

Phototoxic Reaction to Long-term Low-dose Administration of Chlorpromazine in Mice

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Abstract. The mouse tail *in vivo* technique for the study of acute drug phototoxicity was tested in experiments involving prolonged administration of chlorpromazine and ultraviolet light. A phototoxic reaction was induced with lower combined doses of drug and radiation than was the case in single exposures.

Key words: Ultraviolet light; Phototoxicity; Chlorpromazine; Mouse

An experimental method was earlier devised for the study of drug phototoxicity (3). In this method the phototoxic response was evaluated on the basis of wet weight increase of the mouse tail after a single administration of drug and longwave ultraviolet light. Chlorpromazine served as test compound and the minimum dose inducing a phototoxic reaction was 2.5 mg/kg together with 5 hours of blacklight irradiation.

Although the mouse tail technique has proved to be suitable for the study of several phototoxic drugs (4) this single administration procedure differs from the clinical, usually extended-type of medication. We have therefore attempted to construct an *in vivo* model for the study of long-term phototoxicity. In such a model it might even be possible to elicit phototoxic reactions with lower doses of drug and/or radiation than in the single-dose type of treatment.

MATERIAL AND METHODS

Female albino mice (Anticimex, Sollentuna, Sweden) weighing about 30 g were injected intraperitoneally with chlorpromazine chloride (CPZ) ("Hibernal", AB Leo, Helsingborg, Sweden). The tails of the animals were exposed on each injection day to longwave ultraviolet light (UVA) from two blacklight tubes (Philips TL 40W/08) at a distance of 12 cm as described earlier (3). The measured average intensity of radiation was 5.0 mW/cm²/sec.

Erythema of the mouse tails was recorded by a person not knowing the pretreatment of the individual animals. After sacrificing the animals the degree of phototoxic in-

flammation was calculated on the basis of wet weight increase of the tail tissue. An adjacent piece of tail was taken for histological examination. All evaluations took place 24 hours after the last stimulus. For the statistical analysis, Student's *t*-test was used throughout.

EXPERIMENTS

A. Can a phototoxic reaction be induced with a sub-threshold dose of CPZ if this is repeated? CPZ was given in a dose of 1.0 mg/kg to 10 mice which were exposed immediately thereafter to UVA for the regular 5 h. The procedure was repeated on 10 consecutive days. 10 animals receiving CPZ but not UVA served as controls.

Result. There was no phototoxic reaction: 51.4% wet weight ± 1.17 (mean $\pm$ S.D.) vs. 51.9 ± 1.17 in controls.

B. Can a phototoxic reaction be induced with a sub-threshold dose of UVA if this is repeated? CPZ was given in a dose of 5.0 mg/kg to 10 mice which were exposed immediately thereafter to UVA for 1 h only. The procedure was repeated on 10 consecutive days. 10 animals receiving CPZ but not UVA served as controls.

Result. There was no phototoxic reaction as judged on the basis of edematous response: 51.7% ± 1.24 vs. 52.3 ± 2.00 in controls. However, when comparing the two groups of animals with the naked eye, there was a slight erythema in the group that had received both CPZ and UVA.

C. Is the acute phototoxic reaction altered by extended low-dose pretreatment? CPZ was given in a dose of 5.0 mg/kg to 10 mice which were exposed immediately thereafter to UVA for 1 h. The procedure was repeated on 9 consecutive days. On the tenth day the animals obtained the same CPZ dose, but UVA for 5 h. The result in this group was compared with that obtained in 5 animals which received the tenth day treatment only.

Result. A phototoxic reaction was registered in both groups, and the one pretreated did not differ from that receiving a single dose only (55.1% ± 1.40 vs. 54.2 ± 2.28).

D. Can a phototoxic reaction be induced with the sub-threshold dose of UVA if this is repeated for even longer? CPZ was given in a dose of 5.0 mg/kg to 10 mice which were exposed immediately thereafter to UVA for 1 h. The procedure was repeated on 17 consecutive days. 10 animals which received UVA but not CPZ served as controls.

Result. There was a phototoxic reaction in the CPZ+UVA group (wet weight % 52.8 ± 0.91) compared with the UVA group (51.2 ± 1.00). The difference was significant ($p < 0.01$). There was a tail erythema in the CPZ+UVA group in 10/10 animals, in the UVA group in 2/10 animals.

In another experiment CPZ was given in a dose of 1.0 (group II) and 5.0 (group III) mg/kg, each group containing 10 animals. Four hours after the injection¹ the mice were

¹ This delayed exposure was chosen on the basis of a pilot study in which three groups of mice were injected once with 20 mg CPZ/kg and irradiated with one hour UVA at time 0–1 h, 2–3 h and 4–5 h, respectively. The mean wet weight in the three groups was 50.3% ± 1.11 , 50.5% ± 1.07 and 51.7% ± 1.71 . The 4–5 h group gave a significantly ($p < 0.05$) higher wet weight value than the 0–1 h group.

Table 1. Phototoxic reaction in the mouse tail to long-term low-dose treatment with CPZ+UVA

	Groups			
	I	II	III	IV
CPZ, mg/kg/day	0 ^a	1.0	5.0	0 ^a
UVA, h/day	1	1	1	0
n	5	10	10	5
Mean wet weight, %	50.0	50.4	51.9	52.4
S.D. ±	1.04	0.61	0.92	0.56
S.E.M. ±	0.47	0.19	0.29	0.25
Difference against group I		$p > 0.05$	$p < 0.01$	$p < 0.01$
Tail erythema	0/5	0/10	9/10	1/5

^a Group I was injected with vehicle, group IV was not injected at all.

exposed to UVA for 1 h. CPZ injection and UVA exposure were repeated on 17 consecutive days. As controls, 5 animals received UVA and the water vehicle intraperitoneally (group I), as well as 5 animals receiving neither UVA nor any injection (group IV).

Result. (Table 1). Control animals receiving UVA and injection of vehicle had a lower wet weight than entirely untreated animals. A phototoxic reaction was provoked with a CPZ dose of 5.0 mg/kg but not with 1.0 mg/kg. Tail erythema was observed mainly in the group treated with CPZ 5.0 mg/kg. Histopathologic examination of the tails did not reveal any inflammatory reaction in any of the groups of mice.

DISCUSSION

The biological effect in phototoxic reactions is the result of the combined stimulus of the photosensitizer and the ultraviolet radiation. It has been shown for CPZ and UVA that within reasonable dose ranges a reciprocal relationship exists (2). In the present work this principle has been utilized through variations in the dosages of CPZ and UVA, respectively, while keeping the product constant.

It has been shown earlier that the minimum requirement for inducing a phototoxic edema with the mouse tail technique is 2.5 mg/kg CPZ plus 5 h UVA (3). In the present study, no phototoxic response was observed with 1 mg/kg CPZ and 5 h UVA repeated for 10 days, or the reverse treatment with 5 mg/kg CPZ and 1 h UVA (experiment A-B). There was evidently no summation of subthreshold inflammatory stimuli, nor any accumulation of the drug. This was also demonstrated in the experiment (C) in which the acute phototoxic reaction was not influenced by 10 days' pretreatment with drug and UVA.

Saunders et al. (5) performed long-term phototoxic studies with CPZ and blacklight; the response was registered as increasing wet weight of the mouse ear. Their light source seems similar to ours but with CPZ 5 mg/kg and 4 h UVA/day they failed to produce a detectable effect after 3 weeks of treatment. However, with increasing daily CPZ doses up to 15 mg/kg they obtained a wet weight increase, from 54.8% to 61.7% after 4 weeks. The lack of phototoxic reaction with the lower dosage may be explained by irradiation conditions not ensuring a continuous light exposure.

In the present study, a subthreshold phototoxic stimulus for 10 consecutive days did not induce any edema of the mouse tail, but a slight erythema was observed (experiment B). Therefore, in another experiment (D) the stimulation was extended to 17 days which resulted in erythema as well as edema. This erythema was not caused by UVA exposure only (Table 1). It was shown, however, that it is possible to induce a phototoxic reaction with repeated exposures to lower doses of CPZ+UVA than when this combination is given as a single treatment. The phototoxic reactions were weak in all instances, as we attempted to achieve threshold effects by using minimal stimuli in order to avoid acute phototoxic responses.

The phototoxic edema in the 17-day experiment was observed on comparison with control animals treated identically except for vehicle instead of CPZ (Table 1). The wet weight of tail tissue in entirely untreated controls (group IV) was similar to that in phototoxic animals (group III). Thus, a phototoxic reaction would not have been revealed if proper controls had not been used. Apparently, the daily treatment with intraperitoneal injection, fixation under the light source, and the irradiation, reduces the water content of the tail tissue. It has been shown that capillary resistance of the skin may be influenced by physical and mental long-term stress (1).

In these long-term experiments both erythema and edema could be registered. Apparently, erythema develops early and may be observed particularly in weak phototoxic reactions in which the vascular damage does not include a permeability increase which may be registered by the present technique. This may explain the erythematous rather than edematous reaction to tetracyclines arising with this technique (4).

Histopathologically, the mouse tail tissue was not

infiltrated with inflammatory cells, not even when the vascular damage had resulted in edema. This of course speaks against the possibility of an immunological basis of the long-term effect.

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