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High-dose cisplatin with diethyldithiocarbamate (DDTC) rescue therapy: preliminary pharmacologic observations*

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Summary. Diethyldithiocarbamate (DDTC), a chelating agent that is a major metabolite of disulfuram, has been proposed as a potential rescue agent to reduce toxicity following high-dose cisplatin (HDCP) therapy. In the present study, we examined the pharmacologic interaction of HDCP and DDTC given as rescue therapy. Total plasma platinum and ultrafiltrate platinum pharmacokinetics and DDTC levels were determined in six patients with advanced malignancies who received a total of 11 cycles of HDCP with DDTC rescue. HDCP therapy (200 mg/m^2 per cycle) consisted of 100 mg/m^2 reconstituted in 250 cc 3% saline and infused over 3 h on days 1 and 8 of each 28-day cycle. DDTC rescue at a dose of 4 gm/m^2 was given by an i.v. infusion (duration 1.5–3.5 h), beginning 45 min after the completion of cisplatin infusion. Peak total and ultrafiltrate levels and cisplatin pharmacokinetics in this study were indistinguishable from those of previous studies using the same HDCP regimen without DDTC rescue. Ultrafiltrate or unbound plasma platinum was $<10\%$ of total plasma platinum concentrations and demonstrated a biphasic pattern of elimination. Levels of DDTC predicted to be chemoprotective ($>400\ \mu M$) were achieved with the dose and schedule used in this study. These data demonstrate that DDTC can be targeted to protective plasma concentrations without significantly altering plasma cisplatin pharmacokinetics and support the potential usefulness of DDTC as a rescue agent following HDCP therapy.

Introduction

Cisplatin is an antineoplastic agent with activity in a broad range of solid tumors [10, 20]. Both in vitro studies and previous clinical trials have suggested a positive dose-response relationship with this agent [12, 17, 18]. Early attempts to explore this potential by dose escalation were complicated by an unacceptable incidence of nephrotoxicity. Although recent studies confirm that hypertonic saline may substantially reduce this complication, allowing approximately twice the standard dose/cycle of cisplatin to be given, nonrenal toxicities, especially myelosuppression, ototoxicity, and peripheral neuropathy, continue to be dose-limiting [12, 13, 15, 17, 18]. Therefore, new approaches are needed to exploit effectively the dose-response relationship of cisplatin.

In addition to hypertonic saline, other investigational approaches to reducing cisplatin-induced toxicity include the use of potential rescue agents such as thiosulfate and diethyldithiocarbamate (DDTC). Recent studies suggest that although it reduces nephrotoxicity, thiosulfate may fail to increase the therapeutic index of cisplatin except in i.p. therapy [1, 6]. In contrast, extensive animal studies have reported that DDTC is effective in reducing cisplatin-associated renal toxicity, bone marrow suppression, and emesis but has no deleterious effects on antitumor activity [3, 4, 11]. A limited phase I clinical trial confirmed renal protection by DDTC at standard cisplatin doses of 50–120 mg/m^2 and demonstrated the feasibility of this type of rescue therapy [5, 19].

In view of the potential benefits of altered dose scheduling of high-dose cisplatin (HDCP) and the protective effects of DDTC rescue, we carried out a pilot study examining the pharmacologic interaction and clinical effects of a pharmacokinetically designed schedule of HDCP in hypertonic saline with DDTC rescue. The following report summarizes the preliminary observations regarding plasma platinum and DDTC pharmacokinetics in this study.

Methods

Treatment schedule. Before therapy was begun, informed consent was obtained on a protocol approved by the Institutional Review Board. Treatment with cisplatin was preceded by the administration of appropriate antiemetics and i.v. hydration with normal saline. Cisplatin at a dose of 100 mg/m^2 was reconstituted in 250 cc 3% saline and infused over 3 h on days 1 and 8 of each 28-day cycle. Following the completion of cisplatin infusion, hydration with normal saline was continued for approximately 6 h. Administration of DDTC (4 g/m^2 as an i.v. infusion over a planned 1-h period) began 45 min after the completion of each cisplatin infusion. Patients were premedicated with pentobarbital to reduce DDTC-related constitutional side effects (flushing, chest discomfort, anxiety) during the infusion [5, 19].

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Platinum and DDTC pharmacokinetics. *Cis*-Dichlorodiammineplatinum(II) (Platinol for injection) was obtained from Bristol Laboratories (Syracuse, NY). DDTC sodium for injection was obtained from Merieux Institute (Miami, Fla). All other reagents and chemicals used were of analytical grade.

Plasma samples for cisplatin analysis were collected at appropriate times after cisplatin and DDTC infusions and each sample was placed immediately into prechilled, sodium heparinized tubes. After centrifugation, plasma was separated and frozen at −20° C until the day of analysis [12]. Ultrafiltrate plasma was prepared by placing plasma aliquots into Amicon CF-30 filters (mol. wt. cutoff, 30,000) and centrifuging at 5,000 *g* and 4° C for 45 min.

Analysis of platinum by flameless atomic absorption spectrophotometry (FAAS) was accomplished using a Perkin-Elmer model 2280 atomic Absorption spectrophotometer (Norwalk, Conn) equipped with a platinum cathode lamp, and a graphite furnace. Oven programming for platinum analysis was as follows: (1) warming, 60 s at 90° C; (2) drying, 30 s at 110° C; (3) charring, 45 s at 1,500° C; (4) cooling, 15 s at 20° C; (5) atomization, 5 s at 2,500° C; and (6) cleaning, 3 s at 2,650° C. Each sample (20 µl) was pipetted by hand into a pyrocoated graphite tube and assayed in triplicate. The spectrophotometer was set to measure absorbance at 265.9 nm using deuterium background correction. The spectrophotometer was calibrated daily and checked every other sample with standard solutions of cisplatin in 0.9% saline, plasma ultrafiltrate, or plasma. The assay sensitivity is approximately 5 ng/ml and correlation coefficients for linear dilutions of cisplatin standard solutions are >0.99.

Peak DDTC levels were determined using the HPLC method of Lieder and Borch [14]. Plasma samples for DDTC analysis were stabilized with 10 µl 1 *M* sodium hydroxide solution, shipped on dry ice, and analyzed within 24 h of their arrival. Aliquots of plasma (0.5 ml) were vortexed, with the simultaneous addition of 10 µl Meerwein's reagent in methylene chloride (1 g/ml). Chloroform (0.5 ml) containing S-isopropyl-diethyldithiocarbamate (80 µg/ml) was added to each aliquot, which was then vortexed for 30 s. Emulsions were centrifuged at 1,000 *g* for 3 min to aid phase separation; this step was repeated as necessary. The organic phase (100 µl) was removed and diluted with 500 µl acetonitrile, and this solution was passed through a 0.45 µm filter and injected (20 µl) onto a Beckman model 345 ternary HPLC with an Econosphere C18 column (250 × 4.6 mm, 5 µm particle). The isocratic mobile phase consisted of acetonitrile:water (70%:30%) at a flow rate of 2.0 ml/min. Absorbance at 254 nm was recorded using an HP 3390A integrator. The ratio of S-ethyl-diethyldithiocarbamate peak height (3.8 min) to S-isopropyl-diethyldithiocarbamate peak height (4.7 min) was used to construct standard curves and calculate plasma concentrations of DDTC. Standards were prepared by spiking outdated human plasma (obtained from the American Red Cross) with DDTC.

Table 1. Peak total platinum, ultrafiltrate platinum, and DDTC concentrations (*µM*) after high-dose cisplatin on days 1 and 8

	Day 1	Day 8
Total plasma platinum	3.62 (0.84)	4.61 (1.49)
Ultrafiltrate platinum	0.43 (0.150)	0.43 (0.107)
DDTC	466 (229)	442 (281)

[a] Samples represent an average of 6–15 determinations. Standard deviations are noted in parentheses

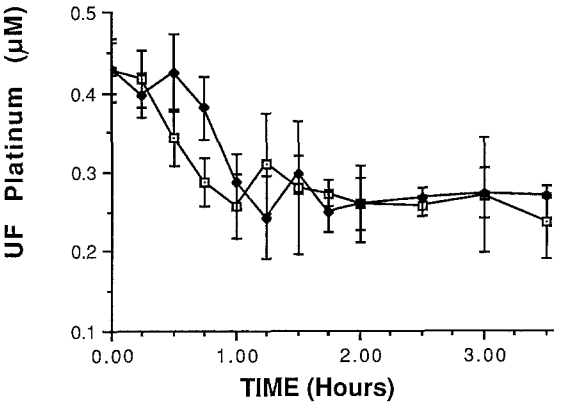

Fig. 1. Mean postinfusion ultrafiltrate platinum (*UF*) concentrations following the start of DDTC infusion on day 1 (⊡–⊡) and day 8 (◆–◆). Bars represent SEM

Results

Peak plasma concentrations for total platinum, ultrafiltrate platinum, and DDTC are shown in Table 1. Peak total platinum and ultrafiltrate platinum occurred at the end of the cisplatin infusion. The mean peak of total plasma platinum increased from 3.62 to 4.61 µ*M* from day 1 to day 8, whereas mean peak ultrafiltrate levels did not change (0.43 µ*M* for both doses), as previously described [12]. Mean peak DDTC concentrations were 466 and 442 µ*M* for days 1 and 8, respectively, with a range of 131–970 µ*M*. Peak DDTC levels >400 µ*M* were obtained in 8 of 13 infusions in which DDTC pharmacokinetics were monitored. The time at which peak DDTC levels occurred varied due to differences in the duration of DDTC infusion (from 1.0 to 3.5 h), related to constitutional side effects of DDTC in some subjects [5].

Plasma platinum ultrafiltrate concentrations from the start of the DDTC infusion are shown in Fig. 1. The biphasic elimination pattern for ultrafiltrate platinum with DDTC rescue was similar to that previously reported for an identical HDCP schedule given without DDTC for both day 1 and day 8 [12]. No apparent accumulation of plasma ultrafiltrate platinum on day 8 was evident from this study [7, 9].

Discussion

DDTC is a heavy-metal-chelating agent that may be unique among proposed cisplatin chemoprotectors in the selectivity of its reactions with cisplatin. The mechanism of action appears to be the chelation and removal of tissue-bound platinum without reversal of the critical platinum-bisguanosine adducts essential for antitumor activity [2, 8]. Animal studies and a prior phase I trial in man have suggested that a peak DDTC concentration >400 µ*M*, requiring a dose of 2–4 g/m^2, is necessary for optimal renal chemoprotection [3, 5, 19]. In one previous study, DDTC at a much lower dose (600 mg/m^2) was reported to change

markedly the pharmacokinetics of ultrafiltrate cisplatin, almost doubling the terminal half-life to 30 h due to circulating DDTC-platinum complexes [16]. This finding, if confirmed, could result in a possible detrimental effect on the antitumor activity of cisplatin.

The present study described the preliminary pharmacologic observations regarding DDTC rescue therapy following a regimen of HDCP that has been well characterized in terms of pharmacokinetics and clinical toxicity patterns [9, 12]. DDTC rescue was delivered at a dose and schedule predicted to achieve chemoprotection based on prior experience with this agent [3, 5, 19]. The pharmacologic goals of this study were to examine plasma platinum pharmacokinetics following DDTC rescue and to determine plasma DDTC levels. The preliminary results show that DDTC does not significantly alter the total platinum or ultrafiltrate platinum kinetic patterns as compared with the results of our previous studies of this HDCP regimen without DDTC [12]. Ultrafiltrate platinum, containing the cytotoxically active fraction, accounted for $< 10\%$ of total peak plasma platinum concentrations and demonstrated a biphasic elimation pattern. During most infusions, peak DDTC concentrations above the level previously shown to provide optimal protection against cisplatin-induced renal toxicity [5] were achieved.

The current study was designed to achieve peak DDTC plasma concentrations of $> 400\ \mu M$, since adequate peak levels during the chemoprotective period may be more important than the total exposure [5]. Even though a wide range of peak DDTC plasma concentrations was observed ($131-970\ \mu M$), the levels achieved were sufficient to test the effectiveness of DDTC as a rescue agent. In addition to possible variability between individual subjects, the range of peak DDTC concentrations in this study may be explained by two other factors. First, DDTC may undergo decomposition in plasma if the latter is not processed and analyzed within a short period after collection. After variability was observed in the initial DDTC specimens in this study, this effect was minimized by alkalinization of subsequent samples with sodium hydroxide. Second, the duration of DDTC infusion during the initial phase of this study was variable, ranging from 1 to 3.5 h due to constitutional side effects of DDTC. Since the half-life of DDTC is approximately 10 min, the longer infusion times would be expected to reduce substantially peak DDTC levels [5]. Nevertheless, both of these factors would lower the apparent peak DDTC levels achieved. Therefore, the DDTC levels measured in this study probably represent conservative estimates of pharmacologic concentrations obtained in some subjects. In summary, we present preliminary evidence showing that DDTC can be targeted to protective plasma concentrations without significantly altering the plasma pharmacokinetics of total or ultrafiltrate platinum. These observations support the potential of DDTC as a chemoprotector following cisplatin therapy.

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