Inflammatory Activity of Polymeric Photoproducts of Chlorpromazine

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To confirm the inflammatory activity of polymeric photoproducts (CPZ-polymers) of chlorpromazine (CPZ), which were obtained by gel filtration of a UV-pre-irradiated CPZ aqueous solution, the histamine release from rat peritoneal exudate cells was studied and the paw-inflammation in mice induced by these CPZ-polymers was examined. CPZ-polymers induced a dose-dependent histamine release at concentrations of 1, 3 and 10 mg/ml. This effect was approximately one-tenth of that of compound 48/80. Furthermore, CPZ-polymers markedly induced lasting paw-edema in a dose-dependent manner, the swellings remaining for at least 96 h. When intraperitoneally injected into mice, CPZ-polymers induced a significant elevation of histamine release in the peritoneal cavity 0.5 h after the injection, compared with a control group. The histamine levels in the cavities returned to normal within the next 0.5 h, and remained normal for at least 23 h, indicating that histamine release may be caused only in the early stages of CPZ-polymer-induced inflammation. The inflammatory activity of the CPZ-polymers suggests that they are inflammatory substances formed from CPZ by UV-irradiation.

Keywords: chlorpromazine; polymeric photoproduct; histamine release; inflammatory action

Chlorpromazine (CPZ), one of the antipsychotic group of drugs, is well known to induce severe phototoxicity in human skin.1,2) The mechanism of this CPZ-induced phototoxicity has been thought to involve a large number of physiological events such as the formation of covalent photo-adducts with DNA and nucleotide bases,3–9) DNA strand breaks,10–12) production of radicals13,14,16) and other species,13,14,16) cell membrane disruption17–19) and depletion of the complement system.20) Recently, it has been reported that pre-irradiated CPZ solutions and photo-products of CPZ exhibit a variety of interesting biochemical actions. Exposure of a CPZ solution to UV irradiation has produced a photoproduction mixture which was toxic to macrophages,17) elicited inflammation when injected into the skin of guinea-pigs21) and also caused the disruption of red blood cell membranes.17–19) Kocchevar and Hom have reported that the hemolysis produced by pre-irradiated CPZ was induced by stable CPZ photoproducts such as dimers and higher molecular weight photoproducts.19) In our previous articles,22,23) we demonstrated that CPZ activated hyaluronidase, known to be an inflammatory enzyme, in the presence of UV light, and this was found to be due to the presence of dimers and higher molecular weight photomultimers. This paper describes a study of the inflammatory effects of the polymeric photoproducts (CPZ-polymers), formed by irradiating CPZ in aqueous solution, in terms of their histamine releasing action.

Materials and Methods

Animals: Male Sprague-Dawley rats (200—250 g) and male ICR mice (22—26 g) purchased from Charles River (Japan) were used. They were housed under conditions of 22 ± 2°C, 55 ± 5% humidity and 12 h light (from 7 a.m. to 7 p.m.), and fed a commercial diet (MF, Oriental Yeast Co., Tokyo) and allowed tap water ad libitum.

Histamine Release in Vitro: Peritoneal mast cells were obtained from male Sprague-Dawley rats by washing the peritoneal cavity with a physiological solution containing: NaCl 136.9 mm, KCl 5.4 mm, NaH2PO4 0.34 mm, KH2PO4 0.44 mm, CaCl2 0.42 mm, MgCl2 0.49 mm, MgSO4 0.41 mm, Na2HCO3 3.0 mm and glucose 5.6 mm. After purification using the method of Enerback and Svensson,24) mast cells of purity of 90% or more were obtained. At least 98% of the cells were viable as estimated by trypan blue exclusion, and the cell suspensions were adjusted to a concentration of 109 mast cells/ml. The cell suspensions were mixed with various concentrations of drugs, incubated for 10 min at 37°C and asphyxiated in reactions terminated by cooling the tubes in ice. The suspensions were centrifuged (200 × g, 10 min, 4°C) and histamine was determined both in the supernatants and cell pellets after extraction according to the fluorimetric method of Shore et al.25) The extraction was carried out using the method of Tasaka and Akagi.26) Histamine release was expressed as a percentage of the total cellular content.

1-Lactate Dehydrogenase (LDH) Activity: The LDH activity in the supernatant was measured by the method of Wroblewski and La Due.27)

CPZ-Polymer-Induced Edema in Mice: The initial hind paw thickness (mm) of male ICR mice was measured using a dissecting microscope (× 10) equipped with a scale. A solution of the CPZ-polymers in sterile saline (0.02 ml/animal) at concentrations of 0.25, 0.5 or 1.0% respectively was injected subcutaneously into the plantar of the hind paw. At various time intervals, the thickness (mm) of each hind paw was determined and the results were represented as percentage hind paw swelling, compared with the initial hind paw thickness.

Histamine Release in Vitro: Male ICR mice were injected intraperitoneally with a solution of 0.5 mg of the CPZ-polymers in 0.05 ml sterile saline. Control animals received sterile saline alone. At various times, the animals were sacrificed and their peritoneal cavities were then washed with 2 ml of the cold physiological solution described above. The peritoneal washings from individual mice were centrifuged for 10 min at 300 × g, 4°C. The histamine in the supernatant was measured by the fluorimetric assay described above.

Preparation of CPZ-Polymers: CPZ-polymers were prepared as described previously.25) Chlorpromazine hydrochloride solution (28 mm in water) was irradiated for 4 h, and then passed through a Sephadex G-50 column (12 × 300 mm) using water as the mobile phase. Irradiation was performed using a 100 W high-pressure mercury lamp filtered with a uranium glass filter to remove UVB radiation. The amount of radiation exposure was 4.5 mW/cm² as determined by a UV radiometer (Toshiba UVR-365, Tokyo, Japan). Fractions (1 ml each) were collected and 200-fold with water in order to monitor the absorbance at 260 nm. Nitroblue tetrazolium (molecular weight = 817) and vitamin B12 (molecular weight = 1355) were used as standards for the gel filtration chromatography. Fractions containing the photoproducts with larger molecular weights than vitamin B12 were collected and evaporated to give a brown powder of CPZ-polymers that was dried in vacuo overnight.

Materials: Chlorpromazine hydrochloride, o-phenaldehyde, trichloroacetic acid, nitroblue tetrazolium and vitamin B12 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Compound 48/80 was from Sigma Chemical Co. (St. Louis, MO, U.S.A.), Sephadex G-50.
was from Pharmacia Fine Chemical (Tokyo, Japan).

**Statistical Analysis**  Mean values of groups were expressed along with S.E.M. The data were evaluated for statistical significance using Student's *t*-test.

**Results and Discussion**

Previously, we reported that CPZ activated the inflammatory enzyme hyaluronidase, in the presence of UV light, and this was found to be due to dimeric and polymeric photoproducts which are present in the UV-irradiated aqueous solution.22,33 Also, we demonstrated that the well known histamine-releaser, compound 48/80, induced extensive activation of hyaluronidase.22 We have examined the histamine release from rat peritoneal mast cells induced by high molecular weight photoproducts (CPZ-polymers) in comparison with compound 48/48 and CPZ. As shown in Fig. 1, CPZ-polymers at concentrations of 1, 3 and 10 mg/ml induced histamine release in a dose-dependent manner, while the histamine release produced by CPZ was weak. Compound 48/80 showed a dose-dependent histamine release at concentrations of 0.1, 0.3 and 1 mg/ml.

Kochevar and Hom have reported that stable CPZ-photoproducts such as the dimers and polymers produced by UV-irradiation, caused disruption of the red blood cell membrane.18,19 Therefore, to investigate whether the CPZ-polymers cause membrane disruption due to induced histamine release, we measured LDH activity in the supernatant of cell suspensions after reaction with CPZ-polymers. LDH activity was not detected in the supernatant. This suggests that the histamine release, induced by CPZ-polymers, may be due to a degranulation similar to that produced by compound 48/80, but not non-specific membrane disruption.

Next, to find out whether CPZ-polymers induce inflammatory actions in vivo, we evaluated CPZ-polymer-induced paw-inflammation in mice. As shown in Fig. 2, CPZ-polymers at concentrations of 0.25, 0.5 and 1% produced marked dose-dependent paw-edema for a period of 96 h after injection of CPZ-polymers. The swelling formed by the 0.25% solution of CPZ-polymers reached a maximum at 2 h after injection. On the other hand, the maximum swelling at 0.5% and 1% was observed 24 h after injection with CPZ-polymers.

In order to investigate the correlation between the inflammatory activity of CPZ-polymers and histamine, the histamine release in mice peritoneal cavities, induced by CPZ-polymers, was investigated. The amounts of histamine recovered from the peritoneal cavities of mice were measured 0.5, 1, 4, 8 and 24 h after intraperitoneal injection of 0.5 mg CPZ-polymers. As shown in Table I, the histamine-recovery from the cavities treated with CPZ-polymers significantly increased 0.5 h after injection of CPZ-polymers as compared with the control group. Thereafter, the histamine level in the cavities rapidly decreased reaching that of the control group within 0.5 h and remaining low for at least 24 h, suggesting the possibility of histamine exhaustion. These results suggest that histamine release may be produced in the early stages of CPZ-polymer-induced inflammation, and also that histamine seems to be involved as one of the initiators of the inflammation. The reason why the inflammation lasted for up to 96 h after injection of CPZ-polymers is not clear and further experiments are now in progress.

From the present studies it is apparent that the stable high molecular weight photoproducts (CPZ-polymers), formed from CPZ in aqueous solution by UV irradiation, induce histamine release and so may play an important role as potent inflammatory substances in vivo.

**References**