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Identification of *cis*-Dichloro-bis-isopropylamine platinum(II) as a Major Metabolite of iproplatin in Humans[1]

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ABSTRACT

Iproplatin is a quadrivalent second-generation platinum complex undergoing clinical evaluation. In plasma and urine of patients receiving this drug, iproplatin- and platinum-containing metabolites of iproplatin were separated by reverse-phase gradient high performance liquid chromatography. One of the metabolites was identified by cochromatography and electron impact mass spectrometry as *cis*-dichloro-bis-isopropylamineplatinum(II), a metabolite formed by reduction of iproplatin. Incubation of iproplatin with ascorbic acid and cysteine, *in vitro*, indicates that iproplatin can be easily reduced to *cis*-dichloro-bis-isopropylamineplatinum(II) by reducing agents. It is hypothesized that the reduction of the quadrivalent complex iproplatin to *cis*-dichloro-bis-isopropylamineplatinum(II) occurs intracellularly.

INTRODUCTION

Iproplatin [*cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine-platinum(IV)] is a second-generation Pt complex currently undergoing Phase II and Phase III clinical evaluation. In clinical trials, the drug has shown effectiveness against small cell carcinoma of the lung (1), carcinoma of the ovary (2), metastatic breast carcinoma (3), and squamous cell carcinoma of the head and neck (4). In a randomized study comparing iproplatin with carboplatin, another second-generation Pt complex, against carcinoma of the cervix, initial data indicate a trend towards superiority of iproplatin over carboplatin (5). A comparative study of cisplatin, carboplatin, and iproplatin in combination with cyclophosphamide in patients with epithelial carcinoma of the ovary showed iproplatin to be more effective than carboplatin while not causing toxic effects induced by cisplatin, such as peripheral neuropathy, hearing loss, or renal failure (6). *In vitro* studies of Alberts *et al.* in carcinoma of the ovary indicated that iproplatin had at least comparable activity to that of cisplatin and carboplatin and that iproplatin may not be cross-resistant to either platinum complex (7). Among the second-generation Pt complexes that have entered clinical trial, iproplatin is structurally unique, in that it is a quadrivalent Pt complex, while all the others tested are divalent square planar Pt complexes. The quadrivalent complexes are octahedral in their structural configuration with two axial ligands projecting at a 90° angle from the square plane. All of the quadrivalent Pt complexes synthesized and tested for antitumor activity were synthesized originally from divalent counterparts which were highly active but poorly water soluble' in an attempt to improve water solubility without affecting antitumor activity (8). Iproplatin is one such complex synthesized by oxidative addition of two OH groups to CIP[3]. The addition of the two axial OH groups indeed resulted in improved aqueous solubility (44.1 mM for iproplatin as opposed to 0.22 mM for CIP), with retention of antitumor activity (1–7).

Studies on the mechanism of action of the quadrivalent platinum complexes have been much more limited than those on the divalent complexes. The redox parameters for Pt IV oxidation states in a chloride system suggest that Pt IV complexes will be reduced to Pt II complexes in a biological milieu (9). However, no direct evidence for this has been obtained. Some early studies have indicated that iproplatin might cleave closed circular DNA (10, 11). Subsequent studies however, have shown that iproplatin itself cannot cleave the DNA, but a perhydrate complex of iproplatin, which was present as an impurity in the earlier preparations, can (12–14). Studies of Blatter *et al.* (13, 14) have shown that iproplatin and another quadrivalent complex, *cis*-dichloro-*trans*-dihydroxy-*cis*-diammineplatinum(IV), do not alter the electrophoretic mobilities of PM2 DNA, while the corresponding divalent forms do. These findings indicate that the reduction to a divalent state may be a necessary prerequisite for Pt binding to DNA. The goal of the present study was to determine whether CIP could be detected in the plasma and urine of patients receiving iproplatin. Part of the study has been published in a preliminary form (15).

MATERIALS AND METHODS

Iproplatin, for clinical use, was formulated in 50-mg vials and kindly supplied by Bristol Laboratories, Syracuse, NY. The analytical grade iproplatin and CIP were also supplied by Bristol Laboratories.

Patient Samples. Plasma and urine used in this study came from patients receiving iproplatin in a Phase I clinical trial. The drug was administered in a 2-h i.v. infusion in 250-ml isotonic saline. Blood and urine were collected as described previously (16) and were stored frozen until the time of study.

Analytical Methodology. The separation of iproplatin from other platinum-containing complexes in plasma and urine was accomplished by a water to methanol gradient on a μBondapak C_{18} column (16). Fractions eluting from the column were collected and analyzed for Pt by FAAS. The chromatographic characteristics of CIP were established with analytical CIP. For structure identification, multiple samples of urine were chromatographed and the fractions eluting under the peak of interest were collected and pooled until 5 μg of Pt containing material had been accumulated. This material was lyophilized and subjected to electron impact mass spectrometry.

***In Vitro* Reactions.** Iproplatin (1 mg/ml) was incubated with ascorbic acid (0.2 M) or cysteine (0.2 or 2 M) at room temperature for 1 h. 10 μl of the reaction mixture was then injected into HPLC. Fractions eluting off of the column were collected and analyzed for Pt by FAAS.

Mass Spectrometry. Mass spectrometric studies were carried out on a Finnigan 4000 quadrupole mass spectrometer interfaced with an Incos data system. 1 ml of chloroform solution containing 2 μg of Pt metabolite was taken up into a flared capillary tube and the solvent was evaporated to dryness under vacuum. The sample tube was then inserted into the ion source of the mass spectrometer via the direct probe. The probe was heated ballistically to a temperature of 300°C. The ion source was maintained at 300°C, and the ionizing voltage was 70 eV.

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[3] The abbreviations used are: CIP, *cis*-dichloro-bis-isopropylamineplatinum(II) complex; FAAS, flameless atomic absorption spectrophotometry; HPLC, high-performance liquid chromatography.

RESULTS

Iproplatin and Its Metabolites in Plasma and Urine. By gradient HPLC analysis and FAAS, three Pt-containing species could be detected in plasma ultrafiltrate (Fig. 1, *A–C*): (*a*) iproplatin which eluted at 11–13 min; (*b*) a polar platinum-containing material, eluting close to the solvent front; and (*c*) another platinum-containing peak which eluted at 21–23 min. Peak b was seen in all samples, while peak c was present in only some, and when present, did not exceed 15% of filterable Pt. In urine (Fig. 1, *D–F*) two or more additional less polar, minor peaks were found. Iproplatin was the major Pt-containing species in plasma up to the end of infusion in all patients. After this time, it varied; while iproplatin remained the major species for up to 1 to 2 h after the end of infusion in some, in others peak b predominated. In urine, iproplatin was the major Pt containing peak up to 2 h after the end of infusion. From then on, the proportion of polar Pt-containing species to the others increased, and by 8–16 h peak b became either the only detectable Pt-containing species or the most significant platinum-containing species in urine (Fig. 1). Occasionally there were urines from patients where iproplatin was either totally undetectable or was found at levels much lower than b and c from the earliest sampling times.

Chromatographic Characteristics of CIP and Reduction Products of Iproplatin Generated *in Vitro*. The CIP standard elutes at 21–23 min in the gradient HPLC system used. When a 1-h incubation mixture of iproplatin with 0.2 M ascorbic acid was chromatographed on HPLC, iproplatin could not be detected but a new Pt-containing peak appeared at 22 min, in the same position as the CIP standard (Fig. 2). When a similar incubation was performed with cysteine, at concentrations of 0.2 and 2 M, a small peak at 22 min was present, but the predominant Pt peak was close to the solvent front (Fig. 3). The reaction was faster at the higher concentration of cysteine.

Cochromatography of CIP with Urine from Patients Receiving Iproplatin. Urine from two patients each containing the 22-min platinum peak as the major platinum-containing species, were cochromatographed with CIP. The results indicate that CIP and the 22-min peak in the urine coeluted, and the urine to which the CIP was added showed additivity in the 22-min peak.

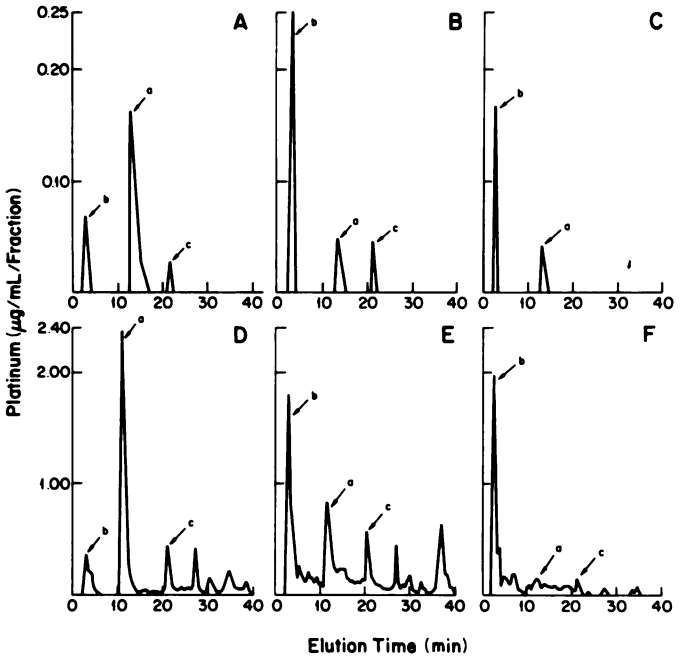

Fig. 1. Platinum-containing compounds in plasma (*A*, 5 min; *B*, 30 min; *C*, 2 h after the end of infusion) and urine (*D*, 0–2 h; *E*, 4–6 h; *F*, 8–12 h after the end of infusion) of a patient receiving iproplatin. *a*, iproplatin, *b*, polar platinum-containing peak, *c*, platinum-containing peak that elutes at 21–23 min.

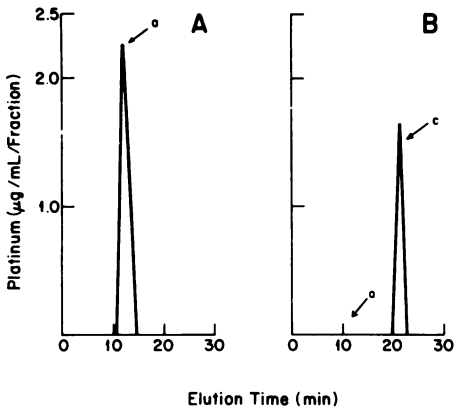

Fig. 2. *In vitro* reaction of iproplatin with ascorbic acid. *A*, iproplatin in water; *B*, iproplatin (1 mg/ml) in water incubated with ascorbic acid (0.2 M) for 1 h at room temperature. The peaks are as defined in Fig. 1.

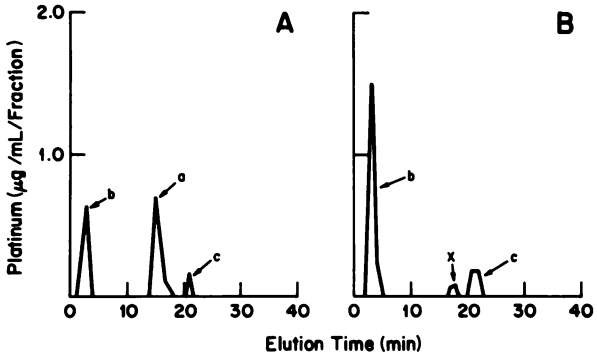

Fig. 3. *In vitro* reaction of iproplatin with cysteine. *A*, iproplatin (1 mg/ml) and cysteine (0.2 M) incubated for 1 h at room temperature; *B*, iproplatin (1 mg/ml) and cysteine (2.0 M) incubated for 1 h at room temperature. The peaks *b* and *c* are as defined in Fig. 1. The identity of peak labeled "*x*" is uncertain.

Structural Identification of the 22-min Peak. The mass spectrum of the CIP analytical standard is shown in Fig. 4*B*. In the molecular ion region, the spectrum shows a cluster of peaks beginning at *m/z* 382, the molecular weight of CIP containing the lowest weight isotope of each of the component atoms in the $PtCl_2$ part of the molecule. The ratio of intensities of the peaks in the cluster (*m/z* 382, 383, 384, 385, 386, 387, and 388) using 384 as 10 are 7.2:7.2:10.0:4.8:5.3:0.4:1.5, which are in agreement with that expected for a complex containing $PtCl_2$, thus supporting the suggested structure of CIP. The cluster of peaks around *m/z* 310 appears to be derived from the loss of two Cl atoms from the molecule. The second cluster of peaks starting at *m/z* 250 apparently corresponds to monoisopropylamine platinum.

Fig. 4*A* shows the mass spectrum of the metabolite collected from the urine, at the 22-min elution time. This mass spectrum shows the same molecular ion cluster (*m/z* 382, 383, 384, 385, 386) in about the same ratio (7.3:6.8:10.0:5.2:4.8) as that for the standard CIP. The peaks for 387 and 388 were too small to be seen with the size of the sample analyzed. The fragmentation pattern also is similar to the standard CIP. The additional minor clusters of different masses seen in this spectrum are presumably due to contaminating material that coeluted with the CIP from urine.

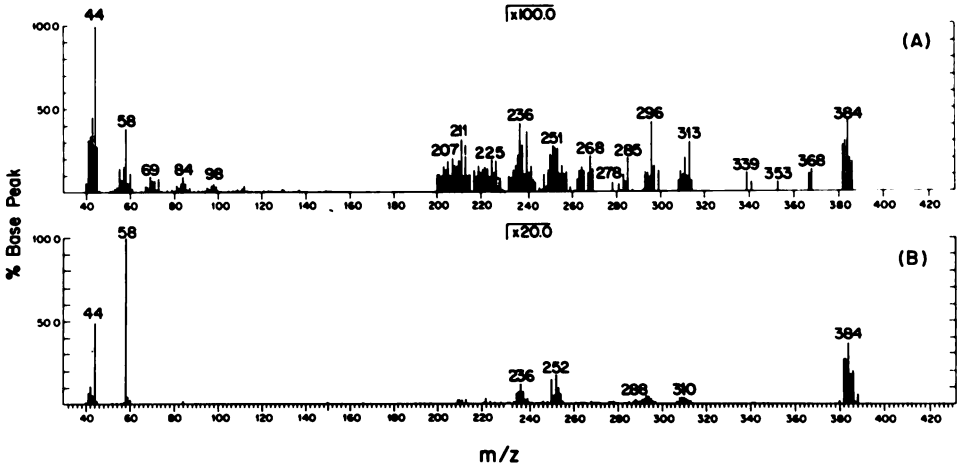

Fig. 4. *A*, electron impact mass spectrum of iproplatin metabolite (22-min retention in our HPLC system) collected from the urine of a patient receiving iproplatin; *B*, electron impact mass spectrum of CIP standard.

DISCUSSION

Iproplatin is a structurally unique, clinically active second generation platinum complex. It is presumed that like cisplatin and other platinum agents, the compound acts by binding to DNA. It is not clear whether two OH groups that project at 90° angles from the square plane should pose a steric hindrance for such an interaction. The question that has never been addressed directly is, whether it is the quadrivalent complex iproplatin or its divalent counterpart CIP, that interacts with the DNA. Measurement of the electrophoretic mobility of DNA after incubation with iproplatin and with CIP suggest that it is CIP which interacts with DNA and not iproplatin. However, until now no data have been available to support the formation of CIP *in vivo*. In this paper we presented evidence that CIP is found in the plasma and urine of patients receiving iproplatin. Quantities of this metabolite were, however, very small in plasma though not in urine. Whether this is a reflection of the higher lipophilicity of CIP [the chloroform/H_2O partition coefficient for CIP is 0.0173, while that for iproplatin is 0.0012 (8)], or higher protein binding capabilities of CIP is not known at this time. It should be noted that in the plasma of patients receiving iproplatin, the bulk of platinum in plasma was protein bound and that iproplatin itself does not bind to proteins as we have previously demonstrated (17).

Our *in vitro* studies demonstrate that iproplatin can be easily reduced to CIP by biological reducing agents such as ascorbic acid and cysteine. The oxidation reduction potential necessary for such reactions (−0.7 V) presumably is not far from that which exists intracellularly (9) and, therefore, such reductions are likely to take place in cells (Fig. 5). Studies on the intracellular fate of iproplatin are ongoing to determine if indeed this complex is reduced to a divalent state in the cell.

At this time the nature of the polar platinum containing peak (peak b), which is always seen in the plasma and urine of patients receiving the drug, is not known. Theoretically, CIP could undergo aquation reactions similar to those reported for cisplatin. These aquated products would be more polar than CIP and would be expected to elute close to the solvent front in a reverse-phase HPLC system. In addition, this peak may consist of CIP adducts formed with other polar biological molecules.

The clinical relevance of the reduction of iproplatin to CIP remains to be determined. There is evidence from early antitumor studies in the ADJ/PC6 plasmacytoma and more recent studies in L210 leukemia that CIP has antitumor activity equal to that of cisplatin (9, 18). In the light of studies that implicate CIP as the DNA interactive agent (12–14), our identification of CIP as an *in vivo* reduction product of iproplatin in patients receiving the drug, suggests that CIP may be the active *in vivo* cytotoxic species of iproplatin.

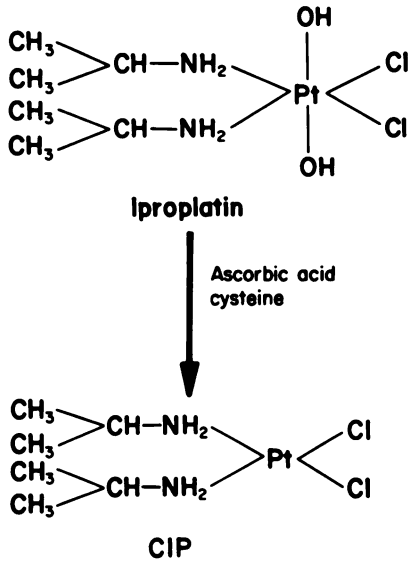

Fig. 5. Schematic representation of the reduction of iproplatin to CIP.

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