by an inhibition of prostaglandin synthesis and/or by an activation of non-opioid antinociceptive mechanisms such as the serotoninergic pathway.

1 Scherrer, R. A., in: Anti-inflammatory Agents, vol. 1, Eds R. A. Scherrer and M. W. Whitehouse. Academic Press, New York 1974.
2 Abraneson, S., Korchack, H., and Ludewig, R., Proc. natl Acad. Sci. USA *82* (1985) 7227.
3 Martini, A., Bondiolotti, G. P., Sacerdote, P., Pierro, L., Picotti, G. B., Panerai, A. E., Restelli, L., Zancaner, F., and Monza, G., J. int. Med. Res. *12* (1984) 92.
4 Sacerdote, P., Monza, G., Mantegazza, P., and Panerai, A. E., Pharmac. Res. Commun. *17* (1985) 679.
5 Sunshine, A., Roure, C., Colon, A., Olson, N. Z., Gonzalez, L., Siegel, C., and Laska, E., Am. J. Med., Suppl. 5A (1988) 16.
6 Paeile, C., Lombard, M. C., Saavedra, H., Bustos, E., and Pelissier, T., Gen. Pharmac. *20* (1989) 519.
7 Cruccu, G., Ferracuti, S., Leardi, M. G., Fabbri, A., and Manfredi, M., Brain Res. *556* (1991) 209.
8 Fabbri, A., Knox, G., Buczko, E., and Dufau, M. L., Endocrinology *122* (1988) 749.
9 Cruccu, G., Agostino, R., Berardelli, A., and Manfredi, M., Neurosci. Lett. *63* (1986) 320.
10 Cruccu, G., Inghilleri, M., Fraioli, B., and Manfredi, M., Neurology *37* (1987) 631.
11 Ongerboer De Visser, B. W., Adv. Neurol. *39* (1983) 727.
12 Kimura, J., in: New Developments in Electromyography and Clinical Neurophysiology, vol. 3, p. 682. Ed. J. E. Desmedt. Karger, Basel 1973.
13 Dauthier, C., Gaudy, J. H., and Bonnet, P., Ann. Anesth. fr. *4* (1981) 317.
14 Delwaide, P. J., Schoenen, J., and Burton, L., Adv. Neurol. *39* (1983) 977.
15 Evinger, C., Sibony, P. A., and Manning, P. A., Exp. Brain Res. *73* (1988) 477.
16 Cruccu, G., Ferracuti, S., Leardi, M. G., and Manfredi, M., Pain, suppl. 5 (1990) 5431.
17 Rossi, B., Risaliti, R., and Rossi, A., Electroenceph. clin. Neurophysiol. *73* (1989) 334.
18 Panneton, W. M., and Burton, H., J. comp. Neurol. *199* (1981) 327.
19 Okuyama, S., and Aihara, H., Jap. J. Pharmac. *35* (1984) 95.
20 Chen, A. C. N., and Chapman, C. R., Exp. Brain Res. *39* (1980) 359.
21 Willer, J. C., De Broucker, T., Bussel, B., Roby-Bramy, A., and Harrewyn, J. M., Pain *38* (1989) 1.
22 Jurna, I., and Brune, K., Pain *41* (1990) 71.
23 Marrazzi, M. A., and Daugherty, J. H., Prostaglandins *14* (1977) 501.
24 Groppetti, A., Braga, P. C., Biella, G., Parenti, M., Rusconi, L., and Mantegazza, P., Neuropharmacology *27* (1988) 499.

0014-4754/92/11-12/1139-04$1.50 + 0.20/0

Protection by chlorpromazine against lethality and renal toxicity of cisplatin in mice *

M. Ishikawa, M. Ozaki, Y. Takayanagi and K. Sasaki

Department of Pharmacology and Toxicology, Cancer Research Institute, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Aoba-ku, Sendai 981 (Japan)
Received 14 April 1992; accepted 9 July 1992

Abstract. The effect of chlorpromazine on acute lethal toxicity and nephrotoxicity induced by cisplatin was studied in mice. Chlorpromazine given (i.p.) 1 h before cisplatin greatly reduced lethal and renal toxicities of cisplatin. Chlorpromazine did not reduce the antitumor activity of cisplatin against Sarcoma 180 in ddY mice or EL-4 Leukemia in C57BL/6J mice.
Key words. Cisplatin; chlorpromazine; lethality; renal toxicity; mice.

cis-Diamminedichloroplatinum (cisplatin; CDDP) is one of the most effective anticancer chemotherapeutic agents used in clinical practice. Its nephrotoxicity is well known as the most important dose-limiting factor[1]. We have been interested in the combined use of routine drugs (e.g., sodium thiosulfate, caffeine, etc.) and CDDP[2,3]. A widely used antiemetic agent, chlorpromazine (CPZ), and related phenothiazines, are routinely used in combination with CDDP to reduce nausea and improve patient compliance[4]. Studies conducted on mice have shown that CPZ offered protection against irreversible renal toxicity produced by nitrosourea and methyl CCNU[5]. The protection mechanism of CPZ is not known. However, it is possible that CPZ could ameliorate some of the more serious side effects of other antitumor agents, including the nephrotoxicity of CDDP. In the following study, we evaluated the possible protective effects of CPZ against CDDP-induced toxicity in mice.

Materials and methods

Male ddY mice weighing 23–25 g and male C57BL/6J mice weighing 22–24 g, obtained from Japan SLC (Hamamatsu), were used. Cisplatin (CDDP) for injection, 10 mg/vial (Briplatin) and chlorpromazine (CPZ) for injection, 25 mg/vial (Wintamine) were purchased from Bristol Myers Co., Tokyo, and Shionogi Pharmaceutical Co., Osaka, respectively.

Acute lethality was recorded over the 8 days following injection. Blood samples were obtained from the orbital vein in heparinized microhematocrit capillary tubes[6]; urea nitrogen levels in serum (BUN) were determined using a kit from Wako Pure Chem. Co., Tokyo. In preliminary experiments, we established that BUN levels were maximally elevated on day 4 following CDDP treatment and we therefore used this time point for BUN determinations in all subsequent experiments. To assess the effect of CPZ on the antitumor activity of CDDP,

ddY mice or C57BL/6J mice were inoculated i.p. with 10^6 Sarcoma 180 cells or 10^6 EL-4 lymphoma cells, respectively. They were treated 24 h later with 0.9% saline solution, CDDP (10 mg/kg, i.p.), or CPZ (6 mg/kg, i.p.) followed by CDDP 1 h later. Animals were observed daily for the incidence of deaths. The experiment was terminated when all mice had died or 60 days had passed after the administration of saline or CDDP. The antitumor activity was evaluated by the mean survival time (MST) of a group of mice or was expressed as the percentage of increased life span (%ILS) of treated mice compared to that of untreated controls, by comparing the mean survival time of the two groups, using the formula:

$$\%ILS = [(MST_{treated} - MST_{controls})/MST_{controls}] \times 100$$

Student's t-test was used to test for the significance of differences, and P = 0.05 was taken as the limit of significance.

Results

The effect of a single administration of CPZ on the lethal toxicity of CDDP in mice is shown in figure 1. In a preliminary experiment, two groups of 20 mice were pretreated with either CPZ (6 mg/kg, i.p.) or saline 1 h before CDDP (15 mg/kg, i.p.) administration (fig. 1 A). This pretreatment regimen with CPZ caused: 1) a delay in the occurrence of death and 2) a decrease in 8-day cumulative lethality compared to injection with saline before CDDP. Furthermore, a dose-dependent protective effect of coadministered CPZ was observed (fig. 1 B). An increase in BUN levels, which is recognized as a suitable index for early renal lesions produced by CDDP, was prevented by CPZ administered 1 h after CDDP administration. Initial studies in mice showed that

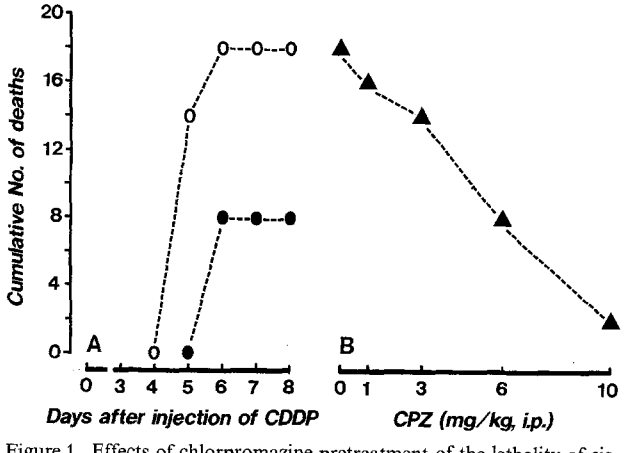

Figure 1. Effects of chlorpromazine pretreatment of the lethality of cisplatin in mice. Groups of 20 animals were used for each experiment. *A* Mice received i.p. injection of chlorpromazine (●, 6 mg/kg) or the saline vehicle (○) 1 h before cisplatin (CDDP, 15 mg/kg, i.p.). Deaths were recorded over the succeeding 8 days. *B* Mice received i.p. injection of chlorpromazine (CPZ, ▲, 0, 1, 3, 6, and 10 mg/kg, i.p.) 1 h before cisplatin (15 mg/kg, i.p.). Lethality was determined 8 days after the administration of cisplatin.

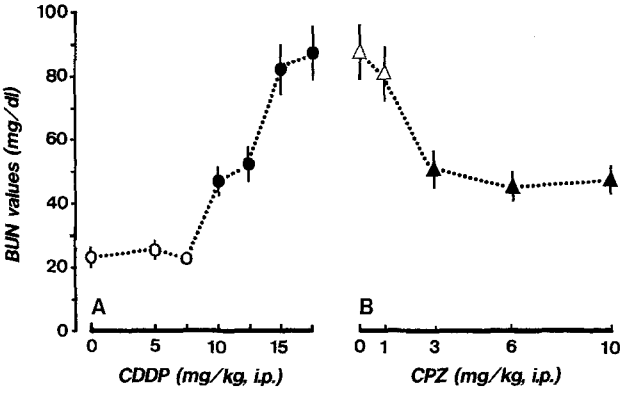

Figure 2. Effects of chlorpromazine on renal toxicity in mice receiving cisplatin. *A* Levels of blood urea nitrogen (BUN) were determined 4 days after treatment with cisplation (CDDP, 0, 5, 7.5, 10, 12.5, 15, or 17.5 mg/kg, i.p.) alone. Controls received saline alone. *B* BUN levels where determined 4 days after treatment with chlorpromazine (0, 1, 3, 6, or 10 mg/kg, i.p.) given 1 h after CDDP (15 mg/kg, i.p.). Controls received CDDP alone. Each point represents the SEM obtained from 6–8 animals. Closed symbols are significantly different from control values (saline alone, *A*; or CDDP alone, *B*), $p < 0.05$.

Influence of chlorpromazine on the therapeutic activity of cisplatin on Sarcoma 180 and EL-4 leukemia in mice

Tumor model	Treatment	MST days	% ILS[a]	Survivors > 60 days
Sarcoma 180	Control	19.6 ± 3.13	0	0/24
	CDDP	34.8 ± 5.64*	77	3/12
	CPZ + CDDP	41.7 ± 6.07*	112	4/12
EL-4	Control	15.5 ± 2.75	0	0/10
	CDDP	32.4 ± 5.14*	109	3/10
	CPZ + CDDP	30.9 ± 4.98*	99	2/10

Mice were inoculated i.p. with tumor cells (10^6 cells/mouse). Cisplatin (CDDP, 10 mg/kg, i.p.) was administered 24 h after the tumor inoculation and chlorpromazine (CPZ, 6 mg/kg, i.p.) was administered 1 h before the CDDP. Survivors were observed over the succeeding 60 days. [a] %ILS is calculated as the $[(T - C)/C] \times 100$, where T = the mean survival in treated animals and C = the mean survival in controls. See Materials and methods for details. *Significantly different from control values ($p > 0.05$).

CDDP produced a dose-dependent increase in BUN levels at doses over 10 mg/kg (fig. 2 A). The maximal increase in BUN levels occurred on day 4; these levels remained elevated for at least 10 days (data not shown). CPZ treatment resulted in a dose-dependent decrease in the CDDP-induced elevation in BUN levels (fig. 2 B). The antitumor activity of CDDP, indicated by prolongation of the survival time of mice inoculated i.p. with Sarcoma 180 cells and El-4 lymphoma cells, was not affected by the coadministration of CPZ (table 1).

Discussion

The parenteral administration of certain drugs has been shown to reduce CDDP toxicity[7]. Pretreatment with CPZ 1 h before CDDP administration depressed not only the lethal toxicity of CDDP but also its renal toxicity, without compromising its antitumor activity against several transplantable tumors in mice. The present investigation demonstrates that CPZ can be added to the list

of compounds that have been shown to be protective against the renal injury resulting from CDDP treatment. The protection mechanism of CPZ is unclear. CPZ and related phenothiazines have previously been shown to have a protective effect against renal injury induced by treatment with the investigational anticancer drugs methyl CCNU (MeCCNU), as well as having a protective effect against renal injury induced by the environmental toxicant mercuric chloride[8]. The protective mechanism of CPZ is not known; however, Harrison et al.[9] have shown that both MeCCNU and $HgCl_2$ share with CPZ the capacity to inhibit calmodulin activity. CPZ and related phenothiazines are among the most potent inhibitors of calmodulin[10]. Protection against $HgCl_2$-induced renal injury correlated with the potency of calmodulin antagonism in a series of phenothiazine analogs[11]. The results suggest that a similar mechanism may be involved in the protective action of CPZ.

The renal protection afforded by CPZ treatment was not accompanied by a decrease in the in vivo antitumor activity of CDDP. This is consistent with in vitro studies showing that phenothiazine and calmodulin antagonists did not affect CDDP cytotoxicity[12]. However, treatment with calmodulin antagonists alone was previously reported to inhibit tumor cell growth in a variety of experimental systems in vitro[11]. Phenothiazine and calmodulin antagonists have been reported to reduce drug efflux from tumor cells and thereby enhance their chemosensitivity, both in vitro and in vivo[13,14]. In the present study, CPZ treatment alone failed to prolong survival in mice bearing Sarcoma 180 cells or EL-4 lymphoma cells (table). These discrepancies in the results may be due to the use of different mouse and tumor strains or populations. From our data it can be concluded that CPZ pretreatment with CDDP is associated with the beneficial effect of decreasing renal toxicity. This suggests, indirectly, that in humans more effective chemotherapeutic treatment of cancer with CDDP may be possible if the chemotherapy includes CPZ. Phenothiazines have numerous pharmacological properties that might account for this observation, and additional studies will be required to establish the mechanism of this protective effect of CPZ against CDDP-induced nephrotoxicity. Further studies on the ability of CPZ to decrease the formation of complexes between CDDP and renal proteins may provide insight into the renal protective mechanism of CPZ.

In conclusion, in mice the preadministration of CPZ (i.p.) 1 h before CDDP (i.p.) injection efficiently depressed not only the lethal toxicity, but also the renal toxicity (indicated by increased blood urea nitrogen values) which was usually observed in mice treated with CDDP alone. Moreover, the preadministration of CPZ had no observed effect on the antitumor activity of CDDP in mice inoculated i.p. with Sarcoma 180 or EL-4 lymphoma cells. The present study suggests that CPZ may be of therapeutic benefit when used with CDDP; this study also provides a rational basis for the selection of antiemetic therapy.

* This work was partly supported by Grants-in-Aid from the Science Research Promotion Fund (Japan Private School Promotion Foundation).

1 Madidas, N. E., and Harrington, J. T., Am. J. Med. *65* (1978) 307.
2 Ishikawa, M., Shiba, Y., Takayanagi, G., and Sasaki, K., A. Rep. Tohoku Coll. Pharm. *34* (1987) 235.
3 Ishikawa, M., Mori, M., Sasaki, K., and Takayanagi, G., A. Rep. Tohoku Coll. Pharm. *35* (1988) 261.
4 Carr, B., Bertrand, M., Browning, S., Dorowshow, J. H., Presant, C., Pulone, B., and Hill, L. H., J. clin. Oncol. *3* (1985) 1127.
5 Harrison, S. D., Coox, J. L., and Giles, R. C., Cancer Chemother. Pharmac. *10* (1982) 62.
6 Ishikawa, M., Sasaki, M., Sasaki, K., and Takayanagi, Y., Res. Commun. chem. Pathol. Pharmac. *64* (1989) 347.
7 Borch, R. F., and Markman, M., Pharmac. Ther. *41* (1989) 371.
8 Harrison, S. D., Cox, J. L., and Giles, R. C., Bull. envir. Contam. Toxic. *34* (1985) 432.
9 Harrison, S. D., Mann, D. M., and Giles, R. C., Cancer Chemother. Pharmac. *14* (1985) 146.
10 Prozialek, W. C., and Weiss, B., J. Pharmac. exp. Ther. *222* (1982) 509.
11 Hait, W. N., and Lazo, J. S., J. clin. Oncol. *4* (1986) 994.
12 Lazo, J. S., Hait, W. N., and Kennedy, K. A., Molec. Pharmac. *27* (1985) 387.
13 Tsuruo, T., Lida, H., Tsukagoshi, S., Sakurai, T., Cancer Res. *42* (1982) 4730.
14 Ganapathi, R., and Grabowski, D., Cancer Res. *43* (1983) 3696.

0014-4754/92/11-12/1142-03$1.50 + 0.20/0