

DRUG PHOTOTOXICITY IN MICE

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Abstract. A series of agents with alleged photosensitizing properties has been studied in mice by a quantitative in vivo method for acute drug phototoxicity. The method, originally developed for phenothiazine studies, was found suitable for several drug groups with varying mechanism of action, such as tetracyclines, protoporphyrin and psoralens. In the sulfa group only sulfanilamide was active, although weakly. Phototoxicity in vivo, hitherto not demonstrated, was observed with griseofulvin, nalidixic acid, imperatorin, kynurenic acid and amiodarone.

Key words: Phototoxicity; Ultraviolet irradiation

Drugs: Sulfonamides; Diazepins; Tetracyclines; Griseofulvin; Nalidixic acid; Psoralens; Protoporphyrin; Kynurenic acid; Amiodarone; Cyclamate

A wide range of drugs induce side-effects with clinical signs of photosensitivity. In the individual case it is often difficult to elucidate the eliciting mechanism, and in recent reviews (25, 26) these agents are sometimes referred to as phototoxic, sometimes as photoallergic. In the present work, several compounds were studied, regarding their phototoxic capacity, by a new quantitative in vivo method. The technique proved highly sensitive in a study with chlorpromazine and related tricyclic drugs (19).

MATERIAL AND METHODS

The in vivo method for the study of acute drug phototoxicity in mice has been described recently (18). Most drugs were dissolved in water and injected intraperitoneally (i.p.). Female albino mice weighing about 30 g were supplied by Anticimex, Sollentuna, Sweden. Some drugs were also administered by gastric tube (p.o.) after suspension in a cellulose solution (sodium carboxymethylcellulose 7.5 g, benzyl alcohol 9 ml, sodium chloride 5.7 g, Tween 80 0.4 ml and distilled water ad 1000 ml). Hereby, the animals were given a brief aether anaesthesia.

This method was also used when testing poorly soluble drugs. Control animals received the vehicle only.

Immediately after this single dose the tails of the animals were exposed to longwave ultraviolet radiation (UVA) for 5 hours with two blacklight tubes (Philips TL 40 W/08) at a distance of 12 cm. The peak of the emission is at 355 nm. The average intensity of radiation was 5.0 mW/cm² sec at a distance of 12 cm as measured with an optometer UDT-40X from United Detector Technology. The animals were sacrificed 24 hrs after the UV exposure was started in all instances except when the psoralens were studied. Here the animals were sacrificed after 48 hrs. The degree of phototoxic inflammation was calculated on the basis of wet weight increase of the mouse tail. The animal was killed by a blow on the head and about 2 cm of the proximal part of the tail excised. Water content was calculated by weighing fresh and repeating after drying at 110°C to constant weight.

In the case of demethylchlortetracycline and of amiodarone the drug was administered not only in a single dose but also on 4 consecutive days followed by a single UVA exposure on the fourth day immediately after the last dose. In the case of sulfa-isodimidine and chlorothiazide, medium-wave (UVB) radiation was also tested, using two 40 W fluorescent tubes (Westinghouse Sun Lamp) for 45 min at 12 cm; the measured average intensity of radiation at this distance in UVB was 2.5 mW/cm² sec, using the same measuring device as above. In one experiment this radiation source was also used with demethylchlortetracycline.

The minimum effective dose was expressed as a significantly increased wet weight over that in control animals treated with the drug but not irradiated. This dose was calculated on the basis of mean values from usually 5 (in a few cases 10) animals. The Student's *t*-test was used and a statistically significant difference vis-à-vis control of $p=0.05$ or less was accepted.

RESULTS

The minimum effective doses of the various agents and using the present method for acute drug phototoxicity are given in Table I. As can be seen, a dose of 600 mg/kg sulfanilamide was required for a

Table I. Drug phototoxicity in the mouse

The minimum effective dose, the mean wet weight (WW) increase, the standard deviation (S.D.) and the degree of significance at this dose level (p) is indicated. In negative cases, the dose range tested is indicated. The result with chlorpromazine is taken from an earlier work (18). i.p. = Intraperitoneally, p.o. = per os

	Min. effect dose (mg/kg)	WW increase (%)	S.D. \pm	p	Dose range tested (mg/kg)
Sulfanilamide p.o.	600	3.0	0.72	<0.02	
Sulfa-isodimidine i.p., p.o.	—	—	—	—	200–2 400
Chlorothiazide p.o.	—	—	—	—	600–1 200
Tolbutamide i.p., p.o.	—	—	—	—	600
Chlorpromazine i.p.	2.5	5.4	2.58	<0.05	
Chlordiazepoxide i.p.	20	4.0	0.34	<0.001	—
Diazepam i.p.	—	—	—	—	10–80
Demethylchlortetracycline i.p.	100	8.1	1.43	<0.01	
Demethylchlortetracycline p.o.	—	—	—	—	200 ^a
Doxycycline p.o.	50	4.9	2.40	<0.05	
Doxycycline i.p.	50	3.5	1.12	<0.02	
Lymecycline i.p.	200	5.8	1.39	<0.05	
Chlortetracycline i.p.	—	—	—	—	50–200
Oxytetracycline i.p.	—	—	—	—	100–200
Tetracycline i.p.	—	—	—	—	25–200
Methacycline i.p.	—	—	—	—	200–400
Minocycline i.p.	—	—	—	—	200
Clomocycline i.p.	—	—	—	—	200
Griseofulvin p.o.	200	7.7	2.75	<0.05	
Nalidixic acid p.o.	50	5.5	1.50	<0.05	
Hydroxynalidixic acid p.o.	25	3.0	1.09	<0.05	
8-methoxypsoralen p.o.	10	32.7	3.48	<0.001	
5-methoxypsoralen p.o.	10	14.3	4.84	<0.02	
Imperatorin p.o.	40	9.6	2.50	<0.01	
Trimethylpsoralen p.o.	40	28.4	9.96	<0.02	
Protoporphyrin i.p.	10	6.6	2.05	<0.05	
Kynurenic acid p.o.	800	5.8	2.18	<0.05	
Amiodarone p.o.	400 ^a	6.8	2.16	<0.01	
Amiodarone p.o.	—	—	—	—	50–800
Cyclamate i.p.	—	—	—	—	1 600

^a Given for 4 consecutive days.

phototoxic response, while the test was entirely negative for sulfa-isodimidine, chlorothiazide and tolbutamide. Of the two diazepam tested, chlordiazepoxide was phototoxic, while diazepam was not (Fig. 1A). Among the tetracyclines tested intraperitoneally, demethylchlortetracycline, doxycycline and lymecycline were found phototoxic (Fig. 1B). Doxycycline was also active by the oral route, not so demethylchlortetracycline, even after repeated doses. Phototoxic responses were also obtained with griseofulvin (Fig. 1D), nalidixic acid and hydroxynalidixic acid (Fig. 1F), the four psoralens tested (Table II and Fig. 2), protoporphyrin (Fig. 1E) and kynurenic acid. Amiodarone was phototoxic after repeated doses only.

UVB alone induced an inflammatory response of $9.2 \pm 4.7\%$ (mean \pm S.D.) wet weight increase. Pretreatment with sulfa-isodimidine 600 mg/kg p.o.

or i.p., as well as with chlorothiazide 400 mg/kg p.o., did not further augment this inflammatory reaction. Actually, with sulfa-isodimidine and UVB a significantly lower response was obtained, $2.5 \pm 3.3\%$ and $3.3 \pm 2.3\%$ wet weight increase for p.o. and i.p. (both $p < 0.05$) resp., than with UVB alone. The degree of inflammatory edema induced by UVB was not influenced by pretreatment with demethylchlortetracycline (Fig. 1C).

DISCUSSION

The sulfa group. It is generally agreed that phototoxic reactions are provoked by longwave ultraviolet radiation and all our positive results were obtained with this wavelength range. Since sulfa-isodimidine and chlorothiazide did not show any phototoxic activity with UVA the drugs were also

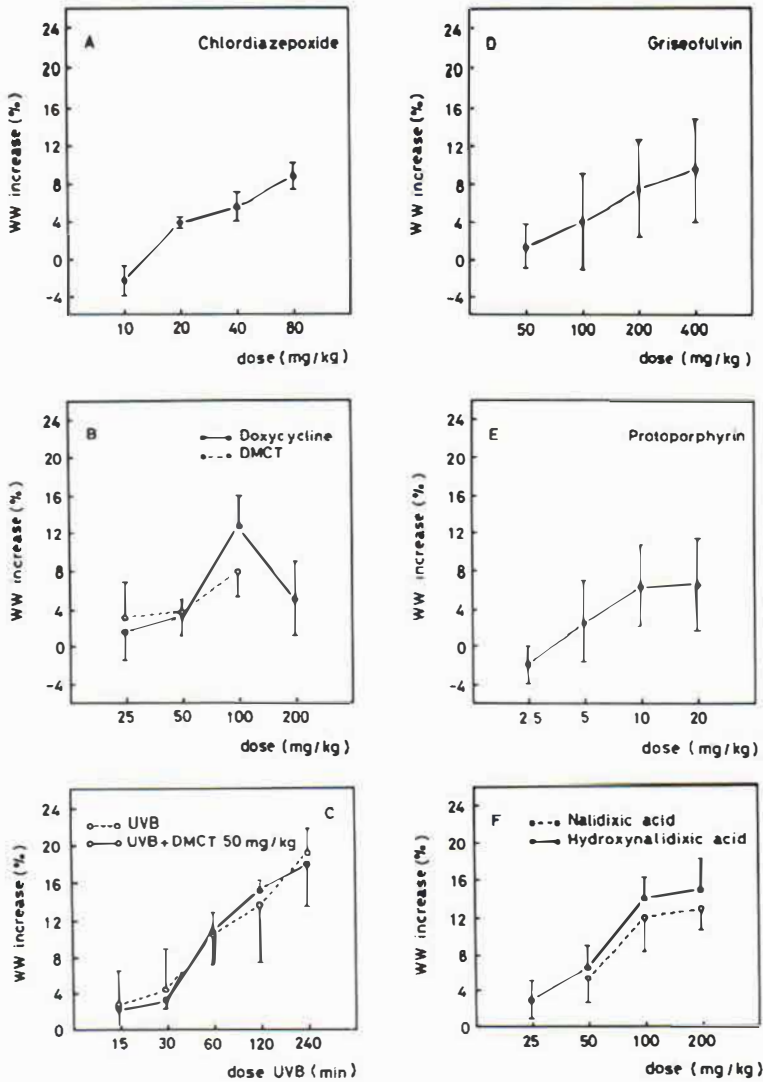


Fig. 1 A–F. The inflammatory response as expressed in mean wet weight (WW) increase of mouse tail after treatment with different drugs and UVA (for 1 C: UVB). DMCT, demethylchlortetracycline. Vertical range bars indicate standard deviation.

tested with UVB—with negative results, however. Sulfa-isodimidine even showed photoprotective properties under these conditions.

In the literature, sulfanilamide is indicated as a phototoxic as well as a photoallergic drug. Several authors have induced a phototoxic response in man by intracutaneous administration (6) but it was not achieved orally in mice (30). In the present study, sulfanilamide was found weakly phototoxic—the high dose of 600 mg/kg was required—although it was the strongest of six sulfa drugs tested intracutaneously by Burckhardt (4). On the basis of these results we conclude that the majority of cutaneous photoreactions reported with sulfa drugs hardly are of a phototoxic nature.

Diazepins. Chlordiazepoxide was found highly phototoxic (Fig. 1 A) in agreement with earlier results in mice (13). The test was negative with diazepam when tested in the highest non-lethal dose. This difference between the two drugs in the experimental animal seems to be in accordance with clinical experience (35).

Tetracyclines. Among the tetracyclines tested, demethylchlortetracycline was very potent, which is in accordance with clinical and experimental experience (15). This result was achieved by i.p. administration only; by the oral route no phototoxicity could be induced, thus agreeing with earlier observations in mice (30). The same potency in this model was demonstrated by doxycycline, working

Table II. Phototoxic activity of 4 psoralens as expressed in wet weight of mouse tail tissue

Mean and standard deviation

Dose (mg/kg)	Compound			
	8-methoxypsoralen	5-methoxypsoralen	Imperatorin	Trimethylpsoralen
2.5	51.1 ± 1.29			
5	52.6 ± 2.14	52.1 ± 0.64		
10	67.8 ± 3.48 ^c	58.4 ± 4.84 ^a		51.5 ± 1.88
20	66.7 ± 3.02 ^c	71.9 ± 1.83 ^c	52.4 ± 1.27	51.5 ± 0.65
40	63.2 ± 4.15 ^c	68.9 ± 1.71 ^c	56.0 ± 2.50 ^b	64.6 ± 9.96 ^a
80	63.6 ± 4.47 ^c	64.7 ± 5.26 ^c	61.3 ± 3.45 ^c	57.0 ± 1.94 ^c
160			68.0 ± 4.28 ^c	

Statistical difference vs. irradiated, non-medicated animals: ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$.

in roughly equal doses by both routes (Fig. 1B). Doxycycline has been reported to be phototoxic also in man (2, 10). Its oral effect may be explained by good penetration properties which also may contribute to the phototoxic activity of lymecycline. The other tetracyclines, tested in sublethal doses, were not phototoxic with the present method although a few isolated clinical reactions with e.g. chlortetracycline and tetracycline, have been reported (29). Methacycline and minocycline were not phototoxic in our model and provocation studies in man have been negative (9, 10).

The difference in potency between chlortetracycline

and its demethylated equivalent, demethylchlortetracycline, is in accordance with earlier findings within the phenothiazine group (20). The enhancing effect from tetracycline to chlortetracycline also agrees with observations on the importance of chlorine substitution (19).

The frequency of clinical photodermatitis to demethylchlortetracycline is fairly high but in the present model much stronger doses were required than with chlorpromazine, for instance. It is possible that the mouse tail technique, based on an increase in vascular permeability, is not optimal for this group of drugs. In fact, by observation of tail erythema at 8 hours with the naked eye a phototoxic reaction was observed at 1/4 of the dose required for inducing a wet weight increase (unpublished observation). This lower dose level was also obtained by Ison & Blank (12) when observing ear and tail erythema in mice injected with demethylchlortetracycline.

The phototoxic responses with tetracyclines were obtained with UVA, thus confirming earlier statements (12, 15, 30). Since some authors claim the action spectrum to be in the UVB (25, 26), this wavelength range too was studied. The results were entirely negative (Fig. 1C) which confirms observations with monochromatic radiation (30).

Griseofulvin. This antimycotic only rarely causes clinical light sensitivity and phototoxic reactions have been difficult to induce in the experimental animal (13, 16). In vitro, it was negative with *Candida albicans* (5) but positive in a photohemolytic system (14). Griseofulvin was clearly phototoxic in the present study (Fig. 1D). This acute reaction after a single dose can hardly be explained by an interference in porphyrin metabolism (28).

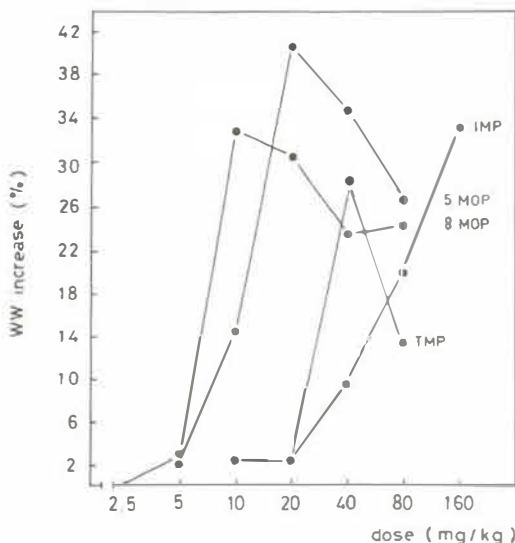


Fig. 2. Phototoxic effect of 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), trimethylpsoralen (TMP) and imperatorin (IMP) as expressed in mean wet weight (WW) increase of mouse tail.

Nalidixic acid. During treatment with this drug for urinary tract infections bullous lesions may occur on light-exposed areas. The nature of this side-effect is not clearly understood. In man, experimental phototoxicity has not been demonstrated after topical application, and not conclusively after oral intake (27). Tests were negative in hairless mice by the intracutaneous and the intraperitoneal route (27) but the drug was phototoxic in a *Candida albicans* system (21).

We found nalidixic acid phototoxic after oral ingestion in albino mice. The minimum effective dose was similar to that of the most potent tetracyclines (Table I). Nalidixic acid is mainly metabolized to a 7-hydroxymethyl product (22) which was available for the present study. The metabolite was phototoxically active in the same dose range as nalidixic acid (Fig. 1F). It is thus possible that the 7-hydroxy metabolite contributes to the phototoxic activity of the drug.

Psoralens. The phototoxic potency of oral psoralens was easily confirmed with the present method in about the same dose range as earlier shown in albino mice by the i.p. route (13). Also confirming clinical experience (33) and experimental findings (24, 34) 8-methoxypsoralen was found more phototoxic than trimethylpsoralen by oral administration (Table I). Also 5-methoxypsoralen was found to be stronger than trimethylpsoralen. A phototoxic reaction to imperatorin, hitherto not demonstrated, was also observed, though weaker than that of the other three psoralens (Table II).

The dose-response curves differed from those of other compounds tested by being very steep (Fig. 2). In higher doses there is a decrease in phototoxic activity similar to that observed with chlorpromazine and some of its metabolites (20); a photoprotective mechanism may explain this phenomenon.

Protoporphyrin. The phototoxic capacity of porphyrins, demonstrated for hematoporphyrin at the beginning of this century (11), was confirmed for protoporphyrin with the present technique (Fig. 1E).

Kynurenic acid. This tryptophan metabolite has been shown to be phototoxic in a hemolytic system (32). Kynurenic acid was now found to be active also in vivo (Table I).

Amiodarone. This diiodinated benzofuran derivative used in coronary diseases has been reported mainly in French literature to cause bluish-grey

pigmentations on light-exposed skin, sometimes preceded by photosensitivity (1, 7, 8). Experimental photosensitization has not been demonstrated earlier and our single-dose test proved negative. When, however, amiodarone was given for 4 consecutive days the drug was found to be phototoxic (Table I), thus possibly demonstrating that amiodarone has slow resorption and skin uptake properties (3).

Cyclamate. Only a few reports on photodermatitis due to this sweetening agent have appeared (17). The reactions have been assumed to be photoallergic, which might agree with our negative results, even at high doses (Table I), for phototoxicity.

General considerations. It should be pointed out that the results presented were obtained in an experimental animal and might not necessarily hold true for the conditions in man. The mouse tail technique has, however, been used for demonstrating phototoxicity of different phenothiazines and related tricyclic drugs (19, 20) with a fairly good correlation between clinical reports and experimental findings. In the present work, this in vivo method was found suitable also when studying other drugs with alleged photosensitizing properties. It was found applicable both to agents probably working by membrane damage, such as protoporphyrin (31), and, as is the case with psoralens (23), to compounds acting on nuclear DNA.

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