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Pharmacokinetics and Toxicodynamics of Cisplatin and Its Metabolites in Rats: Relationship between Renal Handling and Nephrotoxicity of Cisplatin

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Abstract

The renal handling of cisplatin and its metabolites and the relationship between the pharmacokinetics of these platinum species in the kidney and nephrotoxicity in rats were studied by carrying out pharmacokinetic–pharmacodynamic analysis.

Rats received cisplatin intravenously as a bolus (2–10 mg kg^{-1}) or by constant infusion (55 and 140 $\mu g\ min^{-1}\ kg^{-1}$). After intravenous administration of each platinum species, the platinum concentrations of unchanged cisplatin and its mobile and fixed metabolites were determined separately. Nephrotoxicity was estimated by measuring the blood urea nitrogen (BUN) levels and the sigmoid Emax model was used to determine the relationship between pharmacokinetic parameters and BUN levels 5 days after cisplatin administration.

Cisplatin and its mobile metabolites in plasma distributed more rapidly and extensively into the kidney (mean apparent kidney-to-plasma concentration ratios were 2·69 and 7·12 mL (g tissue)$^{-1}$, respectively) than into the liver (less than 1 mL (g tissue)$^{-1}$). Concomitant administration of mobile metabolites did not significantly alter the disposition of cisplatin. Nephrotoxicity, estimated by measuring BUN levels, appeared to be related to the plasma concentration of intact cisplatin, not total platinum, because mobile metabolites formed from cisplatin showed little nephrotoxicity. The sigmoid Emax model showed the maximum BUN level reached after cisplatin administration was related to the area under the renal cisplatin concentration–time curve (AUC_k).

Cisplatin (*cis*-diamminedichloro-platinum (II), cisplatin) is an effective anti-neoplastic agent that has been used to treat various types of solid tumour. However, it is known to cause several side effects and cisplatin therapy is particularly restricted by severe nephrotoxicity. Cisplatin induces marked focal necrosis in the proximal and distal tubules and the damage is localized mainly in the region of the S3 portion of the proximal tubule (Singh 1989).

Cisplatin undergoes ligand exchange reactions, which are virtually irreversible (Daley-Yates & McBrien 1984; Nagai et al 1996). In biological fluids, cisplatin is transformed immediately to aquated cisplatin as a result of release of the chloride ion and equilibrium between cisplatin and its aquated form is maintained. Aquated and unchanged cisplatin also react readily with nucleophiles (Farris et al 1985; Dedon & Borch 1987). Cisplatin is biotransformed through binding to low-molecular-mass substances (such as glutathione, methionine and cysteine) and to high-molecular-mass substances (such as albumin and nucleotides) and the resulting metabolites are known as mobile and fixed metabolites, respectively (Farris et al 1985, 1988). Many investigators have studied the pharmacokinetics of cisplatin by measuring either total or filtered platinum (including cisplatin and its mobile metabolites) concentrations. To monitor the efficacy and safety of drug treatment, it is important to determine the concentration(s) of the active species.

When cisplatin and sodium thiosulphate are administered concomitantly, the nephrotoxicity estimated by blood urea nitrogen (BUN) is ameliorated. In this situation, the plasma filtered platinum (cisplatin + mobile metabolites) concentration changes little, but the plasma cisplatin concentration decreases dramatically, because cisplatin binds

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strongly to sodium thiosulphate (Nagai et al 1995). This suggests that it is important to separate the platinum species into unchanged cisplatin and mobile metabolites and the effect of sodium thiosulphate is associated with the alteration of cisplatin pharmacokinetics. Furthermore, Nagai & Ogata (1997) studied the effects of mannitol and furosemide on the nephrotoxicity and the plasma pharmacokinetics of cisplatin. Although the nephrotoxicity was ameliorated by both drugs, the pharmacokinetics of cisplatin in plasma was little changed, suggesting that both drugs may affect other mechanisms, such as transport of cisplatin into the kidney, intra-renal biotransformation of cisplatin or pharmacodynamics. Although the mechanisms of transport of cisplatin into the kidney and pharmacodynamics of cisplatin have been studied (Safirstein et al 1984; Williams & Hottendorf 1985; Harada et al 1998), the handling and biotransformation of cisplatin in the kidney and the relationship between cisplatin in the kidney and nephrotoxicity are poorly understood.

The aim of this study was to elucidate the pharmacokinetics of cisplatin, especially the manner in which cisplatin and its metabolites are distributed in the kidney and to compare this with the distribution in the liver, and to establish the relationship between the renal handling and nephrotoxicity of cisplatin in rats. Each platinum species was administered separately to rats.

Materials and Methods

Materials

All the reagents and chemicals used were of analytical grade unless otherwise stated. Cisplatin was a gift from Nippon Kayaku Co. Ltd (Tokyo, Japan). Cisplatin was dissolved in 0·9% (w/v) sodium chloride and the solution was stored at 4°C. Male Wistar rats, 235–260 g, were allowed free access to a standard laboratory pellet diet with water in a controlled environment. The rats were fasted for 18 h before the experiment, but were allowed free access to water.

Preparation of metabolites

Mobile metabolites were prepared as follows: freshly-isolated rat serum was ultrafiltered at 4000 *g* and 4°C using a membrane filter (UFC3GC MW cut off of 10 000, Nihon Millipore, Japan) for 30 min and the filtrate was incubated with cisplatin (initial concentration: 1·0 mM) at 37°C for 30 h.

Fixed metabolites were prepared as follows: freshly isolated rat serum was incubated with cisplatin at 37°C for 30 h, dialysed (Spectra/Por, MW cut off of 10 000–12 000, Spectrum Medical Ind., TX) against 10 mM phosphate buffer (pH 7·4) and washed three times at 4°C every 6 h to remove all the platinum species except the fixed metabolites.

In this study, the reaction products were not identified, but the concentration of unreacted cisplatin remaining in each solution was determined. The respective concentrations (as percentages of the initial concentrations) of cisplatin remaining in the serum in the experiments on mobile metabolites and serum in the experiments on fixed metabolites were 6·9 and 0·4%, respectively.

Determination of platinum species in plasma in rats with induced nephrotoxicity

The rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}) and the toxicological study was carried out using the following three different treatment groups: cisplatin alone (2·5, 3·5, 4·0, 5·0 or 10 mg kg^{-1}), mobile metabolites alone (0·325, 1·0 or 2·5 mg platinum kg^{-1}) or saline alone (5 mL kg^{-1}) was administered as a bolus, through a jugular vein. At 1, 2, 3, 5 and 7 days later, blood samples (300 μL) were taken from the other jugular vein, centrifuged at 1000 *g* for 5 min and the plasma was stored at −20°C until analysis.

Distribution of platinum species

The rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.), then cisplatin (2·5, 3·5, 4·0, 5·0 or 10 mg kg^{-1}), mobile metabolites (2·5 mg platinum kg^{-1}) or fixed metabolites (0·5 mg platinum kg^{-1}) were administered as a bolus, through a jugular vein and the rats were killed by withdrawing whole blood from the abdominal artery 1, 3, 5, 15, 30, 60 or 120 min later.

Immediately after death, ice-cold saline was injected quickly into the heart to clear the blood from the other organs, the kidneys and liver were excised quickly, rinsed, blotted on filter paper, weighed and homogenized with ice-cold saline (to produce 1 g/4 mL suspensions) using a Potter-Elvehjem apparatus with a Teflon pestle. The tissue homogenate and blood samples were centrifuged at 105 000 *g* (Beckman L8-60 M) for 65 min and 1000 *g* for 5 min, respectively, at 4°C and the resulting tissue cytosols and plasma were ultrafiltered at 4000 *g* for 30 min at 4°C using a Millipore-filter of the type described above. These ultrafiltrates were stored at −20°C until analysis.

The concentrations of cisplatin in these samples were determined within 3 days.

Renal disposition of cisplatin and its mobile metabolites

The rats were anaesthetized with sodium pentobarbital ($50\,mg\,kg^{-1}$, i.p.). Polyethylene cannula (0·28 mm i.d. × 0·61 mm o.d.) were inserted into the femoral artery and vein of one leg and ureters, and saline containing 0·5% (w/v) inulin was infused constantly into the femoral venous cannula at a rate of $47\,\mu L\,min^{-1}$. When a constant urinary flow rate was attained, a constant infusion of cisplatin or mobile metabolites in saline containing 0·5% (w/v) inulin at a rate of 55 or $140\,\mu g\,min^{-1}\,kg^{-1}$ (cisplatin), or 10 or $20\,\mu g$ platinum $min^{-1}\,kg^{-1}$ (mobile metabolites) was administered through the femoral venous cannula. Urine samples were collected 75–105, 105–135, 135–165 and 165–195 min after starting the cisplatin or mobile metabolites infusion and blood samples were taken at the midpoint of each urine collection period. The rats were killed 90, 150 or 210 min later and the tissues were excised and treated as described above.

Effect of mobile metabolites on the pharmacokinetics of cisplatin

Rats were treated as described above, cisplatin ($50\,\mu g\,min^{-1}\,kg^{-1}$) was infused constantly through the femoral venous cannula and mobile metabolites (1·0 mg platinum kg^{-1}) were administered rapidly through the jugular vein 150 min after starting the cisplatin infusion. Urine samples were collected 90–120, 120–150, 150–180 and 180–210 min after starting the cisplatin infusion and blood was taken from the femoral arterial cannula at the midpoint of each urine collection period. The rats were killed 210 min after and the tissues were excised and treated as described above.

Analytical techniques

The cisplatin concentrations of the biological fluids were determined by an HPLC method as reported previously (Hanada et al 1995). Briefly, the HPLC system consisted of a Shimadzu HPLC apparatus, an LC-6A pump, an SPD-6A spectrophotometric detector operated at a wavelength of 290 nm with a range of 0·01 AUFS and a C-R6A Chromatopac integrator. Cisplatin was separated from other metabolites using an anionic exchange column (150 × 4·6 mm i.d.; 3013-N, Hitachi, Japan) with a mobile phase of 10 mM sodium chloride–acetonitrile (85 : 15, v/v) at a flow rate of $0{\cdot}9\,mL\,min^{-1}$. Cisplatin was detected after post-column derivatization with both $26\,\mu M$ potassium dichromate and 6·6 mM sodium hydrogen sulphite, which were pumped at 0·6 and $0{\cdot}3\,mL\,min^{-1}$, respectively. The lower detection limit of this assay was $20\,ng\,mL^{-1}$ cisplatin in the renal cytosolic ultrafiltrate and the recovery of cisplatin from the kidney homogenate was about 70%. However, the coefficient of variation for renal cytosolic ultrafiltrate cisplatin concentrations in the range $1–10\,\mu g\,mL^{-1}$ was less than 10%. Atomic platinum concentrations were determined using an atomic absorption spectrophotometer (Hitachi Z-9000) (Hanada et al 1995), plasma and urinary inulin concentrations were determined as described elsewhere (White & Samson 1954) and blood urea nitrogen (BUN) levels were determined using a BUN diagnostic kit (Wako Pure Chemical Ind., Tokyo, Japan).

Data analysis

Data are expressed means ± s.d. The platinum levels in both kidney and liver are presented as μg platinum $(g\,wet\,tissue)^{-1}$ (μg Pt/g tissue). The cisplatin concentrations in the tissues were multiplied by a correction factor of 0·7 because cisplatin was partly converted to fixed metabolites during ultracentrifugation (Hanada et al 1995). The concentrations of both metabolites were calculated as follows: fixed metabolites = total platinum − filtered platinum; mobile metabolites = filtered platinum−cisplatin.

Pharmacokinetic analysis

The pharmacokinetic parameters of cisplatin were calculated using the model-independent method. The values of the area under the curve from time zero to the final sampling time (AUC_{0-t1}) were calculated by numerical integration of the plasma and renal cisplatin concentrations from time zero to the final sampling time (t_1) using the trapezoidal rule. The areas under the plasma and renal cisplatin concentration–time curves ($AUC_{p\infty}$ and $AUC_{k\infty}$, respectively), total clearance (CL_t) and renal clearance (CL_r) values were calculated as follows (Gibaldi & Perrier 1982):

$$AUC_{p\infty} = -AUC_{p_{0-t1}} + C_{p1}/k_{ep} \tag{1}$$

$$AUC_{k\infty} = AUC_{k_{0-t1}} + C_{k1}/k_{ek} \tag{2}$$

$$CL_t = Dose/AUC_{p\infty} \text{ or infusion rate}/C_p^{ss} \text{ (at steady state)} \tag{3}$$

$$CL_r = \text{urinary excretion rate}/C_p^{ss} \text{ (at steady state)} \quad (4)$$

where C_{p1} and C_{k1} are the plasma and renal concentrations of intact cisplatin, respectively, at t_1, k_{ep} and k_{ek} are the terminal elimination rate constants for intact cisplatin in the plasma and kidney, respectively, and C_p^{ss} is the plasma concentration of intact cisplatin at steady state. The glomerular filtration rate (GFR) was calculated as the renal clearance of inulin.

Pharmacokinetic–toxicodynamic analysis

The maximum BUN concentration obtained 5 days after administration of cisplatin as a bolus was fitted against the $AUC_{p\infty}$ and $AUC_{k\infty}$ using the sigmoid Emax model and a nonlinear least-square regression program NLS (Sugiyama et al 1987) as follows:

$$\text{BUNlevel(mg/dl)} = \text{BUNbase} + \frac{\text{BUN max} \cdot (\text{AUC})^{\gamma}}{\text{AUC}_{50}^{\gamma} + \text{AUC}^{\gamma}} \quad (5)$$

where BUNbase and BUNmax are the baseline and maximum BUN levels, respectively, and AUC_{50} and γ are 50% of the AUC for the maximum BUN level and the slope factor, respectively.

Statistical analysis

The differences between the pharmacokinetic parameters with different doses and between the pharmacokinetic parameters of cisplatin with and without concomitantly administered mobile metabolites were analysed by the unpaired and paired Student's *t*-tests, respectively. The relationship between platinum doses and BUN levels was analysed by one-way analysis of variance and Dunnett's test was used to compare the treatments when a significant difference among the means was demonstrated. $P < 0.05$ was considered significant.

Results

Nephrotoxicity of platinum species

To ascertain which platinum species in plasma is, or are, responsible for the nephrotoxicity induced by cisplatin administration, BUN levels were determined after administration of various platinum species (Figure 1). Throughout the experimental period, after the administration of saline, the BUN level was virtually constant. After the administration of 2–5 mg kg^{-1} cisplatin, the BUN levels increased strictly, reached peaks 5 days after administration and then returned gradually to the pre-dose values. Five days after the administration of mobile metabolites at a dose of 2·5 mg platinum kg^{-1}, the BUN level was significantly higher than that after saline administration. However, when mobile metabolites were administered at a dose of 0·325 mg platinum kg^{-1}, which produced the same area under the plasma mobile metabolites concentration–time curve as that after the administration of 5 mg kg^{-1} cisplatin, the BUN levels were not significantly different from those after saline administration.

Changes in the plasma concentrations of cisplatin and its metabolites with time

The plasma cisplatin concentration decreased bi-exponentially with a beta-phase elimination half-life of about 14 min. After administration of an intravenous bolus, cisplatin was transformed gradually to both mobile and fixed metabolites. After attaining a peak, the mobile metabolites

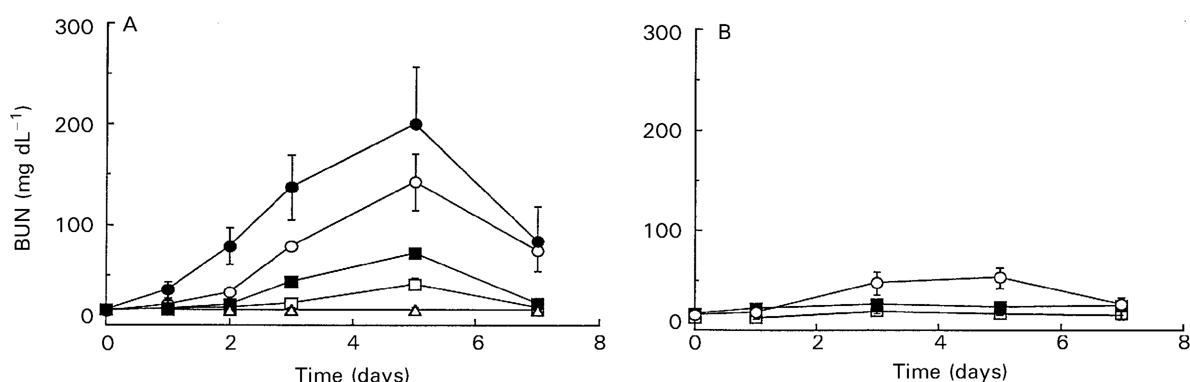

Figure 1. Nephrotoxicity of cisplatin and mobile metabolites after intravenous administration of various doses of (A) cisplatin (●, 5 mg kg^{-1}; ○, 4 mg kg^{-1}; ■, 3·5 mg kg^{-1}; □, 2·5 mg kg^{-1}; △, saline (control)) or (B) mobile metabolites (○, 2·5 mg platinum kg^{-1}; ■, 1·0 mg platinum kg^{-1}; □, 0·325 mg platinum kg^{-1}) to rats. Each point represents the mean ± s.d., n = 5.

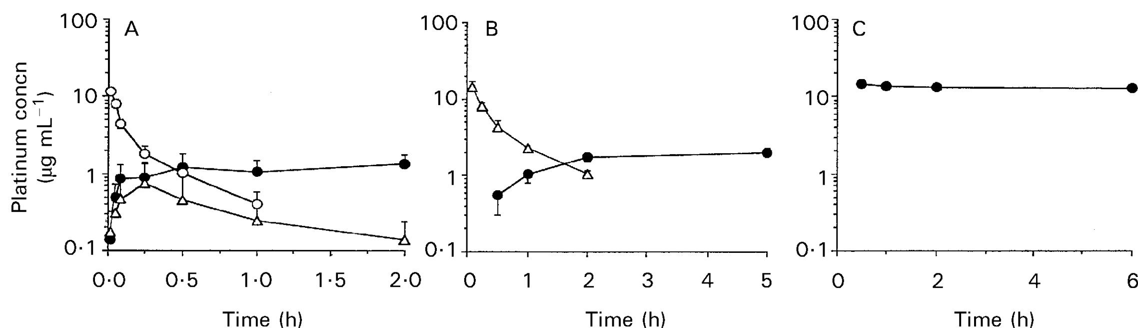

Figure 2. Concentration–time profiles of platinum species in plasma after intravenous administration of (A) cisplatin (5 mg kg^{-1}, n = 7), (B) mobile metabolites (3·5 mg platinum kg^{-1}, n = 3) or (C) fixed metabolites (500 μg platinum kg^{-1}, n = 3) to rats. Each point represents a mean ± s.d. ○, Cisplatin; △, mobile metabolites; ●, fixed metabolites.

were eliminated rapidly and the fixed metabolites were eliminated more slowly (Figure 2A).

After administration of mobile metabolites, the plasma concentration of mobile metabolites decreased bi-exponentially with a beta-phase elimination half-life of about 30 min (Figure 2B). Fixed metabolites were detected in plasma and their concentration gradually increased, whereas cisplatin was not detected, suggesting that mobile metabolites in plasma were partially transformed to fixed metabolites, but not to cisplatin, in-vivo.

Six hours after administration of fixed metabolites, little of the fixed metabolites had been eliminated and neither cisplatin nor mobile metabolites were detected in plasma (Figure 2C).

Distribution of platinum species

After intravenous administration of cisplatin, the patterns of the changes with time of the concentrations of the three platinum species in the kidney and liver were similar (Figure 3). The tissue cisplatin concentrations rapidly attained peaks (within about 3 min) and then declined rapidly in parallel with the decline in the plasma concentrations. The renal and hepatic concentrations of mobile metabolites increased and reached peaks within 30 min, whereas the fixed metabolite concentrations in these tissues increased gradually and reached constant levels, but little was eliminated during the experimental period. Two hours after cisplatin administration, fixed metabolites were the main platinum species in both tissues, as they were in plasma. These results showed that cisplatin is also transformed into both mobile and fixed metabolites in the tissues.

Within 5 min of administration of mobile metabolites, the renal concentration of mobile metabolites also reached a peak and fixed metabolites were detected, whereas platinum was not detected in the kidney or liver when fixed metabolites were administered (data not shown). These results indicated that mobile metabolites were also converted into fixed metabolites in the kidney.

Tissue-to-plasma concentration ratio (Kp)

To assess the renal handling of cisplatin and its mobile metabolites, we studied the plasma and tissue concentrations after administering cisplatin

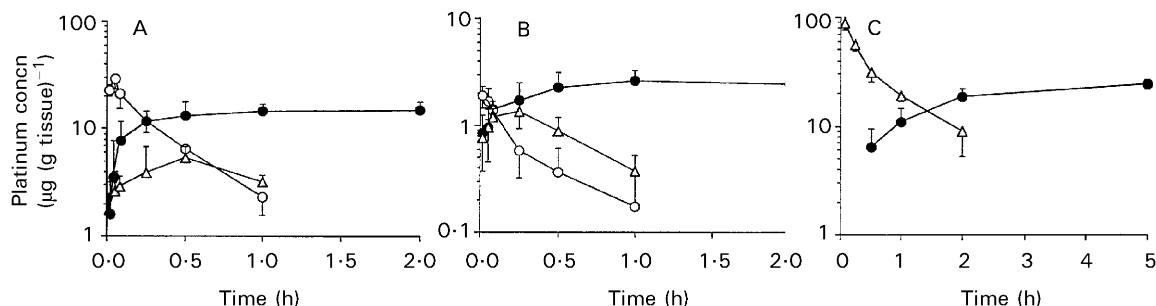

Figure 3. Concentration–time profiles of platinum species in kidneys (A and C) and liver (B) after intravenous administration of cisplatin (5 mg kg^{-1}, n = 7) (A and B) and mobile metabolites (3·5 mg platinum kg^{-1}, n = 3) (C) to rats. Each point represents mean ± s.d. ○, Cisplatin; △, mobile metabolites; ●, fixed metabolites.

as a constant infusion. The plasma, renal and hepatic concentrations of cisplatin and mobile metabolites reached steady state within 1·5 h of starting the infusion, but the concentrations of fixed metabolites continued to increase linearly (data not shown). Table 1 shows the pharmacokinetic parameters of cisplatin and mobile metabolites. The CL_t of cisplatin was about twice that of the mobile metabolites and the urinary excretion of the mobile metabolites was higher than that of cisplatin (50% vs 30% of the dose). Neither parameter was affected significantly by the doses. The mean apparent renal tissue-to-plasma concentration ratio (Kp_{app}) of cisplatin was about 9 times higher than the hepatic Kp_{app} of cisplatin (2·69 vs 0·30 mL (g tissue)$^{-1}$) and dose-independent (Table 1), indicating that the renal handling of cisplatin in rats was subject to linear pharmacokinetics. The Kp_{app} of the mobile metabolites in the kidney was about three times that of cisplatin.

Effect of mobile metabolites on the kinetics of cisplatin

To elucidate the effect of mobile metabolites on the disposition of cisplatin, the plasma cisplatin concentration was maintained at steady state and mobile metabolites were administered intravenously. Neither the CL_t nor the CL_r differed significantly with and without mobile metabolites administration (Table 2). Similarly, the renal Kp_{app} of cisplatin was not significantly reduced by the administration of mobile metabolites.

Pharmacokinetic–toxicodynamic analysis

Nagai & Ogata (1997) determined the quantitative relationship between plasma cisplatin concentrations and nephrotoxicity. They used the sigmoid Emax model and analysed the relation of BUN levels to the AUC of cisplatin and found that differences in the BUN levels were dependent on the dosing rate and the dose administered.

Table 2. Effects of mobile metabolites on the pharmacokinetics of cisplatin in rats.

	Cisplatin alone	Cisplatin with mobile metabolites
C_p^{ss} (μg mL^{-1})	1·72 ± 0·29	1·68 ± 0·72
CL_t (mL min^{-1} kg^{-1})	29·1 ± 3·91	29·8 ± 7·00
CL_r (mL min^{-1} kg^{-1})	8·82 ± 3·08	9·30 ± 3·72
CL_r/GFR	1·97 ± 0·65	2·20 ± 0·96
Kp_{app} (mL (g tissue)$^{-1}$)		3·07 ± 0·32

Each value represents mean ± s.d., n = 4. All parameters were not significantly different before and after mobile metabolites administration. Cisplatin was infused intravenously at a rate of 50 μg min^{-1} kg^{-1}.

In this study, we carried out further analysis of the relationship between BUN levels and renal cisplatin concentrations, because the renal concentration of intact cisplatin corresponds to the cisplatin concentration at the site where toxicity is induced. The relationships between the BUN concentration at 5 days after administration and $AUC_{p\infty}$ and $AUC_{k\infty}$ were also analysed using the sigmoid Emax model (Figure 4). Table 3 shows the toxicodynamic parameters of cisplatin. The slope factors for the AUC_p and AUC_k were almost identical and the AUC_{k50} was about four times higher than the AUC_{p50}.

Discussion

Which platinum species in plasma is the main cause of nephrotoxicity after cisplatin administration is not clear. In this study, to elucidate the relationship between the pharmacokinetics of platinum species and cisplatin-induced nephrotoxicity, mobile metabolites of cisplatin were prepared and administered to rats. Mobile metabolites in plasma

Table 1. Pharmacokinetic parameters of cisplatin and mobile metabolites in rats.

Parameter	Cisplatin		Mobile metabolites	
	50 μg min^{-1} kg^{-1}	140 μg min^{-1} kg^{-1}	5 μg platinum min^{-1} kg^{-1}	20 μg platinum min^{-1} kg^{-1}
CL_t (mL min^{-1} kg^{-1})	25·9 ± 3·66	27·7 ± 4·70	11·0 ± 0·38	9·86 ± 2·50
CL_r (mL min^{-1} kg^{-1})	8·96 ± 2·35	8·35 ± 1·43	5·14 ± 2·91	5·21 ± 2·54
CL_r/GFR	1·38 ± 0·35	1·43 ± 0·28	1·06 ± 0·14	1·34 ± 0·41
Ae (%)	34·7 ± 4·22	30·9 ± 7·69	46·2 ± 24·6	50·5 ± 15·3
Kp_{app} (mL (g tissue)$^{-1}$)				
Kidney	2·84 ± 0·33	2·53 ± 0·45	7·40 ± 0·82	6·84 ± 1·69
Liver	0·28 ± 0·31	0·34 ± 0·41	N.D.	N.D.

Each value represents mean ± s.d. (n = 4). Ae: amount of excertion into urine; Kp_{app}: apparent tissue-to-plasma concentration ratio. N.D., not detected. Each parameter was calculated from constant infusion studies. All parameters between dosing rates were not significantly different ($P < 0·05$).

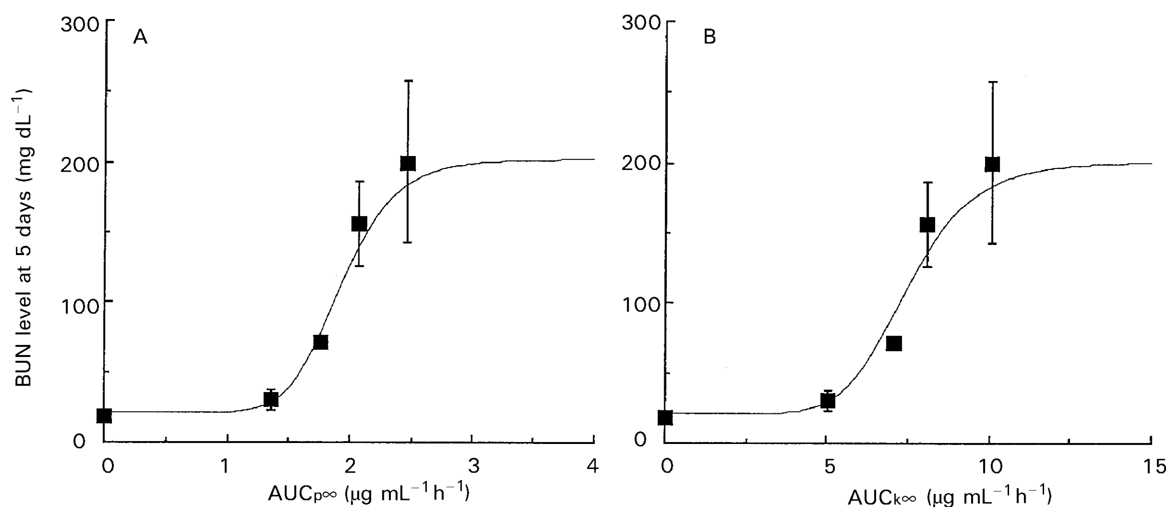

Figure 4. Pharmacokinetic and toxicodynamic analysis of the relationships between the BUN levels in plasma (A) or kidneys (B) 5 days after cisplatin administration to rats and areas under the plasma and renal cisplatin concentration–time curves (AUC_p and AUC_i, respectively). The line shows the computer-simulated line determined according to equation 5 and toxicodynamic parameters (Table 3).

Table 3. Toxicodynamic parameters of cisplatin in rats.

	Plasma	Kidney
BUNbase (mg dL^{-1})	20·0 (3·49)	20·4 (5·34)
BUNmax (mg dL^{-1})	202 (9·96)	197 (12·4)
AUC_{50} (μg mL^{-1} h)	1·93 (4·39)	7·55 (7·47)
γ	8·85 (17·5)	7·73 (15·4)

Each data represents the estimated value (CV% coefficient of variation).

were less toxic than cisplatin, suggesting that intact cisplatin in plasma was the major species responsible for nephrotoxicity after cisplatin administration. However, cisplatin pre-incubated with rat protein-free plasma induced more severe nephrotoxicity than cisplatin itself (Daley-Yates & McBrien 1984). These conflicting results may be attributable to the relatively high concentration of aquated cisplatin, which is more reactive than intact cisplatin (Reishus & Martin 1961). Cisplatin binds strongly to methionine and cysteine in-vitro (Corden 1987; Dedon & Borch 1987) and cisplatin complexed with methionine is not nephrotoxic in rats (Basinger et al 1990; Deegan et al 1994). Takahashi et al (1985) reported that platinum bound to high-molecular-mass substances (mainly albumin) in plasma had no antitumour effect and was not nephrotoxic, results consistent with ours.

In this study, both cisplatin and mobile metabolites were found to obey linear kinetics (Table 1). The CL_t of the mobile metabolites was half of that of cisplatin and the renal excretion fraction of the former was higher than that of the latter. The renal clearance per GFR (clearance ratio) of cisplatin was about 1·4, indicating that cisplatin had undergone renal active secretion as well as glomerular filtration. The existence of renal active secretion of cisplatin in rats has also been suggested by studies in which the filtered platinum concentration was monitored (Safirstein et al 1984; Daley-Yates & McBrien 1985; Klein et al 1991). Reece et al (1989) showed that, in patients, the ratio of the renal clearance of cisplatin to the creatinine clearance exceeded unity and the former was infusion-rate-dependent. We found that the clearance ratio of mobile metabolites was almost unity. It is difficult to reach a conclusion about exactly how mobile metabolites are handled by the kidney, because they are a mixture of platinum species bound to biological substances with low molecular masses. Daley-Yates & McBrien (1985) determined the fractional clearances of several platinum species bound to low-molecular-mass substances in rats and showed that these platinum species underwent renal secretion or reabsorption (or both).

Cisplatin distributed rapidly into both the kidney and liver with peak tissue levels being reached within 1–3 min of intravenous administration of cisplatin and was eliminated in parallel to plasma elimination, indicating that equilibration of cisplatin between plasma and both tissues was attained instantly. Both cisplatin and mobile metabolites were distributed extensively in the kidney and the Kp_{app} values of both platinum species were higher than unity, suggesting that some specific mechanisms may associate to maintain the difference between the plasma and renal concentrations. The Kp_{app} value of the mobile metabolites was about three times higher than that of cisplatin (Table 1).

To establish whether there was an interaction between the renal handling of cisplatin and mobile metabolites in rats, the effect of mobile metabolites on the renal disposition of intact cisplatin was studied. The administration of mobile metabolites did not significantly affect the Kp_{app} of cisplatin in the kidney (Table 2), suggesting that the mechanisms of renal accumulation of these species may differ or that the capacity of the transporter may vary. Studies on renal cortical slices and membrane vesicles showed that the uptake of cisplatin by the kidney is associated with active renal secretion, particularly the organic cation transport systems (Safirstein et al 1984; Williams & Hottendorf 1985).

In this study, we showed that cisplatin in the kidney, as well as in plasma, was transformed to both metabolites and that mobile metabolites were partially transformed to fixed metabolites after cisplatin and mobile metabolites in plasma were taken up by the kidney.

A pharmacodynamic study showed that antitumour activity of cisplatin is dependent on both the exposure time and drug concentration and, therefore, cisplatin is classified as a type I anticancer drug. However, the antitumour activity is independent of, whereas its nephrotoxicity is dependent on, the dosing rate of cisplatin (Nagai & Ogata 1997). Finally, we studied the factor(s) determining the nephrotoxicity of cisplatin. This relationship could be fitted using the sigmoid Emax model, as well as those reported by Nagai & Ogata (1997), and the BUN level was related to the area under the above threshold plasma intact cisplatin concentration–time curve. Therefore, we chose the AUC_p and AUC_k as pharmacokinetic parameters for pharmacokinetic-toxicodynamic analysis. As shown in Figure 4, nephrotoxicity estimated by measuring the BUN levels was related to the AUC of the concentration–time curve for the kidneys as well as to that for the plasma. The estimated BUNbase (about $20\,mg\,dL^{-1}$) was close to the normal BUN range in rats and the BUNmax was also close to those reported previously (Nagai & Ogata 1997; Hanada et al 1999). In this study, we did not change the dosing rate of cisplatin so the threshold values for the plasma and kidneys could not be determined. The slope factor for the AUC_p and AUC_k were virtually the same. The AUC_{k50} was about four times higher than the AUC_p and this can be accounted for by the relationship $AUC_k = Kp_{app} \times AUC_p$. In this study, the pharmacokinetic behaviour of intact cisplatin in plasma and the kidneys showed linear and almost parallel characteristics. Therefore, if such a simple relationship is maintained under various conditions, we should be able to monitor and predict nephrotoxicity by measuring the plasma concentrations of intact cisplatin. However, in situations when the parallel relationship is altered by various factors, such as specific inhibition of cisplatin uptake by the kidney, the plasma cisplatin concentration will result in the wrong interpretation. In other words, from the platinum species corresponding to the nephrotoxicity one should be able to elucidate whether the parallel relationship is altered by various methods. Recently, we reported that organic cation drugs, disopyramide and verapamil, inhibited the renal accumulation of cisplatin and this inhibition resulted in the amelioration of nephrotoxicity, whereas plasma pharmacokinetics of cisplatin were little affected (Hanada et al 1999). In this study, we investigated the relationship between intrarenal pharmacokinetics of cisplatin and nephrotoxicity by concomitantly administering various compounds which may alter the intrarenal disposition of cisplatin.

In summary, cisplatin, but not its mobile metabolites, in plasma appeared to be the major species responsible for nephrotoxicity after cisplatin administration. Only cisplatin and mobile metabolites could be taken up into the kidney and these platinum species were transformed into further metabolites. The pharmacokinetics of cisplatin and mobile metabolites were dose-independent.

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