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Pharmacokinetics and Protein Binding of Cis-dichlorodiammine Platinum (II) Administered as a One Hour or as a Twenty Hour Infusion

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Summary. *The pharmacokinetics of cis-dichlorodiamminoplatinum (II) (cisplatin) have been studied in seven patients, of whom four received the drug as a one hour infusion and three received it as a 20 h infusion. The patients receiving the drug over one hour exhibited biphasic clearance of total platinum with a rapid initial phase (8.7–22.5 min) and a prolonged second phase (30.5–106 h). Free (ultrafilterable) cisplatin was readily detectable in this group and was rapidly cleared (half-life about 22 min). The volume of distribution of the drug was 50.3–65.6 liters and it was 26.6–50% excreted in the urine in 48 h. In the patients receiving the 20 h infusion, a more complex plasma elimination curve was seen, with the appearance of a secondary peak. Free drug was not detectable in these patients and they showed less urinary excretion (21.4–25.9% at 48 h) than the one hour group. Cisplatin was bound to several plasma proteins, including albumin, transferrin, and γ-globulin. The data indicate that cisplatin is retained in the body more extensively after a 20 h infusion than after a one hour infusion.*

Introduction

Cis-dichlorodiammine platinum (II) (cisplatin) is an effective antitumor agent in humans [19]. Its major toxicities are nausea, vomiting, renal damage, and VIII nerve damage. The optimal manner of administering the drug is not established, although investigators have used various schedules such as bolus injection [8], prolonged infusion [14], and fractional daily dosage [5]. It is desirable to evaluate different drug schedules in order to maximize antitumor activity and minimize toxicity, and this evaluation should include comparison of pharmacokinetics and excretion under the different conditions employed.

The pharmacokinetics of cisplatin previously have been studied in animals and man. Plasma elimination is biphasic, with a short initial phase and a long second phase [4, 7, 10, 12, 13, 18]. Also the drug is bound to serum proteins, which markedly influences its disappearance from plasma [4, 12, 18], and the distinction between free (ultrafilterable) and protein-bound (non-filterable) drug is important to its pharmacokinetic description. It is postulated that only the unbound drug has therapeutic activity [7, 18]. However, only a few clinical studies have distinguished between free and bound platinum in plasma and urine, and evaluation of these variables as a function of schedule is still needed. In the present study, cisplatin was administered either as a one hour or as a 20 h infusion. Plasma levels of filterable and total drug were assayed, volumes of distribution were calculated, and urinary excretion was measured for the two schedules. Interactions with specific plasma proteins were also examined.

Materials and Methods

Cisplatin pharmacokinetics were studied in seven patients. Four patients received the total drug dose in a one hour infusion and three received it in a 20 h infusion. The drug dose was 50 mg/m^2 in five patients while two, one in each group, received 100 mg/m^2.

All patients had histologically-proven advanced malignancies and had adequate renal function (blood urea nitrogen < 20 mg/100 ml, serum creatinine < 1.5 mg/100 ml, and creatinine

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clearance > 60 ml/min). The patients were hospitalized and prehydrated with 2000 ml 5% dextrose in normal saline over 24 h prior to drug administration, and were maintained on intravenous fluid for a minimum of 24 h after cisplatin administration. Cisplatin was supplied by the National Cancer Institute and the dose to be administered was dissolved in 100 ml 5% dextrose in normal saline. This dose was given concurrently with 37.5 g mannitol, which was administered over the total duration of the infusion, either one hour or 20 h. A total infusion rate of 125 ml/h was maintained with 5% dextrose in normal saline. Plasma samples were collected at multiple time points up to 72 h following drug infusion. Total urine collections were made for at least 48 h. None of the patients received other cytotoxic drugs simultaneously.

Ultrafilterable and non-filterable platinum were separated by centrifugal ultrafiltration using Centriflo CF-50 filters (Amicon Corp) [1, 12]. Samples of plasma, plasma ultrafiltrate, and urine were analyzed for platinum content using flameless atomic absorption spectroscopy [11]. The limit of detection of platinum by this method is 100 ng/ml. We have previously established that using this method, platinum (Pt) does not bind to the Centriflo membrane. Plasma decay curves for Pt following the infusions were fitted to either one or two-compartment models, using the MLAB computer package at the National Institutes of Health [9] to obtain values of plasma decay constants and volumes of distribution. Volumes of distribution were based upon the ratio of peak plasma level (Po) to total drug dose. These calculations were corrected for infusion time and assumed that a steady state concentration of Pt had not been achieved [6]. Blood samples for patients on the 20 h infusion were taken both during and following drug infusion. Peak plasma levels for this group were estimated from the intercept of the line drawn to fit the early data points. The Pt disappearance curves for these patients were complex and demonstrated secondary peaks. These curves were not fitted exactly to a multicompartment model. Rather, two independent half-times were obtained by fitting early time points (15–210 min) and later time points (240–2880 min) to a single compartment model. For patients on the one hour infusion, blood sampling began immediately as the infusion ended and the value of Po was taken from the first time point.

In order to determine the protein fractions to which drug binding occurred, cisplatin was added to normal plasma to a final concentration of 10^{-4} M. Gel filtration of this sample was then performed on a Sephadex G-200 column (60 cm × 3.2 cm) using an eluting buffer of 0.1 M Tris-HCl, pH 8.0, 1.0 M NaCl. Protein elution was monitored by the absorbance at 285 nm and Pt levels were determined on individual fractions. To confirm the ability of specific proteins to bind Pt, purified samples of human albumin (Sigma Chemical Co.), and human transferrin (courtesy of Dr. J. V. Princiotto, Georgetown University and of Dr. Margaret Hunter, University of Michigan) were co-chromatographed with equimolar (10^{-4} M) quantities of cisplatin on a Sephadex G-200 column (12.5 cm × 0.75 cm), using the same Tris buffer described above. The commercial preparation of albumin was repurified by gel filtration prior to use to obtain a pure monomer fraction.

Results

In general, the disappearance of Pt from plasma (Figs. 1 and 2) showed a rapid initial phase followed by a more prolonged later phase. In several cases, however, secondary drug peaks occurred and the overall pattern was more complex than a simple biexponential function. This was true of all patients

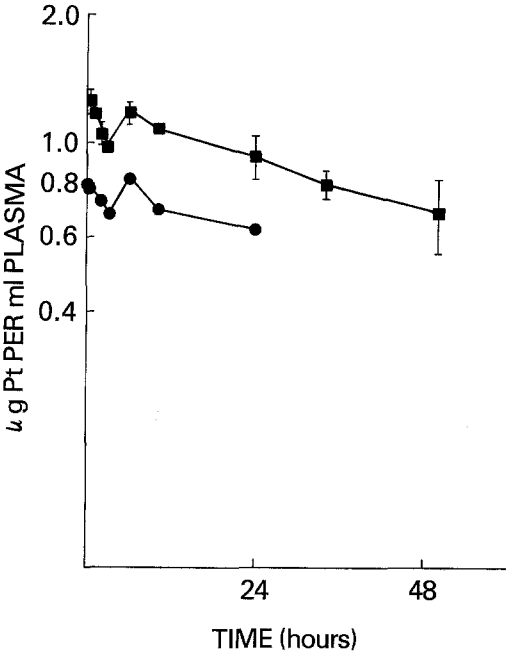

Fig. 1. Plasma levels of elemental platinum following infusion of cisplatin over 20 h. *Squares* represent mean of two patients receiving 50 mg/m^2; *circles* represent a single patient who received 100 mg/m^2

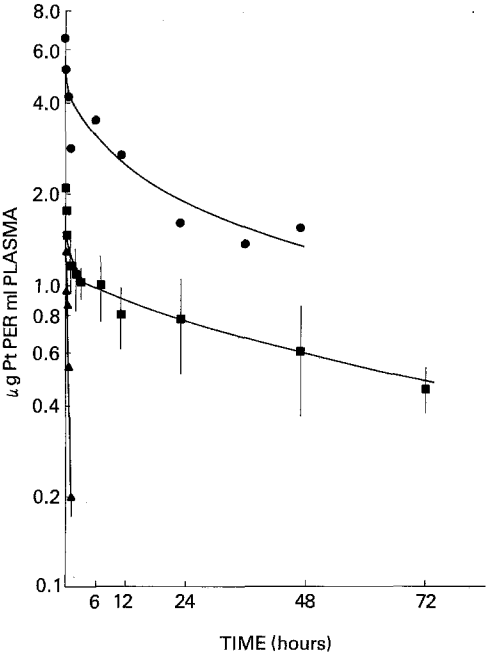

Fig. 2. Plasma levels of elemental platinum following infusion of cisplatin over one hour. *Circles* represent a single patient who received a dose of 100 mg/m^2; *squares* represent the mean ± standard error of three patients receiving 50 mg/m^2; *triangles* represent ultrafilterable platinum in the patients receiving 50 mg/m^2

who received the 20 h infusion (Fig. 1). In each of these patients the initial disappearance was followed by a secondary peak about 200 min after the infusion ended. Thereafter, slow disappearance continued. These curves did not fit simple one or two compartment models, but estimates of the half-lives ($t^1/_2$) of the different curves were obtained by individually analyzing their initial and terminal portions as single exponentials (see below).

In the patients who received a one hour infusion, plasma Pt disappearance was approximately biexponential, especially for those who received 50 mg/m^2 (Fig. 2). The plasma disappearance curve of the patient who received 100 mg/m^2 is shown in Fig. 2 as a least squares fit to a two-exponential function. However, a line directly connecting the first six points on this curve would show a secondary peak.

Peak plasma levels and calculated half-lives of the plasma disappearance curves are shown in Table 1. The three patients in the one hour infusion group who received 50 mg/m^2 had peak Pt levels ranging from 2.26–2.66 μg/ml at the end of the infusion. The patient who received 100 mg/m^2 over one hour had a peak plasma Pt level of 6.6 μg/ml. Among the three patients who received the drug over 20 h, Pt levels drawn during the administration showed that a plateau, steady state concentration had not been achieved at the end of the infusion. In this group, plasma levels at the end of the infusion ranged from 0.80–1.27 μg/ml. The lower value, 0.80 μg/ml, was seen in the patient who received 100 mg/m^2 and the Pt levels for that patient were not significantly different from those of the two who received 50 mg/m^2.

In the one hour infusion group, data for the individual patients were analyzed in two ways. First, the Pt concentration curve for each patient was fitted to a two-exponential function and values for $t^1/_2\alpha$ and $t^1/_2\beta$ were derived. These are shown in Table 1 and range from 8.7 to 22.5 min for $t^1/_2\alpha$ and from 30.5 to 106 h for $t^1/_2\beta$. However, a line strictly connecting the data points in Patient 4 gives a disappearance curve that shows a secondary peak at three hours. Hence in a second analysis in this patient half-lives were calculated for two separate portions of the drug disappearance curve, namely that from 0 to 60 min and that from 3 h to 48 h. Done in this fashion, the initial phase $t^1/_2$ was 18.2 min. This procedure was also carried out for the curves of the patients who received 20 h infusions. An initial $t^1/_2$ was calculated for that part of the curve up to the first trough and another for the period from the second peak to the end of the data. The values (Table 1) ranged from 341 to 912 min for the initial $t^1/_2$ and from 41.6 to 66.8 h for the second $t^1/_2$. These two $t^1/_2$ values were independent of one another and the first was not corrected for the second. The values are estimates of disappearances times and detailed modeling based upon more data points and three or more compartments would yield more complex conclusions.

Filterable plasma Pt in the one hour infusion group decayed in a mono-exponential fashion with half-times of 21–47 min (Table 1), and in three

Table 1. Values of pharmacokinetic variables in plasma urine, and plasma ultrafiltrate from patients receiving cisplatin as a one hour or as a twenty hour intravenous infusion

Patient	Total dose (mg) elemental Pt	Po (μg elemental Pt/ml)	$t^1/_2\alpha$ (min)	$t^1/_2\beta$ (h)	Vd[a] (Liters)	% Urinary excretion 48 h
One hour infusion values for total plasma platinum						
1	62	2.26	22.5	93.9	514.3	26.6
2	56	2.66	22.3	106.0	52.3	50.0
3	62	2.45	8.7	46.6	65.6	35.2
4	130	6.60	17.5	30.5	56.8	44.7
One hour infusion values for plasma ultrafiltrate[b]						
1	62	0.18	47.5	–	81.9	–
2	56	1.40	21.7	–	23.6	–
3	62	1.17	21.7	–	23.6	–
4	130	1.35	23.6	–	44.2	–
Twenty hour infusion values for total plasma						
5	55	1.25	597	41.6	21.2	21.4
6	97	0.80	912	42.8	50.5	25.9
7	49	1.27	341	66.8	27.0	22.0

[a] Vd is corrected for lenght of infusion
[b] No Pt detected in ultrafiltrate from patients receiving 20 h infusion. Limit of sensitivity < 0.1 μg/ml

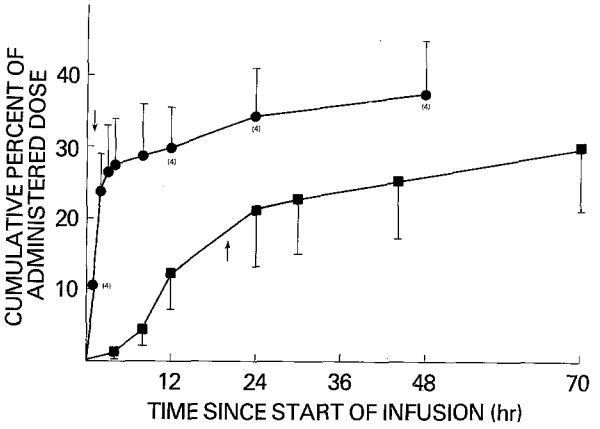

Fig. 3. Cumulative urinary excretion of platinum following the administration of cisplatin by one hour (●——●) or by 20 h (■——■) infusions. Data are represented from the start of the infusion and the *arrows* show the points at which time the two infusions were completed

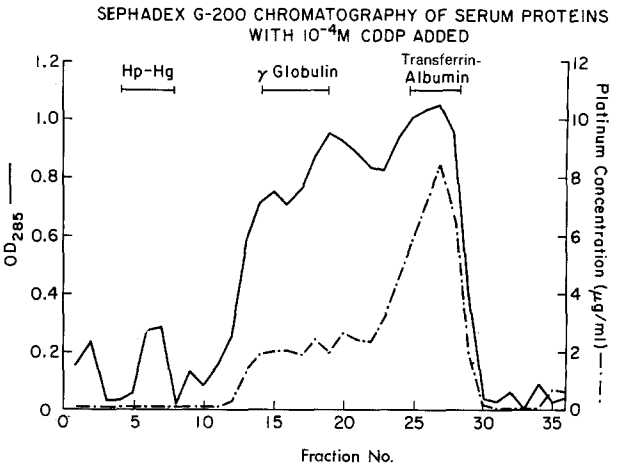

Fig. 4. Chromatography on Sephadex G-200 of serum containing 10^{-4} M cisplatin. Eluting buffer is 0.1 M Tris HCl, pH 8.0, 1 M NaCl. *Solid line* shows protein elution, *broken line* shows platinum concentration in μg/ml in each fraction

of these patients values were very close (21.7–23.6 min). The value of 47 min obtained for the $t^1/_2$ of free drug in Patient 1 may overestimate the actual value. The 60 min time point was out of proportion to the earlier time points. When values only up to 30 min were used, the $t^1/_2$ was 23.1 min. Also in Patient 3, the $t^1/_2\alpha$ of total drug was 8.7 min while the $t^1/_2$ of free drug was 21.7 min. This low value of $t^1/_2\alpha$ resulted from the correction for length of infusion and non-steady state, and the discrepancy between the $t^1/_2$ for total and for free drug does not appear significant. In general there is close agreement between the $t^1/_2$ for total and for free drug. The initial plasma levels of filterable Pt were similar for all four of these patients (0.8–1.4 μg/ml). Platinum was not detectable in the plasma ultrafiltrate of any patient receiving a 20 h infusion.

Volumes of distribution (Vd) of Pt following a one hour infusion do not differ significantly from those obtained for a 20 h infusion. The high values reflect a long residence time and probably depots of Pt in one or more tissues. The Vd calculated for the plasma ultrafiltrate from the one hour patients were in general numerically less than Vd for total plasma Pt in these patients.

Total urinary excretion of Pt was dependent upon the schedule of drug administration (Table 1 and Fig. 3). The patients who received the 20 h infusions exhibited distinctly lower total excretion of drug after 48 h than those who received the one hour infusions. Most of the difference in excretion was accounted for by the initial rapid excretion of 25–30% of the total dose in the first 4 h in the patients receiving the one hour infusion. Apart from this initial burst, the slopes of the excretion curves were quite similar for the two groups. The rate of Pt excretion in the one hour infusion group peaked during the second hour, i.e. during the first hour after the infusion was stopped, and the actual rates of excretion at this peak ranged from 7–12 mg Pt/h. In the 20 h infusion group, the excretion rate peaked during hours 8–12 of the infusion, at which time the numerical rates of excretion were 0.7–1.0 mg Pt/h.

The results of Sephadex G-200 chromatography of a serum sample containing 10^{-4} cisplatin are shown in Fig. 4. Platinum was bound to many, if not all, plasma proteins. High levels were seen in the γ-globulin fraction and in the albumin-transferrin peak. The highest levels appeared to be associated with albumin. In order to ascertain if both albumin and transferrin were capable of binding Pt, additional experiments were performed with purified samples of these proteins. Separate preparations of each protein (10^{-4} M) were treated with equimolar concentrations (10^{-4} M) of cisplatin. The peaks of Pt elution from Sephadex G-200 coincided exactly with the peaks of protein elution, indicating that each protein was capable of binding the drug. Furthermore, dialysis of a solution containing both cisplatin and albumin at 10^{-4} M concentration showed that about 50% of the drug was bound under these conditions.

Discussion

This study has demonstrated several features of cisplatin disappearance from the serum and the body

under two different schedules of administration. The results are in general agreement with previous studies that have shown biphasic elimination and extensive protein binding of the drug. DeConti et al. [4] reported biphasic elimination of cisplatin from plasma following rapid intravenous infusion of doses from 0.15–3.15 mg/kg. The $t^1/_2\alpha$ was 25–49 min and the $t^1/_2\beta$ was 58–73 h. Gormley et al. [7] studied cisplatin disappearance following a 70 mg/m^2 dose given over one hour and found biphasic clearance with half-life values of 23 min and 67 h for the α and β phases respectively. The data for the one hour infusion agree with both of these studies. In addition, the clearance of free (filterable) platinum from the plasma of these patients occurred rapidly, with a half-life of 21.7–23.6 min in three of four patients. These values are in general agreement with those reported by other investigators for both filterable Pt [10] and total plasma Pt [4, 7]. The values of the $t^1/_2\alpha$ of filterable drug are somewhat shorter than those reported [18] for a 15 min bolus infusion but agree with values reported for six hour infusions.

The present data for the 20 h infusion show significant differences from the shorter injection time. No ultrafilterable Pt was detectable at any time in these patients, indicating either that the processes of clearance of free Pt from the plasma are efficient enough to keep free levels below detectability or that all drug is protein-bound at these slow infusion rates. In addition, the plasma decay curve is complex and exhibits a secondary peak during the elimination phase. There were some indications, as discussed above, that this occurred in the short infusion patients also. This phenomenon has been observed by others (Dr. R. Earhart, University of Wisconsin, personal communication). Data on patients receiving five day infusions of cisplatin [14] suggest a secondary peak in concentration, but since only three time points were analyzed during the first day, detailed comparisons with the present are not possible. Secondary peaks or undulations in plasma concentration have been observed for other drugs such as theophylline [21] and chloropromazine [21]. The basis of the phenomenon has been variously ascribed to tissue absorption and release [21] and to enterohepatic recirculation [3, 21]. The present data do not allow more than speculation as to the mechanism in the case of cisplatin, but release of drug from some tissue compartment seems possible.

Cisplatin is bound to several, if not all, serum proteins. These include γ-globulin, transferrin, and albumin. The data in this study have established the fact of binding to these individual proteins but have not detailed the stoichiometry or thermodynamics of the processes involved. Since cisplatin is an electrophilic agent, it may interact with individual amino acids on the protein surface as opposed to binding stereospecifically to sites such as the major hydrophobic binding site on albumin. Our observations on protein binding are in accord with those of Manaka and Wolf [17] that indicated binding of cisplatin to both γ-globulins and albumin, as well as those of Stjernholm et al. [20] showing formation of a transferrin-platinum complex from the compound K_2PtCl_4. The significance of this binding in terms of clinical interactions with other drugs that bind to protein also remains to be determined.

The urinary excretion of platinum is incomplete by both of these infusion schedules, in agreement with other studies [4, 7, 10, 18]. Infusion of drug over 20 h resulted in less cumulative urinary excretion than administration over one hour. While biliary excretion may provide an additional route of drug elimination [2], the data indicate that some fraction of administered platinum has a prolonged residence within the body. This is consistent with the high Vd values obtained. It is of interest that the pattern of toxicity of cisplatin (kidney and VIII nerve) is similar to that of the aminoglycoside antibiotics for which prolonged tissue accumulation is the major index of toxicity [15, 16]. It seems reasonable to postulate that the total accumulation of cisplatin in these target tissues would bear a close relationship to toxicity.

These data do not allow selection of one of these schedules over the other for clinical use. Such a decision must necessarily be made on the basis of comparative clinical trials that establish either amelioration of toxicity or superior antitumor activity for a particular drug schedule. However, the limited clinical observations that were made in these patients indicated that the intensity of acute gastrointestinal toxicity was similar for both schedules. Hence, the choice of a prolonged infusion of the drug should be made predominantly on the basis of improved therapeutic activity by that schedule. The pharmacokinetic data indicate lower urinary excretion and possibly increased deposition of cisplatin in tissues for the 20 h infusion. This could translate into increased drug delivery to tumor tissue and improved therapeutic activity. Randomized, prospective clinical trials comparing the efficacy of the drug by these two schedules in certain signal tumors, e.g., epidermoid carcinoma of the head and neck, would be necessary to establish this possibility.

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