

PHOTOTOXIC REACTION TO CHLORPROMAZINE AS STUDIED WITH THE QUANTITATIVE MOUSE TAIL TECHNIQUE¹

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Abstract. A quantitative in vivo technique for the study of the phototoxic reaction to systemic chlorpromazine has been developed, based on the increasing tissue fluid content of the albino mouse tail. A maximum increase in tissue fluid of about 25% was registered 24 hours after starting the long-wave ultraviolet light exposure. The minimum phototoxic dose of chlorpromazine was 2.5 mg/kg.

Key words: Ultraviolet light; Phototoxicity; Chlorpromazine

Phototoxic dermatitis results from the action of light of certain wavelengths on a photosensitizer present in the tissue. In order to study the phototoxic potency of various drugs a quantitative method approaching in vivo conditions is required. In the following, such a technique is described, using the albino mouse as the experimental animal and systemically administered chlorpromazine as the test substance.

MATERIAL AND METHODS

The experimental animals were female albino mice (BOM/NMR1)—provided by C. W. Friis, Gl. Bomholtgård Ltd. Ry, Denmark—weight 25–35 g. The test substance, chlorpromazine chloride (CPZ)—“Hibernal”, AB Leo, Helsingborg, Sweden—dissolved in about 0.5 ml sterile water, was injected intraperitoneally in the lower left quadrant. The animals were then inserted into horizontal plastic tubes with facilities for ventilation and feeding (Fig. 1) and light exposure was immediately started in such a way that only the tails were exposed to the irradiation from above. The light source, kept at 12 cm distance from the mouse tails, consisted of two black-light tubes (Philips TLA 40 W/08) emitting in the long ultraviolet range (UVA) with a peak at 355 nm. The measured average intensity of radiation was 5.0×10^4

erg/cm²sec. Estimates of UVA flux were calculated from an optometer UDT-40X (United Detector Technology). For quantitative evaluation of the phototoxic reaction the animal was killed by a blow on the head and approximately 2 cm of the proximal part of the tail was excised. The relative water content (WW%) was calculated by weighing the piece of tail before and after drying at 110°C for 3 hours; after this period a steady state of wet weight was obtained. All mean values were obtained from groups of at least 5 animals. For the statistical analysis the Student's *t*-test was used throughout.

RESULTS

The evaporable water content of the mouse tail during phototoxic inflammation constituted the basis of the present method, by which the tissue wet weight in three different control groups was around 53% (Table I). Intraperitoneal injection of the solvent (water) plus exposure to UVA, UVA exposure alone, as well as CPZ administration without light exposure, all resulted in the same percentage wet weight.

The time course of the phototoxic reaction induced by CPZ and 5 hours' UVA is illustrated in Fig. 2. The peak intensity was reached after 24 hrs both with 5 and 20 mg/kg CPZ. The maximum mean wet weight of tail tissue increased by 5.8% and 23.6%, respectively.

With CPZ doses of 5 and 20 mg/kg and sacrifice of the animals 24 hrs after starting the UVA exposure, increasing amounts of irradiation resulted in an increased degree of inflammation up to 5 hours' exposure (Fig. 3). With even higher UVA doses a slight but significant ($p < 0.05$) decline of the curve for 20 mg/kg was observed. With 5 mg/kg the curve continued to rise during the observation period.

When varying the CPZ dose but keeping the

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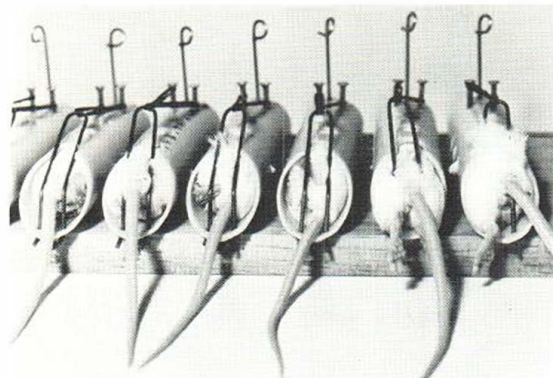


Fig. 1. Device for fixation of the mice during light exposure.

UVA exposure (5 hrs) and sacrifice (24 hrs) constant, a significant phototoxic dermatitis was induced with a lowest dose of 2.5 mg/kg (Fig. 4). A maximum value was recorded at 20 mg/kg CPZ but with 40 mg/kg a significantly ($p < 0.01$) lower figure was obtained.

The histological picture of the phototoxic reaction in the mouse tail (Fig. 5) showed a vasodilatation and intense edema in the lower part of the dermal connective tissue, with a few scattered inflammatory histiocytes and extravasating erythrocytes.

DISCUSSION

Several experimental methods for the determination of phototoxic potency are available (5), *in vitro* (e.g. yeast, photohemolysis) as well as *in vivo* (homo, guinea pig, albino and hairless mice). *In vitro* methods have proved handy, sensitive and

Table 1. Determination of the relative tissue wet weight in three different control groups of 5 animals each

Drug/Dose	0	Aq. ster.	CPZ 20 mg/kg
UVA exposure (hrs)	5	5	0
Start of exposure/sacrifice (hrs)	24	24	24
WW%	54.2	53.2	53.4
	54.1	54.6	53.8
	53.8	53.7	53.0
	51.5	51.9	52.6
	53.2	54.7	53.3
$\bar{m}WW\%$	53.4	53.6	53.2
S.D. $\pm$	1.08	1.15	0.45
S.E.M. $\pm$	0.48	0.51	0.20

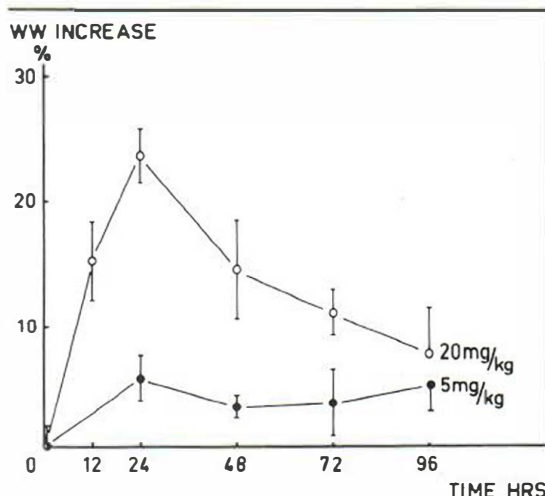


Fig. 2. Phototoxic reaction to CPZ, 5 and 20 mg/kg, and UVA 5 hours, the animals being sacrificed at 5 different time intervals. At each point the mean value from 5 animals and the standard deviation are indicated.

informative, particularly for screening studies. However, in order to approach *in vivo* conditions, animal models are preferable. Experiments in man would be desirable but Epstein (3) has shown a high frequency of photoallergic sensitization in phototoxic studies with CPZ. In the experimental animal the effect of a phototoxic substance has been registered with the naked eye as erythema, edema and/or necrosis. With the exception of an

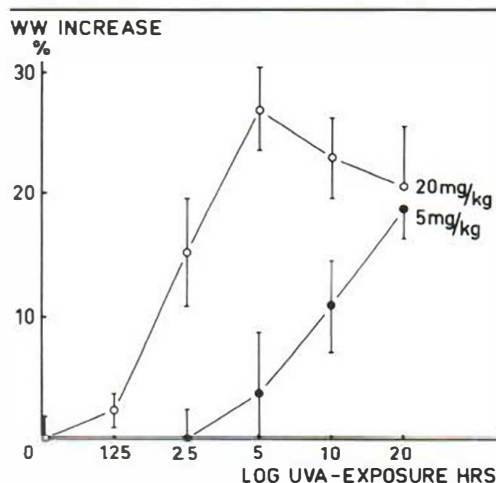


Fig. 3. Phototoxic reaction to CPZ, 5 and 20 mg/kg, in animals sacrificed after 24 hours following increasing UVA doses. At each point the mean value from 5 animals and the standard deviation are indicated.

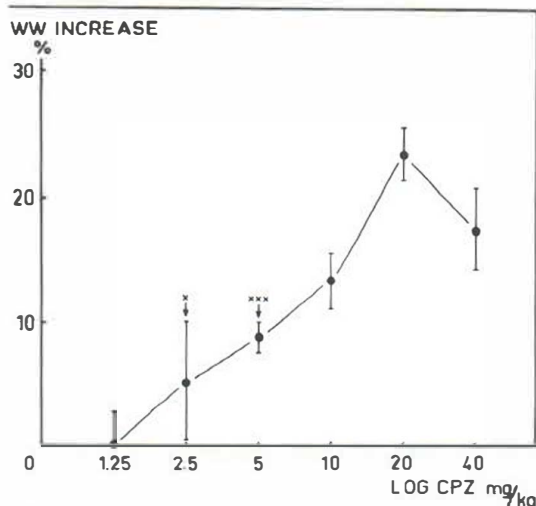


Fig. 4. Phototoxic reaction to increasing doses of CPZ in animals sacrificed after 24 hours following 5 hours UVA. At each point the mean value from 5 animals (at 20 and 40 mg/kg: 10 animals) and the standard deviation are indicated (x = $p < 0.05$; xxx = $p < 0.001$).

attempt to grade the thickness of the inflamed guinea pig ear with a micrometer (14) earlier techniques have thus comprised a qualitative and subjective evaluation.

In the present method, the ordinary albino mouse, a cheap and easily available animal, was found well suited for quantitation of the phototoxic reaction. It was possible to register objectively the inflammatory edema and the wet weight increase in tail tissue was found to be highly reproducible. It should be pointed out that in young animals the tissue water content may be a few percent higher.

Consequently, frequent use of control groups is required. Furthermore, fixation of the animal guaranteed a continuous light exposure, difficult to perform with freely moving animals. With the present experimental set-up, only the tail was irradiated, thus avoiding systemic reactions (1, 13), particularly when using strong doses. A prerequisite for this fixation was a reasonably short exposure time; this was achieved by shortening the skin/lamp distance and avoiding window-glass filtration, which was found to reduce the intensity of UVA irradiation by about 25%. It was considered unnecessary to insert a glass filter, as only 1.6% of the total output from our light source consisted of irradiation less than 320 nm. Accordingly, the control groups exposed to this light did not differ from animals kept in darkness (Table I). Furthermore, the phototoxic action spectrum for CPZ has been demonstrated to be 320–380 nm, irradiation below this range having the same effect as in untreated animals (6).

The inflammatory response induced by CPZ and UVA showed a peak intensity 24 hrs after starting the light exposure (Fig. 2). This time course differs from that observed in the phototoxic CPZ dermatitis of the guinea pig (12) which often reached a maximum after 48–72 hrs. The experimental psoralen dermatitis has also been reported to culminate late (2, 10). A retarded reaction has been observed with threshold doses of psoralen (2) which was not the case in our study with CPZ (Fig. 2). It should be pointed out that in the present work, registration is related to the increased vascular permeability, which may show a different time

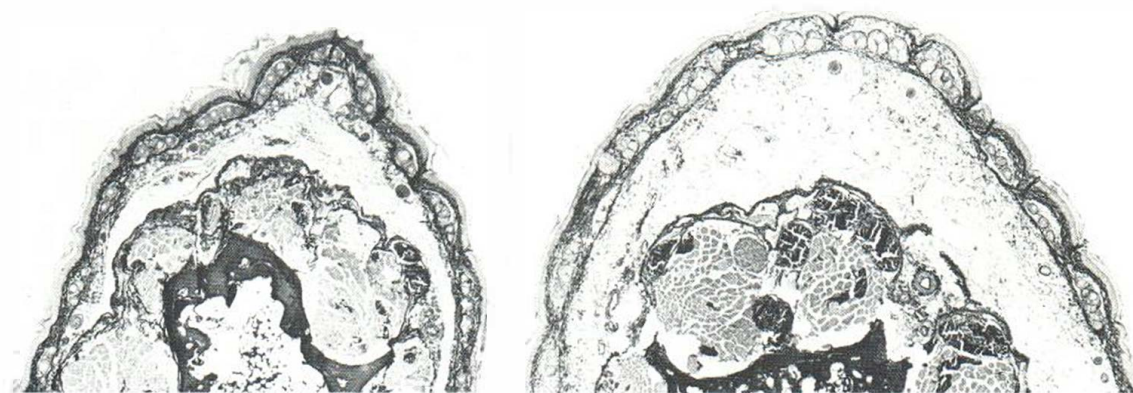


Fig. 5. Section of a mouse tail with a phototoxic reaction to CPZ (right) and from a control animal (left).

course from other inflammatory events, such as erythema, cellular infiltrate, etc.

The maximum reaction implied an increased tissue water content of 25%. This large change, also illustrated in the histologic picture (Fig. 5), should enhance the possibility of grading the phototoxic potency. The sensitivity of the present method was demonstrated by a minimum phototoxic dose of 2.5 mg/kg CPZ (Fig. 4)—markedly below earlier findings in mice (4, 7, 8, 13).

The maximum phototoxic response was obtained with a CPZ dose of 20 mg/kg and 5 hours' UVA. With 40 mg/kg a decreased reaction was registered (Fig. 4), a phenomenon that has earlier been observed with *in vitro* systems (9, 11). This cannot be explained by a central effect of CPZ, since the same decrease was achieved with prolonged light exposure and CPZ 20 mg/kg. With an even lower dose, 5 mg/kg, however, there was a steady increase in the inflammatory reaction. Thus, when testing compounds of a low phototoxic potency an extended exposure time may be required. These results support the assumption that the product of the amount of a phototoxic agent and of the irradiation dose is constant.

The present method has thus proved valuable for the study of CPZ phototoxicity and preliminary results have shown that it may be used for other phenothiazines as well. Nevertheless, its further use may be limited by such factors as toxicity and solubility of the compounds to be tested.

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