# Role of Complement in Chlorpromazine-Induced Phototoxicity

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To evaluate the role of the complement system in inflammation induced by chlorpromazine (CPZ) and ultraviolet-A (UVA) irradiation, the phototoxic response in guinea pigs decomplemented by cobra venom factor was compared with that in saline-treated animals. Phototoxic lesions were induced in animals by intradermal injections of CPZ solution, followed by UVA irradiation. Clinically, the normal animals developed erythema and induration which showed a maximal response at 10 h with a mean value of 1.6 on a scale of 0 to 3+. The complement-depleted animals showed a weaker clinical response than the normal animals 6–24 h postirradiation ($p < 0.05$). These clinical changes were associated with increased vascular permeability, as demonstrated by extravasation of i.v. injected Evans blue in saline-treated animals. In vitro UVA irradiation of serum containing CPZ resulted in a dose-dependent diminution of total complement activity. Such irradiated serum showed immunoelectrophoretic C3 conversion. These results suggest that the complement system is involved in the development of CPZ-induced phototoxic lesions. *J Invest Dermatol 86:142–144, 1986*

The phototoxic properties of chlorpromazine (CPZ) have become well known since this drug was introduced as a tranquilizer. The mechanisms of CPZ phototoxicity are not fully understood although there is evidence suggesting that its in-vivo [1] and in-vitro [2] effects upon red blood cells may be due to toxic photoproducts.

Recently, complement activation has been shown to occur in sera from patients with porphyria [3,4]. Ultraviolet-A (UVA) irradiation of guinea pig serum containing exogenous phototoxic substances, i.e., hematoporphyrin and demethylchlortetracycline, also resulted in complement activation in vitro [5,6]. However, little has been done to study the role of the complement system in such well known CPZ-induced phototoxicity.

In this study, we have evaluated the role of the complement system in CPZ-induced phototoxicity, by: (1) comparing the phototoxic response in guinea pigs decomplemented by treatment with cobra venom factor with that in saline-treated animals; and (2) assessing whether or not in-vitro activation of complement occurs after UVA irradiation in serum containing CPZ.

## MATERIALS AND METHODS

**Light Source** The light sources used were a bank of 10 black lights (Toshiba FL20SBLB; Japan) which emits rays between 300–420 nm, with a maximum at 360 nm (mainly UVA) and a bank of 5 sunlamps (Toshiba FL20SE30; Japan) which emits rays between 280–370 nm, with a maximum at 310 nm (mainly UVB). The energy intensity of the black lights and sunlamps was approximately 7.4 and 2.3 mW/cm$^2$ at a target distance of 20 cm, respectively (Radiometer UVR-305/365; Eisai Co., Japan).

**In Vitro Irradiation of Serum** Normal human sera were obtained from healthy adults on the day of the experiment. Chlorpromazine hydrochloride (Wako Pure Chemical, Japan) was dissolved in isotonic saline at a concentration of 5.0 mg/ml. One-tenth milliliter of this stock solution, or serial dilutions from it, was then added to 0.9 ml of human serum. The ratio of CPZ:serum of 1:9 was used for all the in vitro experiments. The mixtures were transferred to Petri dishes, and placed on ice 20 cm away from the light source. UVA irradiation (6.7, 13.3, and 22.2 J/cm$^2$) was performed, using 1 mm-thick window glass to eliminate wavelengths of <320 nm. To evaluate the effect of UVB irradiation, sera containing CPZ were irradiated in a similar manner with a UVB source (2.1 J/cm$^2$). After irradiation, total complement hemolytic activity ($CH_{50}$) in the sera was determined using Mayer's method [7].

**Immunoelectrophoresis** Two-dimensional immunoelectrophoresis for C3 conversion was performed on microscope slides using 1% agarose (Agarose-I; Wako Pure Chemical, Japan) in veronal buffer (pH 8.6, ionic strength = 0.025), according to the methods of Crowle [8] with minor modifications. Serum activated with zymosan (25 mg/ml of serum; Sigma Chemical Co., U.S.A.) was used as a positive control for complement activation. Goat antiserum to human C3 (Lot 0015) was purchased from Miles Laboratories, Inc., U.S.A.

**Animals** Female albino guinea pigs of the Hartley strain, weighing 300–400 g, were used throughout the experiments. The dorsal area was depilated 3 days before irradiation. Decomplementation was performed in guinea pigs by i.p. injection of 300 U/kg of cobra venom factor (Cordis Laboratories Inc., U.S.A.) 16 h before irradiation [5]. Our pilot studies before CPZ experiments demonstrated that such treatment resulted in a decrease in the total complement hemolytic activity of about 97%. Animals that were injected with saline served as controls.

**In Vivo Induction of Localized Phototoxic Lesions in Animals** The depilated backs of animals were injected i.d. at multiple sites either with 0.1 ml of CPZ in isotonic saline (1.0 mg/ml) or with isotonic saline alone. Thirty minutes later, the left side of the backs of different groups of animals were exposed either to UVA (22.2 J/cm$^2$) or to UVB (0.6 J/cm$^2$), while the right side was covered with a black tape to serve as a nonirradiated control side. Doses of UVA and UVB chosen were 90% of minimal erythema dose in guinea pigs for UVA and UVB, respectively. Clinical changes were evaluated by 2 independent observers at the following times after the completion of irradiation: 1, 6, 10, 17, 24, 48, and 72 h. Skin changes were graded, according

Manuscript received July 8, 1985; accepted for publication September 5, 1985.

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Abbreviations:
CPZ: chlorpromazine
$CH_{50}$: complement hemolytic activity

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to the scoring described [5]. The results were expressed as the mean of clinical response score ± SEM.

**Vascular Permeability** Normal animals were pretreated by i.v. injection of 0.7 ml of 1% Evans blue in isotonic saline. We then injected 0.1 ml of CPZ solution (1.0 mg/ml) i.d. into the depilated skin of the backs of animals. Twenty minutes later, the animals were exposed to UVA (22.2 J/cm$^2$). The degree of blueing was evaluated 30 min after the completion of exposure.

## RESULTS

**In Vitro Complement Activation** When human sera containing varying concentrations of CPZ were exposed to UVA irradiation, there was a progressive decrease in $CH_{50}$ with increasing irradiation energy (Fig 1). Exposure of normal human serum to UVA, in the absence of CPZ, did not alter the complement profile. In contrast to the effect of UVA irradiation, exposure of sera containing CPZ (final concentration; 100 $\mu$g/ml) to 2.1 J/cm$^2$ of UVB radiation resulted in only a small decrease in $CH_{50}$; residual complement activity was 91.0% after UVB exposure. Exposure (13.3 J/cm$^2$) of CPZ (1.0 mg/ml) to UVA radiation before its addition to normal human serum did not cause any alteration of $CH_{50}$ (data not shown).

**Crossed Immunoelectrophoresis** For the direct demonstration of complement activation, immunoelectrophoretic evidence for C3 in serum was sought. Serum containing CPZ resulted in the conversion of C3 from $\beta_1$C to $\beta_1$A after UVA irradiation, in the same way as noted with complement-activated serum by zymosan (Fig 2). Such an extra precipitin peak was absent from CPZ-containing serum without irradiation.

**Intensity and Duration of CPZ Phototoxicity** Intradermal injection of 0.1 ml CPZ (1.0 mg/ml) on the depilated backs of normal guinea pigs, followed by exposure to 22.2 J/cm$^2$ UVA, resulted in the development of erythema and induration at the sites of injection, which peaked at 10 h after irradiation. In the case of cobra venom factor-treated animals, there was a moderate suppression of clinical response 6–24 h after irradiation with 22.2 J/cm$^2$ of UVA. Animals injected with CPZ and kept unirradiated developed no or hardly perceptible erythema with a mean clinical score of 0.4 at 6 h, which subsided by 48 h. At sites injected with isotonic saline and subsequently irradiated, no clinical changes were noted. Those results are shown in Fig 3. Irradiation with UVB (0.6 J/cm$^2$) induced almost no response with a mean clinical score of 0.25 at the site of CPZ injection (data not shown).

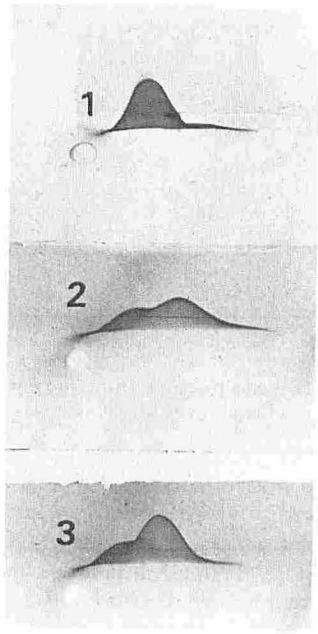


**Figure 2.** Two-dimensional immunoelectrophoresis with anti-C3 antiserum. *1*, Nonirradiated serum containing CPZ. *2*, 22.2 J-irradiated serum containing CPZ. *3*, Zymosan-activated serum. Final concentration of CPZ was 50 $\mu$g/ml.

**Vascular Permeability** We observed the effect of UVA irradiation to CPZ-injected sites on vascular permeability. In normal animals, intense blueing was noted at sites injected with CPZ and exposed to UVA, reflecting a localized increase in vascular permeability (Fig 4). In contrast, blueing was not observed when CPZ-injected sites were kept in the dark.

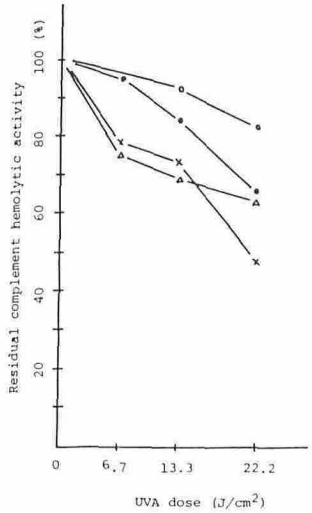


**Figure 1.** Effect of UVA irradiation on the complement activity ($CH_{50}$) in the sera containing CPZ. Final concentration of CPZ was 500 $\mu$g/ml (×——×), 100 $\mu$g/ml (△——△), 50 $\mu$g/ml (●——●), and 10 $\mu$g/ml (○——○), respectively.

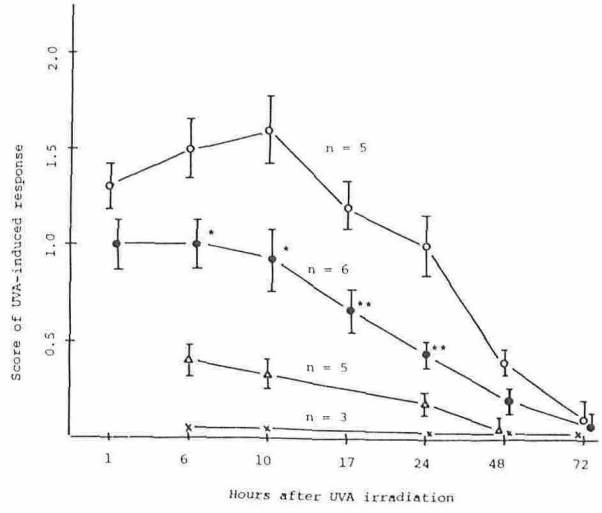


**Figure 3.** Phototoxic response induced by CPZ (1.0 mg/ml) and UVA (22.2 J/cm$^2$) in normal (○——○) or decomplemented guinea pigs (●——●). Statistical difference in clinical response between above 2 groups at the various time points was assessed by Wilcoxon's rank-sum test. $*p < 0.05$; $**p < 0.025$. △——△ = Normal animals injected with CPZ and kept dark; ×——× = normal animals injected with saline and irradiated.

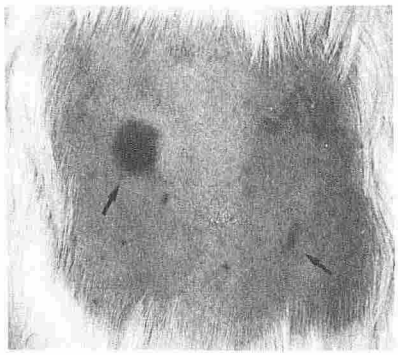

**Figure 4.** Leakage of Evans blue dye at the site injected with CPZ (1.0 mg/ml) after UVA irradiation (*left*). Right side was kept dark.

## DISCUSSION

In the present study, we found that in vitro UVA irradiation of serum containing CPZ induced diminution of $CH_{50}$, which, in turn, resulted in immunoelectrophoretic C3 conversion. Also, decomplemented animals showed weaker inflammatory responses at CPZ-injected sites to UVA irradiation, though normal animals developed strong responses.

CPZ has been demonstrated to be phototoxic in many systems involving DNA, proteins, and membranes. Ljunggren [1] demonstrated that CPZ solutions irradiated prior to injection into the test animals produced toxic responses. CPZ forms covalent photoadducts with proteins and with DNA when irradiated [9]. Bacteriophages were inactivated when they were irradiated with "black light" in the presence of CPZ [10]. CPZ can cause membrane damage by a nonphotodynamic process as indicated by its ability to photosensitize the lysis of red blood cells in both the presence and the absence of oxygen [2,11]. However, oxygen was required for CPZ-photosensitized disruption of liposomes composed of lecithin [12]. The elucidation of the above photochemical reactions contributes to the basic understanding of the pathogenesis of cutaneous phototoxic lesions. However, all these studies were performed in a serum-free environment.

Since edema and erythema are the early findings in the development of phototoxic lesions, chemical mediators that may play a role in such an erythematous response have been investigated. Our examination for Evans blue extravasation confirmed that there is an increase in permeability of the blood vessel wall in CPZ-photosensitivity. Chlorpromazine induces histamine release from guinea pig skin irradiated in vitro [13]. However, mediators sensitive to the effects of protease inhibitors rather than those of antihistamines were suggested to be responsible for the early phase of CPZ phototoxicity in mice [14]. The complement system constitutes a candidate for such protease inhibitor-sensitive, plasma-derived mediators produced in many types of inflammation. Complement activation leads to increased vasodilatation, vasopermeability, and neutrophil chemotaxis. In guinea pigs, administration of CPZ, followed by UV irradiation, results in dermal edema, vasodilatation, and neutrophil infiltrate [15]. These histologic changes are consistent with those mediated by products of complement activation. Our data indicate that decomplementation reduces CPZ-induced phototoxicity in a guinea pig model.

Complement activation has also been shown to occur in sera from patients with erythropoietic protoporphyria and porphyria cutanea tarda after 405-nm irradiation [3,4]. Ultraviolet A irradiation of guinea pig serum containing demethylchlortetracycline also resulted in complement activation in vitro [5]. Moreover, we have shown here that phototoxic lesions by CPZ plus UVA are suppressed in the decomplemented animals. Although our treatment with cobra venom factor in pilot studies induced almost total decomplementation in guinea pigs for the subsequent 5 days, there occurred only a reduction and no elimination of CPZ phototoxicity in such decomplemented animals. These findings suggest that the activation of the complement system is not the sole mechanism for the phototoxic response.

In summary, our present study suggests that the phototoxic potentiation of UVA radiation by CPZ is at least partly mediated by the complement system. Furthermore, we think that the role of the complement system should be studied more extensively in phototoxic responses elicited by other substances, since it may be involved in skin reactions caused by a variety of phototoxic agents.

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