Bjellerup & Ljunggren: unpublished results). The reason for this difficulty may be the lack of UVB in the artificial, as opposed to the natural situation, where UVA and UVB combine. The phenomenon of UVB photoaugmentation in drug phototoxicity has been shown earlier in mice for well known phototoxic drugs, especially chlorpromazine; tetracyclines however were not tested (10).

Therefore it was found to be of interest to study the effect of UVB added to the phototoxic reaction elicited by doxycycline and UVA. The quantitative mouse tail technique (8) was found most convenient for the experiment.

MATERIAL AND METHODS

Animals

Female albino mice (AB Anticimex, Sollentuna, Sweden) weighing around 30 g were used. The mouse tail technique, measuring phototoxic edema, has been described earlier (11).

Doxycycline

Doxycycline (provided by Pfizer, Brussels, Belgium) was dissolved in water and injected intraperitoneally at a concentration of 100 mg/kg bodyweight immediately before UVA irradiation.
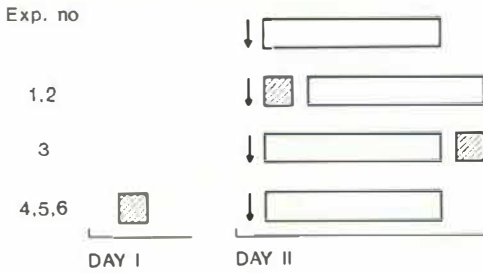
Exp. no

1,2

3

4,5,6

DAY I   DAY II

Fig. 1. UVB given at different time intervals in connection with the phototoxic treatment (doxycyline + UVA) in the 6 experiments. UVA: open bars. UVB: filled bars. Doxycycline injection: ↓. At the top the standard procedure of the quantitative mouse tail technique is shown, i.e. no UVB is added.

Irradiation procedure

During exposure to ultraviolet light the animals were fixed in horisontal plastic tubes allowing only the tails to be exposed. The distance between the light source and the tails was 12 cm. The radiation output at the level of the tails was measured for UVA with a PUVA-meter and for UVB with a UV-meter (Waldmann AG, Schwenningen, GFR).

Light sources

For UVA 2 blacklight fluorescent tubes (Philips TLA 40W/08) with an emission peak at 360 nm were used. A 3 mm windowglass filter was inserted to eliminate wavelengths shorter than 320 nm, giving an output of 2.5 mW/cm² in the UVA region. For UVB 2 fluorescent tubes (FS 40 Westinghouse Sun Lamp, 40 W) emitting continuously from 280–380 nm with a peak at 313 nm were used, giving an output of 1.8 mW/cm² in the UVB region.

Experimental design

The effect of relatively small (in the mouse tail) doses of UVB given in connection with the phototoxic treatment (doxycycline + UVA) was studied in six experiments. According to Fig. 1 UVB was given immediately before the phototoxic treatment in experiments 1 and 2, immediately after in experiment 3 and 24 h before in experiments 4, 5 and 6.

To determine whether the addition of UVB caused an additive or augmentative effect 5 groups of 10 animals were needed in each experiment (groups I to V) (Table I).

Thus group I was given UVA only, serving as a control since previous (10) and fresh pilot experiments had shown that this treatment induces no inflammatory reaction whatsoever. Group II was given UVB only, group III UVB and UVA, group IV doxycycline and UVA and group V UVB, doxycyline and UVA.

Table I. *The treatment of the 5 animal groups in each of the 6 experiments*

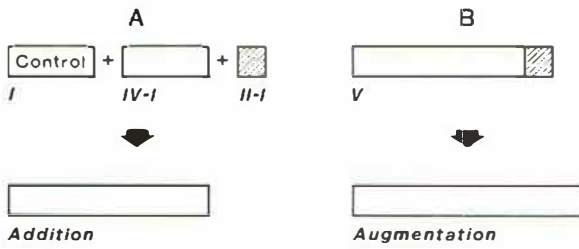|	Group no.				
	I	II	III	IV	V
Exp. 1 and 2					
Day 1 Doxycyline				+	+
Day 1 UVB (40, 20 min)		+	+		+
Day 1 UVA (5 h)	+		+	+	+
Exp. 3					
Day 1 Doxycyline				+	+
Day 1 UVA (5 h)	+		+	+	+
Day 1 UVB (40 min)		+	+		+
Exp. 4, 5 and 6					
Day 1 UVB (40, 20, 10 min)		+	+		+
Day 2 Doxycyline				+	+
Day 2 UVA (5 h)	+		+	+	+

Fig. 2. Comparison between addition of control group I wet weight, UVB-edema (filled bars) and phototoxic edema (open bars) given to separate groups of animals i.e. edema of group $[I+(IV-I)+(II-I)]$ A, compared to group V where all three components were given to one and the same animal B.

Evaluation and statistics

The animals were sacrificed 24 h after the beginning of UVA exposure. A piece of the tail was excised, weighed, dried at 110°C and weighed again. Results are presented as percent wet weight increase over controls. To determine if the inflammatory response in group V (each animal was treated with the full combination UVB, doxycycline and UVA) was greater than expected from simple addition, i.e. augmentation had taken place, this group was compared with an addition of the control group given only UVA (group I), the UVB edema (wet weight % in group II minus I) and the phototoxic edema (wet weight % in group IV minus I), for graphic explanation see Fig. 2. The comparison of group V to the added values $[I + (IV - I) + (II - I)]$ was made using Student's t-test.

RESULTS

Exposure to UVB immediately before the phototoxic reaction

In experiments 1 and 2 UVB 4.3 J/cm^2 (40 min) and 2.2 J/cm^2 (20 min) respectively was given immediately before irradiating doxycycline-treated animals with UVA 45 J/cm^2 (5 h). In experiment 1 the wet weight increase in group V was significantly higher than expected from addition (difference 3.7 percental units) (Fig. 3). In experiment 2 with the lower UVB dose there was a similar difference although not significant (1.9 percental units) (Fig. 3).

Exposure to UVB immediately after the phototoxic reaction

In experiment 3 UVB 4.3 J/cm^2 (40 min) was given immediately after UVA exposure of doxycycline-treated animals. The wet weight increase in group V was significantly higher than expected (difference 4.3 percental units) (Fig. 3).
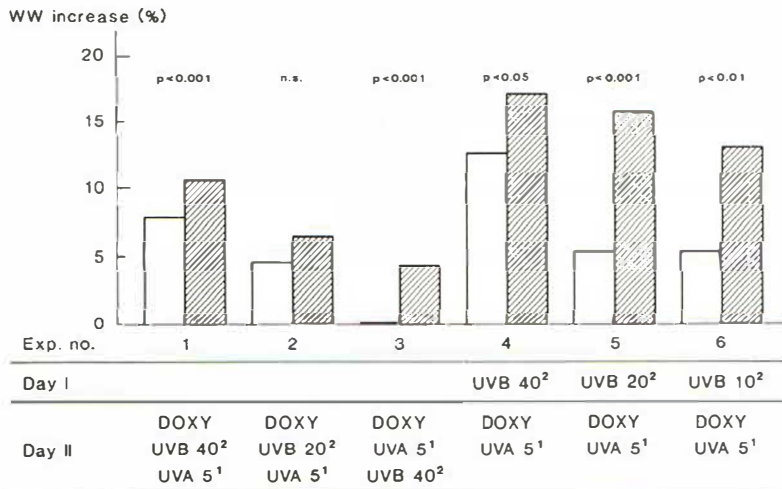

Fig. 3. Results of UVB-addition to the phototoxic reaction from doxycycline + UVA, testing the effect of varying UVB doses and different time intervals. For each of the 6 experiments a comparison between a calculated simple additive effect (open columns) and the *de facto* augmentative effect (black columns) is made. The height of the columns shows the wet weight increase over base line values. For explanation also see Fig. 2.

Exposure to UVB 24 h before the phototoxic reaction

In experiments 4, 5 and 6 UVB 4.3 J/cm^2 (40 min), 2.2 J/cm^2 (20 min) and 1.1 J/cm^2 (10 min) was given 24 h before doxycycline and UVA resulting in wet weight increases significantly higher than expected (difference 4.5, 10.3 and 7.5 percental units respectively) (Fig. 3).

In none of the 6 experiments was there demonstrated any augmentation with the combination of UVA and UVB without doxycycline (group III, Table I).

DISCUSSION

The addition of small doses of UVB to the phototoxic doxycycline reaction thus increased the inflammatory mouse tail edema more than could be explained by simple addition, i.e., a photoaugmentation was demonstrated. The difference was statistically significant in all experiments but one (no. 2, Fig. 3) where the UVB dose evidently was too low to induce augmentation. When UVB was given in immediate connection with the phototoxic reaction the UVB/UVA order was of no importance (experiments 1, 2 and 3).

Photoaugmentation with UVB in drug phototoxicity, and the insignificance of UVB/UVA order, has been shown in mice earlier with chlorpromazine, chlordiazepoxide and 8-methoxypsoralen, however UVB was given only in immediate connection with UVA in these experiments (10).

In the present experiments UVB given 24 h before the phototoxic reaction resulted in an even stronger augmentation, maximally causing a percentual wet weight increase five times higher than when the same UVB dose was given in immediate connection with UVA (Fig. 3, experiments 2 and 5). A possible explanation for this is the increased concentration of tetracycline in inflamed skin as has been reported in man (12). In this situation the augumentation thus is proposed to be a combination of the general augmentative effect independent of UVB/UVA order, seen in the other experiments (nos. 1, 2, 3) and the concentration of drug in UVB-inflamed tissue.

It is thus concluded that UVB, although unable to induce tetracycline-phototoxicity in itself, significantly modifies the phototoxic reaction from doxycycline and UVA in different ways, not only by addition but by augmentation. The lack of awareness of this phenomenon may be responsible for the difficulties in provoking tetracycline phototoxicity in humans using artificial light sources emitting in the UVA only (9, Bjellerup & Ljunggren, unpublished results).

ACKNOWLEDGEMENTS

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