JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 317–322

Received August 20, 2007
Accepted November 12, 2007
DOI 10.1211/jpp.60.3.0006
ISSN 0022-3573

Use of a toxicity factor to explain differences in nephrotoxicity and myelosuppression among the platinum antitumour derivatives cisplatin, carboplatin and nedaplatin in rats

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Abstract

The platinum antitumour drugs cisplatin, carboplatin and nedaplatin differ in their toxicity. The relationships between the pharmacokinetics of these drugs and developed parameters for predicting their nephrotoxicity and myelosuppression were investigated. The drugs were administered to male Wistar rats by intravenous bolus or infusion, and linearity of pharmacokinetics, total clearance and the apparent ratio of tissue concentrations of unchanged drug to plasma concentration (Kp_{app}) at steady state were determined. Apparent hydrolysis rates of each drug were determined in-vitro. Nephrotoxicity and myelosuppression were estimated by blood urea nitrogen (BUN) and platelet count, respectively. Tissue exposure to platinum was estimated as the product of the area under the plasma concentration–time curve for unchanged drug (AUC_p), Kp_{app} and the apparent hydrolysis rate constant ($k_{hydrolysis}$), and toxicity factor was defined as the product of $Kp_{app} \times k_{hydrolysis}$ as an intrinsic drug parameter. The relationship between $AUC_p \times$ toxicity factor and BUN fitted well to an E_{max} model. In bone marrow, this function was also correlated with platelet count. In summary, the product of $AUC_p \times$ toxicity factor is a factor determining the pharmacokinetics of platinum drug-induced nephrotoxicity and myelosuppression in rats, and this toxicity factor may be a useful parameter for predicting the degree of toxicity of platinum antitumour compounds.

Introduction

cis-Diamminedichloro-platinum (II) (cisplatin) is one of the most effective anticancer agents available, but adverse reactions such as nephrotoxicity and nausea frequently restrict the continuation of treatment.

Cisplatin accumulates to the greatest extent in the kidney compared with other tissues, and is taken up by the renal organic cation transport system (Safirstein et al 1994; Harada et al 1998). Concomitant administration of a weakly basic drug inhibits the uptake of cisplatin into the kidney and ameliorates nephrotoxicity, as determined by monitoring blood urea nitrogen (BUN) and serum creatinine (Hanada et al 2000b). The specific uptake of cisplatin by the kidney is thought to be the major factor determining renal damage (Safirstein et al 1994; Leibbrandt et al 1995; Hanada et al 1999, 2000b; Townsend et al 2003).

Cisplatin also undergoes ligand exchange reactions, which are virtually irreversible in biological fluids (Daley-Yates & McBrien 1984; Farris et al 1988). In biological fluids, 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). Many investigators have studied the pharmacokinetics of cisplatin by measuring the concentration of either total platinum or filterable platinum (which includes cisplatin and its mobile metabolites). However, it is difficult to estimate precisely the relationship between pharmacokinetics and pharmacodynamics on the basis of total concentration, because the pharmacokinetics of these platinum species differ markedly, and

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unchanged cisplatin contributes to efficacy and toxicity (Nagai & Ogata 1997; Hanada et al 1999, 2000a, b). Therefore, the pharmacokinetics of platinum derivatives should be evaluated from concentrations of the unchanged drug. We have studied the pharmacokinetic and toxicodynamic relationships of cisplatin under various conditions, such as coadministration of glutathione. We found that the sulfhydryl concentration in the cytosol fraction of the kidney was markedly increased after glutathione administration and, consequently, binding of cisplatin to the sulfhydryl compound was increased, whereas binding to organelles was decreased. This decrease was correlated with amelioration of nephrotoxicity, suggesting that binding of unchanged cisplatin to intracellular organelles is an important factor in cisplatin-induced nephrotoxicity (Hanada et al 2000b). The rate-limiting step for binding of cisplatin to intracellular organelles is thought to be the intracellular rate of hydrolysis (Dedon & Borch 1987; Nagai et al 1996), and therefore the hydrolysis rate constants of platinum drugs should be taken into account when predicting toxicity.

Many platinum derivatives have been developed to improve the toxicity profile of this class of drugs and to enhance antitumour activity. *cis*-Diammine 1-1 cyclobutanedicarboxylato-platinum (II) (carboplatin) is a second generation platinum antitumour agent. *cis*-Diammine glycolate-platinum (II) (nedaplatin) has also been recently developed in Japan (Figure 1). The antitumour mechanisms of these drugs are thought to be identical, but their dose-limiting toxicities are different. The dose-limiting toxicity of carboplatin and nedaplatin is bone marrow suppression (Leyvraz et al 1985; Ariyoshi & Ota 1989), whereas that of cisplatin is nephrotoxicity. Therefore, for effective utilization of these platinum drugs, it is desirable to determine the pharmacokinetic factors that are involved in their nephrotoxicity and other toxic effects.

In the present investigation we focused on the reasons for the differences in toxicity among platinum antitumour derivatives by studying differences in their distribution into the tissues affected by toxicity and also the intracellular reactivity of the drugs. We used these kinetic parameters to calculate a toxicity factor that would explain and predict the different extents of nephrotoxicity and myelosuppression caused by these platinum drugs.

Materials and Methods

Chemicals

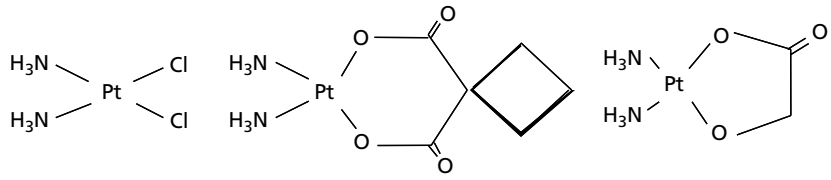
Cisplatin and nedaplatin were kindly donated by Nippon Kayaku Co. (Tokyo, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. Carboplatin was obtained from Sigma Chemical Co. (St Louis, MO, USA). A BUN assay kit was obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Animals

Male Wistar rats (200–240 g) were purchased from Tokyo Laboratory Animal Science Co. (Tokyo, Japan) and were maintained on a standard laboratory pellet diet with free access to water in a controlled environment. The rats (250–300 g) used were handled in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, USA) and the study protocol was approved by Meiji Pharmaceutical University.

In-vivo pharmacokinetic study

An in-vivo renal clearance study was performed using methods reported previously in order to obtain the basic pharmacokinetic parameters (Hanada et al 1999, 2000a, b). In brief, rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). Polyethylene cannulae (0.28 mm i.d. × 0.61 mm o.d.) were implanted into both the femoral vein and artery of one leg and into the ureters. Cisplatin in 0.9% (w/v) NaCl, carboplatin in 5% glucose, or nedaplatin in 5% glucose containing inulin (0.5%) was infused via the femoral venous cannula at a constant rate of 0.037 mL min^{-1} (50, 200 or 50 μg min^{-1}/kg, respectively). Urine was collected at 90–120, 120–150 and 150–180 min after the start of drug infusion, and blood samples were taken from the femoral artery at the midpoint of the urine sampling periods. At 180 min after the start of drug infusion, the rats were killed. The kidneys and bone marrow were quickly excised, blotted on filter paper, weighed and homogenized with 2–5 volumes of 0.9% (w/v) NaCl or 5% glucose. The tissue homogenate was ultracentrifuged at 105 000 *g* for 65 min at 4°C (Beckman L8-60M, Beckman Instruments, Inc., CA, USA). Blood samples were centrifuged at 1000 *g* for 5 min at 4°C, and the plasma and



Cisplatin Carboplatin Nedaplatin

Figure 1 Chemical structures of cisplatin, carboplatin and nedaplatin.

tissue supernatant were ultrafiltered at 4000 *g* for 30 min at 4°C with a membrane filter (Millipore filter UFC3GC, MW cut-off 10 000, Nihon Millipore, Yonezawa, Japan). These samples were stored at −20°C until analysis. The concentrations of unchanged platinum drugs were determined within 3 days. In order to confirm the linearity of pharmacokinetics of carboplatin and nedaplatin, an intravenous bolus administration study was also conducted (carboplatin dose: 20, 40 and 60 mg kg^{-1}; nedaplatin dose: 3, 5, 8 and 10 mg kg^{-1}).

Study of toxicity

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). Saline, cisplatin (1, 2.5, 3, 4 and 5 mg kg^{-1} in saline), carboplatin (20, 40 and 60 mg kg^{-1} in 5% glucose), or nedaplatin (3, 5, 8 and 10 mg kg^{-1} in 5% glucose) were injected as a bolus via the jugular vein. After the rats had regained consciousness they were maintained on a standard laboratory pellet diet with free access to water in a temperature-controlled environment. Blood samples (0.2 mL) were taken from the other jugular vein just before administration and on Days 1, 3, 5, 7, 10 and 14–17 after administration, and 100 μL of the sample was stored at 4°C until determination of platelet counts. An aliquot of the blood was centrifuged at 1000 *g* for 5 min. The plasma was stored at −20°C until BUN analysis.

In-vitro hydrolysis reactions

Phosphate buffer (0.067 M, pH 7.0) containing 14 mM sodium chloride (ionic strength was adjusted to 0.148 by sodium sulfate) and platinum compound (final concentration was 30 μM) was incubated at 37°C as reported previously (Nagai et al 1996). A small volume (50 μL) of the solution was withdrawn just before the start of incubation and again after 0, 2, 4, 6, 8, 12 and 24 h for cisplatin or 0, 12, 24, 48 and 72 h for carboplatin and nedaplatin. The concentration of each unchanged platinum compound was determined.

Analytical methods

The concentrations of unchanged cisplatin and nedaplatin in the plasma, urine and tissues were determined by a previously reported post-column derivatization HPLC method (Hanada et al 1995, 2000b). The concentration of unchanged carboplatin was determined by the method of Duncan et al (1988). The recovery of carboplatin was 67.4 ± 3.1%, but the within- and between-day coefficients of variation were less than 7%. The recovery of cisplatin from the tissue homogenate was about 70%, but the within- and between-day coefficients of variation were less than 10%, as reported previously (Hanada et al 1995, 2000b). The recovery for cisplatin and nedaplatin from plasma was greater than 90%, and the within- and between-day coefficients of variation were less than 11%.

The inulin concentration was determined by the method of White & Samson (1954) and BUN levels were determined with a BUN diagnostic kit. Platelet counts were determined by standard procedures.

Data analysis

Data represent means ± s.d. The pharmacokinetic parameters total clearance (CL_{tot}) and renal clearance (CL_r) of unchanged platinum drugs were calculated as follows:

$$CL_{tot} = \text{infusion rate}/Cp^{ss} \quad (1)$$

$$CL_r = \text{urinary excretion rate}/Cp^{ss} \quad (2)$$

where Cp^{ss} is the mean plasma concentration of unchanged drug at steady state. The Kp_{app} value was calculated as the ratio of the tissue concentration of unchanged drug to the plasma concentration at steady state.

Platinum levels in the tissues were expressed as μg Pt (g wet weight of tissue)$^{-1}$. The apparent hydrolysis rate constant ($k_{hydrolysis}$) in phosphate buffer was calculated by log-linear least-squares regression (Nagai et al 1996). The pharmacokinetic parameters of nedaplatin and carboplatin after intravenous bolus administration were calculated using the model-independent method. The values of the area under the curve from time zero to the final sampling time were calculated by numerical integration of the plasma concentrations from time zero to the final sampling time using the trapezoidal rule. Comparison of pharmacokinetic parameters among doses and the three drugs was analysed by one-way analysis of variance at a significance level of $P < 0.05$. The linear relationships between pharmacokinetic parameters and toxicity grade were analysed using Pearson's correlation test. These statistical analyses were performed using the SPSS computer program (SPSS Inc., Chicago, IL, USA). The relationship between pharmacokinetic parameters and maximum blood urea nitrogen level was analysed with a sigmoid Emax model (Figure 2) using the WinNonlin computer program (Pharsight, IL, USA).

Derivation of toxicity factor

We have already reported that the amount of platinum in renal organelles is correlated with the severity of nephrotoxicity caused by cisplatin (Hanada et al 2000b). Therefore, the amount of platinum in cellular organelles was estimated as follows. The relationship between exposure of intracellular substances to platinum drugs and nephrotoxicity was analysed using a toxicity factor. The rate of formation of a platinum–organelle complex can be expressed by the following equation:

$$\text{amount of complex formed} = \text{formation clearance} \times AUC_r = k \times Vd_r \times AUC_r \quad (3)$$

where k is the formation rate constant of the platinum–organelle complex and Vd_r is the volume of distribution of unchanged compounds in the kidneys. We estimated AUC_r by the following equation, assuming that the concentration of platinum compounds rapidly reaches a state of equilibrium between plasma and intracellular fluid in the kidney:

$$AUC_r = Kp_{app} \times AUC_p = Kp_{app} \times \text{dose}/CL_{tot} \quad (4)$$

k can be replaced by $k_{hydrolysis}$, because the hydrolysis of these compounds is the rate-limiting step for complex formation with intracellular high molecular mass substances. As the compounds studied have similar physicochemical characteristics, their Vd_r values are assumed to be the same. The relative grade of toxicity of each platinum compound was then compared by calculating the product of $k_{hydrolysis}$ and Kp_{app}, which we call the 'toxicity factor' in this study.

Results

We used in-vivo clearance studies to estimate the renal disposition of cisplatin, carboplatin and nedaplatin in rats in terms of renal clearance and tissue distribution at steady state (Table 1). The CL_{tot} of unchanged cisplatin was two or three times greater than that of carboplatin and nedaplatin. However, the fraction excreted in the urine was greater for carboplatin and nedaplatin (88% and 73%, respectively) than for cisplatin (35%), as has been reported by others (Siddik et al 1987; Kawai et al 2005). With regard to tissue distribution, Kp_{app} for kidney was similar among the platinum drugs, whereas distribution of carboplatin into bone marrow was much greater than that of cisplatin or nedaplatin. The mean CL_{tot} of carboplatin and nedaplatin obtained from the intravenous bolus administration study was 17.7, 18.5 and 19.4 mL $min^{-1}kg^{-1}$ (at 20, 40 and 60 mg kg^{-1}) and 5.76, 7.67,

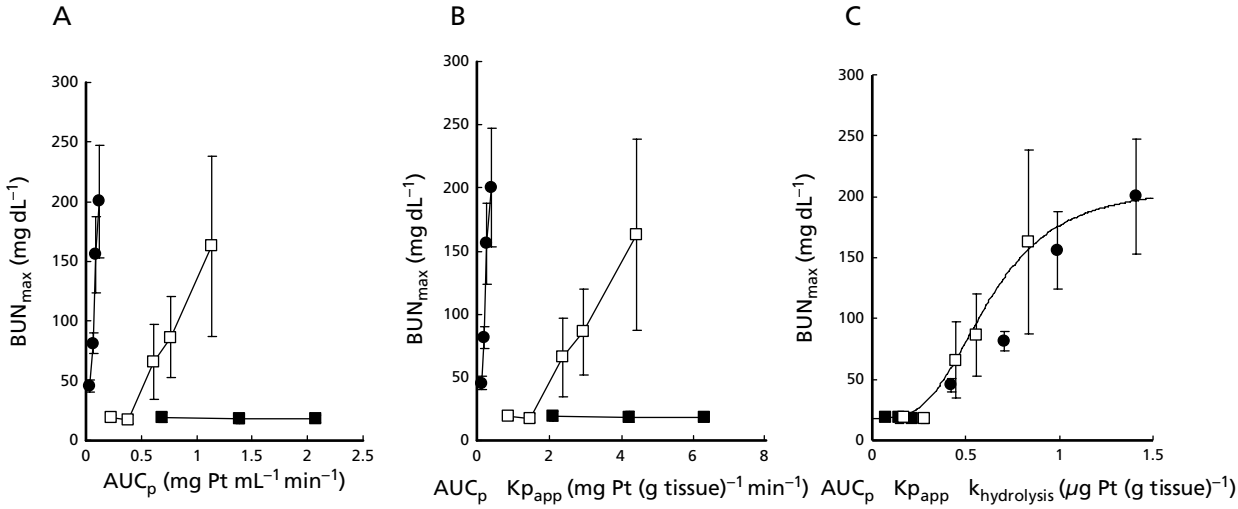


Figure 2 Relationships between the pharmacokinetic parameters, AUC_p (A), AUC_r (B) and $AUC_p \times$ toxicity factor (C) and maximum blood urea nitrogen (BUN_{max}). ●, Cisplatin (n = 5); ■, carboplatin (n = 5); □, nedaplatin (n = 5). The simulation curve was obtained using the following sigmoid Emax model equation: $BUN_{max} = 18.18 + ((190.2 \times (AUC_p \times Kp_{app} \times k_{hydrolysis})^{3.33})/(0.619^{3.33} + (AUC_p \times Kp_{app} \times k_{hydrolysis})^{3.33}))$.

Table 1 Basic pharmacokinetic parameters of cisplatin, carboplatin and nedaplatin in rats

Parameter	Cisplatin	Carboplatin	Nedaplatin
CL_{tot} (mL min^{-1} kg^{-1})	25.9 ± 3.66	15.2 ± 3.23	8.48 ± 3.34
CL_r (mL min^{-1} kg^{-1})	8.96 ± 2.35	13.3 ± 2.30	5.88 ± 2.76
Distribution into tissues			
Kp_{app} (kidney)	3.12 ± 0.47	3.04 ± 0.18	3.89 ± 0.68
Kp_{app} (bone marrow)	0.065 ± 0.014	0.487 ± 0.020	0.292 ± 0.150
Apparent hydrolysis rate constant			
$k_{hydrolysis}$ (min^{-1})	3.60×10^{-3}	3.35×10^{-5}	1.89×10^{-4}
Toxicity factor (mL (g tissue)$^{-1}$ min^{-1})			
Toxicity factor for kidney	1.12×10^{-2}	1.02×10^{-4}	7.35×10^{-4}
Toxicity factor for bone marrow	2.34×10^{-4}	1.63×10^{-5}	5.52×10^{-5}

CL_{tot}, total clearance; CL_r, renal clearance; $k_{hydrolysis}$, apparent first-order rate constant of hydrolysis of unchanged platinum drug; Kp_{app}, apparent ratio of tissue concentration of unchanged drug to plasma concentration under steady-state conditions (kidney, n = 5; bone marrow, n = 3). Toxicity factor values were calculated as $Kp_{app} \times k_{hydrolysis}$.

7.33 and 8.03 mL min^{-1}/kg (3, 5, 8 and 10 mg kg^{-1}), respectively, being linear over the dose range studied.

The concentration of cisplatin in phosphate buffer containing 14 mM NaCl rapidly decreased with a half-life of 3.2 h, as reported by others (Nagai et al 1996). In contrast, the hydrolysis of carboplatin and nedaplatin was relatively slow. The $k_{hydrolysis}$ of cisplatin was about 19 times greater than that of nedaplatin and about 107 times greater than that of carboplatin (Table 1).

In this study, we defined a toxicity factor that was the product of the extent of drug distribution into the affected tissue and the reactivity of the distributed drug with intracellular organelles. The toxicity factors of cisplatin for kidney and bone marrow were much higher than those of the other drugs (Table 1).

The BUN level was markedly increased from 3 days after drug administration and showed a peak at 5 days, as has been observed previously (Hanada et al 1999, 2000a, b). On the other hand, the platelet count in blood gradually decreased, with a nadir at 10 days after drug administration. Therefore, comparative toxicity was estimated from the BUN level at 5 days after drug administration and the platelet count at 10 days after drug administration.

Finally, relationships among pharmacokinetic exposure parameters and extent of toxicity were analysed (Figures 2 and 3). The maximum BUN level was plotted against AUC_p (Figure 2A), which was calculated from the dose and CL_{tot}, against the AUC_r (Figure 2B), which was calculated from AUC_p and Kp_{app}, and against $AUC_p \times$ toxicity factor (Figure 2C). The difference in the nephrotoxicity caused by the three platinum drugs was not explained by the difference in drug exposure in plasma (AUC_p) or kidney (AUC_r), but was fitted by a combination of AUC_p and toxicity factor with an Emax model. Similar relationships were observed between myelosuppression and kinetic parameters, and the percentage platelet count was significantly correlated with $AUC_p \times$ toxicity factor ($r = 0.958$, $P < 0.001$) but not with AUC_p or AUC_b (AUC for bone marrow) (Figure 3).

Discussion

We compared the main processes determining toxicity of the platinum derivatives cisplatin, carboplatin and nedaplatin, in particular the pharmacokinetic properties of the unchanged drug, its tissue distribution, and its reactivity in tissue. We recently reported that, from the viewpoint of a pharmacokinetic–toxicodynamic model, the proportion of cisplatin bound to intracellular organelles is the main determinant of the extent of nephrotoxicity (Hanada et al 2000b). When biotransformation of cisplatin in renal organelles is inhibited by administration of glutathione, the amount of platinum bound to organelles correlates well with the degree of nephrotoxicity, supporting the above finding.

We analysed the relationship between exposure of intracellular substances to platinum drugs and nephrotoxicity by using a toxicity factor, $Kp_{app} \times k_{hydrolysis}$. A good correlation was observed between the maximum BUN level and the product of AUC_p and toxicity factor, suggesting that the toxicity factor may be a useful kinetic parameter for estimating the extent of toxicity caused by these platinum drugs. These results were supported by a report indicating that the morphological changes in the proximal tubule after nedaplatin administration are similar to those caused by cisplatin (Uehara et al 2005). On the other hand, nephrotoxicity, as estimated by BUN analysis, was not observed over the dose range of carboplatin studied. Rats given carboplatin at doses in excess of 100 mg kg^{-1} did not show any increase in BUN level but did not survive in this study (data not shown).

This approach and the calculated toxicity factors were then applied to the myelosuppression caused by platinum drugs, with the extent of depression of the platelet count as a biological marker. All the drugs studied caused a dose-dependent depression of the platelet count, and the extent of myelosuppression caused by the different drugs was strongly correlated with $AUC_p \times$ toxicity factor, but not with AUC_p or AUC_r.

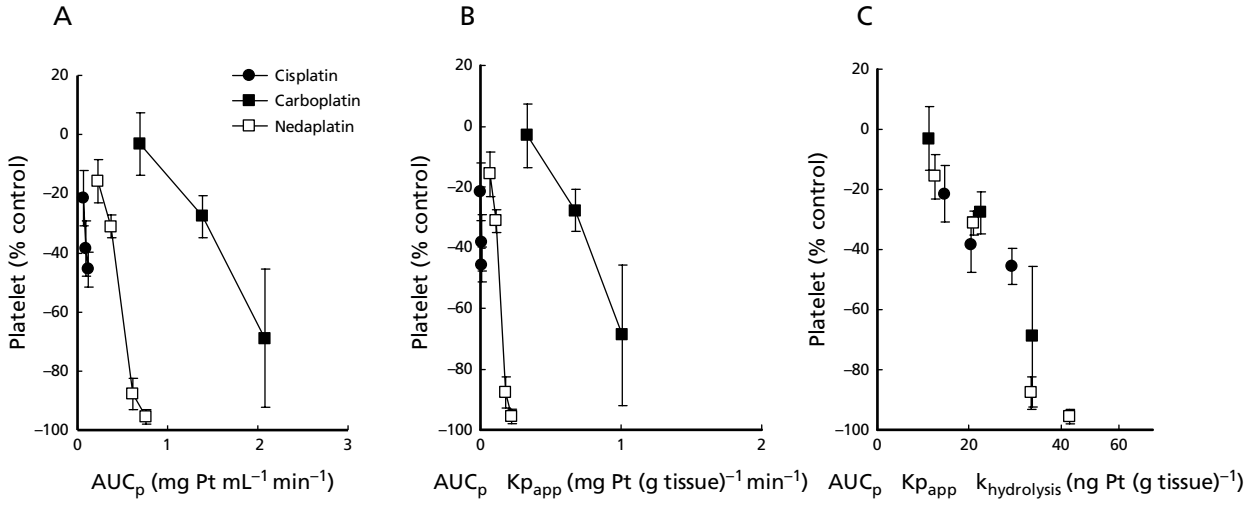


Figure 3 Relationships between pharmacokinetic parameters, AUC_p (A), AUC_b (B), and $AUC_p \times$ toxicity factor (C) and maximum platelet depression (% of control values). ●, Cisplatin (n = 5); ■, carboplatin (n = 5); □, nedaplatin (n = 4–5).

Together, these observations suggest that the extent of platinum binding to intracellular substances is an important factor determining the differences in the toxicity of cisplatin, carboplatin and nedaplatin. They also indicate that the toxicity factor, $Kp_{app} \times k_{hydrolysis}$, would be a useful kinetic parameter for predicting the toxicity of new platinum antitumour compounds as long as the rate-limiting step of platinum binding to intracellular organelles is the hydrolysis rate. Confirmation of the rate-limiting step for the formation of platinum complexes in tissues is a critical issue for validating the use of this toxicity factor for other platinum antitumour drugs.

Recently, Yokoo et al (2007) reported that cisplatin is a substrate of OCT1, which is one of the organic cation transporters in the kidney, whereas carboplatin and nedaplatin are not. Furthermore the localization of organic transporters in the proximal tubule segment (Zhang et al 2002) and the pathological alterations after cisplatin administration are localized to the S3 segment of the proximal tubule situated in the outer stripe of the outer medulla (Safirstein et al 1994). Therefore in order to predict the nephrotoxicity of platinum drugs more precisely, estimation of the extent of their distribution into the specific segment may be required.

Conclusion

Although the dose-limiting toxicities of cisplatin, carboplatin and nedaplatin are different, and these agents show different extents of toxicity, the variations can be explained by the kinetic parameter $AUC_p \times$ toxicity factor, which represents the binding of platinum to intracellular substances associated with intracellular organelles. The toxicity factor may be a useful parameter for predicting the degree of toxicity of platinum antitumour compounds.

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