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

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

Iwao Yamanaka, Isaoishii Ideg, Jiro Ozumi, Yoshiaki Hidaka and Jichi Kumaizama

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

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Abstract: To clarify the differences 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 equitoxic dose (LD50) and were killed at varying 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. Blood and serum creatinine levels in the rats treated with 2.5 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, whereas 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 antitumor agent. However, its nephrotoxicity is one of its major side effects (Weiner & Jacobs 1983, Jones et al. 1985). Carboplatin is a new platinum-containing analogue, with good antitumor activity and decreased nephrotoxicity (Lelieveld et al. 1991). It has been extensively studied (Weiner et al. 1987). We have previously shown that carboplatin is a new platinum-containing analogue, with good antitumor activity and decreased nephrotoxicity (Lelieveld et al. 1991). It has been extensively studied (Weiner et al. 1987).

Materials and Methods

Cisplatin (Nippon Kayaku, Tokyo, Japan) was freshly dissolved in saline and carboplatin (Bristol-Myers Japan, Tokyo, Japan) in 5% glucose prior to use. Male Sprague-Dawley rats weighing 200 to 300 g were housed with free access to water and rat chow throughout the experiment. Animals were killed under light ether anesthesia and the kidneys were removed. The renal cortical and outer medullary tissues were immediately prepared as follows. Both kidneys were removed after perfusion with 0.9% saline, and the cortex and outer medulla were homogenized with a Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 6,000 rpm for 10 min. The supernatant fluid was discarded and the pellet was re-suspended in 20 ml of 0.5 M sucrose, 1 mM CaCl2, and 10 mM Tris-HCl buffer (pH 7.4). One kidney was further homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 20,000 rpm for 20 min. The supernatant fluid was discarded and the pellet was re-suspended in 1 ml of 0.5 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). The DNA content in the nuclei was measured by diphenylamine spectrophotometry (Hill & Tachi, 1981). The DNA content in the nuclei was measured by diphenylamine spectrophotometry.

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 2.5 mg/kg or carboplatin 100 mg/kg intravenously under light ether anesthesia. Each dose was an equitoxic dose and approximate to LD50. These doses were determined by Nippon Kayaku Laboratory (published in a Japanese journal) and Bristol-Myers Laboratory (Doseyma et al. 1985). The treated animals were killed at varying times within 7 days after the injection under light ether anesthesia. 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.). 1 mg CFS-50K ultrafiltration cartridges (Amicon Co.) 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 0.5 M sucrose, 1 mM CaCl2, and 10 mM Tris-HCl buffer (pH 7.4). One kidney was further homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 20,000 rpm for 20 min. The supernatant fluid was discarded and the pellet was re-suspended in 1 ml of 0.5 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 spectrophotometry according to the method of Burton (1955).
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 (Chew & Marchuk 1982) and the alkaline
urate method (Henegar & Tellervik 1973) respectively.

Effect on nuclear DNA synthesis. Effect of cisplatin and carboplatin on nuclear DNA synthesis in vivo were studied according to the method of Littler et al. (1986). Rats were injected intravenously with either cisplatin at a dose of 5.5 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 H-dTTP (5'-d-thyminde-3'-1H-thymid- 
ole-triphosphate) in reaction mixtures (0.5 ml) containing 0.1 M Tris-
HCl (pH 7.4), 4 mM KCl, 4 mM MgCl2, 2 mM 3-mercaptoethanol, 2 mM ATP, 0.08 mM GTP, 0.06 mM dCTP, and 0.01 mM dATP at 37 (or 30) min. Reactions were stopped with 1 ml of 1 M NaOH and DNA was precipitated with 5 ml of saturated (30%) trichloroacetic 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 H-dTTP uptake. The effect of cisplatin and carboplatin on nuclear DNA synthesis in vivo was studied as follows: The rats were injected intravenously with either cisplatin at a dose of 5.5, 10, 25 or 50 mg/kg or carboplatin at 50 mg/kg for 3 hr, and then reincubated with H-dTTP in reaction mixtures for 30 min. DNA synthesis was measured by the procedure previously described. Results are shown as a percent of H-dTTP uptake by control nuclei obtained from untreated rats.

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

Results

Platinum concentrations. Plasma concentration of total platinum after cisplatin or carboplatin injection decayed biexponentially with a rapid ini-
tial 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 injec-
tion but only up to 24 hr after carboplatin injection. Appro-

Fig. 1. Plasma concentrations of total (■) and ultrafilterable (□) platinum in the rat at various times up to 3 days after cisplatin or carboplatin injection. Mean ± S.D.

approximately 30% and 90% of total platinum were ultrafilter-
able for the first 30 min following cisplatin and carboplatin,

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 the first 5 days. 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 following cisplatin injection were not different from those following carboplatin injection. However, at 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 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 stable within the normal range throughout the experimental period. There were significant differences in BUN and crea-
tinine 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 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 nuclei isolated from untreated rats was markedly suppressed after incubation with 10 and 25 µM of cisplatin or carboplatin. Carboplatin also suppressed DNA synthesis in vitro, but to a smaller extent than cisplatin. There were significant differences between cisplatin and carboplatin at both con-
centrations (P < 0.01). The results are shown in fig. 5b.

Discussion

First of all a preliminary experiment was performed to choose the dose to be used. Five rats were injected intra-
venously with either cisplatin or carboplatin at a single dose of the LD100 respectively. Renal dysfunction and weight loss were observed in all cisplatin treated rats and alimentary tract bleeding was observed in all carboplatin treated rats 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, LD100, chosen in the pharmacokinetic study to avoid the influ-
ence of renal dysfunction and gastrointestinal bleeding after drug injection, was shown in the toxic study to dis-
guish the differences in toxicity.

It is well-known that cisplatin produces acute tubular necrosis in the S5 segment of the proximal tubule (Weiner & Jacobs 1985). Jones et al. (1985). On the other hand, carboplatin produces little or no kidney damage (Lelieveld et al. 1986). Fager et al. (1990) reported that human plasma concentra-
tions of total platinum following cisplatin injection decreased exponentially or triexponentially. Littler et al. (1986) and Littler & Magin (1980) reported that the plasma concentrations of total platinum following cisplatin or carboplatin injection decreased triexponentially. Phases of both drugs were of very short duration for the first 10–15 min in rats. The half-lives of total platinum for cisplatin and carboplatin were 1.30 ± 0.06 and 1.20 ± 0.06 hr respectively. Carboxplatin concentrations of total platinum after cisplatin and carboplatin injection decreased biphasically. This may be due to the failure to detect an phase, because the platinum levels for very early phases were not determined. The 1.30 ± 0.06 hr of cisplatin was somewhat longer than the 1.20 ± 0.06 hr of carboplatin and 9.2 ± 0.4 hr for carboplatin, were shorter than in previous reports (Littler et al. 1986; Siddik et al. 1987; Littler & 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 pro-
teins. The unbound platinum in total plasma after cisplatin injection accounted for 25% at 48 min and 0% at one hour after the injection in rats (Siddik et al. 1987). Carboplatin is considered to be slowly bound to plasma protein (Harald et al. 1984; van Balfo et al. 1984; Littler et al. 1986; Siddik et al. 1987). In our study, the proportion of ultrafilterable platinum in total platinum after carboplatin injection was greater than that
of cisplatin was nuclear DNA. However, little information concerning the in vivo effect of cisplatin on DNA function is available (Roberts et al. 1986; Hanuschova & Ujhazy 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 12 and 24 hr after the injection, but DNA synthesis was accelerated at 24 hr after the injection. DNA synthesis by nuclei isolated from untreated rats was markedly suppressed after incubation with 10 or 25 μg/mL 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 euchromatine vacuoles were observed at 72 hr after the injection (data not shown). Cisplatin 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 cisplatin can produce severe nephrotoxicity (Curter 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 & Kohl 1980). Roberts et al. (1986) and Hanuschova & Ujhazy (1987) studied the effect of each drug on DNA synthesis. There may be a similar nuclear component to the mechanism of S nephron cell injury. The difference in nephrotoxicity between cisplatin and carboplatin is related to the difference in the inhibitory effect on nuclear DNA synthesis. The effect of cisplatin on renal cells plays an important role in its nephrotoxicity.

References