

INHIBITION OF ULTRAVIOLET AND PHOTOTOXIC DERMATITIS IN THE MOUSE

Bo Ljunggren

Departments of Dermatology and Experimental Research, General Hospital, Malmö, Sweden

Abstract. The effect of inhibitors on the inflammatory oedema elicited by medium-wave ultraviolet radiation (UVB) and long-wave ultraviolet radiation (UVA) in combination with chlorpromazine has been studied in the mouse, by means of a quantitative technique. Inhibition of the UVB reaction was observed with indomethacin, acetylsalicylic acid and betamethasone valerate, whereas the latter compound only was effective in the phototoxic state. No inhibition was obtained with hydrocortisone, phenylbutazone, ϵ -aminocaproic acid, polyphloretin phosphate, clemastin, α -tocopherol or ascorbic acid. With indomethacin and betamethasone valerate there was no inhibition at high doses when the compound was administered before UVB irradiation. These results are in accordance with a proposed central role for the prostaglandins in UVB inflammation. It is suggested that the phototoxic reaction to chlorpromazine may not be due to mediator action but rather to the effect of toxic photo-products.

Key words: UVB dermatitis; Chlorpromazine phototoxicity; Inhibitors; Corticosteroids; Indomethacin; Acetylsalicylic acid

The mechanisms underlying the vascular response to ultraviolet (UV) radiation are only partly known. The inflammation may arise from a direct action on the vessel wall or from an indirect effect of chemical mediators. Most evidence at present supports the latter theory and data are accumulating, indicating that the prostaglandins may act as mediators in inflammation caused by medium-wave ultraviolet radiation (UVB) (5, 27, 28).

Phototoxic inflammation has been less studied in this respect, and here the mechanism of action seems to be more varied, depending on the type of provoking agent. For some groups of compounds, e.g. the psoralens and the porphyrins, the phototoxic targets on a cellular level are known, but in most other instances little is known about the mode of action.

One way to study these problems is with the help

of compounds blocking the effects of the proposed mediators. It was therefore considered pertinent to test a number of compounds for inhibitory capacity both on the UVB dermatitis and on a phototoxic reaction to chlorpromazine and long-wave ultraviolet radiation (UVA). A recently developed *in vivo* method based on the inflammatory oedema of mouse tail skin was used (17).

MATERIAL AND METHODS

Female albino mice (AB Anticimex, Sollentuna, Sweden) weighing around 30 g were used as experimental animals. The mouse tail technique has been described earlier (17).

Substances. The compounds tested, the dose range and mode of administration are given in Table I. Soluble compounds were dissolved in 0.5 ml sterile water and injected intraperitoneally. Irritating or poorly soluble substances were suspended in 0.5 ml of a carboxymethyl-cellulose solution and administered via a gastric tube during brief ether anaesthesia. All substances were tested in single doses, except α -tocopherol, which was given for three consecutive days before irradiation.

UVB irradiation. A standard UVB dose of 45 min exposure to two fluorescent tubes (Westinghouse Sun-lamp 40W) emitting mainly in UVB with a peak at 313 nm was used. The distance from lamp to tail skin was 12 cm and the emission at this distance 2.5 mW/cm² as measured with an Optometer UDT-40X (United Detector Technology). Irradiation was started one hour after the test compound had been administered. Compounds showing an inhibitory effect were also tested when instead given immediately after the irradiation. For indomethacin, dose-response curves with and without this compound were studied in the dose interval 22.5 to 180 min.

Chlorpromazine + UVA irradiation. Chlorpromazine (Hibernal®, AB Leo, Helsingborg, Sweden) 5 mg/kg in 0.5 ml sterile water was injected intraperitoneally just before the UVA exposure from 2 blacklight fluorescent tubes (Philips TLA 40W/08) started. The emission in the UVA range has a peak at 360 nm. The distance from lamp to tail skin was 12 cm and the output in UVA, 5.0 mW/cm² as measured with the device earlier described. The irradiation lasted for 5 hours. As in the UVB experiment, com-

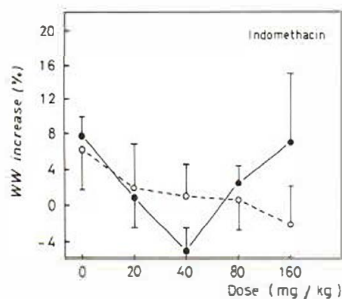


Fig. 1. The effect of indomethacin on the wet weight increase of tail tissue caused by a standard dose of UVB (45 min). Inhibitor administered 1 h before irradiation (●—●), or immediately after irradiation (○---○). At each point the mean of 5 animals and the standard deviation are indicated.

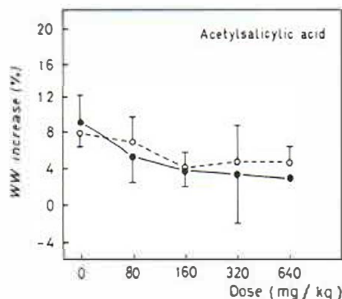


Fig. 3. The effect of acetylsalicylic acid on the wet weight increase of tail tissue caused by a standard dose of UVB (45 min). Inhibitor administered 1 h before irradiation (●—●), or immediately after irradiation (○---○). At each point the mean of 5 animals (for 640 mg/kg the point represents the only surviving animal) and the standard deviation are indicated.

pounds tested for inhibitory activity were administered one hour before the phototoxic stimulus.

Evaluation. Non-irradiated, non-medicated animals and animals treated only with the vehicle and irradiated, served as controls. For indomethacin, acetylsalicylic acid and betamethasone medicated, non-irradiated animals were also studied. 24 hours after commencing UV exposure the animals were killed and the wet weight of tail tissue determined. The results are presented as percentage wet weight increase over non-irradiated controls. All mean values are obtained from groups of at least 5 animals. Student's *t*-test was used for the statistical evaluation, and dose-response curves were treated by regression analysis.

Spectrophotometry. The absorption spectra of indomethacin, acetylsalicylic acid and betamethasone were studied with a Beckman DB-GT spectrophotometer in 5.0×10^{-5} M solutions in ethanol/water.

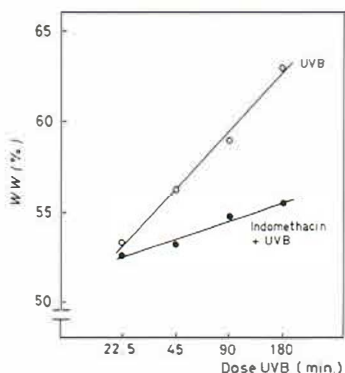


Fig. 2. Dose-response regression lines for UVB in the dose interval 22.5 min to 180 min with and without indomethacin 40 mg/kg administered 1 h before irradiation. Response expressed as percent wet weight of tail tissue.

RESULTS

UVB. The inflammatory oedema was strongly inhibited by indomethacin. The effect was maximal for 40 mg/kg ($p < 0.001$) (Fig. 1). The regression line of the dose-response curve for UVB at 24 hours was skewed to the right and the slope was lowered ($p < 0.001$) when indomethacin 40 mg/kg was given before irradiation (Fig. 2). Both acetylsalicylic acid ($p < 0.02$ for 160 mg/kg) (Fig. 3) and betamethasone ($p < 0.02$ for 10 mg/kg) (Fig. 4a) also inhibited the reaction. Medicated, non-irradiated animals did not differ in wet weight from non-medicated, non-irradiated animals. For indomethacin and betamethasone, decreasing inhibition was noted at high dosages ($p < 0.001$ and $p < 0.01$ respectively). When these compounds were instead given immediately after the irradiation, this declining inhibitory effect did not appear. For acetylsalicylic acid no difference in effect was seen when the substance was administered before or after UVB irradiation. The other compounds studied had no statistically significant effect on the UVB dermatitis in the dose range tested (Table I).

UVA. A significant inhibition of the phototoxic reaction was seen only with betamethasone ($p < 0.05$ for 10 mg/kg) (Fig. 4b). The effect was maintained at high dosages. All other compounds were negative in the dose range tested (Table I).

Spectrophotometry. Indomethacin and acetylsalicylic acid absorbed considerably in UVB, whereas betamethasone was not absorbent in this wavelength range or in UVA (Fig. 5).

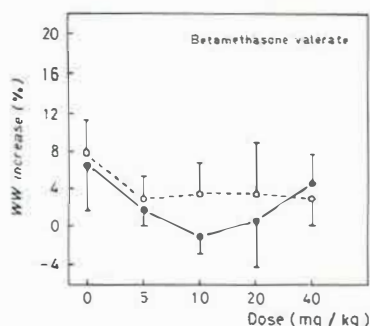


Fig. 4a. The effect of betamethasone valerate on the wet weight increase of tail tissue caused by a standard dose of UVB (45 min). Inhibitor administered 1 h before irradiation (—●—), or immediately after irradiation (---○---). At each point the mean of 5 animals and the standard deviation are indicated.

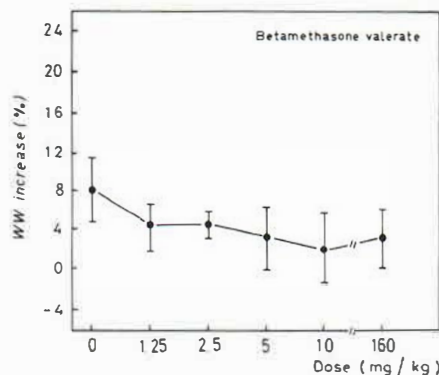


Fig. 4b. The effect of betamethasone valerate on the wet weight increase of tail tissue caused by chlorpromazine 5 mg/kg and UVA 5 h. Inhibitor administered 1 h before irradiation. At each point the mean of 5 animals and the standard deviation are indicated.

DISCUSSION

The mouse tail technique has been shown to be suitable for quantitative studies on phototoxic reactions to various agents (20). Recently the method has also been applied to UVB dermatitis and such parameters as time course and dose-response relationships described (19). In order to investigate the effects of various inhibitory compounds on UVB inflammation a standard UVB dose causing a moderate wet weight increase of 6–8% was chosen. This was achieved with exposure for 45 minutes and sacrifice after 24 hours. Sacrifice after 48 hours would have been optimal for this UV dose, but 24 hours was chosen for practical reasons, and the

difference in response in this dose range has been shown to be small (19). Administration of the inhibitors prior to UV exposure was preferred in order to detect prophylactic activity due to UV absorption for example as well as curative effects. When a modified response was seen, the compound was in most cases also given after irradiation, in order to exclude this protective component.

Indomethacin and acetylsalicylic acid both act on prostaglandin synthesis by inhibiting the enzyme prostaglandin synthetase (7, 31). Indomethacin has been shown to block ultraviolet erythema in man (8, 10, 13, 27), in the guinea pig (27) and in the mouse (26). Hensby et al. (10), when studying the prosta-

Table I. Compounds tested for inhibitory effect on inflammatory mouse tail oedema elicited by UVB for 45 min or by chlorpromazine (CPZ) 5 mg/kg and UVA for 5 hours

The dose range and mode of administration (p.o.=per os; i.p.=intraperitoneally) are indicated

Generic name	Trade name	Dose range mg/kg	Admin.	Effect on	
				UVB	CPZ+UVA
Indomethacin	Indomee (MSD)	20–160	p.o.	+	—
Acetylsalicylic acid	Aspirin (Bayer)	80–640	p.o.	+	—
Betamethasone valerate	Betnovate (Glaxo)	1.25–160	p.o.	+	+
Hydrocortisone	Solu-Cortef (Upjohn)	20–160	i.p.	—	—
Polyphlorethin phosphate ^a		80–640	i.p.	—	—
Phenylbutazone	Butazolidin (Geigy)	20–160	i.p.	—	—
ϵ -aminocaproic acid	Epsikapron (Kabi)	160–1 280	p.o.	—	—
Clemastin	Tavegil (Sandoz)	2.5–20	i.p.	—	—
α -tocopherol		5–40 ^b	i.p.	—	—
Ascorbic acid		80–640	p.o.	—	—

^a Kindly supplied by AB Leo, Helsingborg, Sweden.

^b Administered on 3 consecutive days before irradiation.

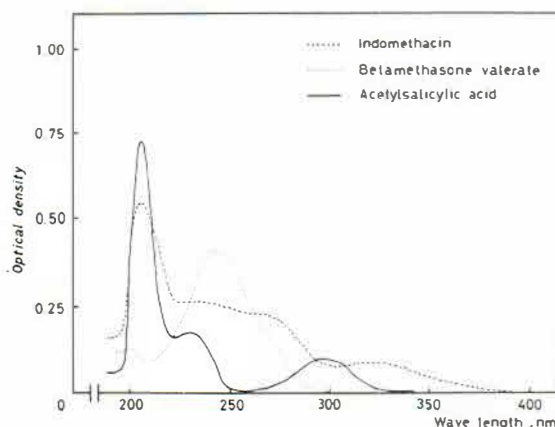


Fig. 5. Ultraviolet absorption curves of 5.0×10^{-5} M ethanol/water solutions of indomethacin, acetylsalicylic acid and betamethasone valerate.

glandin content of suction bullae fluid after UVB irradiation, noted elevated prostaglandin levels after 24 hours but not after 48 hours when erythema was still at maximum intensity. This would appear to suggest that these mediators alone cannot account for the inflammatory response to UVB. In the present study strong inhibition was obtained with indomethacin in the dose range 20–80 mg/kg. With doses in excess of 40 mg/kg the inhibitory effect was successively lost (Fig. 1). When indomethacin was given immediately after irradiation, this behaviour at high doses did not occur and instead the inhibition increased gradually with the dose (Fig. 1). There is no explanation for this phenomenon at present. Part of the prophylactic effect of the compound may be a result of filtration, since indomethacin has a considerable absorptive effect in UVB (Fig. 5). Dose–response curves with and without indomethacin show a skewing of the curve to the right and a shallower slope of the regression line with the inhibitor present, suggesting a mechanism of action other than mere competitive antagonism (Fig. 2).

Acetylsalicylic acid has been demonstrated to inhibit UV erythema in man (8, 27), in the guinea pig (1, 15, 27, 33) and in the mouse (26). The potency of this compound is however weaker than that of indomethacin (27). This was confirmed in the mouse, where inhibition was found only with doses in excess of 160 mg/kg (Fig. 3). No change in activity occurred when acetylsalicylic acid was instead given after irradiation, indicating that the prophylactic effect due to absorption seems to be of minor

importance in spite of the absorption in UVB demonstrable in vitro (Fig. 5).

Much experimental work has been devoted to the influence of corticosteroids on UV inflammation. Their mode of action is not clear, and probably several mechanisms work together. Stabilization of membranes, especially of lysosomes (32), interference with prostaglandin action (2, 24) and vasoconstriction (23) are some effects which may be relevant. Most workers could confirm an inhibitory effect in man (11, 13, 16) whereas animal studies have been negative for the guinea pig (3, 15, 27, 33) and for the mouse, where Sim (26) tested cortisone and prednisolone with no effect.

In the present study hydrocortisone and betamethasone were both tested. Inhibition was seen with the more potent fluorinated compound, while hydrocortisone was quite without effect in doses up to 160 mg/kg (Fig. 4a) (Table I). With betamethasone, as with indomethacin, the inhibitory effect decreased when doses in excess of the optimal were used. When the compound was administered after irradiation this behaviour at a high dosage did not occur. No filtration effects are to be expected, since betamethasone does not absorb in the UVB range (Fig. 5).

Polyphlorethin phosphate exercises prostaglandin blocking activity, probably on the basis of reversible competitive antagonism (6). This compound was shown to inhibit the erythema reaction to intracutaneously injected prostaglandin when administered locally but not when given systemically (29). No significant inhibition of the UVB oedema by polyphlorethin phosphate was observed in this study (Table I), possibly due to inadequate tissue levels; a single dose 24 hours before sacrifice is probably not optimal for this compound.

Phenylbutazone has been shown to inhibit UV dermatitis in the guinea pig (1, 3, 15, 33) after both systemic and topical administration. In the mouse, Sim (26) found this compound effective in doses from 50–100 mg/kg when given 45 min before bluing 24 hours after irradiation. In this study phenylbutazone in single doses up to 160 mg/kg failed to inhibit the UVB oedema when administered one hour before irradiation (Table I).

The finding of Logan & Wilhelm (22) that ϵ -aminocaproic acid has no effect on the late response to UV irradiation, was confirmed by the present findings (Table I).

Antihistamines have been shown not to influence

the late UV-response, either in man or in the experimental animal (25). The antihistamine clemastin had no effect on the late inflammatory response in the mouse (Table I). The early UV response (21) can be blocked by antihistamines in the guinea pig but not in the rat or rabbit (22). Apparently, only the early response is mediated by histamine in certain species.

The anti-oxidant α -tocopherol is capable of preventing red cell hemolysis in vitro (30). No prophylactic effects of this agent in repeated doses, or of ascorbic acid which, because of the formation of a reversible oxidation-reduction system may act as a free radical trap, were seen with the present technique (Table I). Chan & Black, in an in vitro study using short-wave ultraviolet irradiation, found no protective effect with these agents, whereas cell cytotoxicity could be reversed when the compounds were present during the 8 days of incubation after irradiation (4).

Summing up, the results on UVB oedema with the inhibitors tested seem to be in accordance with a proposed central role for the prostaglandins in this type of inflammation.

Pharmacologic inhibition of a phototoxic dermatitis has not been so thoroughly studied. Phototoxic psoralen dermatitis in man was not influenced by indomethacin either systemically or topically (9) or by topical hydrocortisone or betamethasone (16). Kaidbey & Kligman (14) were not able to block phototoxic tar dermatitis with topical fluocinonide or systemical triamcinolone. By contrast, in the present investigation, betamethasone was effective in modifying the phototoxic response to chlorpromazine (Fig. 4b). This discrepancy could be due to species differences, but varying mechanisms of action of these compounds might also be the explanation. Hunter et al. (12) studied chlorpromazine phototoxicity in the mouse with various inhibitors but were unable to establish that any of the agents tested systemically had an inhibitory effect. However, the only corticosteroid assayed was hydrocortisone.

The mode of action of chlorpromazine in the phototoxic process has not been clarified. Recently, in vivo studies have produced evidence that the effect may be attributable to toxic and remarkably stable photoproducts (18). In the present study only betamethasone significantly inhibited the phototoxic reaction to chlorpromazine. All other compounds tested, including the prostaglandin an-

tagonists, were ineffective (Table I), but this is perhaps only to be expected if the reaction is elicited by toxic photoproducts rather than by pharmacological mediators.

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B. Ljunggren, M.D.
Department of Dermatology
General Hospital
S-241 01 Malmö
Sweden