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CORRELATION OF TOTAL AND INTERSTRAND DNA ADDUCTS IN TUMOR AND KIDNEY WITH ANTITUMOR EFFICACIES AND DIFFERENTIAL NEPHROTOXICITIES OF *CIS*-AMMINE/CYCLOHEXYLAMINE-DICHLOROPLATINUM(II) AND CISPLATIN

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Abstract—Mixed amine platinum complexes have been identified as a new class of antitumor agents with activity in some cisplatin-resistant tumor models. *cis*-Ammine/cyclohexylamine-dichloroplatinum(II) is one such analog that we have evaluated *in vivo* and found it to have antitumor activity that was comparable to that of cisplatin in a solid murine fibrosarcoma tumor model. In contrast to the nephrotoxicity observed with cisplatin, the analog was free from inducing this side-effect. Pharmacokinetics of the two compounds administered i.v. at equitoxic dose levels to tumor-bearing mice indicated similar decay kinetics of total platinum in plasma, kidney and the tumor. Furthermore, DNA–platinum adducts of the two agents were similar in the tumor. Total adduct levels in the kidney, on the other hand, were significantly greater ($P < 0.05$) by up to 4-fold for cisplatin compared with the mixed amine analog. Likewise, the levels of interstrand cross-links of the two platinum complexes were comparable in the tumor, but significantly greater ($P < 0.05$) in the kidney for cisplatin. The data indicate that the greater renal levels of total and interstrand DNA–platinum adducts formed by cisplatin correlate with renal damage associated with this agent, and suggest that adduct levels, and not total tissue platinum levels, provide a more useful correlation with pharmacodynamic observations.

Key words: cisplatin; platinum analogs; pharmacokinetics; DNA adducts; antitumor activity; nephrotoxicity

Cisplatin§ is one of the most active antitumor agents available in medical oncology [1]. However, it has several toxicological limitations, the most severe being the cumulative and irreversible nephrotoxicity that has limited the full clinical utility of this inorganic complex. This limitation alone has encouraged the development of several new platinum analogs, and from these has emerged the highly successful carboplatin [2], which does not have nephrotoxicity as a side-effect at tolerated doses [2].

A second major limitation of cisplatin is the development of resistance in initially responsive tumors. Since tumors resistant to cisplatin generally display almost complete cross-resistance to carboplatin [3, 4], chemical efforts have continued in order to identify compounds active in the resistant disease. Currently, tetraplatin (ormaplatin, Fig.1) and oxaliplatin are receiving clinical evaluations for their potential efficacies against such cancers [5]. Both of these compounds contain 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 [5]. Recently, ammine/amine (mixed amine) platinum(IV) congeners, with equatorial chloro and axial carboxylato or hydroxo ligands, have also demonstrated activity against cisplatin-resistant cells *in vitro* [6, 7], and one, specifically ammine/cyclohexylamine - diacetato - dichloroplatinum(IV), has entered clinical trials in Europe as an oral formulation [8].

Despite the intense interest in mixed amine complexes, there is very little information available on their pharmacological properties, including their potential to induce renal damage. Earlier, brief toxicological studies with ammine/isopropylamine-dichloroplatinum(II) and ammine/cyclopentylamine-dichloroplatinum(II) have indicated these compounds to be myelosuppressive, and not nephrotoxic [9]. More recently, McKeage *et al.* [10] have also reported the absence of renal damage associated with oral administration of a series of ammine/cyclohexylamine - dicarboxylato - dichloro - platinum(IV) complexes. It is necessary to note that platinum(IV) complexes themselves are highly inert [11, 12], and reduction of these mixed amine

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§ Abbreviations: cisplatin, *cis*-diamminedichloroplatinum(II); tetraplatin, *trans*-1*R*,2*R*-1*S*,2*S*-diaminocyclohexane tetrachloroplatinum(IV); oxaliplatin, *trans*-1*R*,2*R*-diaminocyclohexane oxalatoplatinum(II); DACH, 1,2-diaminocyclohexane; BUN, blood urea nitrogen; and FAAS, flameless atomic absorption spectrophotometry.

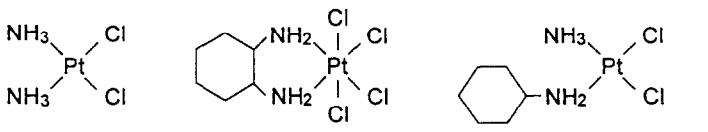

Fig. 1. Structures of platinum complexes.

complexes to the corresponding ammine/cyclohexylamine-dichloroplatinum(II) is expected to be a prerequisite for biological activity [6]. The potential significance of this platinum(II) complex in the pharmacology of ammine/cyclohexylamine-platinum(IV) agents necessitates an investigation into the pharmacokinetic and pharmacodynamic behaviors of ammine/cyclohexylamine-dichloroplatinum(II). In the present report, we have documented briefly its antitumor activity and nephrotoxic potential relative to the structurally resembling parent compound cisplatin (Fig.1), and have investigated in detail the pharmacological basis for the relative activities of the two agents in both the tumor and the kidney.

MATERIALS AND METHODS

Chemicals. Cisplatin and ammine/cyclohexylamine-dichloroplatinum(II) (Fig. 1) were synthesized according to previously published procedures [13, 14]. [*methyl*-^{3}H]Thymidine was purchased from Amersham (Arlington Heights, IL), hyamine hydroxide from ICN Biomedicals, Inc. (Irvine, CA), proteinase K from Boehringer-Mannheim (Germany) and FBS from Bio Whittaker, Inc. (Walkersville, MD).

Animals and tumor system. C3H/He male mice, 21–26 g, were purchased from Charles River Inc., through The National Cancer Institute, U.S.A. The animals had free access to food and water at all times. The FSaIIC murine fibrosarcoma cell line, adapted for growth in culture [15], was provided by Dr. Beverly A. Teicher, Dana-Farber Cancer Institute, Boston, MA. The cells were grown in α-MEM medium (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FBS, 50 μg/mL penicillin, 50 μg/mL streptomycin and 100 μg/mL neomycin, and kept at 37° 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 animals with a take rate of 100%.

Tumor growth delay study. Animals (5/group) bearing FSaIIC tumor were administered 6.5 mg/kg, i.v., or 10 mg/kg, i.p., of each platinum complex on day 6. The tumor size was measured twice weekly by an electronic vernier caliper connected directly to a computer, which recorded the data in a spreadsheet software. The tumor volume was calculated automatically by the software using the formula:

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

where a is the maximal and b the minimal diameter (mm) of the tumor. Tumor growth delay is the time difference in days for tumors in the saline and drug-treated groups to reach 800 mm^3 in size. The use and estimation of this parameter in antitumor evaluations have been reported previously [16]. The experiments were repeated once to confirm the data obtained.

Drug treatment and tissue sampling. Mice were inoculated with the tumor on day 0 as described above, and on day 8, when tumors were approximately 250 mg, animals were injected with [*methyl*-^{3}H]thymidine (3.75 mCi/kg, i.v.; sp. act., 5 Ci/mmol) to determine the DNA labeling index and correct for DNA dilution, if any, during post-drug treatment DNA synthesis [17]. Drug treatment on day 9 involved administration of 20 mg/kg cisplatin or the mixed amine complex via the tail vein. At 0, 3, 12, 24 and 48 hr, animals (4/group) were anesthetized by methoxyflurane (Pitman-Moore, Inc., Mundelein, IL) 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; the plasma was collected following centrifugation of samples at 12,500 g. The tumor and left kidney were excised, and approximately 25 mg of the tissue was transferred to pre-weighed microfuge tubes, which were then reweighed for later analysis of total platinum content. The remaining tissues were transferred to separate containers for later extraction of DNA. All samples were frozen at −70°.

BUN assay. Blood urea nitrogen was determined with a BUN assay kit (Sigma, St. Louis, MO) utilizing the method of Crocker [18]. Briefly, to 4 μL of plasma in a 10 × 75 mm borosilicate glass tube was added 1 mL of reaction mixture (0.6 mL of acid and 0.4 mL of color reagents), followed immediately by placing in a boiling water bath for 10 min. The tubes were then dipped in water at 15°, and absorbance at 535 nm was measured by standard spectrophotometry techniques.

Total tissue platinum level. Plasma platinum level was measured directly by FAAS (model AA300/GTA-96; Varian, Victoria, Australia) following appropriate dilutions with 0.1 N HCl when needed. Tissue samples, however, required processing for platinum determination, as previously described [19, 20]. Briefly, to the tissue (approximately 25 mg) was added 20 μL of hyamine hydroxide per 10 mg of tissue, and the sample was incubated at 55–60° overnight. Four volumes of 0.3 N HCl were added to

the tissue solution, and the platinum content was determined by FAAS.

DNA extraction, total and interstrand DNA adducts, correction for DNA synthesis. Tissue samples (100–200 mg) were homogenized gently with ice-cold PBS (3 mL) and collected in 50-mL conical tubes. The homogenates were centrifuged at 1750 *g* and washed with PBS twice; the pellets were incubated with 10 mL of the extraction buffer (10 mM Tris, 100 mM EDTA, 20 μg/mL RNase, 0.5% SDS, pH 8.0) and 1 mL of a proteinase K solution (1 mg/mL) at 37° for 1 hr and then 50° for 2 hr. DNA was precipitated with 2.5 mL of 10 M ammonium acetate and 26.5 mL of absolute ethanol (total volume, 40 mL). The precipitated DNA was transferred to a 1.5-mL microfuge tube using a pipette with an enlarged orifice, and then washed twice with 70% ethanol. The DNA pellet was partially dried, and dissolved in 250 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) overnight at room temperature. The DNA content and purity were assessed by absorption at 260 and 280 nm, and the amount of platinum in the sample was determined directly by FAAS. DNA interstrand cross-links mediated by the platinum compound were determined by an ethidium bromide interstrand cross-linking assay, previously described by Sriram and Ali-Osman [21]. Briefly, 0.1 mL of the DNA solution (50–100 μg/mL in TE buffer) was dispensed identically in each pair of 13 × 100 mm borosilicate tubes. To this was added 0.4 mL of EB buffer (20 mM potassium phosphate, 2 mM EDTA, pH 11.8), and one of the pairs was heated in a dry bath incubator (Fisher Scientific, Pittsburgh, PA) at 100° for 10 min. After this time, both heated and non-heated tubes were dipped in water at 13° and followed immediately with the addition of 1.0 mL of EB buffer maintained at room temperature. When the temperature of the reaction solution reached 15°, 1.5 mL of ethidium bromide (2 μg/mL) in EB buffer at room temperature was added. The fluorescence of the reaction solution was measured using a fluorometer (model LS-5B, Perkin-Elmer Ltd., Buckinghamshire, UK) at 305 nm excitation (slit width-3) and 590 nm emission (slit width-20) wavelengths. The DNA interstrand cross-link (ISC) index was calculated as follows:

$$\text{ISC index} = (-\ln x_{\text{treated}}) - (-\ln x_{\text{control}})$$

where

$$x = \frac{\text{fluorescence}_{\text{non-heated}} - \text{flourescence}_{\text{heated}}}{\text{fluorescence}_{\text{non-heated}}}$$

Dilution of the total DNA bound platinum and ISC index by DNA synthesis was corrected by dividing with a dilution factor calculated as follows:

$$\text{Dilution factor} = \frac{\text{specific activity of DNA at time t}}{\text{specific activity of DNA at 0 hr}}$$

Pharmacokinetic analysis. 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, CA), as described by the equation:

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

Table 1. Antitumor activity of mixed amine complex and cisplatin against FSaIIC subcutaneous tumor*

Compound	Route	Dose† (mg/kg)	TGD‡ (day)
Mixed amine complex	i.v.	6.5	6.84 ± 1.60
Cisplatin	i.v.	6.5	6.01 ± 1.35
Mixed amine complex	i.p.	10	9.99 ± 0.77
Cisplatin	i.p.	10	7.55 ± 1.11

* FSaIIC cells were grown *in vitro*, and 10^6 cells were inoculated subcutaneously in the right flank area of male C3H/He mice on day 0.

† Doses shown are maximal tolerated for respective schedule and route. The injection was performed on day 6 when tumors weighed 65–150 mg.

‡ Tumor growth delay (TGD) was determined as a difference in time required for the tumor to reach 800 mm^3 between the treated and control groups. Values are means ± SEM, N = 5.

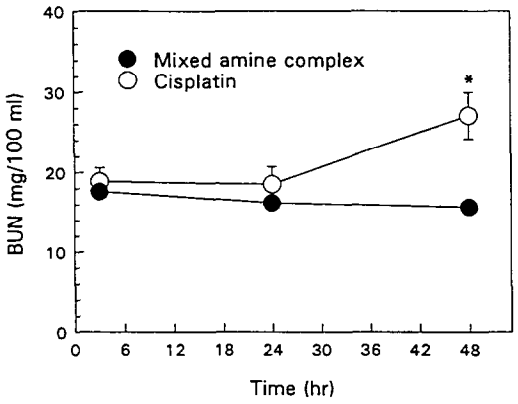

Fig. 2. BUN levels in plasma following i.v. administration of cisplatin or the mixed amine complex in mice. Both compounds were given at a dose level of 20 mg/kg. Data are means ± SEM (errors are shown if larger than symbols); N = 4. Key: (*) $P < 0.05$.

where C_t represents the platinum level at time t, and α is the first-order decay constant [22]. Half-life ($T_{1/2}$) was calculated as follows:

$$T_{1/2} = 0.693/\alpha$$

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

RESULTS

The antitumor activities of ammine/cyclohexylamine-dichloroplatinum(II) and cisplatin were investigated in mice bearing an established solid fibrosarcoma. At maximal tolerated doses (MTD), activities of the two drugs were similar, with tumor growth delays ranging between 6 and 7 days for i.v.

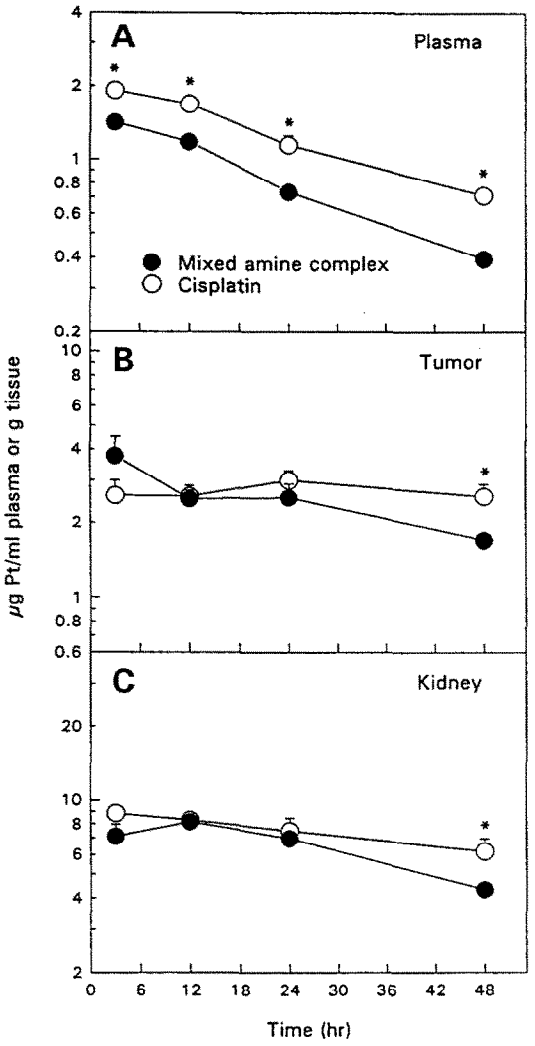

Fig. 3. Kinetic profiles for cisplatin and the mixed amine complex in mouse plasma (A), tumor (B) and kidney (C). Drugs were administered i.v. at a dose of 20 mg/kg. Data are means ± SEM (errors are shown if larger than symbols); N = 4. Key: (*) $P < 0.05$.

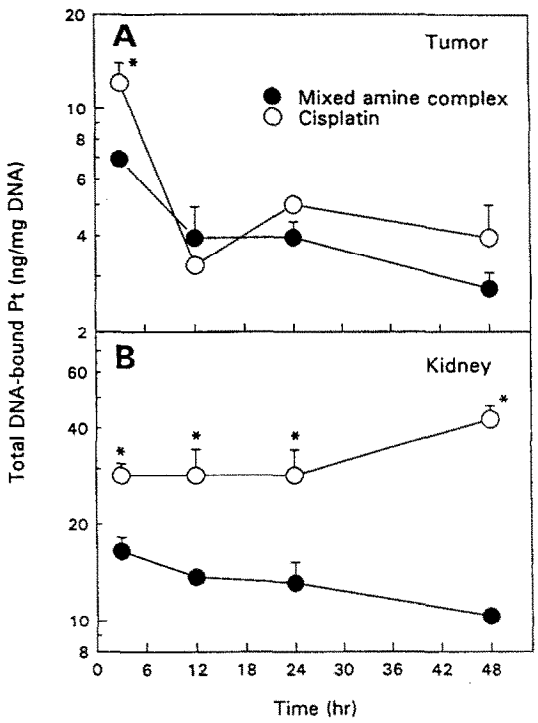

Fig. 4. Kinetic profiles of total DNA-bound platinum in mouse tumor (A) and kidney (B). Cisplatin and the mixed amine complex were administered i.v. at a dose of 20 mg/kg. Data are means ± SEM (errors are shown if larger than symbols); N = 4. Key: (*) $P < 0.05$.

and 8 and 10 for i.p. administrations (Table 1). The nephrotoxic activities of the complexes were determined using equitoxic i.v. doses of 20 mg/kg. At this dose, mice die on day 3 or 4, and, therefore, the study was limited to 2 days. No increase in BUN levels over control values was noted at any time following administration of the mixed amine analog (Fig. 2). Cisplatin, on the other hand, demonstrated no effect at the earlier time points, but on day 2 a significant increase in BUN was observed.

Intravenous doses of 20 mg/kg were also used to investigate drug kinetics. These doses were three times above maximal tolerated doses, and were necessary to facilitate platinum analyses at the whole tissue and DNA levels. In the plasma, only total platinum levels were measurable and were higher following the administration of cisplatin than the mixed amine complex (Fig. 3A). This probably reflects the lower molecular weight of the parent compound (300 vs 382). Decay of platinum in the plasma was similar for both complexes (half-life = 24–31 hr). There were no gross differences in total tissue platinum levels in the tumor or kidney between the two drugs except at the 48-hr time point (Fig. 3, B and C). Half-lives for the mixed amine complex in the tumor and kidney were 47 ± 14 (mean ± SEM) and 57 ± 20 hr, respectively, while the half-life for cisplatin was 86 ± 1 hr in the kidney and immeasurable in the tumor where platinum levels remained steady throughout the 48 hr.

DNA extracted from tissues was analyzed for platinum content to estimate levels at the target macromolecular level. In the tumor, temporal DNA–platinum adduct profiles for the mixed amine complex and cisplatin were essentially the same (Fig. 4A). In contrast, renal adduct levels of cisplatin were significantly greater than those of the mixed amine complex at all time points, and at 48 hr, the difference in levels between the two drugs was 4-fold (Fig. 4B). Interestingly, renal adducts of the mixed amine congener decreased steadily with time, whereas total adducts of cisplatin remained constant over the first 24 hr and appeared to increase at the 48-hr time point. Temporal aspects of DNA interstrand cross-links are shown in Fig. 5. In the tumor, there were no gross differences in interstrand

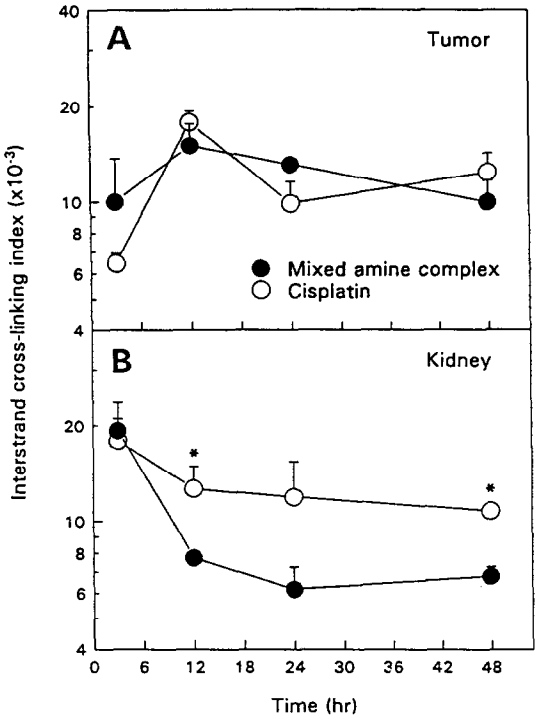

Fig. 5. Kinetic profiles of DNA interstrand cross-links in mouse tumor (A) and kidney (B). Cisplatin and the mixed amine complex were given i.v. at a dose level of 20 mg/kg. Data are means ± SEM (errors are shown if larger than symbols); N = 4. Key: (*) $P < 0.05$.

adduct levels between the two platinum agents. As with total DNA adducts, interstrand adducts were up to 2-fold greater with cisplatin than with the mixed amine complex.

DISCUSSION

Mixed amine platinum(IV) complexes with axial carboxylato and equatorial chloro ligands represent a new avenue in cisplatin analog development [6, 7]. These complexes are expected to be inert and require reduction to the platinum(II) before biological activity can be affected [11]. By analogy with the reduction of other platinum(IV) complexes [12, 23, 24], the expected product of reduction of lead ammine/cyclohexylamine-dicarboxylato-dichloroplatinum(IV) complexes is ammine/cyclohexylamine-dichloroplatinum(II), which indeed has been demonstrated as one of the products in biological studies [6].

We have demonstrated that ammine/cyclohexylamine-dichloroplatinum(II), like cisplatin, has excellent potency and good antitumor activity against the solid fibrosarcoma FSaIIC tumor model. These results are comparable to those reported previously for cisplatin against this tumor [25]. However, unlike cisplatin, the mixed amine analog does not elicit nephrotoxicity even at the high dose that was examined. Although caution may need to be exercised here since BUN used in our study provides only a gross index of renal damage, the result with the analog, nevertheless, is consistent with the reported lack of nephrotoxicity associated with two similar complexes, ammine/isopropylamine-dichloroplatinum(II) and ammine/cyclopentylamine-dichloroplatinum(II) [9]. Similarly, there is good agreement with the data of McKeage *et al.* [10], who have reported absence of renal damage associated with oral administration of a series of ammine/cyclohexylamine-dicarboxylato-dichloroplatinum(IV) complexes. At the dose examined (20 mg/kg, i.v.), the lethal toxicity of cisplatin is renal, and, based on findings of Schurig *et al.* [9] with the two structural homologs, it is assumed that lethal toxicity of our mixed amine analog is to the bone marrow.

Elucidation of the mechanism of cisplatin nephrotoxicity remains a challenge, although we [26] and others [27] have demonstrated that the pharmacological basis for renal damage is probably related to active tubular transport of this platinum-based agent. In contrast, the mechanism of antitumor activity has been relatively well defined and involves cisplatin-induced DNA interstrand and/or intrastrand cross-links in tumor cells [28]. Roberts *et al.* [29] have reviewed data on DNA-bound platinum in tumor cells *in vitro* and *in vivo* to demonstrate that total DNA adducts can also be linked to antitumor response. They proposed that cisplatin-induced renal damage may be similarly explained. Our studies with the FSaIIC tumor indicate that cisplatin and the mixed amine analog produced similar levels of total tissue platinum, tissue total DNA adducts and interstrand DNA cross-links, each of which can be correlated to the observed similar antitumor activities of the two drugs. However, total renal platinum levels are considered unsatisfactory in explaining the differential nephrotoxicity of these agents. This difference in toxicity, on the other hand, correlates well with the significantly higher renal interstrand or total DNA adducts of cisplatin relative to the mixed amine complex, as predicted by Roberts *et al.* [29].

Cisplatin-induced interstrand and intrastrand DNA adducts *in vivo* have been quantitated previously in kidney and other normal tissues [30, 31], but correlations with nephrotoxicity either were not attempted or were very poor. Our studies are more informative as they provide a direct comparison between cisplatin and an analog that was comparable in its potency but lacking nephrotoxic activity. Although our data point to the relatively lower levels of renal DNA adducts as evidence for the absence of nephrotoxicity associated with ammine/cyclohexylamine-dichloroplatinum(II) complex, it is not exactly clear why the analog should be free of this side-effect, considering the close similarities in structures (Fig. 1) and DNA adduct profiles *in vitro* [32] between this compound and cisplatin. One possibility relates to the presence in the mixed amine analog of the alicyclic ring, which may permit more rapid recognition and repair of its DNA lesion in the kidney. Our observation of a greater rate of *in vivo* removal of total and interstrand

adducts of this analog compared with cisplatin supports this contention. However, further studies are needed to investigate this plausible explanation in detail. With regard to the difference in renal DNA adduct levels between the two compounds, the possibility exists that this difference may arise as a result of using a high dose of cisplatin, with the resultant decrease in plasma drug clearance and increase in tissue drug exposure. However, the very short plasma half-life of about 10 min for "free" cisplatin in rodents [26] suggests that renal excretion of the high dose may be complete before nephrotoxicity appears and has any influence on drug clearance. This is supported by data of Smith *et al.* [33], who found that renal platinum levels increase proportionately with dose (ranging from non-lethal to lethal) of cisplatin, tetraplatin or the non-nephrotoxic iproplatin (CHIP).

In conclusion, the complex ammine/cyclohexylamine-dichloroplatinum(II) demonstrated good potency and antitumor activity, while lacking the ability to induce nephrotoxicity. Comparison of data with those of cisplatin suggests that the absence of this side-effect may well be related to lower renal levels of interstrand and total DNA–platinum adducts of the analog in the kidney.

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REFERENCES

1. Prestayko AW, D'Aoust JC, Issell BF and Crooke ST, Cisplatin (*cis*-diamminedichloroplatinum II). *Cancer Treat Rev* **6**: 17–39, 1979.
2. Harrap KR, Preclinical studies identifying carboplatin as a viable cisplatin alternative. *Cancer Treat Rev* **12** (Suppl A): 21–33, 1985.
3. Eisenhauer E, Swerton K, Sturgeon J, Fine S, O'Reilly S and Canetta R, Carboplatin therapy for recurrent ovarian carcinoma: National Institute of Canada experience and a review of the literature. In: *Carboplatin: Current Perspectives and Future Directions* (Eds. Bunn P, Canetta R, Ozols R and Rosencweig M), pp. 133–140. W. B. Saunders, Philadelphia, 1990.
4. Gore M, Fryatt I, Wiltshaw E, Dawson T, Robinson B and Calvert A, Cisplatin/carboplatin cross-resistance in ovarian cancer. *Br J Cancer* **60**: 767–769, 1989.
5. Christian MC, The current status of new platinum analogs. *Semin Oncol* **19**: 720–733, 1992.
6. Kelland LR, Murrer BA, Abel G, Giandomenico CM, Mistry P and Harrap KR, Ammine/amine platinum(IV) dicarboxylates: A novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines. *Cancer Res* **52**: 822–828, 1992.
7. Harrap KR, Kelland LR, Jones M, Goddard PM, Orr RM, Morgan SE, Murrer BA, Abrams MJ and Giandomenico CM, Platinum coordination complexes which circumvent cisplatin resistance. *Adv Enzyme Regul* **31**: 31–43, 1991.
8. McKeage MJ, Mistry P, Ward J, Boxall FE, Loh S, O'Neill C, Ellis P, Kelland LR, Morgan SE, Murrer B, Santabarbara P, Harrap KR and Judson IR, Phase I study of orally administered amminediacetatodichloro(cyclohexylamine)platinum(Pt)(IV) (PO JM 216). *Proc Am Soc Clin Oncol* **12**: 130, 1993.
9. Schurig JE, Bradner WT, Huftalen JB, Doyle GJ and Gylys JA, Toxic side effects of platinum analogs. In: *Cisplatin: Current Status and New Developments* (Eds. Prestayko AW, Crooke ST and Carter SK), pp. 227–236. Academic Press, New York, 1980.
10. McKeage MJ, Morgan SE, Boxall FE, Murrer BA, Hard GC and Harrap KR, Lack of nephrotoxicity of oral ammine/amine platinum(IV) dicarboxylate complexes in rodents. *Br J Cancer* **67**: 996–1000, 1993.
11. Blatter EE, Vollano JF, Krishnan BS and Dabrowiak JC, Interaction of the antitumor agents *cis,cis,trans*-$Pt^{IV}(NH_3)_2Cl_2(OH)_2$ and *cis,cis,trans*-$Pt^{IV}[(CH_3)_2CHNH_2]_2Cl_2(OH)_2$ and their reduction products with PM2 DNA. *Biochemistry* **23**: 4817–4820, 1984.
12. Pendyala L, Arakali AV, Sansone P, Cowens JW and Creaven PJ, DNA binding of iproplatin and its divalent metabolite *cis*-dichloro-bis-isopropylamine platinum(II). *Cancer Chemother Pharmacol* **27**: 248–250, 1990.
13. Vollano JF, Al-Baker S, Dabrowiak JC and Schurig JE, Comparative antitumor studies on platinum(II) and platinum(IV) complexes containing 1,2-diaminocyclohexane. *J Med Chem* **30**: 716–719, 1987.
14. Khokhar AR, Deng Y, Al-Baker S, Yoshida M and Siddik ZH, Synthesis and antitumor activity of ammine/amine platinum(II) and (IV) complexes. *J Inorg Biochem* **51**: 677–687, 1993.
15. Teicher BA and Rose CM, Perfluorochemical emulsions can increase tumor radiosensitivity. *Science* **223**: 934–936, 1984.
16. Begg AC, Principles and practices of the tumor growth delay assay. In: *Rodent Tumor Models in Experimental Cancer Therapy* (Ed. Kallman RF), pp. 114–121. Pergamon Press, New York, 1987.
17. Rosenhoff SH, Bull JM and Young RC, The effect of chemotherapy on the kinetics and proliferative capacity of normal and tumorous tissues *in vivo*. *Blood* **45**: 107–118, 1975.
18. Crocker CL, Rapid determination of urea nitrogen in serum or plasma without deproteinization. *Am J Med Technol* **33**: 361–365, 1967.
19. Siddik ZH, Boxall FE and Harrap KR, Flameless atomic absorption spectrophotometric determination of platinum in tissues solubilized in hyamine hydroxide. *Anal Biochem* **163**: 21–26, 1987.
20. Siddik ZH and Newman RA, Use of platinum as a modifier in the sensitive detection of tellurium in biological samples. *Anal Biochem* **172**: 190–196, 1988.
21. Sriram R and Ali-Osman F, S1-Nuclease enhancement of the ethidium bromide binding assay of drug-induced DNA interstrand cross-linking in human brain tumor cells. *Anal Biochem* **187**: 345–348, 1990.
22. Gibaldi M and Perrier D, *Pharmacokinetics*. Marcel Dekker, New York, 1982.
23. Chaney SG, Wyrick S and Till GK, *In vitro* biotransformation of tetrachloro(*d,l-trans*)1,2-diaminocyclohexaneplatinum(IV) (tetraplatin) in rat plasma. *Cancer Res* **50**: 4539–4545, 1990.
24. Eastman A, Glutathione-mediated activation of anticancer platinum(IV) complexes. *Biochem Pharmacol* **36**: 4177–4178, 1987.
25. Teicher BA, Herman TS, Tanaka J, Eder JP, Holden SA, Bubley G, Coleman CN and Frei E III, Modulation of alkylating agents by etanidazole and fluosol-DA/carbogen in the FSaIIC fibrosarcoma and EMT6 mammary carcinoma. *Cancer Res* **51**: 1086–1091, 1991.
26. Siddik ZH, Newell DR, Boxall FE and Harrap KR, The comparative pharmacokinetics of carboplatin and cisplatin in mice and rats. *Biochem Pharmacol* **36**: 1925–1932, 1987.
27. Daley-Yates PT and McBrien DCH, The renal fractional clearance of platinum antitumour compounds

in relation to nephrotoxicity. *Biochem Pharmacol* **34**: 1423–1428, 1985.
28. Eastman A, Schulte N, Sheibani N and Sorenson CM, Mechanisms of resistance to platinum drugs. In: *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy* (Ed. Nicolini M), pp. 178–196. Martinus Nijhoff, Boston, 1988.
29. Roberts JJ, Knox RJ, Friedlos F and Lydall DA, DNA as a target for the cytotoxic and antitumour action of platinum co-ordination complexes: Comparative *in vitro* and *in vivo* studies of cisplatin and carboplatin. In: *Biochemical Mechanisms of Platinum Antitumour Drugs* (Eds. McBrien DCH and Slater TF), pp. 29–64. IRL Press, Oxford, 1986.
30. Murray D, Jenkins WT and Meyn RE, Kinetics of DNA cross-linking in normal and neoplastic mouse tissues following treatment with *cis*-diammine-dichloroplatinum(II) *in vivo*. *Cancer Res* **45**: 6446–6452, 1985.
31. Poirier MC, Reed E, Litterst CL, Katz D and Gupta-Burt S, Persistence of platinum-ammine-DNA adducts in gonads and kidneys of rats and multiple tissues from cancer patients. *Cancer Res* **52**: 149–153, 1992.
32. Hartwig JF and Lippard SJ, DNA binding properties of *cis*-$[Pt(NH_3)(C_6H_{11}NH_2)Cl_2]$, a metabolite of an orally active platinum anticancer drug. *J Am Chem Soc* **114**: 5646–5654, 1992.
33. Smith JH, Smith MA, Litterst CL, Copley MP, Uozumi J and Boyd MR, Comparative toxicity and renal distribution of the platinum analogs tetraplatin, CHIP, and cisplatin at equimolar doses in the Fischer 344 rat. *Fundam Appl Toxicol* **10**: 45–61, 1988.