EXPERIMENTAL STUDIES ON THE PHARMACOKINETICS AND NEPHROTOXICITY OF CARBOPLATIN (CIS-DIAMMINE-1, 1-CYCLOBUTANE DICARBOXYLATE PLATINUM II) IN RATS

Toyofumi UEDA, Tetsuo YASUMASU and Jiro UOZUMI

Department of Urology, Faculty of Medicine, Kyushu University,
3-1-1 Maedashi, Higashi-ku, Fukuoka 812, Japan

Accepted June 14, 1991

Abstract.....To study the nephrotoxicity of carboplatin (cis-diammine-1, 1-cyclobutane dicarboxylate platinum II, CBDCA), an analogue of cisplatin, we examined its pharmacokinetics and functional and histopathological changes of the kidney in rats that received i.v. injection of carboplatin. Platinum concentrations in the whole plasma rapidly decreased during the first 2 hours and was undetectable at 72 hours following the carboplatin administration. Approximately 90 % of the platinum in the whole plasma was ultrafilterable during the first 30 minutes. The renal tissue concentrations of platinum rapidly decayed during the first 4 hours and then slowly declined up to 120 hours following the carboplatin administration. Platinum concentrations in the renal cortex showed higher levels than those in the renal medulla throughout the experimental period. BUN levels were within the normal range except on day 7. Serum creatinine levels remained stable and normal during the 7 days. Histopathological alterations of the renal tubules were not observed during the experimental period. These results suggest that carboplatin has less nephrotoxicity than cisplatin, because of its rapid excretion through glomerulus and less accumulation in the tubular cells.

Key words : Carboplatin, pharmacokinetics, renal function, histopathology, nephrotoxicity.

INTRODUCTION

Cisplatin demonstrates clinical activity against neoplasmas in a broad spectrum as a single agent. However, its renal toxicity presently limits the dose that can be administered (Talley et al., 1973 ; Von Hoff et al., 1979). The occurrence of its

Correspondence : Dr. Toyofumi UEDA at the above address.

dose-limiting side-effects led to a search for new platinum coordination complexes with a high therapeutic index. It is reported that carboplatin is one of the new platinum-containing analogues which have good antitumor activity and decreased nephrotoxicity (Calvert et al., 1982 ; Curt et al., 1983). Experimental studies concerning pharmacokinetics have been performed by several authors (Litterst, 1984 ; Siddik et al., 1987). However, the exact effects of carboplatin on renal function remains unknown.

To clarify whether carboplatin has a nephrotoxic potential or not, we carried out pharmacokinetic, renal functional and histopathological studies using rats.

MATERIALS AND METHODS

Animal and drug preparation : Male Sprague-Dawley rats weighing 200 to 300 g, were housed with free access to water and food throughout the course of the experiment. Carboplatin was obtained from Bristol Myers Japan (Tokyo), and was dissolved in 5 % glucose by magnetic stirring for 1–4 hours, and then used immediately.

Pharmacokinetics : Rats were given a single i. v. injection of carboplatin at a dose of 80 mg/kg, which is approximate to the 10 % lethal dose (LD₁₀) (Davidson et al., 1985), under light ether anesthesia. Rats were sacrificed at 30 minutes, 1, 2, 3, 4, 6, 8, 24, 72 or 120 hours after the injection. Blood was collected from the aorta for the determination of the platinum level in plasma. Aliquots of plasma samples were immediately centrifuged (1000×g, 15 minutes, 4 °C) using CF50A ultrafiltration cones (Amicon Ltd.) in order to determine platinum levels in the ultrafiltrate. To determine platinum levels of renal tissue, bilateral kidneys were removed after perfusion with a buffer solution containing 0.34M sucrose, 3.3mM CaCl₂ and 10mM Tris (pH 7.4). The excised kidneys were separated macroscopically into cortex and medulla. The separated renal tissues were immediately homogenized with a whirling blender and with twenty strokes in a loose Dounce homogenizer containing 9 parts volume of the same buffer solution at 0°C. Platinum levels of whole plasma, ultrafiltrated plasma and homogenates of renal tissues were measured by flameless atomic absorption spectrophotometry (HITACHI, 180–90, Tokyo, Japan). Protein in the homogenate was measured according to the method of Lowry (Lowry et al., 1951).

Renal function : Rats were given a single i. v. injection of carboplatin at a dose of 100 mg/kg, which is approximately equal to the LD₅₀ (Davidson et al., 1985), under light ether anesthesia. Control rats were given a single i. v. injection of physiological saline at a dose of 2 ml. Rats were sacrificed at 1, 3, 5 or 7 days after the treatment. Blood was obtained from the aorta in order to measure the concentration of blood urea nitrogen (BUN) and serum creatinine. BUN and serum creatinine were determined by the urease method (Chaney and Marbach, 1962) and the alkaline picrate method (Heinegard and Tiderstrom, 1973), respectively.

Renal histopathology : Following the collection of blood for the determinations of renal function, the kidneys were fixed by vascular perfusion with 2 % formaldehyde-1 % glutaldehyde in a phosphate buffer (pH 7.2) and they were then removed. Small rectangular slices of tissue, containing the inner cortex and outer medulla were trimmed and postfixed with 1 % OsO₄ in the same buffer. The samples were dehydrated and mounted in Epon. Semithin sections were cut and stained with toluidine-blue for light microscopy. Ultrathin sections were prepared for electron microscopy.

RESULTS

Platinum concentrations in plasma :

The concentrations of platinum in the whole plasma decayed biphasically ; a rapid phase ($t_{1/2}$: 39 minutes) and a slow phase ($t_{1/2}$: 9.2 hours)(Fig. 1 solid circles). After ultrafiltration of the plasma, platinum was mostly recovered in the ultrafiltrate, especially in the first 30 minutes following the administration, indicating that carboplatin was not bound to plasma proteins as reported previously (Siddik et al., 1987). Platinum concentrations in the ultrafiltrate also decayed biphasically

Fig. 1. Time course of platinum concentrations in the whole plasma(●) and in the ultrafiltrate(○) following a single i. v. injection of carboplatin. The values represent the mean and S. D. of 3 rats.

(Fig. 1 open circles). Its half-life in the rapid falling phase and in the slow decay phase were almost the same as those in the case of the whole plasma. Platinum became undetectable at 72 hours in the whole plasma and at 24 hours in the ultrafiltrate.

Platinum concentrations in renal tissue :

The concentrations of platinum in the renal cortex decayed rapidly within 4 hours and then declined slowly up to 120 hours following the carboplatin injection (Fig. 2 solid circles). Platinum concentrations in the renal medulla declined rapidly within 3 hours, but the decayed fashion was almost the same as those in the case of the renal cortex (Fig. 2 open circles). The concentrations of platinum in the renal cortex were higher than those in the renal medulla throughout the experimental period. Platinum was detectable at 120 hours in both of the renal cortex and medulla.

Fig. 2. Time course of platinum concentrations in the renal cortex(●) and medulla(○) following a single i. v. injection of carboplatin. The values represent the mean and S. D. of 3 rats.

Renal function :

BUN levels were less than 18 mg/dl and within normal range compared to control levels on day 1, 3, and 5, but were elevated to 43 mg /dl on day 7 (Fig. 3 A). Serum creatinine levels were less than 0.9 mg /dl and showed no increase compared to control levels throughout the experimental period (Fig. 3 B).

Renal histopathology :

By light microscopy, glomeruli, proximal and distal tubules in the inner cortex were intact at 1, 3, 5 and 7 days after the carboplatin injections (Photo. 1). At the same time, the third portion of the proximal tubules, which lay in the cortico-medullar junction, showed no histological damage (Photo. 2). By electron microscopy, no histological evidence of cell-organs in the third portion of the proximal tubules were found at 1, 3, 5 and 7 days after the carboplatin injections (Photo. 3).

Fig. 3. Time dependence of BUN (A) and serum creatinine (B) values in rats treated with a single i. v. injection of carboplatin. The values show the mean and S. D. of 5 rats.

Photo. 1. Light micrograph of glomeruli and tubules in the inner cortex, 72 hours following a single i. v. injection of 100 mg/kg (LD_{50}) of carboplatin. No histological evidence of glomerular and tubular damages could be found following this treatment. Toluidine blue ; $\times 120$.

Photo. 2. Light micrograph of the third portion of proximal tubules in the inner cortex, 72 hours following a single i. v. injection of 100 mg/kg (LD_{50}) of carboplatin. No pathological alterations in the tubular cell and lumen were observed following this treatment. Toluidine blue ; $\times 400$.

Photo. 3. Electron micrograph of a cell in the third portion of proximal tubules, 72 hours following a single i. v. injection of 100 mg/kg (LD₅₀) of carboplatin. Ultrastructural alterations of brush border (BB), mitochondria (Mt) and nucleus (N) could not be found in this cell. $\times 8,000$.

DISCUSSION

Cisplatin, a prototype of platinum containing antineoplastic drugs, is reported to bring about a deterioration in renal function as indicated by an elevation of BUN and serum creatinine levels (Yoshimine, 1983). In this study, we showed that carboplatin, a new platinum containing compound, caused no increase in serum creatinine even at the intravenous dose of 100 mg/kg. BUN values also remained within normal range, but increased on day 7. These results suggest that carboplatin has less toxicity on renal function when compared to cisplatin. However, the elevation of BUN on day 7 after the carboplatin administration indicates that the further examination concerning the long-term toxic effect of carboplatin on renal function is recommended.

Morphological nephrotoxicity of cisplatin is characterized by the necrotizing lesions in the proximal tubules of the inner cortex and outer medulla. The affected tubules represent the straight portion, or so-called third segment of the proximal tubules (Ward and Fauvie, 1976). In consistence with this report, our previous electron microscopic study (Ueda et al., 1986) has showed that cisplatin produced morphological alterations, consisting of nucleolar segregation and ribosome dispersion at 12 hours, and denuded brush border with condensed mitochondria and dispersed heterochromatin in the nucleus at 72 hours, in the cells of the third portion of the proximal tubules following the cisplatin treatment. In this study, we showed that

carboplatin did not produce histopathological changes in proximal tubular cells at all by light or electron microscopy during the 7 days after the carboplatin injections.

From the pharmacokinetic characteristics of carboplatin obtained in this study, we discuss the difference in nephrotoxicity between cisplatin and carboplatin. Cisplatin is known to be rapidly bound to plasma proteins. The unbound cisplatin in plasma comprises 25 % at 45 minutes and 0 % at 1 hour after the intravenous injections in rats (Siddik et al., 1987). On the contrary, the unbound fraction of carboplatin showed approximately 90 % during the first 30 minutes and became undetectable at 24 hours following its injection. These results indicate that the major part of carboplatin is present in plasma as a form of protein-unbound platinum and is rapidly filtrated through the glomerulus in the early period after the injection. The renal clearance of carboplatin is similar to that of inulin (Siddik et al., 1987), suggesting that carboplatin is mainly excreted by glomerular filtration and there is no tubular absorption or secretion of the drug. However, cisplatin is excreted not only by glomerular filtration, but also by renal tubular secretion through an organic ion transport system (Jacobs et al., 1980). Concerning the concentrations of platinum derived from cisplatin or carboplatin in the renal tissue, it is reported that both drugs show similar renal level of platinum at 1 and 14 days after the injections despite the 10- to 12- fold difference in the dose administered (Siddik et al., 1986). It is speculated that renal platinum levels may not correlate with the differential nephrotoxicity of the two compounds. In this study, the concentrations of platinum in the renal cortex and medulla were similarly detected 5 days after the carboplatin injections, but functional and morphological nephrotoxicity were not observed. It has already been reported that the protein-bound form of platinum in plasma shows no renal toxicity (Cole and Walf, 1981). Moreover, it is suggested that the high localization of cellular platinum in the cytosol and the presence of a high proportion as non-protein bound species may be important factors in relation to the therapeutic as well as the toxic effects of cisplatin (Sharma and Edwards, 1983). Thus, it may be speculated that despite the presence of a great quantity of the protein-unbound platinum in plasma, carboplatin will be unlikely to contribute to the severe renal toxicity, because of less tubular absorption and secretion in the course of its rapid excretion from the kidney. In summary, it is suggested that the difference in nephrotoxicity between cisplatin and carboplatin may relate to the different mechanism of excretion from the kidney during the early period after the administration of both drugs.

REFERENCES

- Calvert, A. H., Harland, S. J., Newell, D. R., et al. (1982) : Early clinical studies with cis-diammine-1, 1-cyclobutane dicarboxylate platinum II. *Cancer Chemother, Pharmacol.*, **9**, 140-147.
- Chaney, A. L. and Marbach, E. P. (1962) : Modified reagents for determination of

- urea and ammonia. Clin. Chem., **8**, 130–132.
- Cole, W. C. and Wolf, W. (1981) : Renal toxicity studies of protein-bound platinum (CIS). Chem. Biol. Interactions, **35**, 341–348.
- Curt, G. A., Grygiel, J. J., Corden, B. J., et al. (1983) : A phase I and pharmacokinetic study of diammine-cyclobutane dicarboxylatoplatinum (NSC 241240). Cancer Res., **43**, 4470–4473.
- Davidson, T. J., Madissoon, H. and Hoffendorf, G. H. (1985) : Carboplatin (JM-8) : Summary of toxicological studies. p. 9, unpublished report No. MADI/H/20086, Bristol Myers Company, PF & DD, Syracuse, New York.
- Heinegard, D. and Tiderstrom, G. (1973) : Determination of serum creatinine by a direct colorimetric method. Clin. Chim. Acta, **43**, 305–310.
- Jacobs, C., Kalmlman, S. M., Tretton, M., et al. (1980) : Renal handling of cis-dichloroplatinum (II). Cancer Treat. Rep., **64**, 1223–1226.
- Litterst, C. L. (1984) : Plasma pharmacokinetics, urinary excretion, and tissue distribution of platinum following i.v. administration of cyclobutane-dicarboxylatoplatinum-II (CBDCA) and cis-platinum to rabbits. In Platinum Coordination Complexes in Cancer Chemotherapy (M. P. Hacker., E. B. Douple, and I. H. Krakoff, eds.). p. 71–81, Martinus Nijhoff, Boston.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L., et al. (1951) : Protein measurement with the folin phenol reagent. J. Biol. Chem., **193**, 265–275.
- Sharma, R. P. and Edwards, I. R. (1983) : Cis-platinum : Subcellular distribution and binding to cytosolic ligands. Biochem. Pharmac., **32**, 2665–2669.
- Siddik, Z. H., Dible, S. E., Boxall, F. E., et al. (1986) : Renal pharmacokinetics and toxicity of cisplatin and carboplatin in animals. In Biochemical Mechanisms of Platinum Antitumor Drugs (D. C. H. Mc Brien, and T. F. Slater, eds.). p. 171–198, IRL Press, Oxford.
- Siddik, Z. H., Newell, D. R., Boxall, F. E., et al. (1987) : The comparative pharmacokinetics of carboplatin and cisplatin in mice and rats. Biochem. Pharmac., **36**, 1925–1932.
- Talley, R. W., O'Bryan, R. M., Gutterman, J. U., et al. (1973) : Clinical evaluation of toxic effects of cis-diamminedichloroplatinum (NSC-119875)-phase I clinical study. Cancer Chemother. Rep., **57**, 465–471.
- Ueda, T., Mihara, Y. and Yoshimine, K. (1986) : Mechanism of cis-platinum nephrotoxicity. Jpn. J. Nephrol., **28**, 940–942.
- Von Hoff, D. D., Schilsky, R., Reichert, C. M., et al. (1979) : Toxic effects of cis-dichloro-diammine platinum (II) in man. Cancer Treat. Rep., **63**, 1527–1531.
- Ward, J. M. and Fauvie, K. A. (1976) : The nephrotoxic effects of cis-diammine-dichloroplatinum (II). Toxicol. Appl. Pharmacol., **38**, 535–537.
- Yoshimine, K. (1983) : Experimental study of nephrotoxicity after administration of cis-diamminedichloroplatinum (II). Nishinohon J. Urol., **45**, 511–525.