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Disposition of total and free cisplatin on two consecutive treatment cycles in patients with ovarian cancer*

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Summary. The disposition of total and ultrafilterable cisplatin was determined in 12 women with ovarian carcinoma receiving cyclophosphamide 500 mg/m^2, adriamycin 50 mg/m^2 and cisplatin 50 mg/m^2 during their first and second course. Plasma samples were obtained over 96 h following the completion of the cisplatin infusion and assayed for total platinum by atomic absorption spectroscopy. Plasma samples obtained up to 4 h after cisplatin infusion contained measurable ultrafilterable (free) cisplatin. The mean disposition of free cisplatin conformed to a two-compartment model with a mean terminal half-life ($\pm$SD) of 46.2 ± 20.2 min during the first course and 37.8 ± 18.0 min during the second course of therapy.

The mean disposition of total cisplatin conformed to a three-compartment model with a mean terminal half-life ($\pm$SD) of 57.8 ± 19.3 h during the first course and 86.6 ± 33.3 h during the second course of therapy. We found that the mean total cisplatin levels were significantly higher during the second course than the first course and the total body clearance of total platinum decreased from the first to the second course. Divided urine collections were obtained over 24 h after completion of cisplatin infusion, but cisplatin was not always detectable at all time intervals. The total fraction recovered was 0.14 and 0.12 of administered dose after the first and the second course, respectively. Renal clearance was 0.61 ± 0.32 l/h/m^2 and 0.45 ± 0.16 l/h/m^2 for the first and the second course, respectively.

We conclude that: (1) urinary platinum excretion is variable between patients and with time; (2) a trend to decreased renal clearance of platinum from first to second course may be due to a decrease in renal excretion of cisplatin; and (3) the body's elimination pathways clear less platinum upon repeat administration.

Introduction

Cisplatin is a heavy metal complex demonstrating significant activity in the treatment of many solid tumours, including testicular and ovarian cancers in adults and neuroblastoma and osteogenic sarcoma in children [11]. Extensive experimental studies of drug effects have related cisplatin cytotoxicity to damage of DNA manifested by DNA interstrand crosslinking [15].

Clinical studies of cisplatin have analyzed biological fluids for total, unbound and intact cisplatin by flameless atomic absorption spectroscopy [1, 10] and high-pressure liquid chromatography [3]. The published literature would suggest that unbound cisplatin disappears from plasma in a mono- or bi-exponential manner with a variable terminal half-life and that total platinum disposition may be described by a bi- or triphasic curve with a long but poorly defined half-life [4, 7, 8, 9, 12]. Urinary excretion of this drug appears to be limited and occurs during a short interval after drug administration. Although the dose-limiting renal toxicity is cumulative, limited information regarding the drug's disposition on repeated courses has been reported.

We undertook to determine the pharmacokinetics of total and unbound cisplatin in a homogenous population of previously untreated patients with one tumour type receiving the same antineoplastic drug combination, i.e. cyclophosphamide, adriamycin and cisplatin on the same schedule of treatment during two consecutive courses in order to define clearly: (1) the terminal half-life of total platinum; (2) patient-to-patient variation during any treatment course; (3) course-to-course variation for the population; and (4) the urinary clearance of platinum.

Methods and meterials

Patient selection. Patients with FIGO stage IIb-IV ovarian carcinoma who were admitted to The Ontario Cancer Institute for their first course of chemotherapy with cyclophosphamide 500 mg/m^2, adriamycin 50 mg/m^2 and cisplatin 50 mg/m^2 were eligible for entry into this study. Requirements for entry included serum creatinine <2 mg/dl or creatinine clearance >40 ml/min, WBC ≥ 3500/mm^3, granulocyte count ≥ 2000/mm^3 and platelet count ≥ 100000/mm^3, Karnofsky performance status ≥ 60 and written informed consent.

Drug administration and patient monitoring. The following sequence of drug administration was used in each patient: (1) adriamycin; (2) cyclophosphamide; and (3) cisplatin, which was given over 15 min into the side arm of a free-running intravenous. Patients were prehydrated with 0.3% NaCl and 3.33% dextrose 4 h prior to drug administration.

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Samples of blood were collected through a heparin lock placed in the arm opposite that in which drug was administered and 10 ml of heparinized blood was collected prior to drug administration, immediately after drug dosing, at 5, 10, 15, 30, and 60 min and at 2, 4, 6, 12, 24, 48 and 96 h. For samples obtained up to 4 h after drug administration the plasma was immediately separated by centrifugation at 4 °C and plasma removed within 15 min of sampling. A volume of this plasma was processed immediately for unbound platinum by centrifugation in Centriflo Ultrafiltration Cones CF-50 (Amicon, Toronto, Canada) for 10 min at 1000 *g* in a refrigerated centrifuge (4 °C). The remaining plasma was frozen at −20 °C for analysis of total platinum.

Urine was collected for 1 h prior to drug administration and then in divided collections over 0–3, 3–6, 6–12 and 12–24 h following cisplatin administration. The urine was frozen for later analysis after the excretion had been determined. Patients were monitored on the first and second courses of treatment, which were separated by at least 3 weeks.

Platinum assay. Total and ultrafilterable platinum was measured by injecting samples into a carbon rod furnace (varian CRA-90) interfaced with an atomic absorption spectrophotometer. The platinum 265.9-nm line at 7 mA and 0.2 nm was monitored. A three-stage heating program included drying at 100 °C for 9 s, ashing at 1300 °C for 20 s, and for atomization, the temperature was ramped at 600 °C/s to 2300 °C for 1.5 s hold time. Background correction was not necessary. In preparing samples for total platinum measurement, 50 µl plasma was diluted with two volumes of a 0.1% Triton X solution. For ultrafilterable platinum, samples were first centrifuged as described above. The volumes injected for the total and ultrafilterable platinum amounted to 5 µl ([13, 14].

Total urinary platinum was assayed using a procedure identical to that with plasma. Urine samples were diluted 1:3, 1:10 and 1:20 with water, and 5 µl of each dilution sample was injected into the carbon rod. Samples were compared to urine standards (0.5–10 mg/l) prepared in the same manner.

Pharmacokinetic analysis. Pharmacokinetic parameters were computed by standard methods [6] for individual patient data. Total and free cisplatin clearances were calculated by dividing the administered dose by the total area under the concentration-vs-time curve (AUC) for total plasma and ultrafiltrate cisplatin, respectively. The area was determined via the geometric mean-trapezoid method from time zero to the last sample, and from there on to time infinity by the formula:

$$AUC_{t(last)} \text{ to infinity} = C_{last}/\text{terminal rate constant}$$

The mean residence time (MRT) was based upon the area under the first moment curve (AUMC) and the AUC according to:

$$MRT = AUMC/AUC$$

The time-averaged renal clearance (CLr) of cisplatin was calculated from the urinary recovery of cisplatin (ΣAe_t) and the AUC by:

$$CLr = \Sigma Ae_t/AUC_t$$

The cisplatin concentration-vs-time data were examined to establish which exponential equation best exemplified the observations. One-, two- and three-compartment models with elimination from the central compartment were considered in this evaluation. The data were analyzed with a nonlinear fitting program originally described by D'Argenio and Schumitsky [5] and modified to run on an LSI 11/23 computer. The statistical method of Boxenbaum et al. [2] was used to discriminate between exponential equations and thus to establish whether additional compartments improved the fit significantly. From the fitting of the data to the appropriate model-dependent equations, parameter estimates could be obtained for the elimination and intercompartmental mass transfer constants, as well as the apparent distribution volumes. The equations also permitted an estimation of the anticipated concentrations during the second course of cisplatin, based upon possible residual levels remaining from the first course. The various data obtained from these experiments were evaluated statistically using the paired Student's *t*-test.

Results

Pharmacokinetic studies of cisplatin were performed in 12 patients with ovarian carcinoma during the first and second courses of therapy. The median age was 56 (range 39–68) and two patients were stage IIb, eight Stage III and two stage IV. Three patients had received prior radiation therapy to the pelvis. The mean cisplatin dose (±SD) was 70±26.1 mg on the first course and 69.2±24.1 mg on the second course. Doses of cisplatin ranged from 25 mg/m^2 to 100 mg/m^2.

The detection limit of the platinum assay was 0.05 mg/l and 0.02 mg/l for plasma and ultrafiltrate respectively. The spectrophotometer gave a linear response up to 10 mg/l. At three concentrations (0.5, 1.0 and 2.5 mg/l), the recovery of platinum added as cisplatin to plasma averaged 98% ($n=10$, CV=4.5%). The between-day precision at these three concentrations was >90%. The ultrafilterable platinum gave an average recovery of 55% ($n=10$, CV=9%) at whole blood concentrations of 0.25, 1.0 and 2.5 mg/l, and the between-day precision ($n=20$)

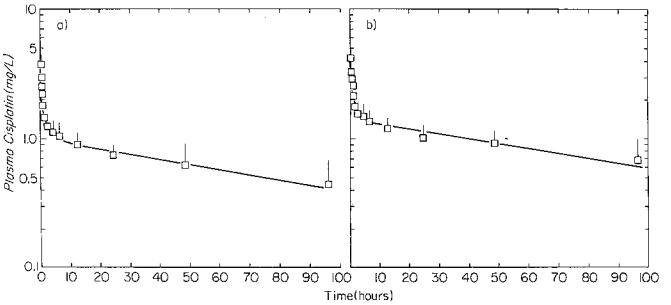

Fig. 1. a Mean (±SD) plasma cisplatin concentrations observed following the first course. The line represents the best computer fit using the three-compartment model equation. Final fitted parameter values were: $V_c = 9.4$ l/m^2, $\alpha = 0.42$ h^{-1}, $\beta = 5.1$ h^{-1}, $\gamma = 0.0092$ h^{-1}, $K_{21} = 0.22$ h^{-1}, and $K_{31} = 1.87$ h^{-1}. **b** Mean (±SD) plasma cisplatin concentrations observed following the second course. The line represents the best computer fit using the three-compartment model equation. Final fitted parameter values were: $V_c = 7.5$ l/m^2, $\alpha = 1.29$ h^{-1}, $\beta = 9.9$ h^{-1}, $\gamma = 0.0087$ h^{-1}, $K_{21} = 5.04$ h^{-1}, and $K_{31} = 0.54$ h^{-1}

Table 1. Summary of mean parameters (±SD) obtained from the analysis of individual patient total cisplatin concentrations

Course	Number of patients		α[a] (h^{-1})	β (h^{-1})	γ (h^{-1})	AUC (mg h/L)	TBC (l/h/m²)	MRT (h)	V_C (l/m²)
	2-cpt	3-cpt							
1	5	7	16.0 (26.3)	2.0 (1.9)	0.012 (0.006)	99.6 (70.8)	0.52 (0.22)	127.2 (117.2)	9.3 (3.9)
2	3	9	7.1 (13.2)	7.1 (13.3)	0.008 (0.005)	293.3 (263.3)	0.22 (0.14)	311.5 (225.4)	8.0 (3.0)

[a] For 3-cpt model only

averaged 82%. Using aqueous solutions of cisplatin at 0.5 and 2.5 mg/l, the recovery of platinum in the ultrafiltrate was 75% ($n=10$, CV=5.9%). There was no interference in the analysis of total platinum from plasma specimens containing a high concentration of BUN, creatinine, bilirubin, hemoglobin and lipids. We also observed no matrix effect in the measurement of ultrafiltrate supplemented with cisplatin. Platinum in both plasma and ultrafiltrate could be recovered quantitatively even after storage of the samples over a period of 4 months at 4 °C. The detection limit was 0.5 mg/l in urine with a between-sample variation <10% at 1.25 mg/l when urine was diluted to 1:20. All plasma and urine sample measurements were the mean of triplicate analyses performed by a single operator.

The average data for plasma cisplatin vs time in courses 1 and 2 are displayed in Fig. 1. A tri-exponential equation indicative of a three-compartment model was the minimal requirement to provide a reasonable fit to the mean data. For the individual patients, a bi- or tri-exponential equation was needed for a similar fit. A summary of the parameter values for the patients can be found in Table 1. The mean terminal half-life (±SD) was 57.8±19.3 h during the first course of therapy and 86.6±33.3 h during the second course of therapy. Although there was no significant difference in the administered dose for the two courses ($p<0.10$), Student's t-test showed that the total body clearance (TBC) was significantly lower during the second treatment course ($p<0.01$). Considering individual patient data rather than averaged data, 11/12 showed a decrease in TBC; the mean (SD) percentage change in TBC between courses was −41.3% (56.1%). This change was associated with a significant prolongation in the MRT ($p<0.05$). Ten of 12 patient showed an increase in MRT; the mean (SD) change in MRT between courses was +282.2% (403.1%). Finally, the central distribution volume of cisplatin showed no significant difference between courses 1 and 2 ($p>0.05$). This reflects the small change coupled with the large variability observed in individual patient data; 9/12 showed a decrease in volume, and the mean (SD) change between the courses was +14% (111.6%). The other compartmental hybrid or micro constants occasionally exhibited large and inconsistent changes, and have thereby not been considered noteworthy of this report.

A comparison of the mean plasma data for the two courses was made and is displayed as a plot of mean cisplatin concentration, course 2/course 1, in Fig. 2. Student's t-test showed the differences to be significant ($p<0.05$) at all time points except at the second post-dosing collection time (20 min).

The average ultrafiltrate cisplatin-vs-time data for courses 1 and 2 are displayed in Fig. 3. A bi-exponential equation indicative of a two-compartment model was minimally required to provide a reasonable fit to the mean data. For the individual patients, a mono- or bi-exponential equation was needed for a similar fit. A summary of the

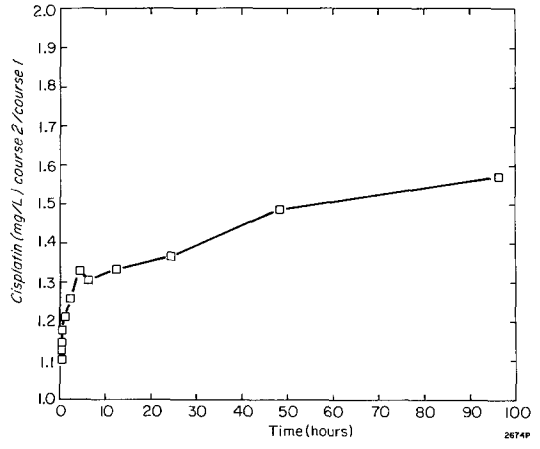

Fig. 2. Ratios of mean plasma cisplatin concentrations during course 2 to mean plasma cisplatin concentrations during course 1

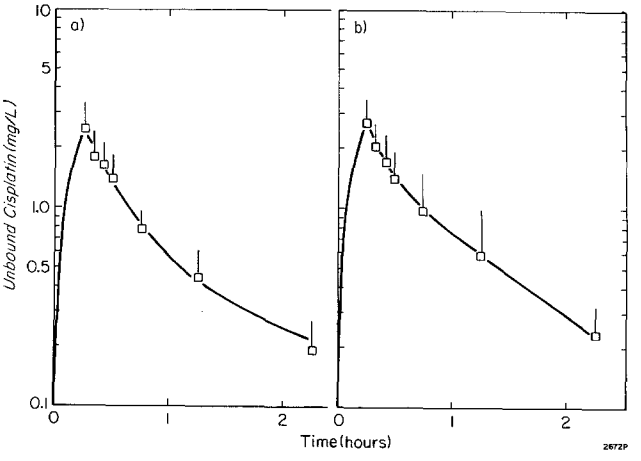

Fig. 3. a Mean (±SD) ultrafiltrate cisplatin concentrations observed following the first course. The line represents the best computer fit using the two-compartment model equation. Final fitted parameter values were: $V_c=15.1$ l/m², $\alpha=3.38$ h^{-1}, $\beta=0.55$ h^{-1}, and $K_{21}=1.12$ h^{-1}. **b** Mean (±SD) ultrafiltrate cisplatin concentrations observed following the second course. The line represents the best computer fit using the two-compartment model equation. Final fitted parameter values were: $V_c=12.2$ l/m², $\alpha=6.28$ h^{-1}, $\beta=0.94$h^{-1}, and $K_{21}=3.11$ h^{-1}

Table 2. Summary of mean parameters (±SD) obtained from analysis of individual patient ultrafiltrate cisplatin concentrations

Course	Number of patients		K[a] (h^{-1})	α[b] (h^{-1})	β[b] (h^{-1})	AUC (mg h/L)	TBC (l/h/m^2)	V_D (l/m^2)
	1-cpt	2-cpt						
1	8	4	23.9 (26.1)	23.9 (26.1)	0.9 (0.7)	1.49 (0.76)	31.7 (10.3)	20.2 (9.0)
2	9	3	44.8 (36.1)	44.8 (36.1)	1.1 (1.0)	1.50 (0.56)	30.4 (17.1)	16.8 (6.2)

[a] For 1-cpt model only
[b] For 2-cpt model only

parameter values for the patients can be found in Table 2. The mean initial disposition half-life (±SD) and the mean terminal half-life were 46.2±20.2 min respectively 1.7±0.21 min during the first course of therapy and 0.9±0.4 min and 37.8±18.0 min during the second course of therapy. Student's *t*-test discerned no significant difference in V_D ($p>0.05$) or in TBC ($p>0.75$). The *t*-test also showed that the differences between the ultrafiltrate cisplatin concentrations in courses 1 and 2 were insignificant ($p>0.05$) at all time points.

Although urine was collected over 24 h in the intervals of 0–3, 3–6, 6–12, and 12–24 h following each course of treatment in all patients, cisplatin levels in the urine were not always measureable in all of the intervals. In some patients the cisplatin could be quantified only in the first sample. Nevertheless, the available information permitted an expansion of the pharmacokinetics information, and the results are summarized in Table 3. On the basis of Student's *t*-test, the fraction of the cisplatin dose recovered and the renal clearance did not differ significantly between courses 1 and 2 ($p>0.05$). This reflects the small average change coupled with considerable variability observed in individual data; 7/12 patients showed a decrease in renal clearance and the mean change between the courses was −7.3% (61.6%). The major fraction (0.11) was recovered during the 0–3 h interval after drug administration during each course.

Since the serum creatinine and creatinine clearance was determined in each patient for both courses, the cisplatin renal clearance was examined in the light of renal function. A plot of renal clearance against either serum creatinine or creatinine clearance resembled a scattergram and exhibited no discernible relationship (data not shown). There might be a perceived reduction in renal function, because on the basis of individual data 8/12 patients showed an increase in course 2 pre-drug serum creatinine [mean (SD) change of +7.3% (16.3%)], and 8/10 patients (only 10 were measured) showed a decrease in course 2 creatinine clearance [mean (SD) change of −7.7% (30.3%)]. On the basis of the *t*-test, changes were not significant ($p>0.05$). Hepatic enzyme levels and serum bilirubin did not change from course to course for individual patients.

Table 3. Summary of mean parameters (±SD) obtained from the analysis of individual patient urinary and plasma data

Course	Dose (mg)	Recoverable cisplatin (mg)	Fraction recovered	CLr (l/h/m^2)
1	70.0 (26.1)	10.5 (6.5)	0.14 (0.06)	0.61 (0.32)
2	69.2 (24.1)	8.8 (4.1)	0.12 (0.03)	0.45 (0.16)

Discussion

Cisplatin disposition has been described by other investigators employing high-pressure liquid chromatography and flameless atomic absorption [4, 7, 8, 9, 14]. Whereas the former technique can differentiate between parent and other forms of the compound, the latter assay method only measures platinum. This loss of specificity is associated with an increase in sensitivity compared to the high-pressure liquid chromatography technique. This suited our need to assess in detail the terminal portion of the total platinum disposition, and therefore we employed flameless atomic absorption in our studies. Although other reports of cisplatin kinetics have suggested the presence of a long third-terminal half-life, this has been poorly documented. Our ability to obtain plasma samples 96 h after drug administration has allowed us to define the terminal phase clearly. The opportunity to study the same patients on two consecutive courses makes this a unique study.

We found that the total platinum disposition was represented by a multicompartment model. Some patients studied required a bi-exponential equation to describe their data and others required a tri-exponential equation (Table 1). Nevertheless, three exponentials were required to best fit the mean data for these patients, and the cisplatin disposition would therefore be associated with a three-compartment model. Whether this number of compartments actually reflects the pattern of cisplatin distribution is not entirely clear. The tri-exponential fit could simply reflect the nature of the total cisplatin elimination process. For example, if the free cisplatin plasma levels were the driving force for elimination and free cisplatin was relatively high during the early period of drug administration, cisplatin would appear to be eliminated at an accelerated rate during the early period. Further studies detailing the elimination of cisplatin would be necessary to clarify this issue.

Urinary elimination of platinum was defined by analyzing samples of urine collected after drug administration. The fraction of urinary platinum recovered was similar in the first and second courses (Table 3). However, the range of concentrations of detectable platinum in the different urine aliquots varied widely from patient to patient and

for any given patient, suggesting variable urinary elimination with time. No correlation could be found between the renal clearance of the drug on each course and the creatinine clearance. The reasons for this variation and lack of relationship to creatinine clearance are unknown at present, but may reflect more than one renal excretion process for different platinum species [4] and a changing renal clearance with course.

One of the most striking aspects of the data is the decrease in total clearance of cisplatin during course 2. The average decrease of about 60% between course 1 and course 2 coincides with an average increase in mean residence time of about 145% (Table 1). Since the total clearance was calculated from dose and area under the curve, it was important that the apparent decrease was not due to cisplatin remaining in the body from course 1. Computer simulations using the three-compartment model for the mean course 1 data demonstrated that essentially no cisplatin would be left in the body at the time of the second course of therapy. While this evidence is not unequivocal, it is sufficient to conclude that the observations of Fig. 2 cannot be attributed to residual drug. Thus, it appears that the body removes cisplatin more slowly during the second course. Further evidence for the slowed elimination during course 2 comes from the urinary data. Although the average decrease in renal clearance and recoverable urinary platinum from course 1 to course 2 was not significant, the trend towards reduction is in keeping with the observed decrease in the total body clearance and would point to a decrease in the kidney's ability to excrete the drug.

The mean ultrafiltrate data were best fit by a bi-exponential curve (Fig. 3), consistent with other studies reported in the literature [9, 12]. However, in the individual patient, a mono- or bi-exponential equation was needed for an adequate fit (Table 2). These differences may be due to the extremely rapid decrease in total cisplatin levels which leads to undetectable levels of free cisplatin in plasma ultrafiltrates and difficulty in adequately assessing the terminal phase in some cases. In addition, the short elimination half-life of ultrafiltrate makes calculation of free or unbound platinum renal clearance meaningless.

We conclude from these studies that the body's elimination pathways clear less platinum upon repeated administration together with cyclophosphamide and adriamycin. This new finding is worthy of further study because it has practical implications on drug dosing of patients on repeated courses. Continued administration of cisplatin does not simply re-expose malignant cells and normal tissue to drug, but exposes them to higher levels for a greater length of time. Such drug behaviour clouds the understanding of cisplatin pharmacodynamics and may explain the occurrence of cumulative drug toxicity.

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