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安増, 哲生

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Comparative Study of Cisplatin and Carboplatin on Pharmacokinetics, Nephrotoxicity and Effect on Renal Nuclear DNA Synthesis in Rats

Tetsuo Yasumasu, Toyofumi Ueda, Jiro Uozumi, Yukitaka Mihara and Joichi Kumazawa

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

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Abstract: To clarify the difference in nephrotoxicity between cisplatin and carboplatin, the pharmacokinetics of platinum, renal function and nuclear DNA synthesis in renal cortical and outer medullary cells were studied in rats which had received cisplatin or carboplatin. Male Sprague-Dawley rats were given either cisplatin or carboplatin intravenously at an equi-toxic dose (LD_{10} or LD_{50}) and were killed at various times within 7 days after the injection. Cisplatin bound to plasma proteins more avidly than carboplatin. Much more platinum was detectable in the renal nuclei after cisplatin injection than after carboplatin injection. BUN and serum creatinine levels in the rats treated with 8.5 mg/kg of cisplatin were significantly higher than in those treated with 100 mg/kg of carboplatin. Cisplatin markedly suppressed the renal nuclear DNA synthesis both *in vivo* and *in vitro*, when compared with carboplatin. It is concluded that the differences in nephrotoxicity between cisplatin and carboplatin are related to their different inhibitory effects on nuclear DNA synthesis in the renal cells.

Cisplatin is a widely used and highly effective antitumour agent. However, its nephrotoxicity is one of its major side effect (Weiner & Jacobs 1983; Jones *et al.* 1985). Carboplatin is a new platinum-containing analogue, with good antitumour activity and decreased nephrotoxicity (Lelieveld *et al.* 1980; Calvert *et al.* 1982; Curt *et al.* 1983; Boven *et al.* 1985; Smith & Brock 1988). Cisplatin-induced acute renal failure has been extensively studied (Weiner & Jacobs 1983; Daley-Yates & McBrien 1985; Jones *et al.* 1985; Siddik *et al.* 1986 & 1987). Pathological alterations following cisplatin are localized to the S_3 segment of the proximal tubule (Weiner & Jacobs 1983; Jones *et al.* 1985) and the first changes in S_3 are nucleolar segregation and ribosome dispersion (Jones *et al.* 1985). It is generally accepted that DNA is the target responsible for the cytotoxic action of platinum compounds (Zwelling & Kohn 1980; Roberts *et al.* 1986; Hanušovská & Ujházy 1987). These findings suggest that the effect of cisplatin on renal cell nuclear function is related to tubular cell injury. In the present study, the differences in pharmacokinetics between cisplatin and carboplatin and their effects on the renal nuclei were examined in order to investigate the mechanism of cisplatin-induced nephrotoxicity.

Materials and Methods

Drugs. Cisplatin (Nippon Kayaku, Tokyo, Japan) was freshly dissolved in saline and carboplatin (Bristol Myers Japan, Tokyo, Japan) in 5% glucose prior to use.

Animals. Male Sprague-Dawley rats weighing 200 to 300 g, were housed with free access to water and rat chow throughout the experiment.

Platinum concentrations. The experimental animals received either cisplatin at a single dose of 6.5 mg/kg or carboplatin 80 mg/kg intravenously under light ether anaesthesia. Each dose was an equitoxic dose and approximate to LD_{10}. These doses were determined at Nippon Kayaku Laboratory (published in a Japanese journal) and Bristol Myers Laboratory (Davidson *et al.* 1985). The treated animals were killed at various times within 7 days after the injection under light ether anaesthesia. Blood was drawn from the aorta to obtain plasma samples for the determination of the level of platinum. Aliquots of plasma samples were immediately centrifuged (1,000 × g, 15 min., 4) using CF50A ultrafiltration cones (Amicon Ltd.) for the determination of ultrafilterable platinum levels. To determine platinum levels, renal tissue and renal nuclei were prepared as follows. Both kidneys were removed after perfusion with a buffer solution containing 0.34 M sucrose, 3.3 mM $CaCl_2$ and 10 mM Tris (pH 7.4). One kidney was separated macroscopically into cortex and medulla. The separated renal tissues were immediately homogenized with a wharing blender and with twenty strokes in a loose Dounce homogenizer containing 9 parts volume of buffer solution (described above) at 0 . The other kidney was carefully trimmed, and cortical and outer medullary tissue was removed. The removed renal cortical and outer medullary tissues were immediately homogenized in the same manner, and the homogenate was passed through four sheets of gauze, and the filtrate was centrifuged at 700 × g for 10 min. The supernatant fluid was discarded and the pellet was resuspended in 20 ml of 2 M sucrose, 1 mM $CaCl_2$ and 10 mM Tris buffer with five strokes in a loose Dounce homogenizer. The suspension was then laid over 20 ml of 2.2 M sucrose, 10 mM $MgCl_2$ and 10 mM Tris buffer and the nuclei were sedimented by centrifugation at 68,420 × g for one hour. Finally, the nuclei were suspended in 1 ml of 0.3 M sucrose. Platinum levels of whole plasma, ultrafiltrated plasma, homogenates and renal nuclei were determined by flameless atomic absorption spectrophotometry (HITACHI, 180-70, Tokyo, Japan). Protein in the homogenate was determined according to the method of Lowry *et al.* (1951) and the DNA content in the nuclei was measured by diphenylamine according to the method of Burton (1955).

Assessment of renal function. Renal function was assessed by blood urea nitrogen (BUN) and serum creatinine levels. The animals received either cisplatin at a dose of 8.5 mg/kg or carboplatin 100 mg/kg intravenously. The doses were approximately equal to LD_{50} to distinguish the difference in nephrotoxicity. Control animals

received 2 ml saline. The rats were killed at 1, 3, 5 or 7 days after treatment. Blood was drawn from the aorta to determine BUN and serum creatinine. BUN and serum creatinine were determined by the urease method (Chaney & Marbach 1962) and the alkaline picrate method (Heinegard & Tiderstrom 1973) respectively.

Effect on nuclear DNA synthesis. Effects of cisplatin and carboplatin on nuclear DNA synthesis *in vivo* were studied according to the method of Lynch *et al.* (1970). Rats were injected intravenously with either cisplatin at a dose of 8.5 mg/kg or carboplatin 100 mg/kg, and killed after 8, 12, 24 or 72 hr in order to prepare the fraction of the renal nuclei as described earlier. Isolated nuclei were incubated with 0.016 mM ^{3}H -dTTP (^{3}H-deoxythymidine-triphosphate) in reaction mixtures (0.5 ml) containing 0.1 M Tris-HCl (pH 7.4), 4 mM KCl, 16 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 2 mM ATP, 0.08 mM dGTP, 0.08 mM dCTP and 0.08 mM dATP at 37° for 30 min. Reactions were stopped with 1 ml of 1 M NaOH and DNA was precipitated with 5 ml of ice-cold 10% trichloracetic acid. The DNA was washed three times in 1 ml of 1 M NaOH. The radioactivity was measured in the liquid scintillation mixture to determine ^{3}H-dTTP uptake. The effects of cisplatin and carboplatin on nuclear DNA synthesis *in vitro* were studied as follows. The renal nuclei isolated from untreated rats were incubated with 5, 10 or 25 μM of cisplatin or carboplatin at 37° for 1 hr, and then re-incubated with ^{3}H-dTTP in reaction mixtures for 30 min. DNA synthesis was measured by the method previously described. Results are shown as a percent of ^{3}H-dTTP uptake by control nuclei obtained from untreated rats.

Statistical analysis. The values are given as means ± S.D. Statistical differences were evaluated by Student's *t*-test. A significance level of $P < 0.05$ was accepted.

Results

Platinum concentrations.

Plasma concentrations of total platinum after cisplatin or carboplatin injection decayed biphasically with a rapid initial phase and a prolonged second phase (fig. 1). The initial and second half-life were 38 min. and 12.1 hr for cisplatin, 39 min. and 9.2 hr for carboplatin. Platinum in the whole plasma was detectable for up to 3 days after cisplatin injection but only up to 24 hr after carboplatin injection. Approximately 30% and 90% of total platinum were ultrafilterable for the first 30 min. following cisplatin and carboplatin,

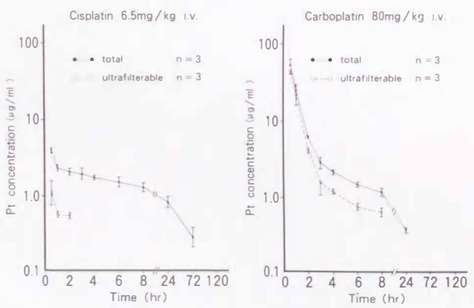

Fig. 1. Plasma concentrations of total (●) and ultrafilterable (○) platinum in the rat at various times up to 5 days after cisplatin or carboplatin injection. Mean ± S.D.

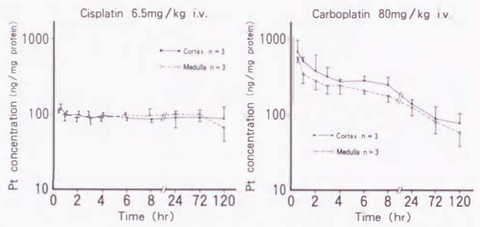

Fig. 2. Renal cortex (●) and medulla (○) concentrations of platinum in the rat at various times up to 5 days after cisplatin or carboplatin injection. Mean ± S.D.

respectively. The proportion of ultrafilterable platinum in total platinum after carboplatin injection decreased slowly and accounted for 53% at 8 hr. Ultrafilterable platinum was detectable for only up to 2 hr after cisplatin, but 8 hr after carboplatin injection.

Renal tissue concentrations of platinum after cisplatin injection were stable up to day 5 (fig. 2). The concentrations following carboplatin injection decreased during this period. Platinum in the kidney was detectable 5 days after both cisplatin and carboplatin injection, and platinum levels in the cortex were almost the same as in the medulla.

Renal cortical and outer medullary nuclear concentrations of platinum (fig. 3) for the first 4 hr following cisplatin injection were not different from those following carboplatin injection. However, 8 to 72 hr after the injection, renal nuclear levels of platinum were significantly higher after cisplatin injection than after carboplatin injection ($P < 0.05$).

Assessment of renal function.

BUN levels of cisplatin treated rats began to rise on day 3 and continuously rose up to day 7. Those of carboplatin

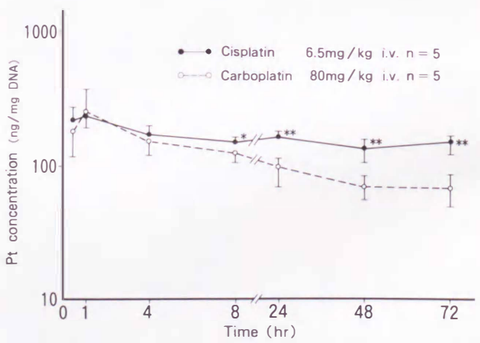

Fig. 3. Renal cortical and outer medullary nuclear concentrations of platinum in the rat at various times up to 72 hr after cisplatin (●) or carboplatin (○) injection. Mean ± S.D. Asterisks indicate significant differences between cisplatin and carboplatin with a P value of < 0.05 (*) or < 0.01 (**).

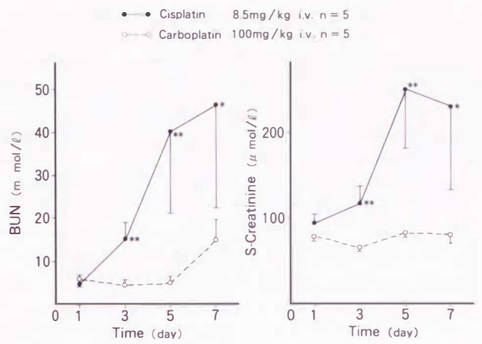

Fig. 4. Changes in BUN and serum creatinine levels up to 7 days after cisplatin (●) or carboplatin (○) injection. Mean ± S.D. Asterisks indicate significant differences between cisplatin and carboplatin with a P value of < 0.05 (*) or < 0.01 (**).

treated rats were almost stable, except on day 7. Serum creatinine levels of cisplatin treated rats were elevated with a peak on day 5. Those of carboplatin treated rats were within the normal range throughout the experimental period. There were significant differences in BUN and creatinine levels between cisplatin and carboplatin treated rats on days 3, 5 and 7 ($P < 0.05$). The results are shown in fig. 4.

Effect on nuclear DNA synthesis.

The synthesis of nuclear DNA in the renal cortical and outer medullary cells was suppressed at 8, 12 and 24 hr but was accelerated at 72 hr after cisplatin injection. Carboplatin did not suppress DNA synthesis at any time after the injection. There were significant differences between cisplatin and carboplatin at 12 and 72 hr after the injection. The results are shown in fig 5a.

DNA synthesis by renal cortical and outer medullary

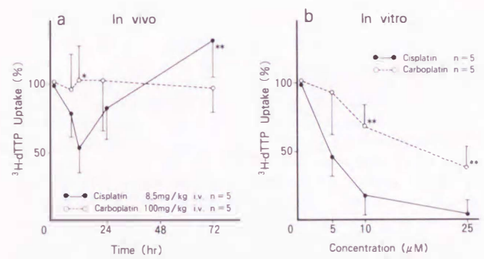

Fig. 5a. ^{3}H-dTTP uptake in isolated renal cortical and outer medullary nuclei in the rats after cisplatin (●) or carboplatin (○) injection *in vivo*. b. ^{3}H-dTTP uptake in isolated nuclei after incubation for 1 hr with cisplatin (●) or carboplatin (○) *in vitro*. Mean ± S.D. Asterisks indicate significant differences between cisplatin and carboplatin with a P value of < 0.05 (*) or < 0.01 (**).

nuclei isolated from untreated rats was markedly suppressed after incubation with 10 and 25 μM of cisplatin *in vitro*. Carboplatin also suppressed DNA synthesis *in vitro*, but to a smaller extent than cisplatin. There were significant differences between cisplatin and carboplatin at both concentration ($P < 0.01$). The results are shown in fig. 5b.

Discussion

First of all a preliminary experiment was performed to choose the doses to be used. Five rats were injected intravenously with either cisplatin or carboplatin at a single dose of the LD_{50}, respectively. Renal dysfunction and weight loss were observed in all cisplatin treated rats and alimentary tract bleeding was observed in all carboplatin treated rats at 7 days after the injection. One of the cisplatin treated rats died at 7 days, but no death were observed in carboplatin treated rats during 7 days. Based on these results, LD_{10} was chosen in the pharmacokinetic study to avoid the influence of renal dysfunction and gastrointestinal bleeding after drug injection. LD_{50} was chosen in the toxic study to distinguish the differences in toxicity.

It is well-known that cisplatin produces acute tubular necrosis in the S_3 segment of the proximal tubule (Weiner & Jacobs 1983; Jones *et al.* 1985). On the other hand, carboplatin produces little or no kidney damage (Lelieveld *et al.* 1980).

Foster *et al.* (1990) reported that human plasma concentrations of total platinum following cisplatin injection decreased biexponentially or triexponentially. Lázničková *et al.* (1986) and Litterst & Magin (1988) reported that the plasma concentrations of total platinum following cisplatin or carboplatin injection declined triexponentially and the α phases of both drugs were of very short duration for the first 10-15 min. in rats. The half-lives of total platinum for α, β and γ phases in rats were 1.4–3.0 min., 10–16 min. and 40 hr for cisplatin and 2.0–6.5 min., 25–41 min. and 51 hr for carboplatin, respectively (Lázničková *et al.* 1986; Siddik *et al.* 1987; Litterst & Magin 1988). In our study, plasma concentrations of total platinum after cisplatin and carboplatin injection decreased biphasically. This may be due to the failure to detect an α phase, because the platinum levels for very early phases were not determined. The $t_{1/2}$ β of 38 min. for cisplatin was somewhat longer and the $t_{1/2}$ γ of 12.1 hr for cisplatin and 9.2 for carboplatin, were shorter than in previous reports (Lázničková *et al.* 1986; Siddik *et al.* 1987; Litterst & Magin 1988). These results may be due to the small number of the plots of the plasma levels.

Cisplatin is known to be rapidly bound to plasma proteins. The unbound platinum in total plasma platinum after cisplatin injection accounted for 25% at 45 min. and 0% at one hour after the injection in rats (Siddik *et al.* 1987). Carboplatin is considered to be slowly bound to plasma proteins (Hariand *et al.* 1984; van Echo *et al.* 1984; Lázničková *et al.* 1986; Siddik *et al.* 1987). In our study, the proportion of ultrafilterable platinum in total plasma platinum after carboplatin injection was greater than that

of cisplatin. The unbound fraction of carboplatin accounted for 90% during the first 30 min. and 53% at 8 hr after the injection. These results indicate that the major part of carboplatin is present in the plasma as a form of protein unbound platinum for the first 8 hr. Eighty–90% of the carboplatin dose was excreted in the urine within 4 hr mainly as unchanged compound (Siddik *et al.* 1987). On the other hand, urinary excretion of cisplatin during the first 4 hr was much lower than that of carboplatin (Siddik *et al.* 1987). This difference in urinary excretion seems to be due to the difference in binding to plasma proteins.

The renal clearance of carboplatin seems to be similar to that of inulin (Siddik *et al.* 1986 & 1987), suggesting that carboplatin is mainly excreted by glomerular filtration. On the other hand, cisplatin is considered to be excreted not only by glomerular filtration but also by renal tubular secretion through the organic ion transport system (Williams & Hottendorf 1985). Probenecid and cimetidine are actively secreted through the organic ion transport system and reduce the nephrotoxicity of cisplatin as a competitive antagonist for the secretion of cisplatin on renal tubular level (Ross & Gale 1979; Sleijfer *et al.* 1987). These previous reports suggest that the differences in tubular secretion between cisplatin and carboplatin might be related to the greater nephrotoxicity of cisplatin compared to carboplatin.

Siddik *et al.* (1986) investigated the concentration of platinum in kidneys of rats receiving an equitoxic dose of cisplatin and carboplatin and reported that cisplatin and carboplatin produced similar renal tissue levels of platinum, 1 day and 14 days after the injection, despite the ten-fold difference in the doses utilized. They also speculated that renal platinum levels did not correlate with the differential nephrotoxicities of the two compounds. In our study, renal tissue levels of platinum after cisplatin injection were stable for the first 8 hr. The renal tissue level of platinum after carboplatin injection was about 3 times higher than that of cisplatin, 2 hr after the injection. However, renal platinum levels after carboplatin injection declined rapidly and showed almost the same values as those of cisplatin, 3 to 5 days after the injection. The cause of such differential pharmacokinetics may be due to differences in the irreversible protein binding in tissue and our results agree with the previous report (Siddik *et al.* 1986) indicating that renal tissue platinum levels have no relation to nephrotoxicity. The subcellular distribution of cisplatin and carboplatin in the kidneys was investigated (Weiner & Jacobs 1983; Curt *et al.* 1983). The mitochondrial and the cytosolic fractions had the highest concentrations of both cisplatin and carboplatin and the distribution of both drugs to the nuclei were similar in the rats (Curt *et al.* 1983). Our results indicate that renal nuclear levels of platinum, 8 to 72 hr after cisplatin injection were significantly higher than after carboplatin injection. This result suggests that cisplatin platinum may be able to enter the renal nuclei more easily than carboplatin platinum, considering that the injected carboplatin resulted in a ten-fold dose of platinum compared to cisplatin.

Zwelling & Kohn (1980) clearly showed that the target of cisplatin was nuclear DNA. However, little information concerning the *in vivo* effect of cisplatin on DNA function is available (Roberts *et al.* 1986; Hanušovská & Ujházy 1987). Based on previous studies and our pharmacokinetic findings, we examined the effect of each drug on the synthesis of nuclear DNA in renal cells. Cisplatin suppressed the synthesis of nuclear DNA *in vivo* at 8, 12 and 24 hr after the injection, but DNA synthesis was accelerated at 72 hr after the injection. DNA synthesis by nuclei isolated from untreated rats was markedly suppressed after incubation with 10 or 25 μM of cisplatin *in vitro*. These results indicate that cisplatin affects renal cell nuclear function. The acceleration of synthesis at 72 hr might be caused by renal tubular regeneration, because regenerative cells which had clear nuclei and autophagocytic vacuoles were observed at 72 hr after the injection (data not shown). Carboplatin did not suppress the synthesis of nuclear DNA *in vivo*, however suppressed it *in vitro*, but to a smaller extent than cisplatin. These findings support previous results showing that high-dose carboplatin can produce severe nephrotoxicity (Curt *et al.* 1983; Siddik *et al.* 1986). The results suggest that differential nephrotoxicity between cisplatin and carboplatin reflects the difference in the inhibitory effect on nuclear DNA synthesis in renal cells.

In conclusion, DNA is the target responsible for the cytotoxic action of platinum compounds (Zwelling & Kohn 1980; Roberts *et al.* 1986; Hanušovská & Ujházy 1987). There may be a similar nuclear component to the mechanism of S_3 tubular cell injury. The difference in nephrotoxicity between cisplatin and carboplatin seems to be related to the difference in the inhibitory effect on nuclear DNA synthesis. The effect of cisplatin on nuclei in renal cells plays an important role in its nephrotoxicity.

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