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Kinetics of tissue disposition of *cis*-ammine/cyclohexylamine-dichloroplatinum(II) and cisplatin in mice bearing FSaIIC tumors

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Abstract The clinical potential of mixed amine platinum(IV) complexes has been identified, and interest in this new class of antitumor agents has been heightened by demonstration of their activity in cisplatin-resistant neoplasms. These tetravalent platinum agents are expected to undergo a reductive reaction to form the corresponding platinum(II) drug prior to eliciting biological activity. *cis*-Ammine/cyclohexylamine-dichloroplatinum(II) is one such product that we evaluated with cisplatin in vivo, and we found the two complexes given i.v. or i.p. to have comparable activities against a solid murine fibrosarcoma. Following i.v. administration of the two compounds at equitoxic dose levels (20 mg/kg) to tumor-bearing mice, platinum levels in the plasma were consistently higher for cisplatin. Tissue platinum levels, in contrast, were comparable between the agents or higher for the mixed amine analog at the earliest (3-h) time point. The temporal profiles determined for the concentrations over 48 h were tissue- and/or drug-specific and could be described by terminal-phase constants or half-lives of platinum in most tissues. In the plasma, kidney, lung, and jejunum, platinum levels arising from both compounds decayed with half-lives of 24–92 h. The terminal-phase constants of platinum determined in the heart for the two complexes were not significantly different from zero, indicative of levels remaining steady, whereas the constants were negative in the spleen, indicative of an increase in tissue drug concentration. In the tumor, liver, and testes, positive values for the decay-phase constants corresponding to half-lives of 47, 256, and 79 h, respectively, were seen with the mixed amine complex; this pattern contrasted with that found for cisplatin, for which the terminal-phase constant was either zero or negative. In vitro binding studies demonstrated the mixed amine complex to be more reactive. Thus, the presence of one ammine and one cyclohexylamine carrier ligand in the mixed amine complex, as opposed to the diammine ligands in cisplatin, leads to an increase in drug distribution and an alteration in the kinetics of tissue binding and removal of platinum.

Key words Alicyclic mixed amine complex · Pharmacokinetics · Tissue distribution

Abbreviations *DACH* 1,2-Diaminocyclohexane · *FAAS* flameless atomic absorption spectrophotometry · *FBS* fetal bovine serum

Introduction

Cisplatin is the first inorganic complex to demonstrate potent antitumor activity against several human cancers, including those of the head and neck, ovary, testes, and bladder [19]. Its clinical utility, however, is limited due to several toxicities, with renal damage being the most notable [19]. This limitation has encouraged the development of several new platinum analogs, and from these has emerged the highly successful carboplatin, which lacks nephrotoxicity at tolerated doses [9].

Development of drug resistance in initially responsive tumors is a recognized feature in the clinical application of cisplatin. Carboplatin has not helped in alleviating this drawback, as cross-resistance between this agent and cisplatin is apparent [6, 8]. In an effort to provide more effective therapy for resistant disease, tetraplatin and oxaliplatin have been introduced into clinical trials [4]. These compounds contain 1,2-diaminocyclohexane (DACH) as a carrier ligand, which when coordinated to the central platinum atom, has proved to be highly effective in circumventing cisplatin resistance in selected tumor models [4]. Ammine/amine (mixed amine) platinum(IV) congeners, with equatorial chloro and axial carboxylato or

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hydroxo ligands, have also demonstrated such an ability in vitro [10, 11].

Mixed amine complexes are currently receiving close attention, due partly to one such analog, ammine/cyclohexylamine-diacetato-dichloroplatinum(IV), entering clinical trials in Europe as an orally formulated preparation [14, 20]. However, very little information is available on their pharmacokinetic and toxicological properties. Earlier studies with ammine/isopropylamine-dichloroplatinum(II) and ammine/cyclopentylamine-dichloroplatinum(II) have indicated these compounds to be myelosuppressive and nonnephrotoxic [21]. The lack of nephrotoxicity is consistent with the more recent data of McKeage et al. [15], who have reported the absence of renal damage associated with oral administration of ammine/cyclohexylamine-dicarboxylato-dichloroplatinum(IV) complexes. Platinum(IV) complexes are highly inert and require reduction to the platinum(II) form for activity [2, 18]. As further support, the clinical trial with ammine/cyclohexylamine-dicarboxylato-dichloroplatinum(IV) has demonstrated only traces (<2%) of the intact drug in the plasma, whereas the expected reduction product, ammine/cyclohexylamine-dichloroplatinum(II) (Fig. 1), was present as the major metabolite [20]. The potential significance of this platinum(II) analog in the pharmacology of ammine/cyclohexylamine-platinum(IV) agents makes it necessary to determine the pharmacokinetic profile of the ammine/cyclohexylamine-dichloroplatinum(II) congener. As part of this profile, we examined the tissue disposition of this divalent complex and compared it with that of the structurally similar parent compound cisplatin (Fig. 1).

Materials and methods

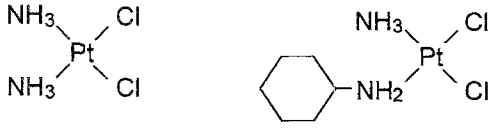
Chemicals

Cisplatin and ammine/cyclohexylamine-dichloroplatinum(II) were synthesized according to previously published procedures [12, 27]. Hyamine hydroxide was purchased from ICN Biomedicals, Inc. (Irvine, Calif.) and fetal bovine serum (FBS) was obtained from BioWhittaker, Inc. (Walkersville, Md.).

Animals and tumor system

C3H/He male mice weighing 21–26 g were purchased from Charles River Inc. through the National Cancer Institute (Washington, D.C., USA). The animals were allowed free access to food and water at all times. The FSaIIC murine fibrosarcoma cell line, adapted for growth in culture [25], was kindly provided by Dr. Beverly A. Teicher of the Dana-Farber Cancer Institute (Boston, Mass.). The cells were grown in α-minimum essential medium (Life Technologies, Inc., Grand Island, N.Y.) supplemented with 10% FBS, 50 μg penicillin/ml, 50 μg streptomycin/ml and 100 μg neomycin/ml and kept at 37 °C in a humidified atmosphere of 5% CO_2 in air. One million tumor cells in 0.1 ml of Hanks' buffered salt solution were inoculated subcutaneously in the right flank of mice with a take rate of 100%.

Fig. 1 Structures of platinum complexes



Cisplatin ***cis*-Ammine/cyclohexylamine-dichloro-Pt(II)**

Tumor growth-delay study

Animals bearing FSaIIC tumors were given 6.5 mg/kg i.v. (tail vein) or 5 mg/kg i.p. of either platinum complex on day 6. The i.p.-treated group received additional treatments on days 10 and 14. The tumor size was measured twice weekly by an electronic vernier caliper directly connected to a computer that recorded the data in a spreadsheet. The tumor volume was calculated automatically by the software using the formula:

$$\text{Tumor volume } (mm^3) = \frac{ab^2}{2},$$

where a is the maximal and b, the minimal diameter (mm) of the tumor. Tumor growth delay was defined as the time difference in days required for tumors in the saline-treated (control) and drug-treated groups to reach a volume of 800 mm^3. The use and estimation of this parameter in antitumor evaluations has been discussed previously [1].

Drug treatment and tissue sampling

Animals, inoculated with the fibrosarcoma on day 0, received 20 mg/kg of i.v. cisplatin or the mixed amine complex via the tail vein on day 9, when the tumor volume was about 300 mm^3. At 3, 12, 24, and 48 h after drug administration, animals were anesthetized by methoxyflurane (Pitman-Moore, Inc., Mundelein, Ill.) inhalation and exsanguinated by severing the left axillary vessels. Blood was collected into a heparinized 1-ml tuberculin syringe and transferred to 1.5-ml microfuge tubes, and the plasma was isolated following centrifugation of samples at 12,500 *g*. The tumor, liver, kidney, lung, heart, spleen, jejunum, and testes were excised, and approximately 25 mg of each tissue was transferred to preweighed microfuge tubes, which were then reweighed for accurate determination of sample weights. Samples were frozen at −70 °C for later assessment of the tissue platinum content.

Platinum analysis

Plasma samples were analyzed directly by flameless atomic absorption spectrophotometry (FAAS) on a Varian AA300 instrument equipped with a graphite furnace (model GTA 96) and an autosampler [22]. Thawed tissue samples, however, required processing by first being incubated overnight at 55°–60 °C with 25 μl of hyamine hydroxide/20 mg of tissue and then being acidified with 4 vols. of 0.3 *N* HCl prior to analysis by FAAS as described previously [23].

Protein-platinum binding in vitro

To 450-μl aliquots of FBS was added 50 μl of a freshly prepared solution (50 μg/ml) of each platinum compound. Aliquots (50 μl) were removed and added to 450 μl of 0.1 *N* HCl to confirm the final drug concentration. The samples were incubated in a shaking water bath at 37 °C, and 100-μl reaction aliquots were removed at selected time points and added immediately to 200 μl of cold 10% trichloroacetic acid, and then vortexed. After 10 min on ice, the precipitated protein was pelleted by microfugation at 12,500 *g*, and the supernatant was collected for analysis of protein-free ("free") platinum by FAAS.

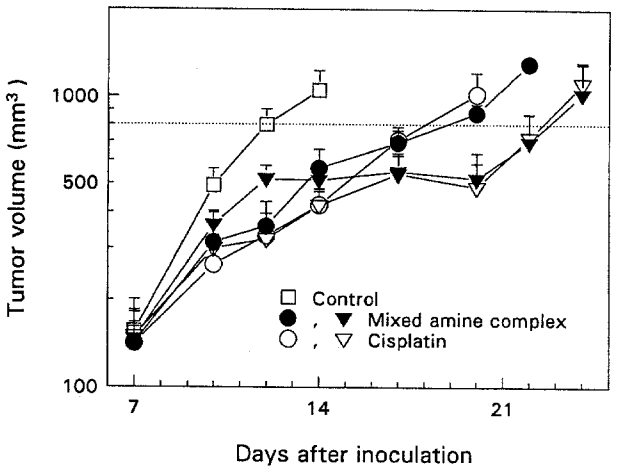


Fig. 2 Growth profile of fibrosarcoma FSaIIC following treatment with platinum complexes. The mixed amine complex or cisplatin was injected i.v. (*circles*; 6.5 mg/kg on day 6 via the tail vein) or i.p. (*triangles*; 5 mg/kg on days 6, 10, and 14) at the maximal tolerated dose. Data represent mean values ± SE, $n = 5$

Determination of pharmacokinetic parameters

A one-compartment open model was fitted to platinum concentrations using a weighted (by $1/C^2$) nonlinear least squares computer program (GraphPAD Inplot, San Diego, Calif.) as described by the equation:

$C_t = Ae^{-\alpha t}$,

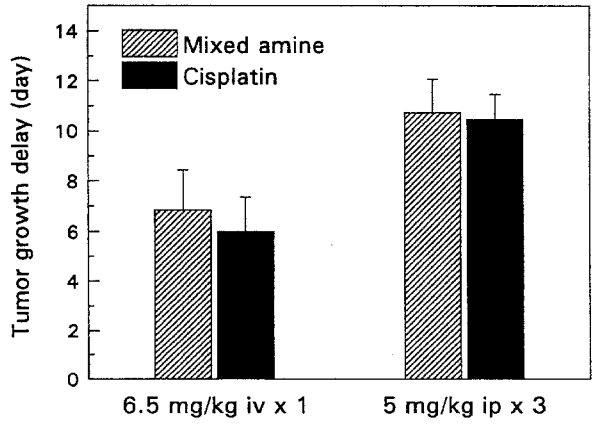
where C_t represents the platinum level at time t, A is a constant, and α is the first-order decay constant [7]. Half-lives ($t_{1/2}$) were calculated as follows:

$t_{1/2} = 0.693/\alpha$.

Statistical analysis

Differences between groups were examined by Student's t-test, with $P < 0.05$ being considered significant.

Fig. 3 Antitumor efficacy of the mixed amine complex and cisplatin against fibrosarcoma FSaIIC. The data were derived from the curves presented in Fig. 2 (see the legend to Fig. 2 for details of the doses used). Data represent mean values ± SE, $n = 5$



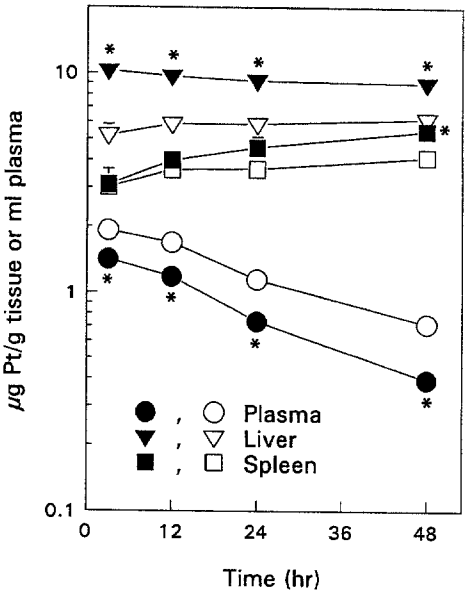


Fig. 4 Pharmacokinetic profiles of the mixed amine complex (*solid symbols*) and cisplatin (*open symbols*) in plasma, liver, and spleen. The complexes were given i.v. to mice at the 20-mg/kg dose level. Data represent mean values ± SE, $n = 4$

Results

The growth of the fibrosarcoma in control and treated mice is shown in Fig. 2. This tumor has a fairly short doubling time, taking only 6 days in control groups to grow from a volume of about 100 mm³ at the onset of treatment on day 6 to the predetermined cutoff volume of 800 mm³. Administration of either the mixed amine complex or cisplatin at maximal tolerated doses delayed tumor growth to this limit by about 6 days for the single i.v. treatment and 10 days for the triple i.p. injections (Fig. 3).

Intravenous doses of 20 mg/kg were used to investigate drug kinetics in tumor-bearing mice. These doses were 3 times the maximal tolerated doses and were necessary to facilitate platinum analyses at the microanalytical level in tissues, particularly at the later time points, and to allow the possibility of estimations of free drug levels in plasma. The rapid clearance of free drug, however, precluded such an estimation in the plasma. Typical kinetic curves generated for total platinum levels following i.v. administration of the compounds are shown in Fig. 4. More detailed information on tissue distribution is presented in Table 1. Among the tissues, the testes had the lowest platinum concentration, whereas the liver and kidney had the highest. In the plasma, platinum levels were higher following administration of cisplatin than after injection of the mixed amine complex. This finding most likely reflects in part the lower molecular weight of cisplatin (300) relative to that of the mixed amine complex (382), resulting in a 27% higher molar dose of cisplatin relative to the analog. However, in most tissues, total platinum concentrations detected at the 3-h time point following administration of the mixed amine complex and cisplatin were similar. In the liver, lung, and heart, on the

Table 1 Tissue distribution of mixed amine complex and cisplatin

Tissue	Compound	Platinum concentration (μg/ml plasma or μg/g tissue)[a]			
		3 h	12 h	24 h	48 h
Plasma	Mixed amine	1.42±0.02*	1.18±0.03*	0.74±0.02*	0.40±0.03*
	Cisplatin	1.91±0.10	1.69±0.06	1.14±0.11	0.71±0.04
Tumor	Mixed amine	3.76±0.74	2.50±0.22	2.54±0.35	1.70±0.08*
	Cisplatin	2.59±0.40	2.57±0.27	2.99±0.28	2.56±0.32
Kidney	Mixed amine	7.12±0.87	8.16±0.22	6.99±0.34	4.33±0.29*
	Cisplatin	8.86±0.58	8.30±0.49	7.49±0.96	6.19±0.80
Liver	Mixed amine	10.24±0.40*	9.67±0.74*	9.22±0.39*	8.99±0.17*
	Cisplatin	5.20±0.64	5.86±0.27	5.85±0.34	6.18±0.28
Lung	Mixed amine	4.30±0.19*	4.44±0.52	3.60±0.39*	3.17±0.18*
	Cisplatin	2.06±0.36	3.39±0.50	2.61±0.23	1.80±0.10
Heart	Mixed amine	3.35±1.15*	1.99±0.37	2.24±0.12*	1.85±0.12*
	Cisplatin	0.88±0.06	1.20±0.39	1.09±0.19	1.16±0.10
Jejunum	Mixed amine	3.14±0.52	2.27±0.26	1.83±0.18	1.36±0.12
	Cisplatin	2.83±0.40	2.42±0.37	1.65±0.18	0.98±0.23
Spleen	Mixed amine	3.10±0.55	3.99±0.42	4.56±0.54	5.46±0.56*
	Cisplatin	3.02±0.04	3.61±0.51	3.64±0.27	4.13±0.34
Testes	Mixed amine	0.66±0.09	0.71±0.16	0.63±0.03	0.46±0.07*
	Cisplatin	0.56±0.14	0.72±0.13	0.68±0.10	0.95±0.13

* $P < 0.05$
[a] The tissue platinum levels are expressed as mean values ± SE, $n = 4$

Table 2 A one-compartment pharmacokinetic analysis of platinum in tissues following administration of mixed amine complex or cisplatin in male mice

Tissue	Compound	Pharmacokinetic parameters[a]		
		A (μg/ml)	α (h^{-1})	$t_{1/2}$ (h)
Plasma	Mixed amine	1.57±0.81	0.029±0.002*	23.8 ± 1.53*
	Cisplatin	2.08±0.10	0.023±0.002	30.7 ± 2.46
Tumor	Mixed amine	3.44±0.41	0.015±0.004*	46.6 ± 13.7
	Cisplatin	2.65±0.19	0.000±0.003	ND[b]
Kidney	Mixed amine	8.30±1.01	0.012±0.004	56.5 ± 19.6
	Cisplatin	9.10±0.03	0.008±0.0001	86.4 ± 1.23
Liver	Mixed amine	10.1 ±0.23*	0.003±0.001*	256 ± 77.5
	Cisplatin	5.36±0.20	–0.003±0.001	ND[b]
Lung	Mixed amine	4.51±0.23	0.008±0.002*	91.8 ± 22.6*
	Cisplatin[c]	4.08±0.56	0.017±0.002	40.1 ± 3.71
Heart	Mixed amine[c]	2.17±0.29*	0.003±0.004	256 ±399
	Cisplatin[c]	1.16±0.10	0.000±0.003	ND[b]
Jejunum	Mixed amine	2.97±0.26	0.017±0.003*	40.0 ± 7.44*
	Cisplatin	3.07±0.12	0.024±0.001	28.7 ± 1.73
Spleen	Mixed amine	3.22±0.23	–0.012±0.003	ND[b]
	Cisplatin	3.12±0.16	–0.006±0.002	ND[b]
Testes	Mixed amine	0.73±0.05*	0.009±0.003*	79.2 ± 23.5
	Cisplatin	0.56±0.04	–0.011±0.003	ND[b]
FBS[d]	Mixed amine	97.9 ±2.24	0.114±0.003*	3.79± 0.14*
	Cisplatin	101.0 ±1.09	0.183±0.007	6.08± 0.17

* $P < 0.05$ (Student's t-test)
[a] Pharmacokinetic parameters are expressed as mean values ± SE, $n = 4$, except for FBS
[b] Not determined because α values were ≤0
[c] Values generated using data obtained at the final three time points
[d] Binding of mixed amine complex and cisplatin (5 μg/ml) to FBS in vitro, $n = 3$

other hand, levels were 2- to 4-fold greater following treatment with the mixed amine analog than after cisplatin injection. During the 12- to 48-h period, platinum levels deriving from both agents generally declined (plasma, kidney, lung, and jejunum), remained unchanged (heart), or increased (spleen). In the tumor, liver, and testes, levels detected after administration of the mixed amine compound decreased during the same period, whereas they remained unchanged or increased after administration of cisplatin.

Plasma and tissue platinum levels were fitted to the exponential function representing the terminal decay phase of the concentration versus time curve, and the results are shown in Table 2. The constant A provides an estimate for tissue platinum concentration upon completion of the initial distribution phase, and the differences in values found for

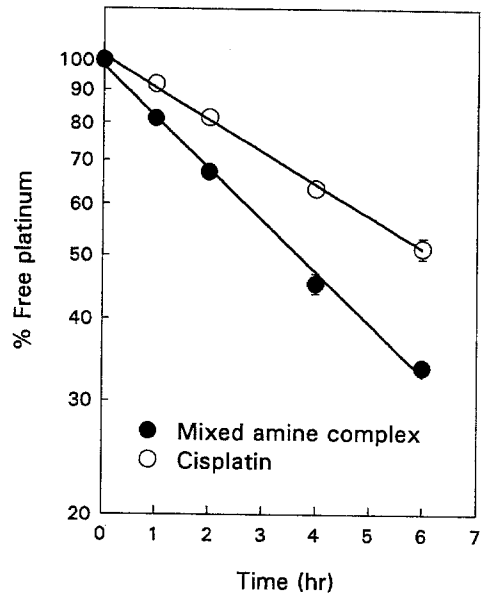


Fig. 5 Kinetics of in vitro binding of the mixed amine complex and cisplatin to FBS. Data represent mean values ± SE, $n = 3$. Lines are linear following regression analysis of data points obtained for each complex. Correlation coefficient (r) = 0.998 for mixed amine and 0.999 for cisplatin

the two drugs in each tissue reflected the differences in concentrations observed at 3 h. The terminal-phase constants (α) for tissues with decreasing concentrations over time allowed estimation of platinum half-lives. Terminal decay was fastest in the plasma, with the half-lives of the two drugs being 24–31 h. Half-lives were similar in the jejunum ($t_{1/2}$ = 29–40 h) and longer in the kidney ($t_{1/2}$ = 57–86 h) and the lung ($t_{1/2}$ = 40–92 h). In the tumor, liver, and testes, half-life estimations were possible only for the mixed amine complex and ranged from 47 h in the tumor to 256 h in the liver. In the remaining tissues, kinetic constants were not significantly different from zero or were negative. Significant differences in decay constants and, therefore, half-lives were found between the two drugs in the plasma, tumor, liver, lung, jejunum, and testes.

Figure 5 demonstrates the monoexponential decay of free platinum during in vitro incubations of the platinum complex with FBS. The kinetic constants and corresponding half-lives are included in Table 2. With cisplatin, platinum levels decayed with a half-life of 6.1 h. In comparison, the mixed amine complex gave faster kinetics, with the half-life being 3.8 h for the decline of free platinum.

Discussion

Mixed amine platinum(IV) complexes with axial carboxylato and equatorial chloro ligands represent a new area in cisplatin analog development [10, 11]. These complexes are expected to be inert and require reduction to the platinum(II) form before eliciting biological effects [2]. By analogy with the reduction of other platinum(IV) complexes [3, 5, 18], the expected product of reduction of the lead ammine/cyclohexylamine-dicarboxylato-dichloroplatinum(IV) complexes is ammine/cyclohexylamine-dichloroplatinum(II), which indeed has been demonstrated as an early major product in plasma in a clinical trial of the ammine/cyclohexylamine-diacetato-dichloroplatinum(IV) analog [20]. We have independently confirmed by high-performance liquid chromatography (HPLC) that the in vitro disappearance of 200 μM ammine/cyclohexylamine-diacetato-dichloroplatinum(IV) complex in fresh mouse plasma is rapid ($t_{1/2}$ = 1.9 h) and precedes irreversible protein binding, which is relatively slower ($t_{1/2}$ = 5.2 h; unpublished observations).

In the present study, we compared the kinetics of tissue platinum levels following administration of ammine/cyclohexylamine-dichloroplatinum(II) complex and cisplatin. Since it was essential to relate the doses used to the therapeutic outcome, the antitumor activities of these complexes were evaluated in a fibrosarcoma FSaIIC tumor model. Fortunately, the doses used in antitumor evaluations for cisplatin and the ammine/amine analog were not only maximally tolerated but also equieffective against this tumor, which facilitated pharmacokinetic comparisons between the drugs. These antitumor results are comparable with those reported previously for cisplatin against this tumor [26].

Comparison of pharmacokinetics between the mixed amine complex and cisplatin revealed some interesting differences. Although plasma platinum levels attained with the mixed amine complex were lower than those achieved with cisplatin, levels measured in tissues at the earliest time point were comparable with or higher than those seen with cisplatin. The greater tissue distribution of the analog may be due in part to a difference in physicochemical properties between the two compounds. In particular, the difference in lipophilicity between cisplatin, whose partition coefficient is about −1.9 [13], and the more lipophilic mixed amine complex, whose coefficient value is −0.3 (unpublished data), is about 40 orders of magnitude and may facilitate greater penetration of the analog into tissues. Such differences in lipophilicity have resulted in substantially greater in vitro cellular accumulation of lipophilic mixed amine platinum(IV) compounds as compared with cisplatin [11, 17]. The greater concentration of the mixed amine analog in tissues may also relate to its greater reactivity, resulting in a more rapid covalent binding of the platinum species to the macromolecule and its intracellular fixation.

The kinetic profiles observed for the compounds depended to some extent not only on the tissue type but also on the drug itself. For instance, platinum in some tissues provided the expected decay profile, whereas in others the levels increased progressively over time. Although the increases observed with time in the spleen are consistent with previous observations with cisplatin [24] and can be attributed to decreases in spleen size, the opposing direction of slopes of the kinetic curves generated for the two complexes in the liver and testes is unexplained. Clearly,

the difference in chemical structure between the two complexes is the basis for these differential observations, and it is possible that the bulkier cyclohexylamine ligand in the mixed amine analog may facilitate intracellular mechanisms to remove irreversibly bound drug from target sites. In any event, the significance of such tissue-specific differences in kinetics between the two drugs cannot be ascertained at this stage because drug effects in these tissues have not been fully defined. Indeed, it is possible that the significance, if any, at the pharmacodynamic level may be minimal, since similar but less pronounced differences in kinetics between the drugs were also observed in the tumor, which nevertheless was equally responsive toward both drugs. Likewise, the similarity in the renal kinetics of the two agents does not provide immediate clues to suggest why cisplatin would be nephrotoxic [28] and the mixed amine complex would be projected to be free of this activity [15, 21]. This, however, remains to be confirmed.

The most recent study of McKeage et al. [16] provides an opportunity to compare the tissue distribution data obtained following oral administration of 50% of the maximally tolerated doses of ammine/cyclohexylamine-diacetato-dichloroplatinum(IV) and two other ammine/cyclohexylamine-dicarboxylato homologs with those obtained in our studies following i.v. administration of the major reduction product, the ammine/cyclohexylamine-dichloroplatinum(II) complex. The reported study examined distribution in mice at only the 48-h time point for tissue comparison, and at this time the liver contained the highest platinum concentration (range, 9.1–13 μg/g), followed by the kidney (3.0–4.5 μg/g), spleen (0.7–2.1 μg/g), lung (1.3–1.4 μg/g), and heart (0.6–1.5 μg/g) [16]. Interestingly, the respective 48-h values of 9.0, 6.2, 5.5, 3.2, and 1.9 μg/g obtained by us are in the same rank order. The quantitative differences between these and the reported values are not gross and probably reflect differences in the doses and routes of drug administration. Nevertheless, the qualitative similarity in the data suggests that the dichloroplatinum(II) species formed in the plasma may dictate the tissue-distribution pattern of the corresponding dicarboxylato-dichloroplatinum(IV) analogs.

In conclusion, the ammine/cyclohexylamine-dichloroplatinum(II) complex demonstrated good potency and antitumor activity. Comparison of its kinetic data with those of cisplatin suggested that the cyclohexylamine ligand in the mixed amine complex can have substantial effects on platinum levels in certain tissues. The drug levels and their temporal profiles were probably a net effect of distribution and fixation of drug, of the capacity of tissues to remove the bound platinum, and/or of tissue turnover rates. This study may prove useful in the pharmacological understanding of ammine/cyclohexylamine platinum(IV) complexes.

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