Cisplatin or cis-diamminedichloroplatinum(II) is a platinum coordination compound showing clinically useful antitumor activity, but the major dose-limiting factor is a dose-dependent cumulative nephrotoxicity. At the electron microscopic level, platinum has been localized in the nucleus, microsomes and cytoplasm of cells of the kidney and liver in rats. This report confirms the subcellular localization of platinum and adds one more site of platinum accumulation, the microbody, based on results obtained with an energy-dispersive X-ray microanalyzer (EDX). After daily administration of cisplatin (0.5 mg/ml/kg body weight) successively for 5 weeks, accumulated platinum was detected in microbodies of hepatocytes and epithelial cells of proximal convoluted tubules of the rat. The major site of metal deposition in the kidney was the matrix of many microbodies in the epithelial cells of proximal convoluted tubules. EDX revealed the presence of platinum in those metallic deposits. The matrix of the nucleus also had metallic deposits but they were rather diffuse and platinum could not readily be detected on individual grains in the nucleus. In the liver, major damage was concentrated in hepatocytes, and other types of cells such as Kupffer cells, Ito cells and endothelial cells of capillaries were less affected. Metallic fine grains were localized in the cisterna of smooth-surfaced endoplasmic reticulum and in the matrix of microbodies. The nucleus of hepatocytes had few, if any, metallic precipitates. A specific type of metallic deposition was round aggregations of dense tubules in which platinum was detected by EDX. There was no evidence of platinum precipitation in mitochondria or Golgi complex. These findings suggest that the microbody may play an important role in degradation of the platinum complex both in hepatocytes and epithelial cells of proximal convoluted tubules.

Key words: Cisplatin — Platinum complex — Nephrotoxicity — Microbody — Hepatocyte
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MATERIALS AND METHODS

Cisplatin (Biplatin, Bristol-Myers) solution (0.5 mg/ml/kg body weight) was orally administered daily for 5 weeks to 24 male Wistar rats (body weight 200–220 g, 7 to 8 weeks old). Each animal was sacrificed by decapitation and tissue samples were obtained from the liver and kidney. The samples were immersed in 4% cold glutaraldehyde solution buffered with 0.1M sodium cacodylate-HCl at pH 7.4. After a 2 hr fixation, these samples were briefly washed with the same buffer and refixed with 2% osmium tetroxide in 0.1M cacodylate buffer for an additional 2 hr. After being dehydrated with a series of graded ethanols, specimens were soaked in propylene oxide and embedded in Poly/Bed 812. Ultrathin sections (approximately 40nm thick) were mounted on copper grids and stained with lead citrate and uranyl acetate. For X-ray microanalysis, sections without such staining were mounted on beryllium grids to avoid prominent peaks due to Cu and Pb in the EDX spectra. Analytical conditions were kept identical throughout this experiment as far as possible: specimen current, approximately 0.5×10^-10 A; accelerating voltage, 15 kV; live counting period, 100 sec. To identify each spectrum, some of the operating conditions (such as live counting time) were marked in the upper corner of the spectra.

RESULTS

The main site of cytotoxicity caused by the platinum compound in the liver of rats was the cytoplasm of hepatocytes. Damage to other types of cells in the liver such as Kupffer cells, Ito cells, endothelial cells of capillaries, lining cells of the bile duct and interstitial cells was milder than that to hepatocytes. The indices of cytotoxicity in hepatocytes included large empty space, small vacuoles, dilated cisterna of the smooth-surfaced endoplasmic reticulum (s-ER) and swollen mitochondria.

Fine-grain precipitates of platinum compound were localized in small vesicles and in the matrix of microbodies (Fig. 1). The distribution of such fine grains in microbodies was variable and there was no specific pattern to indicate affinity of platinum for a certain substructure in the microbody. Precipitates of platinum complex were unexpectedly sparse, if any, in the matrix of the nucleus of hepatocytes. An interesting finding was a specific type of dense bodies, 0.15 μm in diameter, in the cytoplasm of hepatocytes (Fig. 2). The appearance of this type of specific body was similar to that of the siderosome in iron-overloaded hepatocytes.19) The substructure of the specific body was composed of dark tubules of comparatively uniform diameter. Despite the lack of direct evidence of components, this substructure could be a form of platinum-binding protein (PtBP). By means of EDX, Pt and Fe were detected in this type of specific body in hepatocytes (Fig. 3). Other elements such as P, S, and Cl are intrinsic components of the cytoplasm of many types of cells, including hepatocytes. Cu peaks in Fig. 3 were due to the copper grids used in that case. Out of several theoretical peaks of Pt, at least two could be discerned: the left side of Fig. 3 includes Mα 2051 eV and the right shows Lα 9360–9441 eV. However, characteristic peaks from Pt (Mα 2051–2127 eV, Os (Mα 1910–1978 eV) and P (Kα 2014–2015 eV) could not be discerned in practice. Peaks of Fe could be identified as Kα 6390 eV and Kβ 6403 eV. The significance of Fe in the specific body remains to be investigated.

One location of metallic fine grains in the kidney was the outer surface of the basal lamella of the glomerulus.4) Convoluted15) or straight16) proximal tubules have been reported as a main site of nephropathy due to platinum compounds. In this experiment, metallic grains were dispersed in the matrix of the nucleus of many epithelial cells of the proximal tubule (Figs. 7 and 8). Finer metallic grains were found in the matrix of some microbodies of the epithelial cells (Figs. 4 and 5). They often aggregated in certain parts of the matrix (Figs. 4 and 5) and also along the limiting membrane of microbodies (Figs. 7 and 8). EDX over the aggregated fine grains revealed the presence of Pt in them (Fig. 6). On the other hand, detection of Pt on individual fine grains in the nucleus was not possible by EDX. The fine grains in the nucleus were mainly distributed in the matrix without specific association with chromatin (Fig. 8). The basal lamella of the epithelial cells of the proximal tubule appeared to be free from metallic precipitate.

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Fig. 1. The cytoplasm of a hepatocyte of a rat after successive administrations of cisplatin (0.5 mg/ml/kg body weight/day) for 5 weeks. Fine grains are localized in the microbody (arrow) and also in the cisterna of smooth-surfaced endoplasmic reticulum (arrowheads). ×41000 (without lead staining).

Fig. 2. An aggregate of tubular and fine grains found in the cytoplasm (diameter approximately 0.15 µm). This structure resembles siderosomes in iron-overloaded rat liver. The substructure could represent a type of platinum-binding protein (PtBP). ×150000.

Fig. 3. An energy-dispersive X-ray microanalytical spectrum obtained by point analysis over the substructure in Fig. 2. Pt Lα 9360–9441 eV (right arrowhead), Pt Mα 2051 (left arrowhead). P, S, and Cl are intrinsic elements of liver. Cu: copper grid. Fe Kα 3690–6405 eV, Kβ 7057 eV. 15 kV, 100 sec, 0.5 × 10^{-10} A.

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Fig. 4. A general view of the cytoplasm of an epithelial cell of a proximal convoluted tubule of the rat. Patchy precipitates of fine grains (arrowheads) are located in microbodies (MB). Golgi complex (G) and mitochondria (M) appeared to be free of such precipitates. ×51000.

Fig. 5. Aggregate of platinum grains similar to those in Fig. 4 (arrowhead), on which energy-dispersive X-ray microanalysis was performed. ×38500.

Fig. 6. EDX spectrum from the substructure of the microbody in Fig. 5. Operating conditions of the instrument were identical with those in Fig. 3. The left arrowhead indicates the peak of Pt Mα and the right one indicates Pt Lα. 100 sec, 15 kV, 0.5 × 10⁻¹⁰ A.
Fig. 7. Many microbodies are apparent in some epithelial cells of the proximal tubule. These microbodies contain metallic fine grains in the matrix and also along the limiting membrane. Metallic fine grains could also be seen in the matrix of the nucleus (N). Basal lamella (B) and mitochondria (M) appeared to be devoid of such precipitates. ×16500.

Fig. 8. A higher magnification of part of an epithelial cell of a proximal convoluted tubule. Metallic fine grains (arrowheads) are visible in the matrix of the nucleus. Microbodies (MB) contain similar fine grains while mitochondria (M), basal lamella (B) and endothelial cell (E) show little indication of platinum precipitates. ×24000.
All of the findings indicated that the main site of accumulation of the platinum compound after long-term administration is the microbodies. A large single injection of cisplatin did not clearly result in accumulation of fine grains in microbodies of the kidney or liver. However, the accumulation of the platinum complex after various doses and administration periods is being investigated in this laboratory.

**DISCUSSION**

The aim of this experiment was to identify the location of residual platinum complex after long-term administration of cisplatin to elucidate the mechanism of its nephrotoxicity. Subcellular localizations of platinum so far reported include the nucleus, microsomes and cytosol. The microbody has not previously been identified as a target site of cisplatin; this report is, to our knowledge, the first to describe the accumulation of platinum in microbodies in the liver and kidney. By using EDX, Pt was detected in aggregated fine grains in the matrix of the microbodies (Figs. 3 and 6), though individual fine grains were too small for the characteristic X-ray radiation to be detectable. EDX detection of Pt has been reported in a study of the root of water hyacinth treated with (NH₄)₂[PtCl₆]. Despite many reports on the nephrotoxicity of cisplatin, no direct attempt has previously been made to detect Pt at the ultrastructural level.

Besides the ultrastructural localization of platinum in microbodies, an interesting form of precipitation of platinum complex was a specific round body containing tubular substructure (Fig. 2). A similar form of metallic precipitates has been found in the ferritin overloaded hepatocytes of rats. In another report, 2 types of iron, i.e. small granules and large aggregates, were described; the aggregated iron was often situated in membrane-bound vacuoles. The tubular substructure found in the present work might represent platinum-binding protein (Pt-BP) rather than free platinum metal. Some investigators have reported that platinum combined with protein on entering the circulation, and only free platinum had antitumor activity. If this is so, the siderosome type of precipitate could be a residual form of platinum complex. It has been established in studies on the metabolism of cisplatin that platinum tends to combine with DNA and it is reasonable to expect precipitates in the nuclear matrix at the electron microscopic level. In fact, metallic fine grains were localized in the nucleus of epithelial cells of convoluted tubules of the nephron (Figs. 7 and 8) but the amount of precipitates was not as great as we had expected. The nucleus of hepatocytes contained few, if any, grains of platinum.

In dogs and rats, cumulative excretion of cisplatin after daily administration has been reported. After 21 days of administration to rats, 35 to 40% of cisplatin was excreted in urine but only 1% in feces. The highest tissue level of cisplatin in rats was recorded in the kidney.

Subacute toxicity tests of cisplatin in rats and dogs and clinical treatment of human patients resulted in cytotoxicity in the nephron, liver, thymus and sensory organs such as the organ of Corti. The distribution of platinum and Pt-BP in other organs such as the ovary, testis and brain at the electron microscopic level remains to be studied. In attempts to reduce the side effects of cisplatin on the nephron, liposomal encapsulation of cisplatin and simultaneous administration of cisplatin and fosfomycin are being investigated in this laboratory.

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**REFERENCES**


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