Exacerbation of Doxorubicin Toxicity by Chlorpromazine in Male ddY Mice

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ABSTRACT—We examined whether chlorpromazine (CPZ) modulates the lethality of doxorubicin (DOX, 12 mg/kg, i.p.) in mice treated with CPZ (6 mg/kg, i.p.) 1 hr before administration of DOX. Pretreatment with CPZ produced: 1) earlier deaths and 2) an increase in the number of deaths, compared to the controls. Potentiation of the bone marrow toxicity of DOX by CPZ was also reflected in the drug lethality towards the animals.

The antitumor antibiotic doxorubicin (DOX) is considered to be a very effective and useful chemotherapeutic agent for the treatment of many human tumors (1, 2). However, its extensive use in humans and animals at doses adequate for effective antitumor therapy is restricted by the risk of severe cardiotoxicity or leukopenia (1-3). Its toxic action is well-known to increase with temperature, both in cultures (4, 5) and in vivo (6, 7). Chlorpromazine (CPZ)-related phenothiazines and a number of other substances can affect the toxicity of various anticancer drugs (8-13). CPZ increases the toxicity of DOX to cells in culture, probably by increasing DOX uptake, especially by resistant cells (11, 12).

Since in the intact organism, CPZ produces hypothermia, we examined two of its effects in vivo: 1) its direct effect to increase DOX toxicity and 2) its indirect, hypothermia-mediated protective effect.

Male ddY mice, 5 weeks old and weighing 25-27 g, obtained from Japan SLC, Hamamatsu, were used. Animals were housed on a 12 hr light/dark cycle in a room maintained at 25 ± 1°C and 55 ± 5% relative humidity, and were given free access to standard laboratory food (CE-2, Japan Clea, Tokyo) and tap water throughout the experiments. Each group contained 8-12 mice. CPZ (Wintamine for injection) and DOX (Adriacin injection) were obtained from Shionogi Pharmaceutical Co. (Osaka) and Kyowa Fermentation, Inc. (Tokyo), respectively. CPZ (6 mg/kg) was given by i.p.-injection 1 hr before DOX. This interval was chosen because it allows a profound hypothermia to develop. Acute lethality was recorded over the succeeding 21 days. The number of leukocytes in the blood was counted using a Coulter counter (Particle counter Model PC-604A, ERMA Optical Works, Ltd., Tokyo) as an indicator of bone marrow toxicity. Student's t-test was used to test for significance of difference, and P = 0.05 was taken as the limit of significance.

Rectal temperature was recorded from a thermometer connected to a thermistor probe (Shibaura Electronics, Co., Ltd., type PtS006). Mice having rectal temperatures of 37 to 38°C were used in this experiment after two measurements were made at 15 min intervals. The animals were given the drugs, and their body temperature was monitored every 30 min
for 3 hr. The rectal temperature 1 hr after CPZ administration (6 mg/kg, i.p.) was 32.6 ± 1.3°C, compared to 37.7 ± 1.5°C for the controls. Figure 1A shows mortality from DOX (12 mg/kg, i.p.) with and without CPZ (6 mg/kg, i.p.) pretreatment. In this experiment, two groups of 20 mice were pretreated with either CPZ or saline at 1 hr before DOX administration. The results indicate that this pretreatment regimen with CPZ produces: 1) earlier deaths and 2) an increase in their number. No mouse died at a dose of 15 mg/kg CPZ (data not shown). In the CPZ-pretreated group, 12 of 20 mice were dead by the 7th day. On the other hand, the vehicle-pretreated mice suffered only 4 deaths during the entire 21-day period after DOX (12 mg/kg, i.p.). The lethality induced by a higher dose of DOX (15 mg/kg, i.p.) during the entire 14-day period after injection was 60%, which was close to the percent of death observed with CPZ plus DOX (12 mg/kg, i.p.). In addition, the lethality after 21 days was 0, 10, 20, 60, 90 and 100% in the groups treated with DOX alone at 8, 10, 12, 15, 17 and 19 mg/kg, respectively. In CPZ-treated mice, the lethality was 0, 40, 70 and 100%, which occurred in the groups treated with relatively smaller doses of DOX; i.e., 8, 10, 12 and 15 mg/kg, respectively (Fig. 1B). Figure 2 shows the effect of CPZ on bone marrow toxicity following injection of DOX. In a preliminary experiment, bone marrow toxicity was observed in mice 4 days after administration of DOX at 8 mg/kg, i.p. In subsequent studies, therefore, mice were killed 4 days administration of DOX to determine the bone marrow toxicity. The total number of leukocytes, taken as an indicator of bone marrow toxicity, was potentiated by pretreatment with CPZ. Namely, 4 days after DOX administration (8 mg/kg, i.p.) in animals pretreated with CPZ (6 mg/kg, i.p.) 1 hr before DOX injection, there was a significant decrease in the total number of leukocytes from 6480 to 2780 cells/mm³. The total number of leukocytes in groups treated by DOX (12 mg/kg, i.p.) was 2980 cells/mm³, which was close to the percent of the number of leukocytes observed in the group treated with CPZ plus DOX (8 mg/kg, i.p.). The mice treated with CPZ alone showed no bone marrow toxicity.

![Fig. 1. Effect of chlorpromazine pretreatment on doxorubicin-induced lethality in mice. A) Mice were given saline with doxorubicin (DOX, 12 or 15 mg/kg, i.p.), or DOX (12 mg/kg, i.p.) 1 hr after injection of chlorpromazine (CPZ, 6 mg/kg, i.p.). ○: DOX (12 mg/kg) alone, ●: CPZ plus DOX (12 mg/kg) alone. Δ: DOX (15 mg/kg) alone. B) Mice were given DOX after injection of CPZ (6 mg/kg, i.p.). ○: DOX alone, ●: CPZ plus DOX. Animals were observed for the following 21 days. Each point represents the percent mortality of 10–20 animals.](image-url)
Fig. 2. Effect of chlorpromazine on doxorubicin-induced bone marrow toxicity in mice. Mice were given saline with doxorubicin (DOX, 8 or 12 mg/kg, i.p.), DOX (8 mg/kg, i.p.) 1 hr after injection of chlorpromazine (CPZ, 6 mg/kg, i.p.) or CPZ plus saline. Control animals were treated with saline. Bone marrow toxicities were measured 4 days after saline, CPZ, DOX or CPZ plus DOX. Each column and vertical bar represents the mean ± S.E. of 6–8 animals. Data were analyzed by Student's t-test; †, P < 0.05 with respect to the control and †††, P < 0.05 with respect to the group given DOX (8 mg/kg) alone.

The data presented in this study indicate that CPZ pretreatment increases the acute lethal toxicity of DOX in the mouse, and this was reflected by the enhancement of bone marrow toxicity. These results are in accord with studies of the cytotoxic effect of DOX using calcium channel blockers and calmodulin inhibitors such as verapamil, trifluoperazine and CPZ (9, 11–13). However, the present results contrast with data from several investigators (4–6), who reported decreased DOX lethality when CPZ was used. The apparent discrepancy between their data and our own findings may be related to differences in species, strain, sex and/or age of the animals. The mechanism by which CPZ increases DOX-induced mortality is not clear. However, there is evidence suggesting that calcium channel antagonists- or calmodulin inhibitor-chemotherapy may be clinically useful (9, 11–13). CPZ is among the most potent inhibitors of calmodulin (14). It has been demonstrated that CPZ can increase DOX uptake by some cells in vitro and in vivo (9, 11, 13). Ganapathi et al. (15) have shown that CPZ increases the sensitivity of cells to DOX. It has also been suggested that CPZ may have a DOX-like action (16). These characteristics would lead us to expect increased toxicity from the CPZ-DOX combination. The reason for this may be because the direct cellular effect of DOX is much more important than the effect of hypothermia, at least under the present experimental conditions.

It is clear that calmodulin plays an important role in the cytotoxicity of a variety of antitumor drugs. Inhibition of calmodulin by CPZ has been shown to sensitize tumor cells to certain chemotherapeutic agents (8–12). Moreover, recently it has been demonstrated that the ability for tumor cells to develop resistance to anthracycline and vincaalkaloid antitumor drugs (DOX, daunomycin, vinblastine and vincristine etc.) may in certain instances be related to increased calmodulin function (indicated by stimulated P-glycoprotein function). However, the potential use of CPZ and related phenothiazines or other calcium channel blockers and calmodulin inhibitors depends not only on a thorough understanding of possible drug interactions and/or host toxicities that may arise from combining various antitumor drugs, but also on an understanding of calmodulin function in the tumor systems against which these therapies may be used. Although CPZ and related phenothiazines are frequently used to relieve antitumor drug-induced emesis, CPZ had not been reported to increase the clinical incidence of toxicity. When CPZ and related phenothiazines are used as a treatment in
psychotherapy or as a drug to relieve antitumor drug-induced emesis, one must carefully consider that the effect of this type of drug may be altered when it is given in combination with another drug. It is apparent that the influence of calmodulin on DOX toxicity remains in question, and further studies are needed to clarify the CPZ-induced enhancement of DOX lethality.

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