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Protection against cisplatin nephrotoxicity by prochlorperazine

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Summary. Prochlorperazine (Compazine; PCPZ) is often used to limit cisplatin (CDDP)-induced emesis. However, recent studies in mice have shown that PCPZ protects against renal injury produced by treatment with various nephrotoxicants (e.g., MethylCCNU, mercuric chloride). Because renal toxicity remains a serious limitation to the effective use of CDDP, we conducted the present study to determine whether PCPZ could also protect against CDDP-induced renal injury. PCPZ treatment was shown to ameliorate CDDP-induced renal lesions in both rats and mice at doses and treatment schedules that were comparable with those used for alleviating chemotherapy-induced emesis. A PCPZ dose of 10 mg/kg × 2 offered complete protection against CDDP-induced increases in blood urea nitrogen (BUN) levels in mice, with significant protection occurring at a PCPZ dose as low as 5 mg/kg. Similarly, PCPZ ameliorated CDDP-induced increases in BUN, glucosouria, and enzymuria in F344 rats. PCPZ treatment did not affect the urinary excretion or renal tissue levels of total platinum or the plasma pharmacokinetics of free platinum. However, it did cause a marked reduction in the concentration of total plasma platinum (free platinum + protein-bound platinum). PCPZ was not found to affect the in vivo antitumor activity of CDDP against P388 leukemia. The present study suggests that PCPZ may be of therapeutic benefit when used with CDDP and provides a rational basis for the selection of antiemetic therapy.

Introduction

Cisplatin (CDDP) is an important anticancer drug that is widely used in the treatment of a variety of cancers [19]. However, the clinical usefulness of this drug is limited by the development of nephrotoxicity [10, 17]. Clinical attempts to prevent this dose-limiting toxicity have included hydration and diuresis [9], hypersalination [20], diethyldithiocarbamate [18], and disulfiram [21]. In spite of these efforts, renal injury remains a major clinical problem.

Another serious side effect associated with the use of cisplatin is a debilitating nausea. Although this form of toxicity is not life-threatening, gastrointestinal discomfort can be so severe that patients may abandon this potentially successful form of therapy [13]. A variety of antiemetic agents are routinely used in conjunction with CDDP to reduce nausea and improve patient compliance [2]. However, several recent reports suggest that a widely used antimetic agent, prochlorperazine (PCPZ; compazine), may also protect against some of the more life-threatening toxicities associated with the use of chemotherapy. These studies, conducted in mice, have shown that PCPZ offered protection against irreversible renal toxicity prooduced by the nitrosourea, MethylCCNU [6], and decreased the lethality of doxorubicin [4]. The mechanism of protection of PCPZ is not known. However, it is possible that PCPZ may ameliorate some of the more serious side effects of other antitumor agents, including CDDP nephrotoxicity. In the following preclinical study, we evaluated the possible renal protective effect of PCPZ against CDDP-induced renal injury in mice and rats.

Materials and methods

Animals and treatment. Male $B_6D_2F_1$/J mice (BDF; 20–30 g) were supplied by Jackson Laboratories (Bar Harbor, Me). Male Fisher 344 rats (F344; 150–200 g; Taconic Farms, Germantown, NY) were housed singly in metabolic cages (Sybron/Nalge) to enable the separation of urine and feces. A 4-day acclimation to the metabolic cages was allowed before initiation of the experiment as described by Kramer and Boyd [12]. On the 5th day, pretreatment values (day 0) were obtained and animals were given CDDP and/or PCPZ. The animals used in these studies were maintained in accordance with institutional guidlines as prescribed by the *Guide for the Care and Use of Animals* (DHHS Publication 86-23, revised 1986). CDDP (Bristol Laboratories) was dissolved in saline immediately prior to a single i.v. injection of 5, 8, 10, 14, 18, and 26 mg/kg for mice and 1–5 mg/kg for rats. PCPZ (1–10 mg/kg) was given by i.p. injection 0.5 h before and 3 h after CDDP. All injections were given in a volume of 10 ml/kg body weight.

Blood analysis. Blood was collected by orbital sinus puncture with the animals under ketamine anesthesia and then analyzed for blood urea nitrogen (BUN) by the urease method (Sigma Chemical Co., St. Louis, Mo). In preliminary experiments, we established that BUN levels were maximally elevated on day 4 following CDDP treatment

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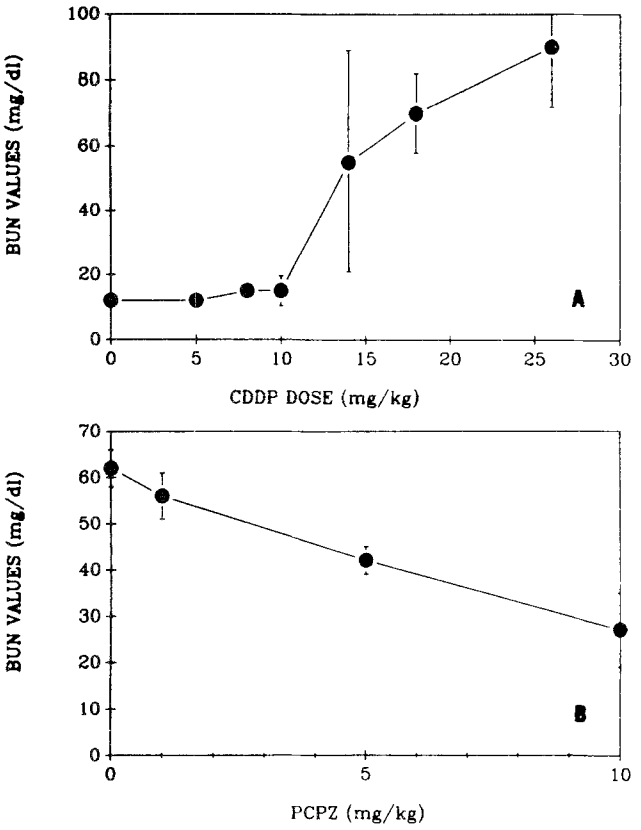

Fig. 1. Effect of CDDP and PCPZ on BUN concentrations in mice. **A** BUN levels were determined 4 days after treatment with 0, 5, 8, 10, 14, 18, and 26 mg/kg CDDP alone or **B** after treatment with PCPZ (1, 5, or 10 mg/kg) given 0.5 h before and 3 h after CDDP (14 mg/kg). Each point represents the mean ± SD for four animals

(data not shown) and therefore used this time point for BUN determinations in all subsequent experiments. Plasma glucose was quantitated by the hexokinase/glucose-6-phosphate dehydrogenase coupled assay (Sigma Chemical Co.).

Urinalyses. Urinalyses were conducted on individual 16-h urine samples from F344 rats, collected over ice. Urinary volume was recorded and the following measurements were made: specific gravity was determined with a clinical refractometer; protein, by the Bradford Coomassie blue-dye binding method (Bio-Rad); and glucose, by the coupled hexokinase assay (Sigma Chemical Co.). An aliquot of each urine sample was subjected to Sephadex G-25 column chromatography (Pharmacia Fine Chemicals, Uppsala, Sweden) and eluted with 0.9% saline to remove interferring substances for the determination of lactate dehydrogenase activity [15], as described by Kramer and Boyd [12].

Pharmacokinetics. The effect of PCPZ on the pharmacokinetic disposition of CDDP was determined in BDF male mice. PCPZ (10 mg/kg, i.p.) was given 0.5 h before an i.v. dose of CDDP (14 mg/kg). Plasma, renal, and urinary CDDP levels were determined by atomic adsorption spectrophotometry (Perkin Elmer). Kidney and blood samples were obtained at 0.25, 0.5, 1, 2, 4, 6, and 24 h after CDDP. Blood samples were obtained by intracardial puncture with animals under ketamine anesthesia, following which the rodents were euthanized by cervical dislocation and their kidneys were removed. At each time point, the blood of three animals were pooled and plasma ultrafiltrate was prepared using a MPS-1 micropartition system (Amicon Laboratories, Danvers, Mass) for the determination of free platinum. Plasma and ultrafiltrate were stored at −20° C until analysis. Kidney samples were frozen in liquid nitrogen immediately after excision. Animals used for obtaining the 24-h time point were placed in groups of three in metabolic cages for collection of urine to measure total excreted CDDP. Tissue, total plasma, plasma ultrafiltrate, and urine samples were prepared for analysis by the procedure of Litterst et al. [16]. Standards were made up in plasma (CDDP, 1,001 μg/ml, Sigma Chemical Co.).

AUC calculation. The trapezoidal rule was used to calculate the AUC and was based on the mean concentration vs time curve at a CDDP dose of 14 mg/kg.

Antitumor studies. The effect of PCPZ on the antitumor activity of CDDP was determined using the P-388 leukemia model in BDF mice. Each mouse was innoculated with 1×10^6 cells and treated 24 h later with i.v. doses of CDDP (0.5, 1.0, 3.0, and 5.0 mg/kg) with and without PCPZ given as two i.p. injections as described in the nephrotoxicity studies (below). Survival was assessed twice daily and expressed as the percentage of increased life span (ILS) vs control values.

Results

Nephrotoxicity experiments

Initial studies in mice showed that CDDP produced a dose-dependent increase in BUN levels at doses of >10 mg/kg (Fig. 1 A). The maximal increase in BUN levels occurred on day 4; these levels remained elevated for at least 12 days (data not shown). PCPZ treatment resulted in a dose-dependent decrease in the CDDP-induced elevation in serum BUN levels (Fig. 1 B). A PCPZ dose of 10 mg/kg offered complete protection against the CDDP-induced increase in BUN levels. Slight protection was observed at PCPZ doses as low as 1 mg/kg. CDDP also caused elevations in BUN levels in F344 rats at doses of >3 mg/kg; these were also maximal on day 4 after CDDP treatment (data not shown). PCPZ treatment was also shown to protect against CDDP-induced increases in BUN in F344 rats (Table 1).

Urinalyses

Renal function was assessed in male F344 rats by urinalyses. CDDP did not produce a significant change in urinary volume or specific gravity (data not shown). However, CDDP treatment resulted in a slight proteinuria and a marked increase in the urinary excretion of glucose and LDH activity (Fig. 2). The maximal increase in urinary protein, glucose, and LDH occurred 3–4 days after CDDP administration, which correlated with the increase in BUN levels. CDDP treatment produced maximal increases in the urinary excretion of protein, LDH, and glucose of approximately 3-, 20- and 100-fold, respectively. Plasma glucose levels were slightly decreased by CDDP

Table 1. BUN values in serum of rats 4 days after treatment with PCPZ and CDDP

Treatment	BUN (mg/dl)
Control	18 ± 4[a]
CDDP (3 mg/kg)	40 ± 4*
CDDP + PCPZ (10 mg/kg)	27 ± 1*, **

[a] Values represent the mean ± SEM; $n = 4$
* Significantly different from control values ($P < 0.05$)
** Significantly different from values obtained for CDDP alone ($P < 0.05$)

treatment (data not shown), demonstrating that the increase in urinary glucose was due to decreased tubular reabsorption rather than an elevation in glucose concentration in the glomerular filtrate. PCPZ treatment offered complete protection against CDDP-induced increases in the urinary excretion of glucose and LDH activity but caused a slight increase in protein excretion on days 2 and 3 after treatment. This increase in protein excretion was transient and returned to control levels by day 4. PCPZ treatment alone did not increase urinary volume or affect any other parameter of renal function used in these studies (data not shown).

Pharmacokinetic studies

The effect of PCPZ on CDDP pharmacokinetics was determined in BDF mice. Semilogarithmic plots of total platinum and free platinum plasma concentrations are shown in Figs. 3 A and 3 B. The concentration of total platinum in the kidney is shown in Fig. 3 C, and the pharmacokinetic data is summarized in Table 1. Free plasma platinum was cleared rapidly ($t_{1/2}$, 15 min) and could not be detected beyond 2 h. PCPZ treatment did not affect the plasma pharmacokinetics of free platinum or the renal tissue levels or urinary excretion (Table 2) of total platinum. However, PCPZ did produce a marked decrease in total plasma platinum levels observed between 2 and 6 h after CDDP treatment. The AUC (concentration × time) for total platinum was reduced by nearly 50% by PCPZ treatment (Table 2). Neither peak plasma levels (not shown) nor the initial half-life of total platinum was affected by PCPZ (Table 2).

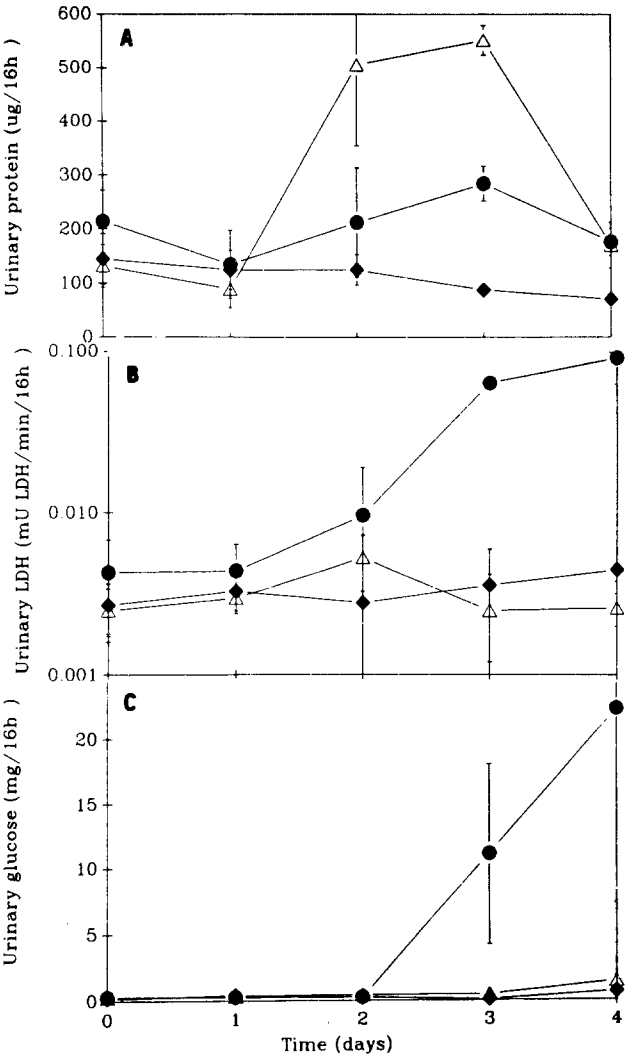

Fig. 2. Effect of CDDP and PCPZ treatment on the 16-h urinary excretion of **A** protein; **B** LDH activity, and **C** glucose. Rats were treated with PCPZ (10 mg/kg, i.p.) 0.5 h before and 3 h after treatment with CDDP (4 mg/kg; i.p.). Urine was collected as described in *Materials and methods*. ◆, control; ●, CDDP-treated; △, CDDP + PCPZ-treated. All values represent the mean ± SEM for four animals, assayed in duplicate

Table 2. Effect of PCPZ treatment on the pharmacokinetics of CDDP (14 mg/kg)

	Plasma[a]						
	Total CDDP:			Free CDDP:		Total kidney[b]	Total urine[c]
Treatment	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (h)	AUC (0–6 h)	$t_{1/2}\alpha$ (min)	AUC (0– h)	AUC (0–24 h)	(mg/24 h)
Control	23	24.5	5.9	15	0.46	10.4	3.95
PCPZ	20	1.0	3.2	10	0.44	10.9	4.27

[a] Equal volumes of plasma were pooled from three mice for determination of total and free CDDP
[b] Determined from the mean concentration vs time curve for individual kidneys obtained from three animals
[c] Urine samples from three animals were pooled for determination of urinary CDDP

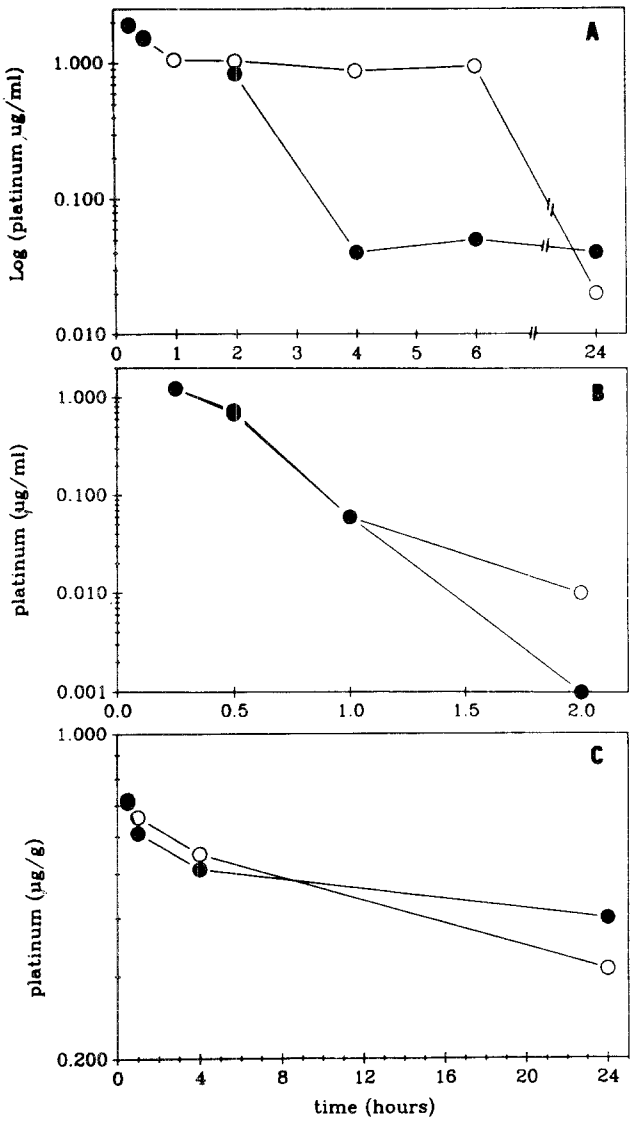

Fig. 3. Concentration vs time curves for **A** total plasma platinum; **B** free plasma platinum, and **C** total renal platinum in BDF mice following CDDP (14 mg/kg; i. v.) administration. *Open symbols* represent control values; *closed symbols* denote PCPZ pretreatment. PCPZ was given as a single injection (10 mg/kg; i. p.) 0.5 h before CDDP. Equal volumes of plasma from three animals per time point were pooled for the determination of total and free plasma platinum. Kidney values represent the mean ± SEM for three animals.

Antitumor studies

CDDP (0.5–5 mg/kg, i. v.) produced a small but dose-dependent increase in the life span of mice bearing the P388 murine leukemia (Table 3). PCPZ alone had no antitumor activity, nor did PCPZ treatment affect the antitumor activity of CDDP (Table 3).

Discussion

The present investigation demonstrates that PCPZ can be added to the list of compounds that have been shown to protect against renal injury resulting from CDDP treatment. Chemoprotection by PCPZ was observed in both rats and mice at doses and treatment schedules that were comparable with those used for alleviating chemotherapy-induced emesis. For example, a dose of PCPZ as low as 5 mg/kg (15 mg/m^2) given 0.5 h before and 3 h after CDDP produced a significant reduction in CDDP nephrotoxicity in mice (Fig. 1 B). This dose of PCPZ was equivalent to a 25-mg dose in patients (assuming a body surface area of 1.7 m^2).

PCPZ and related phenothiazines possess a number of pharmacologic effects that may account for the observed protection against CDDP-induced renal injury. These include diuresis and increased renal blood flow [1], hypothermia [4], and calmodulin antagonism [22]. In the present study we found that PCPZ did not affect urinary volume in rats (data not shown) at the doses used to protect against renal injury. Studies on the disposition of CDDP in mice demonstrated that PCPZ affected neither the urinary excretion of CDDP (Table 2) nor the concentration of CDDP in the kidney (Fig. 3 C). These observations argue against a role for diuresis or altered renal blood flow as the mechanism of renal protection. PCPZ also did not affect the free plasma concentration of platinum in mice (Fig. 3 B), suggesting that pharmacokinetic drug interactions were probably not responsible for the protective action of PCPZ.

However, PCPZ treatment did result in a marked decrease in the AUC for total plasma platinum (Table 2). The biological significance of this finding is difficult to interpret because total platinum levels mainly reflect protein-bound drug. Several studies have shown that platinum loses its biological activity when bound to serum protein [3, 11]. Although it is possible that the PCPZ-induced decrease in total platinum was due to displacement of protein-bound platinum by PCPZ this decrease was not accompanied by an increase in the concentration of either plasma free platinum or urinary platinum. Further studies on the ability of PCPZ to decrease the formation of complexes between CDDP and renal proteins may provide insight into the renal protective mechanism of PCPZ.

PCPZ has previously been shown to protect against renal injury produced by treatment with the investigational anticancer agent MethylCCNU (MeCCNU) as well as the environmental toxicant mercuric chloride [6]. The

Table 3. Effect of PCPZ and CDDP treatments on survival in mice bearing P388 murine leukemia

Treatment	Mean survival (days)	%ILS[a]
Control	10.6 ± 0.9[b]	0
PCPZ (10 mg/kg)	10.6 ± 1.9	0
CDDP (0.5 mg/kg)	11.9 ± 2.1	10
+ PCPZ[c]	10.1 ± 0.2	0
CDDP (1 mg/kg)	12.6 ± 1.3	16
+ PCPZ	11.6 ± 2.3	8
CDDP (5 mg/kg)	14.8 ± 2.0	28
+ PCPZ	13.4 ± 2.9	21

[a] %ILS is calculated as the T-C/C × 100, where T = the mean survival in treated animals and C = the mean survival in controls
[b] Values represent the mean ± SEM for six BDF mice innoculated i. p. with 1 × 10^6 cells
[c] PCPZ was given i. p. 0.5 h before and 3 h after a single i. v. injection of CDDP

protective mechanism of PCPZ is not known; however, Harrison et al. [8] have shown that both MeCCNU and $HgCl_2$ share with PCPZ the capacity to inhibit calmodulin activity. PCPZ and related phenothiazines are among the most potent inhibitors of calmodulin [22] Protection against $HgCl_2$-induced renal injury correlated with the potency of calmodulin antagonism among a series of phenothiazine analogs [7]. However, renal calmodulin activity was not affected by $HgCl_2$ or MeCCNU treatment [7, 8], and it is not at all clear what role calmodulin antagonism plays either as a cause for renal injury or as a mechanism of protection against renal injury.

The renal protection afforded by PCPZ treatment was not accompanied by a decrease in the in vivo antitumor activity of CDDP (Table 3). This is consistent with in vitro studies showing that phenothiazine calmodulin antagonists did not affect CDDP cytotoxicity [14]. However, treatment with calmodulin antagonists alone was previously reported to inhibit tumor cell growth in a variety of experimental systems in vitro [5]. The in vivo antiproliferative activity of calmodulin antagonists has not been clearly demonstrated [5]. In the present study, PCPZ treatment alone failed to prolong survival in mice bearing P388 murine leukemia (Table 3).

Although PCPZ is frequently used for alleviating CDDP-induced emesis, it has not been reported to affect the clinical incidence of renal injury. It is possible that a thorough retrospective analysis of patients given both CDDP and compazine may reveal a decrease in the frequency and/or severity of toxic side effects relative to that observed in patients receiving other forms of antimetic therapy. However, a more complete understanding of the chemoprotective effects of PCPZ requires further evaluation in a clinical setting. Thus, although hydration and diuresis remain an effective front-line treatment for reducing CDDP renal injury, the present study suggests that PCPZ may be of therapeutic benefit when used with CDDP and provides a rational basis for the selection of antiemetic therapy.

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