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Pharmacokinetic and biotransformation studies of ormaplatin in conjunction with a phase I clinical trial

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Abstract. Ormaplatin is a second-generation platinum (Pt) analogue with in vitro activity against some cisplatin-resistant malignant cell lines. We have evaluated the pharmacokinetics and biotransformations of ormaplatin during a phase I trial in which ormaplatin was administered by daily 30-min infusions on 5 consecutive days every 28 days. Sixteen patients received 25 courses at doses ranging from 5.0 to 11.6 mg/m^2 per day. Pharmacokinetic parameters determined for ultrafilterable Pt measured by atomic absorption spectrophotometry revealed a short half-life ($t_{1/2}$ 16 min), moderate volume of distribution (Vd 12 l/m^2), and relatively fast systemic clearance (Cls 544 ml/min per m^2). Cls and percentage of drug unbound decreased during the 5-day administration period. Average systemic exposure increased with dose; however, inter-individual variability in Cls produced overlap in systemic exposure between the dose levels. The major active biotransformation product [$PtCl_2$(dach)] was evaluated at the highest dose level by HPLC. This product decayed monoexponentially with a mean $t_{1/2}$ of 13 min and a higher degree of pharmacokinetic variability than that of ultrafilterable Pt at this dose. No unreacted ormaplatin was detected; however, several inactive biotransformation products persisted for at least 120 min. Approximately 32% of the dose was excreted in the urine during the first day, one-third of this during the initial 1.5 h. The human pharmacokinetic characteristics of ormaplatin resemble those of cisplatin; however, additional study will be required to discern which analyte of ormaplatin correlates best with clinical effects.

Introduction

Ormaplatin (tetrachloro(*d,l-trans*)1,2-diaminocyclohexane platinum(IV); NSC 363812; formerly known as tetraplatin) is a second generation platinum (Pt) analogue synthesized and evaluated in the search for Pt compounds with lower toxiciy and unique properties compared with cisplatin [*cis*-diamminedichloroplatinum(II)]. Ormaplatin was chosen for clinical development because of its broad spectrum of activity [1, 23, 35, 46], including significant efficacy against L1210 murine leukemia cells [1, 50] and human ovarian carcinoma cells [23, 35] which were resistant to cisplatin, and also its aqueous solubility and stability [1]. Recent data from human tumors also suggest the potential for synergism with other Pt analogues [36]. While ormaplatin has been shown to be effective against many cisplatin-resistant cell lines, it is clearly not effective against all resistant cell lines [3, 23, 25, 35, 42]. Preclinical studies of ormaplatin have demonstrated significantly less nephrotoxicity than is found with cisplatin [15, 43, 44], though myelotoxicity and gastrointestinal toxicity are comparable [9, 43]. Neurotoxicity was not seen in the preclinical studies, but ormaplatin has been shown to inactivate choline acetyltransferase in vitro [24]. Animal studies have demonstrated that altering the systemic concentration of ormaplatin by manipulation of the infusion rate over a relatively short period could modulate the drug's therapeutic efficacy [22]. Thus, investigation of the pharmacokinetic disposition of ormaplatin is of interest during trials in humans.

The pharmacokinetics and biotransformations of ormaplatin have been best characterized in pre-clinical studies with rats. These studies have shown that once ormaplatin enters the circulation it is very rapidly ($t_{1/2}$ 3 s at 37° C) reduced to the corresponding platinum(II) compound, dichloro(*d,l-trans*)1,2-diaminocyclohexane-platinum(II) [$PtCl_2$(dach)] [6, 7]. The major reducing agent in the blood appears to be protein sulfhydryl [7], which is present in excess at therapeutic doses of ormaplatin [6]. $PtCl_2$(dach) can be considered the major "active" biotransformation product of ormaplatin, since it crosses cell membranes readily [30] and is rapidly ($t_{1/2}$ 15 min) converted to active

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biotransformation products inside the cell [31]. The other major biotransformation products of ormaplatin in rat plasma are $[Pt(H_2O)(Cl)(dach)]^+$, Pt(methionine)(dach), Pt(cysteine)(dach) or Pt(ornithine)(dach), and Pt(urea)(dach) or Pt(citrato)(dach) [6, 7]. $[Pt(H_2O)(Cl)(dach)]^+$ is positively charged and thus is unlikely to readily cross the cell membrane [31]. However, because it is in equilibrium with $PtCl_2$(dach) [27], it can also be considered an active biotransformation product. Pt(methionine)(dach), Pt (cysteine)(dach), Pt(ornithine)(dach), and Pt(urea)(dach) are positively charged complexes that are unreactive [7, 31, 32] and do not appear to cross cell membranes to a significant extent [32]. Thus, they are likely to be inactive biotransformation products of ormaplatin. The Pt(citrato)(dach) complex is known to have some therapeutic efficacy [45]. However, it could only be detected in rat plasma in vitro after relatively long periods of incubation [7]. It was not observed in rat plasma in vivo [6].

In rats treated with therapeutic doses of ormaplatin, both total and ultrafilterable Pt decay from the plasma with biphasic kinetics [6, 37]. For example, at 3 mg/kg the initial and terminal half-lives for ultrafilterable Pt are 10.9 min and 7.47 h, respectively [37]. The active biotransformation products ($PtCl_2$(dach) and $[Pt(H_2O)(Cl)(dach)]^+$), on the other hand, decay from rat plasma in a monoexponential fashion with a $t_{1/2}$ of 4 min [6], while the inactive biotransformation products are persistent in the circulation for at least 3 h and probably account for most of the terminal $t_{1/2}$ for filterable Pt [6]. Thus, pharmacokinetic parameters derived from the measurement of active ormaplatin biotransformation products appear to be significantly different from those derived from analysis of filterable Pt.

This report characterizes the pharmacokinetics of total Pt, ultrafilterable Pt and major Pt biotransformation products during a phase I clinical trial in which ormaplatin was administered by short daily infusions. Because previous studies in rats showed a lack of correlation between the pharmacokinetics of the active platinum biotransformation products and ultrafilterable Pt, we have focused on that comparison in this study.

Materials and methods

Patients and schedule. Adult patients were eligible to receive ormaplatin if they had a histologically confirmed diagnosis of a solid tumor malignancy that was refractory to conventional therapy. Adequate liver (SGOT < 4 times normal and total bilirubin <2.0 mg/dl), renal (serum creatinine < 1.6 mg/dl and creatinine clearance > 59 ml/min), and bone marrow function (granulocyte count > 1500, platelet count > 100,000 and hemoglobin > 9.9 g/dl) and adequate performance status (CALGB grade 0–2) were prerequisites. All patients gave written informed consent according to federal, state and institutional guidelines.

Ormaplatin (NCI, Bethesda, Md.) was administered as a 30-min i.v. infusion, daily for 5 consecutive days per course. Dose levels were determined by a standard, modified Fibonacci approach (Table 1). Patients were eligible to receive additional courses at the same dose level every 28 days in the absence of progressive disease or severe toxicity.

Heparinized blood was collected prior to and at the following times from starting the 30-min infusion on days 1 and 4 of the first course in order to determine ormaplatin pharmacokinetics: 30, 45, 60, 90, 120, 180, 240, 360, and 480 min. In addition, pharmacokinetic samples were collected prior to doses 2, 3, and 5 in course 1 and in at least one patient at each dose level during the second course. All samples were immediately placed on ice and centrifuged at 4° C to separate the plasma. Four aliquots of plasma were subsequently placed in Amicon Centrifree tubes (MWCO 30 kd; Amicon, Beverly, Mass.) and centrifuged for 30 min at 4° C to obtain ultrafiltrates. The ultrafiltrates and remaining plasma were stored at –70° C until analysis.

Urine was collected at intervals of 0–1.5 h, 1.5–6 h, 6–12 h, and 12–24 h from the start of the infusion on days 1 and 4. The volume was recorded and 2 ml aliquots of each sample were stored at –70° C

Analysis of platinum concentrations. Standards were compounded from Pt atomic absorption solution (Sigma, St. Louis, Mo.). A stock solution was prepared in 0.9% saline. Fresh, single-donor plasma (Red Cross, Durham, N.C.) was used to make the final dilutions for plasma standards of 25, 50, 100, 200 and 400 ng/ml. Plasma samples and standards were diluted 1:1 with 0.25% Triton X100 (Sigma) prior to injection. Ultrafiltrate and urine standards were prepared daily in 0.9% saline at final concentrations of 10, 25, 50, 150 and 250 ng/ml.

Plasma ultrafiltrate, plasma, and urine samples were analyzed for Pt content by an atomic absorption spectrophotometer (AAS) equipped with a graphite furnace and automated sampler (Models 2380, HGA 400, and AS40, respectively; Perkin-Elmer, Norwalk, Conn.). The furnace program consisted of three drying steps, an 1100° C char, and a 2700° C atomization. Fifty-microliter aliquots were injected onto pyrolytically coated graphite tubes for AAS analysis. Pt was detected using a wavelength of 265.9 nm.

Analysis of platinum biotransformation products. For in vitro evaluation of ormaplatin biotransformation products, ^{3}H-ormaplatin (20 μM; 21 mCi/mmole, prepared as described previously [51]) was incubated with freshly prepared rat or human plasma for 6 or 12 h at 37° C. Following incubation, the samples were cooled to 4° C and plasma ultrafiltrates obtained by filtration through Amicon YMT membranes (MWCO 30 kd). For in vivo determination of ormaplatin biotransformation products, plasma ultrafiltrates were analyzed from all patients on day 1 at the highest dose level. In both assays ormaplatin was resolved from $PtCl_2$(dach) and other biotransformation products with the reverse phase, ion pair HPLC system we have described previously [7, 29]. The major biotransformation products were tentatively identified on the basis of the retention times of the 37 platinum(II) and 13 platinum(IV) standards we have previously prepared [7, 8, 20, 29, 31, 32]. The HPLC column was calibrated with ormaplatin, Pt(serine)(dach), $PtCl_2$(dach), $[Pt(H_2O)(Cl)(dach)]^+$ and Pt(methionine)(dach) standards prior to the analysis of each set of plasma ultrafiltrates [29].

Each HPLC fraction was analyzed for Pt content by a modification of the technique described by Riley et al [41] using a Perkin-Elmer Model 560 atomic absorption spectrometer with Zeeman correction, a HGA graphite furnace and an AS-1 autosampler. The presence of heptane sulfonate in the HPLC fractions caused splattering during the drying stage and incomplete removal of volatile residue during the charring stage. These difficulties were overcome by reducing the drying temperature to 95° C and increasing the drying time to 95 s, the ramp time to 20 s, and the charring temperature to 1500° C. Because of the low levels of Pt in many of the HPLC fractions, it was necessary to repeat the inject, dry and char cycle 3–10 times before each burn. Thus, the volatility of the methanol became a problem when multiple samples were analyzed. This problem was overcome by lyophilizing all samples to dryness and resuspending in water. This step also removed effects of methanol on the efficiency and precision of analysis. The optimized procedure allowed quantitation of Pt with an efficiency of 49.8 ± 8.2% (n = 5 separate experiments) at a Pt level of 10 ng/ml, 69.1 ± 5.8% (n = 3 separate experiments) at a Pt level of 50 ng/ml, and 80.0 ± 3.2% (n = 3 separate experiments) at a Pt level of 100 ng/ml.

Pharmacokinetic modeling and statistics. Selection of the appropriate pharmacokinetic model and initial parameter estimations for each patient were performed by curve stripping techniques (RSTRIP V.4.03, MicroMath, Salt Lake City, Utah.). Subsequent evaluation of in-

Table 1. Patient characteristics

Patient no.	Sex	Age (years)	Diagnosis	Dose level (mg/m²)	Courses given
1	M	54	Colon	5.0	1
2	M	48	Head & neck	5.0	2
3	F	64	Head & neck	5.0	2
4	M	73	Head & neck	6.5	1
5	M	71	Head & neck	6.5	2
6	F	36	Colon	6.5	2
7	M	46	Colon	8.7	1
8	M	42	Head & neck	8.7	2
9	M	50	Colon	8.7	4
10	M	51	Renal	11.6	1
11	F	72	Melanoma	11.6	1
12	M	44	Colon	11.6	2
13	M	68	Melanoma	11.6	1
14	F	50	Head & neck	11.6	1
15	M	71	Head & neck	11.6	1
16	M	68	Colon	11.6	1

Table 2. Number of patients with clinical toxicity[a]

	Dose level (mg/m² per day)			
	5.0	6.5	8.7	11.6
Total number treated	3	3	3	7
Hematologic				
Neutropenia	1	0	1	4
Anemia	2	3	2	5
Thrombocytopenia	0	0	1	4
Nonhematologic				
Nausea and vomiting	3	2	3	4
Phlebitis	1	1	1	3
Paresthesias	0	0	1	1
Hypomagnesemia	0	1	1	0

[a] In addition, 1 patient each with diarrhea, headache, ileus

dividual data sets was conducted by weighted non-linear least-squares regression using a one-compartment model with zero-order input and a first-order elimination process (PCNONLIN V.3.0, Statistical Consultants, Lexington, Ky.). Drug clearance was calculated by dividing the dose by the area under the concentration × time curve (AUC). Systemic exposure to the metabolites was calculated by the trapezoidal rule.

Statistical analysis was performed using the Wilcoxon test for paired analysis and the Mann-Whitney U test for non-paired group comparison.

Results

Twenty-five courses of ormaplatin were administered to 16 patients with various solid tumors, as shown in Table 1. All patients had prior chemotherapy, and 4 had prior radiation therapy. No responses were observed. Neutropenia, thrombocytopenia and anemia appeared dose-related over the dosage range studied (Table 2). Only one episode of grade ≥ 3 myelosuppression was noted; this consisted of neutropenia in a patient at the 11.6 mg/m² dose level.

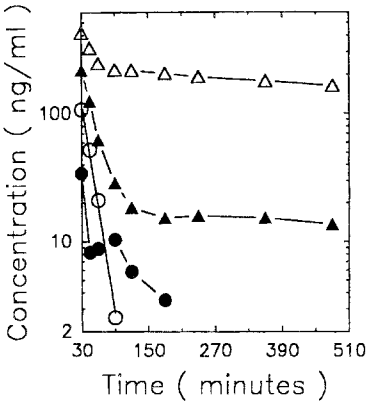

Fig. 1. Pharmacokinetics of total platinum (Pt), ultrafilterable Pt, and plasma biotransformation products from seven patients treated with ormaplatin at 11.6 mg/m². Mean plasma concentrations versus time are shown for total Pt (△), ultrafilterable Pt (▲), [dichloro(*d,l-trans*)-1,2-diaminocyclohexaneplatinum(II)] $PtCl_2$(dach) (○), and all other biotransformation products combined (●). The data for the biotransformation products other than $PtCl_2$(dach) were obtained from two patients only

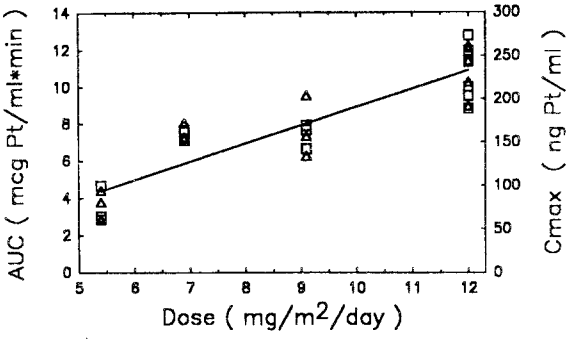

Fig. 2. Daily ultrafilterable Pt systemic exposure (AUC □; C_{max} Δ) during the first course of therapy in all 16 patients. The *line* represents linear regression of dose and AUC ($r^2 = 0.81$)

The most common nonhematologic toxicity was nausea and vomiting, which occurred in 12 of the 16 patients despite premedication with prochlorperazine (Table 2). Premedication with ondansetron appeared to result in improved drug tolerance. This toxicity was of grade 1 or 2 in all cases except for 1 patient at 11.6 mg/m², who was refractory to antiemetic treatment. All other nonhematologic toxicities were of grade ≤ 2 and included phlebitis (6 patients), peripheral neuropathy (2 patients), and hypomagnesemia (2 patients). One patient each experienced diarrhea, headache and ileus.

The clinical course of patients with neuropathy is of interest. One patient at the 8.7 mg/m² dose level received a total of four courses of drug. He had onset of grade 1–2 paresthesias over his feet during the third course of drug and these persisted for over 5 months. The other patient was treated with the 11.6 mg/m² dose level and suffered from transient paresthesias during prior treatments with vinblastine and navelbine. After one course of ormaplatin he experienced grade 1–2 peripheral paresthesias that resolved within a month. Following reports of severe cumulative neurotoxicity in two other ongoing ormaplatin trials, a decision was made to stop accrual to this trial even

Table 3. Ultrafilterable platinum (Pt) pharmacokinetic data[a]

	Course 1		Course 2	
	Day 1	Day 4	Day 1	Day 4
Number	12	16	5	4
Pt $t_{1/2}$ (minutes)	16.2 (11–20)	20.0 (13–27)	18.2 (11–23)	16.9 (13–23)
Clearance (ml/min per m^2)	543.6 (441–625)	468.6 (367–758)	667.6 (527–696)	620.7 (440–685)
Vd (l/m^2)	12.4 (9–18)	12.9 (9–22)	17.4 (11–19)	12.7 (12–21)
% Uf[b]	57.1 (39–87)	38.0 (25–56)	n/a	n/a

[a] Median (range)
[b] Percent in ultrafiltrate at 30 min (C_{max})

though a maximally tolerated dose had not been reached [34, 47].

Ultrafilterable Pt rapidly decayed from plasma samples following the infusion (Fig. 1); however a small, but relatively stable, concentration persisted, which was thought to be indicative of Pt bound to small-molecular-weight species (see below). This residual ultrafilterable Pt concentration was reached by approximately the 120-min sampling point. A one-compartment pharmacokinetic model adequately described the data from each patient set when truncated to include samples from the start of the infusion to the 90-min point. A summary of the pharmacokinetic disposition parameters is displayed in Table 3. Systemic clearance values decreased by an average of 63 ml per min/m^2 over the first four doses of cycle 1 ($P = 0.025$), increasing the half-life by 3.6 min ($P = 0.011$). This trend was not evident in the second cycle; however, the limited amount of data for that cycle prevents a firm conclusion. Pharmacokinetic determinants of systemic (ultrafiltered) Pt exposure (AUC; C_{max}) were linearly correlated to dose ($r^2 = 0.81$ and 0.77, respectively; Fig. 2). Inter-patient variability in systemic clearance was demonstrated over only a one- to twofold range; however, this was enough to provide overlapping systemic exposure between the dosage levels.

Plasma Pt concentrations increased over the 5 days of drug administration, with mean pre-dose concentrations of 74, 136, 193, and 230 ng/ml at 24, 48, 72, and 96 h, respectively ($r^2 = 0.4$, $P < 0.001$). This phenomenon accounted for an average 19% reduction in the fraction of ultrafilterable Pt, from 57% of the total plasma Pt following dose 1 to 38% by dose 4 ($P < 0.001$; Table 3). Pt was not detectable in plasma immediately before the second cycle in 4 out of 5 patients studied.

Prior to analysis of clinical samples, the in vitro biotransformations of ormaplatin were compared in human and rat plasma. For these experiments, ^{3}H-ormaplatin was incubated in vitro with freshly prepared human or rat plasma for 6 or 12 h at 37° C and the biotransformation products were resolved as described in "*Materials and methods*". The incubation times chosen were based on the

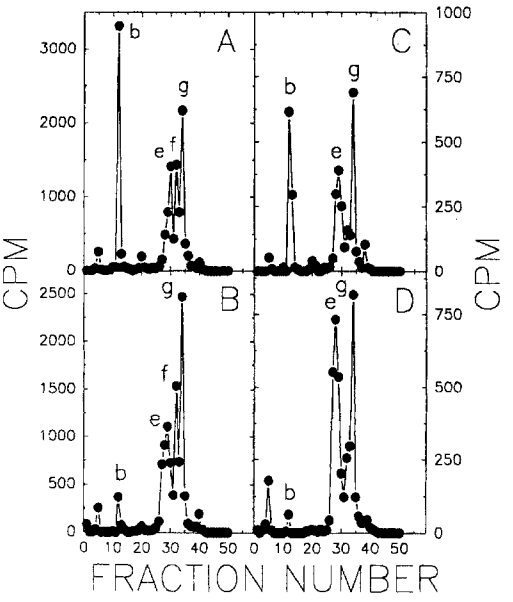

Fig. 3 A–D. Comparison of in vitro reactions of ormaplatin in rat and human plasma. ^{3}H-Ormaplatin (20 μM) was incubated with freshly prepared rat (**A, B**) or human (**C, D**) plasma for either 6 (**A, C**) or 12 (**B, D**) h at 37° C and analyzed by HPLC. The individual peaks are identified in the text

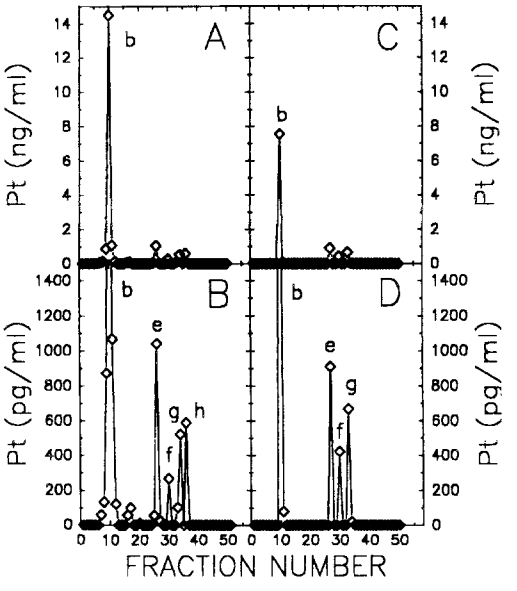

Fig. 4 A–D. HPLC analysis of biotransformation products in plasma ultrafiltrates obtained from a patient treated with ormaplatin. Plasma ultrafiltrates were obtained at 30 min (**A, B**) and 45 min (**C, D**) from beginning an ormaplatin infusion of 11.6 mg/m^2. The HPLC profile is shown full scale (**A, C**) to show the decrease in $PtCl_2$(dach) (*peak b*) between 30 and 45 min and at an expanded scale (**B, D**) to show the stability of *peaks e, f,* and *g* over the same time period

times that gave maximum resolution of biotransformation products in rat plasma [7]. The HPLC patterns obtained from the reaction of ormaplatin with both rat (Fig. 3 A, B) and human (Fig. 3 C, D) plasma were very similar with respect to $PtCl_2$(dach) (peak b), the cysteine/ornithine complex (peak e) and the methionine complex (peak g).

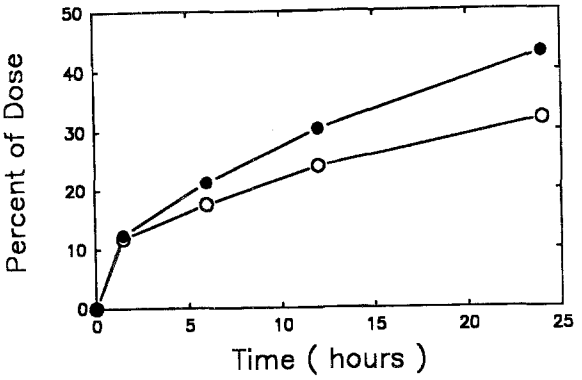

Fig. 5. Cumulative urinary Pt excretion following ormaplatin administration in 16 patients. Median values for sample collection intervals of 0–1.5 h, 1.5–6 h, 6–12 h, and 12–24 h are shown for doses 1 (○) and 4 (●) of the first course

Table 4. Median ultrafilterable Pt and $PtCl_2$(dach) data for 7 patients receiving 11.6 mg/m² ormaplatin

	$PtCl_2$(dach)	Ultrafilterable Pt
$t_{1/2}$ (minutes)	12.9 (9.1–23.1)	16.0 (11.4–19.4)
AUC (μg Pt/ml per min)	3.77 (1.74–4.31)	9.12 (8.01–11.32)
C_{max} (ng Pt/ml)	127 (63–143)	227 (206–248)

$PtCl_2$(dach), dichloro(*d,l-trans*)1,2-diaminocyclohexane-platinum(II)

However, the reaction of ormaplatin with human plasma produced significantly less of peak f. Based on our previous experiments [29, 32] this peak most probably corresponds to the Pt(dach) complex with serine, threonine, asparagine or glutamine at the time of these incubations.

Since the in vitro biotransformations of ormaplatin in human plasma did not suggest the presence of any unusual biotransformation products that might have required further identification, the same approach was used to analyze ormaplatin biotransformation products in vivo. Typical HPLC profiles from the 30- and 45-min plasma ultrafiltrates of a patient at the 11.6 mg/m² dose are shown in Fig. 4. No unreacted ormaplatin was detected at any time. $PtCl_2$(dach) (peak b) was the major biotransformation product at early times. Figures 4 A and C show the profiles at full scale to emphasize the rapid decrease in plasma levels of $PtCl_2$(dach)between 30 min (Fig. 4 A) and 45 min (Fig. 4 C). The same data are shown on expanded scale in Fig. 4 B and D, to allow identification of the other biotransformation products. Peaks e, f and g vary little in concentration between 30 and 45 min, while peak h was found only in the 30-min sample. The likely identity of peaks e, f and g has been discussed above. Peak h most probably corresponds to the Pt(citrato)(dach) complex previously found in rat plasma in vitro [7].

The time course for the disposition of the plasma biotransformation products identified above is also included in Fig. 1. The open circles show the plasma disposition of $PtCl_2$(dach) which clearly showed a monoexponential decay pattern. No slower, secondary decay (beta $t_{1/2}$) was detectable in these experiments. The closed circles show the disposition of all other low molecular weight biotransformation products combined (peaks e–h in Fig. 4). Following an initial, rapid decline, these biotransformation products were more persistent in the circulation than $PtCl_2$(dach). Based on previous studies of ormaplatin biotransformations in rat plasma [6, 7], the initial rapid decline between 15 and 30 min was most likely due to the presence of the $[Pt(H_2O)(Cl)(dach)]^+$ complex, which is known to be in rapid equilibrium with $PtCl_2$(dach) [27] and which is cleared from the circulation in parallel with $PtCl_2$(dach) in rat plasma [6]. Those remaining biotransformation products that persisted in the circulation beyond 60 min most probably consist of dach-Pt complexes with cysteine, methionine and other amino acids and have been shown to be biologically inert in previous studies [6, 7]. At times beyond 90 min, a significant portion of the ultrafilterable Pt exists in a form that does not elute from the reverse-phase column. These biotransformation products remain unidentified at present. However, they most probably represent Pt bound to low-molecular-weight proteins and/or peptides, since all Pt complexes with amino acids and other plasma metabolites tested to date elute from this column under the conditions of analysis [6, 7, 20, 29, 31, 32]. Such Pt complexes should also be biologically inert [28, 48]. The pharmacokinetic parameters for $PtCl_2$(dach) and ultrafilterable Pt derived from all patients at the 11.6 mg/m² dose are summarized in Table 4. From these data, it is clear that the disposition of $PtCl_2$(dach), the major active biotransformation product present in plasma, is substantially different from the disposition of ultrafilterable Pt. Moreover, inter-patient variability was also much greater for the $PtCl_2$(dach) species (2.4-fold; CV 25%) than for ultrafilterable Pt (1.4-fold; CV 13%).

Approximately 32% of the ormaplatin dose was excreted in the urine as Pt equivalents during the first 24 h of the regimen (Fig. 5). Over one-third of the total urinary excretion occurred within the first 1.5 h of beginning the infusion. The urine disposition pattern during the fourth dose mirrored the pattern of dose 1, although, concentrations were significantly higher with the later dose ($P = 0.007$).

Discussion

This phase I trial of ormaplatin administered on a daily x5 schedule did not reveal dose-limiting toxicity through daily doses of 11.6 mg/m². The trial was terminated early because of the disabling peripheral neuropathy associated with cumulative doses of ormaplatin in the range of approximately 165 mg/m² or higher noted in other studies [47]. The toxicity profile observed on this study was consistent with that reported by other investigators at cumulative ormaplatin exposures less than 165 mg/m².

A variety of analytic methods have been utilized to determine the clinical pharmacokinetic disposition of Pt compounds. Theoretically, evaluation of ultrafilterable or "free" Pt is considered the "gold standard"; however, most of the published data relating systemic exposure and clinical effect for highly reactive compounds such as cisplatin have been derived from total plasma platinum concentrations [5, 12, 26]. Fournier et al [18] have reported a correlation ($r = 0.76$) between the AUC for ultrafilterable Pt

and the concentration of Pt bound to plasma proteins at 24 h. However, no studies have convincingly demonstrated a correlation between the AUC for ultrafilterable Pt and either clinical efficacy or Pt-DNA adduct levels in peripheral leukocytes (which has been suggested as a prognostic indicator of clinical efficacy [38, 39]). In addition, previous studies of both cisplatin [11] and ormaplatin [6] biotransformations in rat plasma have explicitly shown that the ultrafilterable Pt fraction consists of a mixture of active and inactive biotransformation products that are cleared from the circulation with very different kinetics. Thus, it is not clear that ultrafilterable Pt is a good indicator of active Pt species in the plasma. Measurement of the diethyldithiocarbamate (DDTC)-reactive Pt in plasma ultrafiltrates has been suggested as an alternative approach for quantitating active Pt species [4, 21, 40]. However, a detailed analysis of that method has shown that DDTC reacts with some inactive plasma biotransformation products [2]. Because of these considerations, HPLC separation techniques appear to offer the most reliable means of separating and quantitating the active and inactive plasma biotransformation products of Pt anticancer agents [13]. Thus we thought it was of importance to evaluate the clinical pharmacokinetics of ormaplatin by the use of both standard and novel quantitation methods.

It was evident by the second dose level that a dramatic reduction in the ultrafilterable Pt concentrations occurred within 120 min of the infusion, while thereafter a low, but detectable, concentration of Pt remained for extended periods of time. Since prior studies of similarly reactive Pt analogues have found these late samples to have relatively little Pt compound with active binding capacity compared with immediate post-infusion concentrations [21], we chose to truncate our ultrafilterable Pt data to include time points up to 90 min for subsequent pharmacokinetic modeling. The half-life of ultrafilterable Pt was approximately 16 min follwing the first dose, increasing to 20 min by dose 4. Pt clearance was reduced by approximately 14% within patients over the course of several days of administration. Similar effects have been noted for other Pt analogues [19, 33]. The average systemic ultrafilterable Pt exposure increased with the ormaplatin dose; however inter-patient variability in systemic clearance produced overlapping AUC and C_{max} values between dose levels.

Rahman et al. evaluated the disposition of ultrafilterable Pt in rats following i.v. administration of ormaplatin [37]. Early plasma protein binding was 33%, similar to the average value in our human experiment. The initial Pt half-life of 10.9 min was also similar to the value reported here. Approximately 28% of the Pt was excreted in the rats' urine over 24 h, compared to 32% in the current human study.

The pharmacokinetic disposition of ultrafilterable Pt following intermittent infusion of ormaplatin is qualitatively similar to cisplatin. However, studies which use similar infusion and analytic methodology show a slightly longer half-life (approximately 30 min) and higher volume of distribution (approximately 50 l/m^2) values for cisplatin [10, 14, 17, 49].

These data represent the first extensive use of HPLC methodology to characterize the pharmacokinetics of active and inactive Pt biotransformation products of ormaplatin in conjunction with a clinical trial. Our data show that little or no ormaplatin was present even at the earliest sampling times. This is consistent with previous rat data showing that ormaplatin is rapidly ($t_{1/2}$ 3 s) reduced to $PtCl_2$(dach) by plasma protein sulfhydryl [7]. $PtCl_2$(dach) disappeared monoexponentially with a $t_{1/2}$ (12.9 min) that was slightly less than the truncated initial $t_{1/2}$ (16 min) for decay of ultrafilterable Pt. No slower, terminal decay phase was observed for $PtCl_2$(dach). These data alone cannot distinguish whether a terminal decay phase exists for this compound due to its rapid and irreversible biotransformations [7, 20, 31] or whether the terminal decay portion of the curve was beneath the limits of detection at this dose. However, these data are fully consistent with the pattern of clearance of $PtCl_2$(dach) from rat plasma [6], suggesting that no terminal decay phase exists for this compound. In contrast, the stable biotransformation products were very persistent in the circulation and appeared to account for much of the terminal decay of ultrafilterable platinum, an observation that is also fully consistent with previous data recorded in rats treated with ormaplatin [6]. Limited HPLC analyses have implied that the pharmacokinetics of cisplatin and its active biotransformation products are similarly related to the pharmacokinetics of ultrafilterable Pt [11]. These data suggest that determinations of $t_{1/2}$ beta for ultrafilterable Pt may be of little relevance in quantitating systemic exposure to active Pt species, and that the truncated, single-compartment model used in these experiments probably provides a valid first approximation for systemic exposure to active Pt species.

However, even with utilization of the truncated, single-compartment model, the pharmacokinetic parameters for $PtCl_2$(dach), the major active biotransformation product of ormaplatin, are clearly different from the pharmacokinetic parameters obtained for ultrafilterable Pt. The $t_{1/2}$ for $PtCl_2$(dach) was 80% of the truncated $t_{1/2}$ for ultrafilterable Pt; and the C_{max} and AUC for $PtCl_2$(dach) were both 50% of the corresponding values for ultrafilterable Pt. More importantly, as seen in Table 4, there is more inter-patient variability in the AUC and C_{max} for $PtCl_2$(dach) than for ultrafilterable Pt. Previous studies have also shown considerable inter-patient variability in Pt-DNA adduct levels in cisplatin-treated patients [16, 38, 39] and a correlation between Pt-DNA adduct levels and clinical outcome [38, 39]. This suggests that measuring the disposition of active Pt biotransformation products may be more representative of inter-patient variability for systemic exposure to reactive Pt species than measuring the disposition of ultrafilterable Pt. Thus, assuming that systemic exposure to active Pt species is an important determinant of Pt drug efficacy and/or toxicity, these data suggest that HPLC analysis of the active plasma biotransformation products is an important part of the pharmacokinetic characterization of Pt anticancer agents. This assertion should be more rigorously tested in future clinical trials.

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