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Comparison of the Pharmacokinetics of Ultrafilterable Cisplatin Species Detectable by Derivatization with Diethyldithiocarbamate or Atomic Absorption Spectroscopy

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Abstract—*The pharmacokinetics of the cisplatin (DDP) species detected by measurement of diethyldithiocarbamate (DDTC)-reactive species (DDTC-DDP) were compared to the pharmacokinetics of the species detected by measurement of total ultrafilterable platinum in patients receiving DDP alone or in combination with the nephroprotective agent sodium thiosulfate. The doses of DDP studied were* 100 mg/m^2 (11 *courses given to eight patients) and* 202.5 mg/m^2 (*five courses given to four patients) given as* 2 h *i.v. infusions, the latter with concurrent thiosulfate. When DDP was given alone* (100 mg/m^2) *the two assays yielded the same area under the curve (AUC) values for DDTC-DDP and total ultrafilterable platinum during the first* 4 h *after the start of infusion; however, beyond* 4 h *post-infusion, the AUC for total ultrafilterable platinum was consistently greater than that for DDTC-DDP. When DDP was given with thiosulfate* (202.5 mg/m^2), *the AUC for total ultrafilterable platinum was significantly greater than that of DDTC-DDP during the whole sampling period. The ratio of the AUC for total ultrafilterable platinum to DDTC-DDP, when DDP was given with thiosulfate, was barely significantly greater than that when DDP was given alone. These data indicate that during and immediately following a short infusion of DDP the major platinum-containing species present in plasma ultrafiltrate are still capable of reacting with nucleophilic sites on molecules such as DDTC; however, as the reactive species are eliminated, longer half-lived non-reactive ultrafilterable platinum species begin to predominate. They also indicate that although thiosulfate does neutralize a measurable amount of DDP in the plasma on the schedule employed, this degree of neutralization is not sufficient to explain the protection against DDP-induced nephrotoxicity produced by thiosulfate.*

INTRODUCTION

CISPLATIN (DDP) is predominantly cleared from plasma by covalent reaction with proteins and renal excretion [1–8]. The original studies of DDP pharmacokinetics were accomplished by measuring total platinum in plasma or urine by graphite furnace atomic absorption spectrophotometry [1–3, 9], or by monitoring the radioactivity from ^{193m}Pt-labeled DDP [4]. More recently most investigators have used the amount of platinum in plasma ultrafiltrate to estimate the concentration of free DDP [6, 7, 10]. However, ultrafiltrate may contain both native DDP and several metabolites as well [7]. We have made extensive clinical use of an assay [11] originally developed by Bannister *et al.* [12] and Borch *et al.* [13] that permits high pressure liquid chromatographic (HPLC) quantitation of those DDP species capable of reacting with the strong nucleophile DDTC. In order to further assess the utility of this convenient DDTC assay, we have compared the pharmacokinetics of the DDP species detected by DDTC derivatization to the pharmacokinetics of the species detected by measurement of total ultrafilterable platinum in patients receiving DDP alone or in combination with the nephroprotective agent sodium thiosulfate.

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MATERIALS AND METHODS

Studies were conducted on 11 patients during a total of 16 courses of treatment with DDP administered as a 2 h i.v. infusion at a dose of either 100 mg/m^2 without concurrent sodium thiosulfate (11 courses to eight patients) or 202.5 mg/m^2 with thiosulfate (five courses to four patients; one patient was given one course of each dose). When thiosulfate was used it was administered i.v. in the arm opposite to that receiving the DDP infusion at a dose of 10 g/m^2 as a 3 h infusion starting 1 h prior to the start of the 2 h DDP infusion. The patients did not receive any other medications except antiemetics. DDP was supplied by Bristol-Myers Co. (Syracuse, NY) as well as by the Pharmacologic Resources Branch of the National Cancer Institute (Silver Springs, MD). Sodium thiosulfate was supplied by Eli Lilly and Company (Indianapolis, IN).

Blood samples (7 ml) were collected before treatment, and then at a maximum of 22 further time points over the 2 h DDP infusion and for 6 h thereafter. Samples were drawn into chilled heparinized tubes, and the plasma was immediately centrifuged at 991 ***g*** through CF 25 A filter cones (Amicon Corp., Danvers, MA) for 30 min at 4°C. A 0.5 ml aliquot of the ultrafiltrate was used for immediate DDTC derivatization, and the remainder was frozen at −20°C for analysis by atomic absorption spectroscopy (AA).

Ultrafilterable DDP species reactive with DDTC were quantitated using the assay whose characteristics have been reported by Andrews *et al.* [11]. Five μl of 841 μM nickel chloride was added to the 0.5 ml aliquot of ultrafiltrate as an internal standard. Fifty μl of 10% DDTC in 0.1 N NaOH was then added, and the mixture was incubated at 37°C for 30 min, then chilled on ice and extracted with 0.2 ml of chloroform. A 20 μl aliquot of the chloroform layer was injected on to a Waters Associates (Milford, MA) high pressure liquid chromatograph; a 10 cm × 8 mm Radial-Pak C_{18} cartridge (10 μm particle size) was used as the column. The mobile phase consisted of a 4:1 methanol–water solution (vol/vol) pumped at a flow rate of 1.5 ml/min. Detection was accomplished at 254 nm at a sensitivity of 0.01 absorbance units full scale.

Total platinum in the ultrafiltrate was quantitated by graphite furnace atomic absorption spectroscopy using a Perkin–Elmer 373 atomic absorption spectrophotometer equipped with an HGA-2200 graphite furnace, with a lamp current of 15 mA and monitoring of the 265.9 nm platinum line. Injection volumes of 2–20 μl of thawed ultrafiltrate were analyzed using the following temperature program: dry at 100°C for 50 s, ramp to 1300°C over 10 s, char at 1300°C for 15 s, and atomize at 2350°C for 7 s. The limit of sensitivity (signal to noise ratio greater than 2) for both assays was 0.05 μg/ml.

Pharmacokinetic analyses were carried out separately for each course of treatment. No parameters were to be assumed to operate in common, either within a patient, dose level, or assay level. The parametric models fitted were of a standard idealized form for constant infusion over a limited time, followed by one compartment clearance. The model is represented by the equation:

$$Y(t) = C_{ss}(1 - e^{-k[\min(t,T)]})\,(e^{-k[\max(t-T,0)]})$$

where t is time in minutes, T is the infusion time, $Y(t)$ is the drug concentration at time t, k_e is the elimination constant, C_{ss} is the projected steady-state concentration of DDP assuming an infinitely long continuous infusion at the same dose rate, min (t,T) denotes minimum t and T, and max $(t-T,0)$ denotes maximum $[(t-T),0]$. The estimate is nonlinear, and an approximte maximum likelihood procedure was used assuming log normal errors on the responses.

The total area under the fitted concentration versus time curve (time 0 to infinity) takes on the simple form TC_{ss} (equivalent to $120C_{ss}$ in this study). Each AUC determined by this method was checked against the AUC calculated by the trapezoidal method. The half-life was calculated as $0.693/k_e$. The clearance was calculated as the absolute dose divided by $120C_{ss}$, the volume of distribution as absolute dose/$120C_{ss}k_e$, and the mean residence time as $AUMC_{120-\infty}/AUC_{120-\infty}$ (this is equivalent to the standard formula given by $[(AUMC_{0-\infty}/AUC_{0-\infty}) - (\text{infusion duration}/2)]$). For the purpose of testing whether the assay methods were sytemically different, k_e and C_{ss} were treated as random effects, and the 32 sampled estimates of k_e and C_{ss} were subjected to an unbalanced analysis of variance with a two-way layout, according to the factors dosage and assay method. Each case was weighted according to the standard error of estimation, to take account of variable reliability from some curves having more sampled points than others. The analyses of variance were calculated using special software (PC-ISP, Artemis Systems, 1985). The standard errors were derived from delta method calculations.

RESULTS

The concentration versus time data for total ultrafilterable platinum and DDTC-DDP obtained in patients receiving DDP alone (100 mg/m^2) are plotted in Fig. 1. The data for patients receiving DDP in combination with sodium thiosulfate are presented in Fig. 2. Each point represents the arithmetic mean concentration for all courses given. The pharmacokinetic parameters calculated from these curves are presented in Table 1.

The two assays were compared with respect to the AUC (as determined by the trapezoidal method)

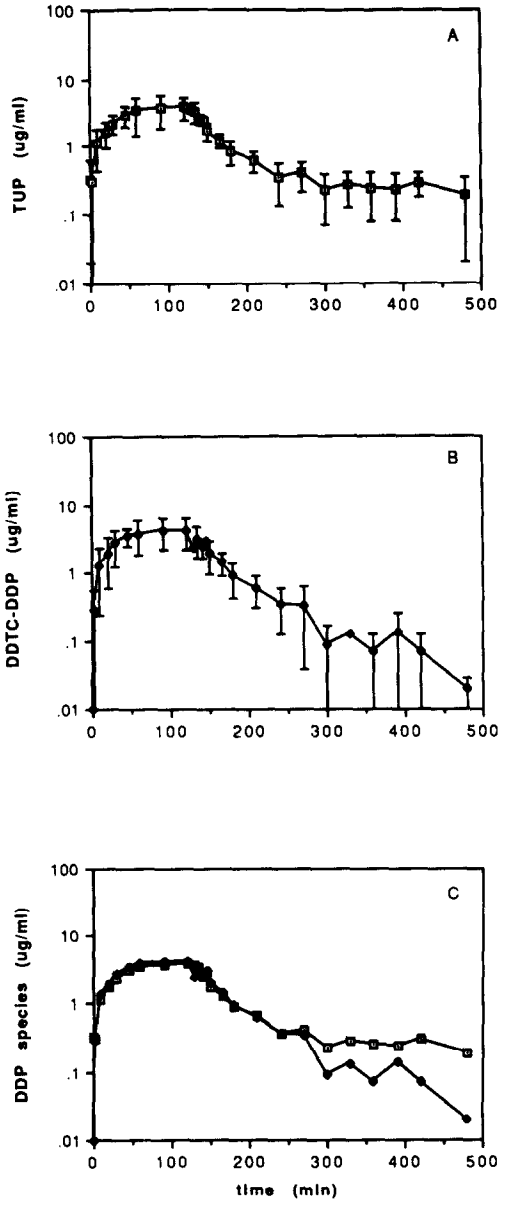

Fig. 1. Mean total ultrafilterable platinum (TUP) concentration (A) and DDTC-DDP concentration (B) in plasma ultrafiltrates of 11 patients receiving DDP 100 mg/m² as a 2 h infusion without concurrent thiosulfate. The vertical error bars represent standard deviations. The two curves are superimposed in panel C so that a comparison of the concentrations of the DDP species measured by atomic absorption spectroscopy (i.e. TUP) can be made with those measured by HPLC (i.e. DDTC-DDP).

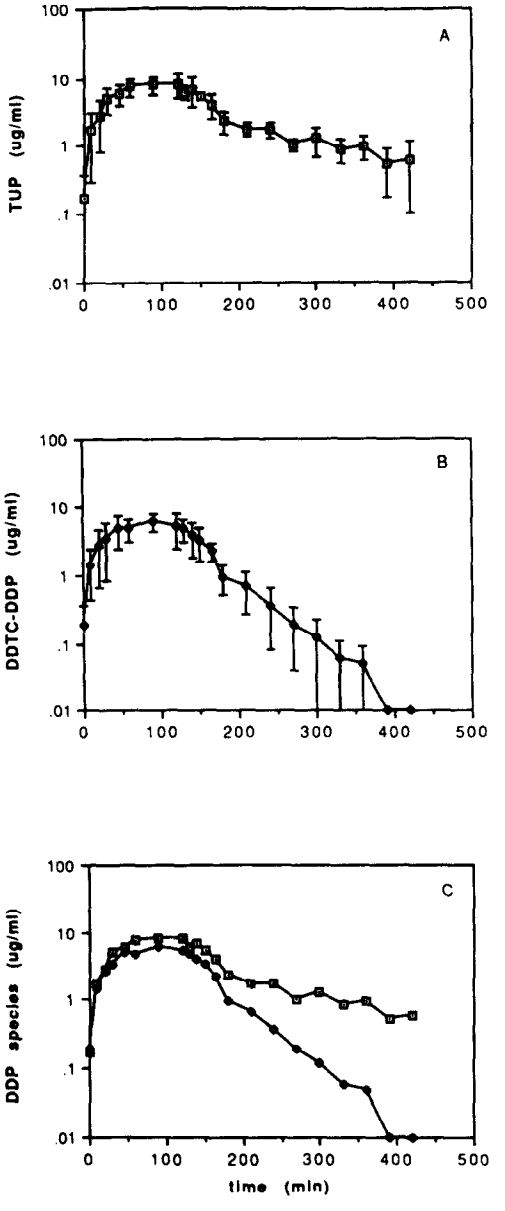

Fig. 2. Mean total ultrafilterable platinum (TUP) concentration (A) and DDTC-DDP concentration (B) in plasma ultrafiltrates of five patients receiving DDP 202.5 mg/m² over 2 h concurrently with thiosulfate. The vertical error bars represent standard deviations. The two curves are superimposed in panel C so that a comparison of the concentrations of the DDP species measured by atomic absorption spectroscopy (i.e. TUP) can be made with those measured by HPLC (i.e. DDTC-DDP).

that they yielded for the full period of sampling, and for each 1 h interval from the beginning of the infusions. They were also compared with respect to the AUC values obtained when DDP was infused alone at 100 mg/m² as opposed to when it was infused at 202.5 mg/m² in combination with sodium thiosulfate.

When DDP was infused alone, the two assays yielded esssentially the same cumulative AUC for the first 4 h. Thereafter the AUC for the DDTC-DDP was a progressively smaller fraction of the AUC for total ultrafilterable platinum for each additional hour until the end of the sampling period. During the period from 4 to 8 h, the AUC for DDTC-DDP was only 45 ± 50% (S.D.) of the AUC for total ultrafilterable platinum, and this difference was statistically significant ($P < 0.05$, paired t test). Since the cumulative AUC during the 4–8 h time period was only 10 ± 11% (S.D.) of the total AUC for total ultrafilterable platinum and 4 ± 5% (S.D.) for DDTC-DDP, it was not unexpected to find no statistically significant difference in total AUC values over the 8 h sampling period for the two assays given the variance observed between patients.

When DDP was infused in combination with thiosulfate (202.5 mg/m²), the same general pattern

Table 1. Pharmacokinetic parameters

Dose(mg/m²)	100	100	202.5	202.5
Assay	HPLC	AA	HPLC	AA
Species	DDTC-DDP	TUP*	DDTC-DDP	TUP*
Thiosulfate given	No	No	Yes	Yes
Number of patients	8	8	4	4
Number of courses	11	11	5	5
Nonlinear fitted curves				
k_e (min^{-1})†				
(mean)	0.016	0.0088	0.019	0.0083
(±S.E.)	±0.001	±0.0006	±0.001	±0.0005
C_{ss} (μg/ml)†				
(mean)	4.29	4.72	5.80	11.9
(±S.E.)	±0.26	±0.34	±0.46	±0.5
Calculated parameters				
AUC(μg-min/ml)				
Nonlinear method		(0 to infinity)		
(mean)	515	566	696	1422
(±S.E.)	±3	±41	±55	±65
Trapezoidal method‡	(0–8 h)		(0–7 h)	
(mean)	601	588	839	1450
(±S.D.)	±199	±146	±279	±366
Half life (min)†				
(mean)	44	79	36	84
(±S.E.)	±3	±6	±2	±6
Volume of distribution (l)				
(mean)	26	35	32	32
(S.E.)	±2	±4	±3	±2
Clearance(ml/min)				
(mean)	347	304	584	268
(±S.E.)	±21	±22	±46	±12
Mean residence time (min)				
(mean)	74	132	55	125
(±S.E.)	±4	±8	±3	±8

*Total ultrafilterable platinum.

†Both parameters are significantly different, when compared between doses (within a given assay method), and when compared between assays (within a given dose). The P values are all less than 0.001, except that the P value is less than 0.01, when k_e and $t_{1/2}$ are compared between doses, by AA. The analysis of variance are used on various subsets of parametric fits, weighted for their own standard errors.

‡The total AUCs are compared between assays for the two doses. They are not significantly different ($P > 0.05$, paired t test) at 100 mg/m², but are significantly different at 202.5 mg/m² ($P < 0.01$).

was observed with the assays yielding more and more divergent AUC values with increasing time. However, in this case the AUC values for the two assays were significantly different during the first 4 h ($P < 0.01$, paired t test), the final 3 h ($P < 0.05$), and also during the whole 7 h sampling time ($P < 0.01$). The total AUC for DDTC-DDP was 57 ± 24% (S.D.) of the AUC for total ultrafilterable platinum when measured over the full 7 h period.

When DDP was infused alone (100 mg/m²) the plasma half-life for DDTC-DDP was 44 ± 3 (S.E.) min, whereas for the total ultrafilterable platinum it was 79 ± 6 (S.E.) min. When DDP was infused in combination with thiosulfate (202.5 mg/m²) the half-life of the DDTC-DDP was 36 ± 2 (S.E.) min, and that for total ultrafilterable platinum was 84 ± 6 (S.E.) min. For both doses the half-life of the DDTC-DDP was statistically significantly less than that for the total ultrafilterable platinum ($P < 0.001$, analysis of variance). Thiosulfate also significantly affected the half-lives calculated using the two different assay methods: it increased the half-life of total ultrafilterable platinum and decreased the half-life of DDTC-DDP ($P < 0.01$ and $P < 0.001$ respectively, analysis of variance).

The difference in the steady-state concentration that would have been attained if the DDP infusion was continued indefinitely was projected based on the data obtained from the 2 h infusion for each assay. The projected steady-state concentrations of total ultrafilterable platinum and DDTC-DDP were significantly different at both doses ($P < 0.001$, analysis of variance on various subsets of parametric

fits, weighted for their own standard errors). When DDP was infused alone the projected steady-state concentration of total ultrafilterable platinum was 0.4 μg/ml higher than that projected for DDTC-DDP. When DDP was administered with thiosulfate, the projected steady-state concentration for total ultrafilterable platinum was 6.0 μg/ml higher than that for DDTC-DDP.

DISCUSSION

In this study the plasma pharmacokinetics of those DDP species measured as total ultrafilterable platinum were compared to the pharmacokinetics of those DDP species detected by derivatization with DDTC. Like prior reports, the total ultrafilterable platinum [13–16] and DDTC-DDP data [7, 17] fit a one compartment model. When DDP was infused without thiosulfate (100 mg/m^2), both assays gave the same cumulative AUC for the first 4 h. However, thereafter the curves diverged, and at subsequent times the concentration of DDTC-DDP was significantly less than that of total ultrafilterable platinum. The AUC from 4 to 8 h for DDTC-DDP was only 45% of that for total ultrafilterable platinum. This result indicates that during the first 4 h after the start of DDP infusion, the species present in the plasma are those capable of rapidly reacting with a nucleophilic site on a molecule such as DDTC, and are thus potentially cytotoxic forms of the drug. These species have a plasma half-life that averages 44 min. However, as the shorter half-life DDTC-reactive species are eliminated (3–4 half-lives, or approximately 3 h), non-reactive species with longer half-lives come to dominate the plasma kinetics of total ultrafilterable platinum. These species are presumably formed through the reaction of DDP with soluble thiols and other small molecules in the plasma. These species have a prolonged plasma half-life [7], and because of this they significantly affect total clearance and AUC values for total ultrafilterable platinum.

Several earlier studies followed the plasma concentration of total ultrafilterable platinum for only 1.5–3 h beyond the end of drug administration [14, 15]. If sampling in the study reported in this paper stopped at 2–3 h, the curve obtained from both assays would have been identical, and our data would have appeared to show that both assays were measuring the same DDP species. The half-life of DDTC-DDP of 44 ± 3 min is similar to the initial half-life reported for total ultrafilterable platinum by other investigators [14, 15]. The similarity of total ultrafilterable platinum and DDTC-DDP concentrations during the immediate period following drug administration has been reported by Drummer *et al.* [18]. The relatively short plasma half-life of the DDTC-DDP suggests that the vast majority of the total renal exposure to these species will have occurred within 2 h after the end of DDP infusion, and that thereafter renal excretion of DDTC-reactive species should be essentially nil even though non-reactive non-protein bound forms of platinum may continue to be delivered to the kidney and excreted for some time.

Reece *et al.* [8, 19] have recently reported kinetic parameters for native DDP following i.v. infusion. They determined a half-life of 31.6 ± 6 (S.D.) min [8] which is significantly shorter than the value of 44 ± 10 (S.D.) min for DDTC-DDP when DDP was given at the 100 mg/m^2 dose level ($P < 0.01$, both tails, Student's t test), but not shorter than the value of 36 ± 7 (S.D.) min for DDTC-DDP when DDP was given at 202.5 mg/m^2 with thiosulfate. This comparison suggests that DDTC-DDP consists both of native DDP and other species from which DDP can be displaced by DDTC, and that DDTC-derivatization results in at least a 25% overestimation of the concentration of native drug. The similarity of the values in the presence of thiosulfate can be explained if one hypothesizes that the availability of thiosulfate in the plasma results in the formation of DDP-thiosulfate metabolites not derivatizable with DDTC to the exclusion of the longer half-life metabolites from which DDO can be displaced by DDTC. However, several other explanations are also possible, and further study will be required to clarify this issue. The exact species of DDP detected by derivatization with DDTC are not known. However, it appears that quantitation of DDTC-DDP represents an improvement over measurement of total ultrafilterable platinum when trying to estimate the pharmacokinetics of the native drug.

Sodium thiosulfate blocks the nephrotoxicity of DDP [20] permitting the administration of approximately twice as large a dose as is tolerated in the absence of thiosulfate [21]. Thiosulfate can neutralize DDP by direct covalent reaction [22, 23], however, at the concentrations of DDP and thiosulfate found in the plasma the half-life of this reaction is far slower than the rate of loss of DDP from plasma by other mechanisms [24]. Substantial neutralization of DDP in plasma would produce large differences in the measured values of total ultrafilterable platinum relative to DDTC-DDP since the DDP-thiosulfate metabolite dose not react readily with DDTC under the conditions of the DDTC assay [11]. In this study, the AUC ratio (calculated by the trapezoidal rule) of total ultrafilterable platinum to DDTC-DDP was 1.7 ± 0.7 (S.D.) when DDP was infused with thiosulfate (202.5 mg/m^2), and 1.0 ± 0.4 (S.D.) when DDP was infused alone (100 mg/m^2). This difference was just barely statistically significant ($P < 0.05$, unpaired t test). In addition, the total AUC for DDTC-DDP at 202.5 mg/m^2 was 135% of that for 100 mg/m^2 (by the non-linear method, Table 1). At

202.5 mg/m^2, it would be expected to be just over double that for 100 mg/m^2, assuming no neutralization of DDP by thiosulfate was taking place and the kinetics of DDP were first-order. These findings, though not conclusive in and of themselves, suggest that neutralization of DDP in plasma is not the only mechanism by which thiosulfate protects the kidneys. Other studies [20, 23, 25] seem to confirm this hypothesis, and indicate that the observed protection against nephrotoxicity may be due mainly to rapid inactivation of DDP in the kidneys themselves where thiosulfate is extensively concentrated.

It is particularly important to note that, when using thiosulfate to improve the therapeutic index of DDP, the dose of thiosulfate is critical. Elferink *et al.* [26] have recently confirmed that the rate of reaction between DDP and thiosulfate is highly dependent on thiosulfate concentration. With a DDP dose of 202.5 mg/m^2 and a sodium thiosulfate infusion of 10 g/m^2 over 3 h, the data presented here suggest that there is a measurable neutralization of DDP in the plasma. Thus, the relative increase in exposure of unneutralized drug is less than the relative increase in dose of DDP. Nevertheless, the use of thiosulfate permits the use of larger doses of DDP, and the net effect is an increase in total exposure to the native drug. It is perfectly possible to further impair the antitumor activity by using a larger dose of thiosulfate, or a dose schedule that results in a higher plasma thiosulfate/cisplatin concentration ratio [27–29].

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