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Effect of diethyldithiocarbamate rescue on tumor response to *cis*-platinum in a rat model

[*cis*-dichlorodiammineplatinum(II)]

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ABSTRACT **The nephrotoxic effects of *cis*-dichlorodiammineplatinum(II) (NSC-119875) (DDP) in female F344 rats were effectively inhibited by administration of sodium diethyldithiocarbamate (DDTC) in doses of 750 mg/kg intraperitoneally or 100 mg/kg intravenously 2 hr after administration of DDP. Rats were inoculated with mammary tumor 13762 and treated after 10 days with DDP (2.0 or 8.0 mg/kg) with or without DDTC rescue (750 mg/kg intraperitoneally or 100 mg/kg intravenously). Initial reductions in tumor size were identical with or without rescue in all experiments. High-dose intraperitoneal rescue, however, resulted in earlier relapse and more rapid progressions at both DDP doses than was observed in the absence of rescue. Low-dose intravenous rescue led to a tumor response identical to that observed without rescue. Urinary excretion of free DDTC was increased by prior administration of acetazolamide; however, this combination was more toxic to rats after DDP administration than was DDTC alone. Intravenous administration of DDTC appeared to be the most effective route for delivery of this ligand to the kidney. These results support our earlier mechanistic hypothesis and demonstrate the feasibility of inhibition of *cis*-platinum toxicity by DDTC without inhibition of the antitumor effect.**

cis-Dichlorodiammineplatinum(II) (DDP) has demonstrated impressive clinical efficacy against a number of human tumors (1). Dose-related nephrotoxicity (2, 3) and a variety of other side effects (4) are frequently observed with this drug, however. Although intravenous prehydration with or without mannitol apparently ameliorates acute nephrotoxicity at modest DDP doses (5, 6), the loss of renal function either from acute high-dose or chronic low-dose therapy constitutes a major limitation on the drug's future potential. We recently postulated a mechanism for this nephrotoxicity based upon irreversible binding of platinum to sulfhydryl groups in tubular membrane-bound enzymes and reported the efficacy of sodium diethyldithiocarbamate (DDTC) as an inhibitor of nephrotoxicity in a rat model (7). This report describes an effective tumor response to the combined DDP–DDTC "rescue" treatment in a rat model and suggests that nephrotoxicity can be inhibited without adverse effects on tumor response.

MATERIALS AND METHODS

Female F344 (Fischer) rats, initial weight 100–150 g, were obtained from the Charles River Breeding Laboratories. They were housed in methacrylate cages and given Purina rat chow and water ad libitum for at least 1 week. Mean weight of the rats was 150 g at the onset of experiments unless otherwise noted.

DDP (NSC-119875) was kindly provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Solutions of DDP were prepared immediately prior to injection in isotonic saline (0.9% NaCl) at a concentration of 0.2–1.0 mg/ml. DDTC was obtained from Sigma, and solutions were prepared immediately before use at a concentration in mg/ml equal to the dose expressed in mg/100 g; thus, injection volumes were identical at all dose levels. Sodium acetazolamide (Diamox) was obtained from Sigma and prepared as a 1.0 mg/kg solution in isotonic saline. Mammary tumor 13762 was obtained in the form of an implanted tumor in a female F344 rat from W. Hrushesky (University of Minnesota Medical School, Minneapolis). All intravenous injections were carried out under brief ether anesthesia.

Rats were inoculated with tumors as follows. Two rats bearing tumors having a mean diameter of 2.5–3.5 cm were killed by ether anesthesia. The tumors were excised immediately, finely minced, combined, and resuspended in 20–40 ml of isotonic saline. This suspension was filtered through a gauze pad to remove coarse pieces of tissue and the resulting suspension was inoculated subcutaneously (0.2–0.5 ml per injection) into the left flank of each rat. Tumors were palpable within 7 days in >98% of inoculated animals and generally reached a mean diameter of 2.5–3.5 cm within 10–14 days.

Comparative tumor-response experiments were carried out by inoculating randomly selected control and rescue groups with tumor as described above. On day 10, DDP at the appropriate dose was administered intravenously to both control and rescue groups. Two hours after DDP administration, the rescue group was treated with DDTC at a dose of 750 mg/kg given intraperitoneally or 100 mg/kg given intravenously. Tumor size was determined by measuring the longest and shortest diameters with a calipers and recording the arithmetic average. The rats were monitored for 50 days after tumor inoculation or until tumor size exceeded 4 cm. Comparative nephrotoxicity experiments were carried out as described (7). In those experiments using acetazolamide, the drug was administered in a dose of 10 mg/kg intraperitoneally 30 min prior to administration of DDP, and rescue was carried out 3 hr after administration of DDP.

Excretion of free and conjugated DDTC in the urine of rats was measured as follows. DDTC was administered in selected doses via intraperitoneal or intravenous injection (see Fig. 3). Acetazolamide was administered (10 mg/kg intraperitoneally) in selected cases 30 min prior to DDTC administration. Rats were then placed in metabolic cages and urine was collected and analyzed at 30-min intervals. Urine samples (0.1–5.0 ml) were added to 2 ml of a solution containing ferric nitrate (20 mM) and sodium citrate (200 mM). The dark $Fe(DDTC)_3$ complex was extracted into chloroform (4 ml) by mixing on a Vortex for 5 min. The mixture was chilled and centrifuged for 1 min, and the chloroform layer was removed. To this chloro-

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Abbreviations: DDP, *cis*-dichlorodiammineplatinum(II); DDTC, sodium diethyldithiocarbamate.

form solution was added saturated aqueous cupric sulfate (2 ml); the mixture was agitated on a Vortex for 5 min, chilled, and centrifuged for 1 min. The chloroform solution was removed and the $Cu(DDTC)_2$ absorbance was measured at 435 nm. Free DDTC was determined by comparison of absorbance to a standard curve. Total DDTC was determined by addition of 0.05–1.0 ml of urine to 3 ml of saturated cupric sulfate followed by incubation at 45°C for 30 min. The resulting $Cu(DDTC)_2$ complex was extracted into chloroform and analyzed as described above. DDTC-glucuronide excretion was determined by subtraction of free DDTC from total DDTC.

RESULTS

DDP was administered in doses of 2, 4, and 8 mg/kg to duplicate groups of eight rats (mean weight 125 g) at each dose level 10 days after injection of tumor cells. DDTC rescue (7) was carried out with an intraperitoneal dose of 750 mg/kg 2 hr after administration of DDP in one group at each dose level. Tumor responses at DDP doses of 2 and 8 mg/kg are summarized in Figs. 1 and 2, respectively; response to the 4 mg/kg dose was intermediate between these two (data not shown). At a DDP dose of 2 mg/kg without rescue, the mean tumor size diminished progressively until day 24, when two out of eight rats were free of tumors and six out of eight rats bore tumors that reached a mean minimal diameter of 1.6 cm. At day 30, the mean tumor diameter in these six rats had increased to 2.4 cm. Treatment with 2 mg of DDP per kg followed by 750 mg of DDTC per kg 2 hr later gave an initial response identical to that of the unrescued group; disappearance of tumor was not observed in this rescued group, however, and mean minimal tumor diameter reached 1.2 cm at day 21 with relapse to a mean tumor diameter of 3.0 cm at day 30. The rescue thus leads to an identical initial response but with earlier onset of relapse and more rapid subsequent tumor growth than in the absence of rescue. Qualitatively similar results were obtained at DDP doses of 4 and 8 mg/kg. In the latter case, however, three out of eight rats died as a result of DDP nephrotoxicity and three out of eight were free of tumors by day 28 in the unrescued group; with DDTC rescue there were no deaths and one out of eight was tumor free by day 28.

When DDTC (500 mg/kg) was administered intraperitoneally to rats, total DDTC excreted by the kidneys within 2 hr represented 18–20% of the dose given. Of this DDTC analyzed in the urine, <0.1% of the initial dose was present as the free ligand; the remainder was the *S*-glucuronide conjugate. When acetazolamide (Diamox; 10 mg/kg intraperitoneally) was administered 30 min before DDTC, the amount of excreted glucuronide was unchanged but free DDTC excretion increased 12-fold (data not shown). Reduction of the DDTC dose to 100 mg/kg intraperitoneally with prior administration of acetazolamide resulted in greater excretion of free DDTC than observed with 500 mg of DDTC per kg without acetazolamide (see Fig. 3). Finally, administration of DDTC as an intravenous bolus of 100 mg/kg with or without acetazolamide increased renal excretion of free DDTC in the first 30 min by 5- to 10-fold over that observed with an intraperitoneal dose of 500 mg/kg.

The effects of DDTC at 100 mg/kg with and without acetazolamide upon rescue from DDP nephrotoxicity were

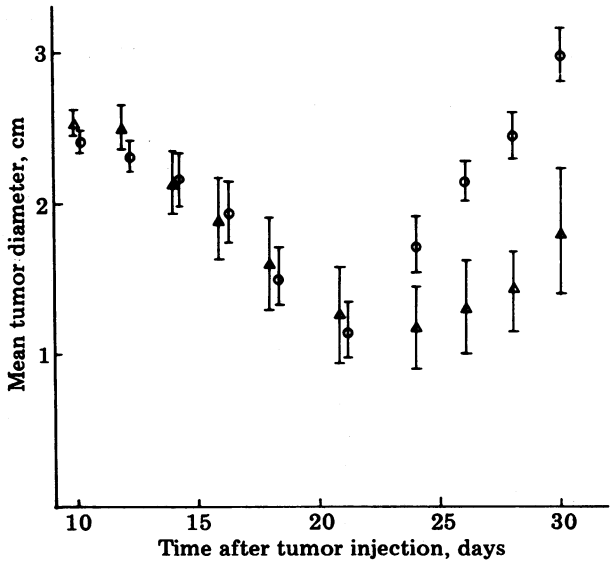

FIG. 1. Response of mammary tumor 13762 in female F344 rats to 2.0 mg of DDP per kg with (○) and without (△) rescue by 750 mg of DDTC per kg intraperitoneally 2 hr after administration of DDP (n = 8; mean ± SEM).

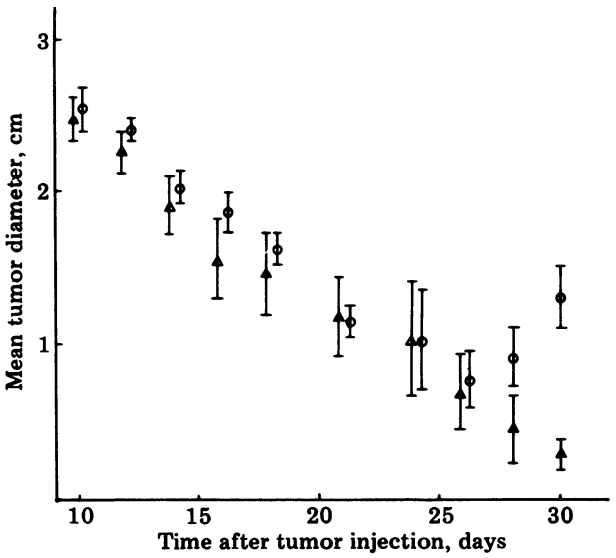

FIG. 2. Response of mammary tumor 13762 in female F344 rats to 8.0 mg of DDP per kg with (○) and without (△) rescue by 750 mg of DDTC per kg intraperitoneally 2 hr after administration of DDP (n = 8; mean ± SEM).

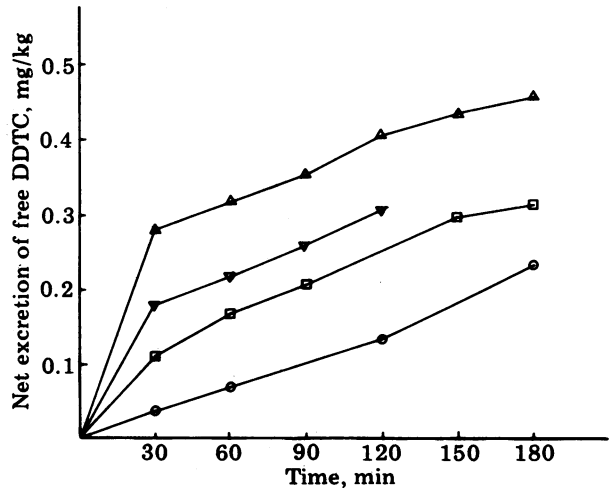

FIG. 3. Urinary excretion of free DDTC in female F344 rats. ○, DDTC (500 mg/kg intraperitoneally); □, acetazolamide (10 mg/kg) followed by DDTC (100 mg/kg intraperitoneally) 30 min later; ▽, DDTC (100 mg/kg intravenously); △, acetazolamide (10 mg/kg) followed by DDTC (100 mg/kg intravenously) 30 min later.

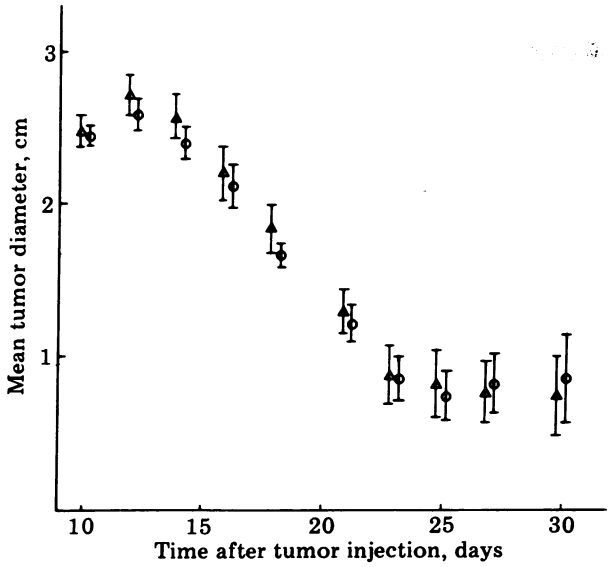

FIG. 4. Response of mammary tumor 13762 in female F344 rats to 2.0 mg of DDP per kg with (○) and without (△) rescue by DDTC (100 mg/kg intravenously) 2 hr after administration of DDP (n = 8; mean ± SEM).

studied as described (7). A group of 16 rats was treated with DDP (8 mg/kg intravenously); half of these rats (control group) received DDTC (500 mg/kg intraperitoneally) 3 hr later, and the other half received acetazolamide (10 mg/kg intraperitoneally) 2½ hr and DDTC (100 mg/kg intraperitoneally) 3 hr after administration of DDP. Although weight loss was comparable in the two groups, blood urea nitrogen levels on day 5 were 120 ± 9 (SEM) in the control group and 250 ± 24 (SEM) in the acetazolamide-treated group. In the acetazolamide-treated group three out of eight rats died on days 5–7 compared to no deaths in the control group. Necropsy revealed a grossly normal gastrointestinal tract in both groups.

Comparison of low-dose intravenous with high-dose intraperitoneal DDTC rescue showed that protection against nephrotoxicity was essentially equivalent for both routes. DDP (6 mg/kg intravenously) was administered to three groups of eight rats each. The control group received no further treatment, the intravenous group received 100 mg of DDTC per kg intravenously, and the intraperitoneal group received 750 mg of DDTC per kg intraperitoneally 2 hr after administration of DDP. Weight loss on day 5 was similar in the three groups (11%, 14%, and 9%, respectively); diarrhea was noted in one out of eight rats in the control group but was not observed in the intravenous or intraperitoneal groups. Blood urea nitrogen values on day 5 for the control, intravenous, and intraperitoneal groups were (mean ± SEM) 92 ± 15, 43 ± 5, and 73 ± 4, respectively. Kidney sections from the control group revealed a zone of moderate necrosis and degeneration at the corticomedullary junction similar in type to, but to a lesser degree than, that observed previously at DDP doses of 8 mg/kg (7). Sections from the intravenous and intraperitoneal rescue groups were similar and showed mild hydropic changes with occasional evidence of degeneration.

Finally, tumor responses to DDP in the absence of rescue and in the presence of low-dose intravenous and high-dose intraperitoneal rescue were compared. DDP (2 mg/kg intravenously) was administered on day 10 after tumor inoculation. The control group (n = 8) received no further treatment; the intravenous (n = 8) and intraperitoneal (n = 11) groups received DDTC 2 hr after DDP in doses of 100 mg/kg intravenously and 750 mg/kg intraperitoneally, respectively. Tumor response in the intraperitoneal rescue group was qualitatively similar to that described previously; minimal tumor diameter (1.3 cm) was reached on day 23, with relapse occurring in nine out of eleven rats to a mean tumor diameter of 2.2 cm by day 30 (data not shown). Tumor responses in the control and intravenous rescue groups, however, were equivalent in all respects (see Fig. 4). The initial response was identical in both groups; relapse was observed in two out of eight rats in each group between days 25 and 30, with the other rats remaining essentially free of tumor through day 50, when the study was terminated.

DISCUSSION

We have demonstrated previously that intraperitoneal administration of DDTC in doses of 500–750 mg/kg would afford protection from the nephrotoxic effects of DDP at doses as high as 8 mg/kg (7). This rescue protocol was tested in the rat mammary tumor 13762 model to determine its effect on inhibition of tumor growth by DDP. This tumor model is unusual in that spontaneous relapse at the original site occurs approximately 15 days after DDP administration in 25–75% of animals. Although initial tumor response to 2, 4, and 8 mg of DDP per kg was identical with or without DDTC rescue, local recurrence commenced earlier and tumors grew more rapidly in those animals receiving DDTC at intraperitoneal doses of 750 mg/kg.

Two approaches were then examined in an attempt to deliver larger quantities of free DDTC to the likely site of toxicity in the renal tubule; urinary excretion of DDTC was monitored as a means of estimating changes in tubular levels of free DDTC. The standard assay for DDTC uses a spectrophotometric determination following the reaction with cupric ion to form the colored $Cu(DDTC)_2$ complex (8). Unfortunately, the affinity of cupric ion for sulfur ligands is such that this metal ion will catalyze the decomposition of the DDTC-glucuronide and, therefore, will not discriminate between free and conjugated DDTC. Ferric ion reacts selectively with the free ligand, however, so that we now have a convenient assay for both free and total DDTC in urine. DDTC is unstable at pH values below 6 (9); we have demonstrated that DDTC decomposition is first order with respect to H^+ concentration in the pH range 5–8. Acetazolamide, a carbonic anhydrase inhibitor, decreases renal bicarbonate absorption and increases urine pH. The combination of DDTC and acetazolamide resulted in significant increases in renal excretion of DDTC as expected, even when the DDTC dose was reduced by 80%. Notwithstanding this increase in urinary DDTC levels, the combination of DDP, DDTC, and acetazolamide was clearly more toxic to rats than the combination DDP and DDTC alone. Conceivably the site of toxicity occurs proximal to the site of major carbonic anhydrase activity in the tubule; acetazolamide would only increase levels of free DDTC distal to the site of normal urinary acidification and would not potentiate rescue at proximal sites.

Because DDTC is rapidly conjugated in the liver (10), intravenous administration should be more effective than the oral or intraperitoneal route for delivery of free DDTC to the kidney. In fact, 100 mg of DDTC per kg administered intravenously cleared more than 6 times as much free DDTC in the urine at 30 min than 750 mg/kg given intraperitoneally. Rescue experiments demonstrated that 100 mg of DDTC per kg given intravenously protects against the nephrotoxicity of 6 mg of DDP per kg as well as 750 mg of DDTC per kg given intraperitoneally. This rescue protocol was ultimately tested for its effects on tumor response to DDP at a dose of 2 mg/kg; this DDP dose was selected because of its maximal sensitivity to the effects of DDTC noted in earlier experiments. Under these

conditions, no adverse effects of DDTC administration on tumor response to DDP were observed. Thus we have demonstrated that DDTC can be administered in doses that effectively inhibit platinum-induced nephrotoxicity without deleterious effects on tumor response.

Comparison of the (nonrescued) control groups in Figs. 1 and 4 shows very different late responses to an identical dose of DDP, with significant differences in minimal tumor size, incidence of relapse, and subsequent growth rate. The only significant differences between these two groups were in rat weight (mean of 125 g in contrast to 150 g) and, presumably, in age. Because this is a mammary tumor and may in fact be hormone dependent, age and maturation level may be important variables in assessing tumor response in this model.

The basis for the earlier and more rapid growth of locally recurring tumors in the high-dose intraperitoneal rescue is not clear. If this does in fact represent a reversal of platinum–DNA binding in tumor cells, it is not obvious why initial tumor response to DDP is not affected. Because the low-dose intravenous rescue presumably gives higher plasma levels of free DDTC, one might expect greater inhibition of the antitumor effect with this protocol if tumor cell–DDTC interactions were involved. Conceivably the DDTC-glucuronide or another unidentified hepatic metabolite is acting as a carrier or transport form which provides access to the intracellular milieu; preliminary experiments in our laboratory, however, suggest that improbably high intracellular levels of DDTC must be maintained for hours to reverse platinum–DNA binding in tumor cells.

Evidence has appeared recently supporting our hypothesis (7) that nephrotoxicity results from platinum binding to sulfhydryl-containing tubular enzymes; in this case, renal tubule Na^+,K^+-ATPase is specifically implicated. Guarino *et al.* (11) also provide evidence that this mechanism operates in DDP-induced ototoxicity and suggest that it might be the mechanism for gastrointestinal and neurotoxicity as well.

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