Kinetics of tissue disposition of cis-ammine/cyclohexylamine-dichloroplatinum(II) and cisplatin in mice bearing F3H1C tumors

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...
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 non-nephrotoxic [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-dicarboxyldichloroplatinum(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-dicarboxyldichloroplatinum(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 FSaIC 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° in a humidified atmosphere of 5% CO2 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%.

Tumor growth-delay study

Animals bearing FSaIC 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 mm3. 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 mm3. 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 frozen at −70°C 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 12,000 g, and the supernatant was collected for analysis of protein-free ("free") platinum by FAAS.