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ORIGINAL ARTICLE

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Quantitative relationship between pharmacokinetics of unchanged cisplatin and nephrotoxicity in rats: importance of area under the concentration-time curve (AUC) as the major toxicodynamic determinant in vivo

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Abstract *Purpose*: The major pharmacokinetic parameters of unchanged cisplatin (CDDP) related to nephrotoxicity were evaluated in rats in vivo using a pharmacodynamic model. *Methods*: CDDP was administered according to various dosing schedules (single bolus, intermittent bolus, or continuous infusion). Unchanged CDDP in plasma and urine was quantified using high-performance liquid chromatography (HPLC). The pharmacokinetics were assessed by model-independent methods. The relationship between pharmacokinetics and BUN levels was evaluated using a sigmoid maximum response (E_{max}) model. *Results*: Unchanged CDDP showed linear pharmacokinetics after single bolus injections of 1 to 5 mg/kg CDDP. Nephrotoxicity was ameliorated following intermittent bolus injection (1 mg/kg per day for 5 days) and continuous infusions (over 2 and 3 h) of the same CDDP doses (5 mg/kg), although these dosing schedules did not change the area under the concentration-time curve (AUC), total clearance (Clt), urinary excretion of unchanged CDDP or kidney platinum levels significantly. The maximum BUN level, as a nephrotoxicity marker, showed dose-related increases after single bolus injection of 1 to 5 mg/kg CDDP and after 3-h infusion of 5 to 25 mg/kg. The pharmacodynamic relationship between the maximum BUN level and C_{max} and between the maximum BUN level and AUC were apparently different between single bolus injection and 3-h infusion. The maximum BUN level was related to the AUC calculated by plasma concentrations of unchanged CDDP greater than the threshold level ($AUC_{>Cmin}$), a relationship most successfully described by the signoid E_{max} model, regardless of CDDP dose and schedule. The plasma threshold level of unchanged CDDP was determined as 0.9 μgPt/ml in rats. *Conclusions*: The present results substantiated the importance of C × T (AUC) value as an indicator of CDDP-induced nephrotoxicity in vivo as well as of tumor cell-killing effect of CDDP in vitro. The $AUC_{>Cmin}$ of unchanged CDDP was found to be an important pharmacokinetic parameter predicting CDDP nephrotoxicity.

Key words Unchanged cisplatin · Nephrotoxicity · Pharmacokinetic/pharmacodynamic relationship · Area under the concentration-time curve (AUC)

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Introduction

Cisplatin (CDDP) is of great clinical value in the management of a variety of tumors, although its optimal use is often complicated by its dose-related nephrotoxicity [9, 18]. Appropriate use of intravenous hydration, osmotic diuretics and antidotes have reduced both the incidence and severity of CDDP-induced nephrotoxicity [14, 15]. Clinically, CDDP is administered as an intravenous dose over infusion times ranging from minutes to several days. Modification of the infusion time, for example by using continuous infusion and fractional daily dosage schedules, has been found to produce less nephrotoxicity than a single bolus dose [4, 5, 28, 32].

In order to clarify the correlation between infusion time and nephrotoxicity, the pharmacokinetics of CDDP after various dosing schedules have been compared. Some studies have shown significant schedule-dependent differences in the pharmacokinetics of CDDP [4, 13, 25], whereas others have indicated that the pharmacokinetics are independent of the dosing schedule [23, 33, 34]. However, in only a few of these pharmacokinetic studies were the levels of biologically active unchanged CDDP monitored and the pharmacokinetics with different dosing schedules compared [23, 25].

Preclinical in vitro studies using cultured tumor cells have demonstrated a lack of schedule-dependency of the antitumor activity of CDDP [1, 8, 17]. Thus, CDDP is a cell cycle phase-nonspecific agent, the cell killing (antitumor) action of which is dependent on the $C \times T$ (concentration-time) value or clinically on the AUC (area under the concentration-time curve) [22, 29]. However, the most plausible reason suggested for the amelioration of nephrotoxicity by modification of the infusion time is that continuous infusion and fractional dosing schedules result in lower peak CDDP levels than a single bolus injection of the same dose [10, 33, 34].

In the present study, CDDP was administered to rats using various dosing schedules (single bolus injections, intermittent bolus injections or continuous infusion) and the relationship between the pharmacokinetics of unchanged CDDP and nephrotoxicity was evaluated using pharmacodynamic models. The most informative pharmacokinetic parameters of unchanged CDDP relating to nephrotoxicity are discussed.

Materials and methods

Materials

Cisplatin (Randa) was a gift from Nippon Kayaku Co. (Tokyo, Japan). It was dissolved in 0.9% w/v sodium chloride (NaCl) solution at a concentration of 1 mg/ml to produce a standard solution. All the other chemicals and reagents used were of analytical grade.

Pharmacokinetic study

After fasting overnight, male Wistar rats (210–264 g) were anesthetized with ethyl carbamate (1.0 g/kg). A polyethylene catheter (PE-50) was inserted into the left jugular vein for hydration and continuous infusion of CDDP and another into the left femoral artery for blood sampling. A polyethylene catheter (PE-10) was inserted into the right and another into the left ureter for urine sampling. A blood sample (0.15 ml) was taken as a blank plasma sample for the inulin assay. Inulin solution (5 mg/ml in 0.9% w/v NaCl solution) was infused at a constant rate of 0.05 ml/min until a constant urine output was obtained and then CDDP was administered as a bolus or by continuous infusion.

For the bolus injections, CDDP (1, 2.5, 3.5 and 5 mg/kg) was injected rapidly via the right femoral vein. Blood samples (0.6 ml) were drawn into heparinized microtubes 5, 15 and 30 min before CDDP injection and 1, 5, 15, 30, 50 and 75 min thereafter. Urine samples were collected during the periods 0–10, 10–20 and 20–40 min before and 0–10, 10–20, 20–40, 40–60 and 60–90 min after CDDP injection. For the continuous infusions, the standard CDDP solution (1 mg/ml) was diluted with inulin solution (5 mg/ml) to produce doses of 5 mg/kg in 6 and 9 ml (2- and 3-h infusions, respectively), which were infused via the left jugular venous catheter at a constant flow rate of 0.05 ml/min. Blood samples (0.6 ml) were taken during the 2-h infusion, 30, 60, 90 and 120 min after its start, and 5, 15, 30 and 50 min after its completion. Urine samples were collected during the periods 15–45, 45–75, 75–105, 110–130, 130–140 and 140–160 min after starting the CDDP infusion. Blood samples (0.6 ml) were taken during the 3-h infusion, 30, 90, 150 and 180 min after its start, and 5, 15 and 30 min after its completion. Urine samples were collected during the periods 15–45, 75–105, 135–165, 170–190, 190–200 and 200–220 min after starting the CDDP infusion.

Urine samples were collected into ice-cold microtubes and the collection period was limited to 30 min to avoid decomposition of unchanged CDDP during collection. After taking the final sample, the rats were sacrificed and their kidneys were excised and homogenized with 0.25% w/v Triton X-100 (1:4 w/v) for platinum analysis (described below). The blood samples were centrifuged (1000 *g*) for 3 min at room temperature immediately after being taken and 50 µl of the plasma obtained was transferred to another microtube for inulin assay. The rest of the plasma was ultrafiltered (4000 *g*) for 30 min at 4 °C using a membrane with a molecular weight cut-off pore size of 10 000 Da (UFC 3GC membrane; Japan Millipore, Tokyo, Japan). The samples (plasma, ultrafiltered plasma, urine and kidney homogenates) were stored below −20 °C until analyzed.

Evaluation of CDDP nephrotoxicity

Male Wistar rats (224–320 g) were anesthetized lightly with 40 mg/kg pentobarbital and assigned to the following dosing schedules: single bolus injections, intermittent bolus injection or continuous infusions. For the single bolus injection schedules, 1, 2.5, 3.5 and 5 mg/kg CDDP solution was injected rapidly via the right femoral vein. The rats in the control group were injected with 0.9% w/v NaCl solution (5 ml/kg). For the intermittent bolus injection schedules, bolus injection of the standard CDDP solution (1 mg/kg) or 0.9% w/v NaCl solution (1 ml/kg, control) was administered after a small incision, which was then closed with a single stitch. CDDP was injected daily for 5 days via different parts of the same femoral vein. For the continuous infusion schedules, CDDP solution (5, 6, 10 and 25 mg/kg in 9 ml) was infused through the left jugular venous catheter at a constant rate of 0.05 ml/min over 3 h, or 5 mg/kg in 6 ml over 2 h in the same manner. The control rats received the same values of 0.9% w/v NaCl solution (6 and 9 ml over 2 and 3 h, respectively).

The rats' body weights were monitored daily and blood samples (0.4 ml) were taken immediately before and 1, 3, 4 and 5 days after completing each treatment for determining blood urea nitrogen (BUN) levels. The rats were sacrificed 5 days after completing each treatment, and their kidneys were removed and homogenized with 0.25% w/v Triton X-100 (1:4 w/v) for platinum analysis (described below).

Analyses

The concentrations of unchanged CDDP in ultrafiltered plasma and urine were determined by high-performance liquid chromatography (HPLC), as reported previously [16] with a slight modification. Although unchanged CDDP was stable during sample processing and storage [16], HPLC analysis was finished the same day that the animal experiment was performed. A 50-µl aliquot of each biological sample (ultrafiltered plasma or urine diluted with water, 1:100 v/v) was injected directly into the analytical column (Hitachi No. 3013-N, Chromato Research, Tokyo, Japan) and eluted with acetonitrile/10 m*M* NaCl (15:85, v/v) at a constant flow rate of 0.9 ml/min. The unchanged CDDP separated by the analytical column was subsequently derivatized with both 26 µ*M* potassium dichromate and 6.6 m*M* sodium hydrogen sulfite, then monitored at 290 nm. Calibration curves showed good linearity up to 30 µg/ml with a detection limit of 80 ng/ml (100 µl injection) in ultrafiltered plasma and urine. Good accuracy with a coefficient of variation of $<8\%$ was obtained [16].

The platinum concentration of each sample was determined using a Hitachi Model Z-9000 atomic absorption spectrometer (Hitachi, Tokyo, Japan). A 20-µl aliquot of each kidney homogenate was

injected directly into the tube cuvette of the atomic absorption spectrometer and its platinum concentration was analyzed at 265.9 nm using a four-stage temperature program consisting of a 30-s dry stage at 80–120 °C, a 17-s ash stage at 1300 °C, a 15-s atomizing stage at 2800 °C and a 5-s clean stage at 3000 °C [16].

The concentrations of inulin in plasma and diluted urine were determined using the method of Davidson and Sackner [7] and BUN was measured using a diagnostic kit (Urease Test Wako, Wako Co., Osaka, Japan).

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using a model-independent method. The AUC_{0-t1} and Ae_{0-t2} were calculated by numerical integration of the plasma concentration- and urinary excretion rate-time data from time zero to the final sampling time using the trapezoidal rule. The AUC, cumulative amount excreted in urine from time zero to infinity (Ae), mean residence time (MRT), total clearance (Clt) and renal clearance (Clr) values were calculated as follows [12]:

$$AUC = AUC_{0-t1} + Cp_{t1}/kel_p$$

$$Ae = Ae_{0-t2} + (dAe/dt)_{t2}/kel_u$$

$$Ae\ (\%) = Ae/D \cdot 100$$

$$AUMC = AUMC_{0-t1} + Cp_{t1}/kel_p \cdot (1/kel_p + t_1)$$

$$MRT = AUMC/AUC - T/2$$

$$Clt = D/AUC$$

$$Clr = Ae/AUC$$

where Cp_{t1} is the plasma concentration at the final sampling time (t_1), kel_p is the plasma terminal elimination rate constant, $(dAe/dt)_{t2}$ is the urinary excretion rate at the final sampling time, t_2 is the midpoint of the final urine sampling interval, kel_u is the terminal elimination rate constant calculated from the urinary excretion rate-time profile, Ae (%) is the cumulative percentage of unchanged CDDP excreted in the urine from time zero to infinity, AUMC is the area under the first moment curve from time zero to infinity, T is the infusion time.

Pharmacodynamic analysis

The pharmacodynamic analysis was modeled using a sigmoid maximum response (E_{max}) model.

$$BUN = BUN_0 + \frac{BUN_{max}(AUC_{>Cmin})^{\gamma}}{(AUC_{50})^{\gamma} + (AUC_{>Cmin})^{\gamma}}$$

where BUN_0 and BUN_{max} represent the baseline level without CDDP treatment and the maximum attainable effect estimated. $AUC_{>Cmin}$ represents the AUC calculated using plasma concentration of unchanged CDDP greater than the threshold level (C_{min}). C_{min} was assumed to be 0.5–1.2 μgPt/ml in the present analysis. The AUC_{50} is the value of $AUC_{>Cmin}$ that produces 50% of BUN_{max}. The exponent γ is a constant, representing the shape of the response curve. The computer program NLS [30] was used to estimate parameters (BUN_0, BUN_{max}, AUC_{50} and γ) for the combined data of all dosing schedules. The selection of the parameters which described the pharmacodynamic relationship most successfully was performed by comparing Akaike's information criterion [35].

Statistics

All the data were expressed as means ± SD. The differences among pharmacokinetic parameters, kidney platinum and BUN levels after administration of CDDP according to various dosing schedules were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis *H*-test [27]. The multiple range test (Scheffe method) was carried out if a significant difference between means was found [27]. The mean BUN levels of the CDDP-treated and corresponding control groups were compared using Student's *t*-test for unpaired data. The relationships for CDDP dose with C_{max}, AUC and kidney platinum were examined by Pearson's correlation coefficient (*r*). Differences at $P < 0.05$ were considered to be significant.

Results

Effects of dosing schedule on pharmacokinetics of unchanged CDDP, kidney platinum levels and nephrotoxicity

The time-courses of plasma concentration of unchanged CDDP after administration of CDDP (5 mg/kg) by single bolus injections and 2- and 3-h continuous infusions are shown in Fig. 1. The plasma concentration of unchanged CDDP after a 1 mg/kg dose was determined in order to calculate the pharmacokinetic parameters for the intermittent bolus injections. Plasma concentration-time curves of unchanged CDDP after a single bolus injection were biphasic and the mean terminal elimination half-life of unchanged CDDP was about 18 min. Steady-state plasma conditions were attained within 60 min of starting the continuous infusions, and the ranges for unchanged CDDP were 1.61–2.55 and 1.11–1.57 μgPt/ml for the 2- and 3-h infusions, respectively.

Figure 2 shows the BUN levels during the 5 days after administration of the same total dose (5 mg/kg) by bolus injections, intermittent bolus injections and 2- and 3-h infusions. The mean BUN level 5 days after 5 mg/kg as a bolus was significantly higher (218.6 mg/dl) than the levels after dosing by other schedules. After the intermittent bolus injections and

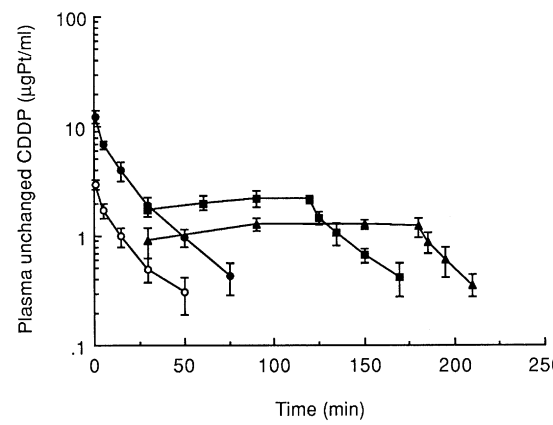

Fig. 1 Plasma concentration-time curves of unchanged CDDP after single bolus injections, and 2- and 3-h infusions of CDDP (○ 1 mg/kg bolus, ● 5 mg/kg bolus, ■ 5 mg/kg 2-h infusion, ▲ 5 mg/kg 3-h infusion). Values are means ± SD of the data obtained from four to six rats

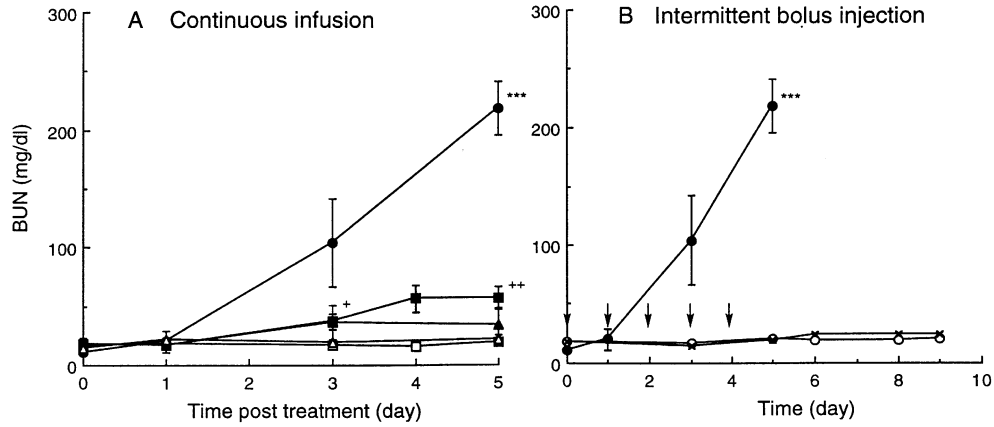

Fig. 2A, B Time-courses of BUN levels during the 5 days after administration of CDDP (5 mg/kg) by continuous infusion (**A**) and intermittent bolus injection (**B**) to rats. ● CDDP bolus; ■ CDDP 2-h infusion; □ 0.9% w/v NaCl solution 2-h infusion (control); ▲ CDDP 3-h infusion; △ 0.9% w/v NaCl solution 3-h infusion (control); ○ CDDP bolus, 1 mg/kg/day for 5 days; 1× 0.9% w/v NaCl solution 1 mg/kg/day for 5 days (control); ↓ time of 5-day bolus dosing. Values are means ± SD of the data obtained from three to five rats. $^{***}P < 0.001$ vs other groups; $^{\dagger,\ddagger}P < 0.05$, 0.01 vs control group

Table 1 Comparison of pharmacokinetic parameters calculated according to a model-independent method of unchanged CDDP, kidney platinum and maximum BUN levels in rats after adiminstration of CDDP (5 mg/kg) by single bolus injection, intermittent bolus injection and continuous infusion (*CI2* 2-h infusion, *CI3* 3-h infusion, *IB* intermittent bolus injection, *Kidney Pt* kidney platinum levels 90 min (bolus), 180 min (CI2) or 240 min (CI3) after CDDP administration, *Kidney* Pt_{5day} kidney platinum levels 5 days after CDDP administration)

	Dosing schedule				ANOVA
	Bolus	CI2	CI3	IB	
C_{max} (μgPt/ml)	16.37 ± 4.67	2.28 ± 0.23	1.35 ± 0.18	3.07[a] ± 0.75	Bolus > *IB* > *CI2* > *CI3* (**)
AUC (μgPt/ml·min)	210.5 ± 37.5	245.0 ± 18.0	224.2 ± 23.5	52.35 × 5 ± 12.2	NS
Ae (%)	34.6 ± 10.5	37.6 ± 6.1	29.8 ± 4.7	Unobtainable	NS
Clt (ml/min/kg)	15.8 ± 2.8	13.3 ± 1.0	14.6 ± 1.5	Unobtainable	NS
Clr (ml/min/kg)	5.29 ± 0.71	4.86 ± 1.16	4.27 ± 0.74	Unobtainable	NS
MRT (min)	23.2 ± 3.2	24.6 ± 2.6	28.5 ± 7.2	26.9 ± 4.7	NS
Kidney Pt (μgPt/g tissue)	17.25 ± 4.01	18.71 ± 3.37	14.50 ± 3.10	Unobtainable	NS
Kidney Pt_{5day} (μgPt/g tissue)	9.90 ± 1.74	7.27 ± 1.56	5.26 ± 0.55	7.45 ± 1.08	Bolus > *IB* > *CI2* > *CI3* (**)
BUN (mg/dl)	218.6 ± 22.9	56.5 ± 9.9	33.6 ± 14.5	24.0 ± 4.0	Bolus > *CI2* > *CI3* > *IB* (***, ***, ***)

[a] Value obtained after single bolus injection of 1 mg/kg CDDP
$^{**}P < 0.01$, $^{***}P < 0.001$, *NS* not significant

3-h infusion, the BUN levels were maintained almost within the normal range and did not differ significantly from those in the corresponding control groups (0.9% w/v NaCl solution, 1 ml/kg daily bolus injection for 5 days and 9 ml over 3-h infusion). The maximum BUN level after the 2-h CDDP infusion was significantly higher than that in the corresponding control group (0.9% w/v NaCl, 6 ml over 2 h).

The pharmacokinetic parameters of unchanged CDDP, kidney platinum and maximum BUN levels after administration of the same total dose (5 mg/kg) by single bolus injection, intermittent bolus injection and 2- and 3-h infusions are summarized in Table 1. The C_{max} values differed significantly, but the AUC, Ae, Clt, Clr and MRT values of unchanged CDDP did not differ significantly among these dosing schedules. The kidney platinum levels 90 min after the single bolus injection and 180 and 240 min after starting the 2- and 3-h infusions did not differ significantly. However, the kidney platinum levels 5 days after dosing and the maximum BUN levels during the 5-day postadministration period differed significantly and tended to be

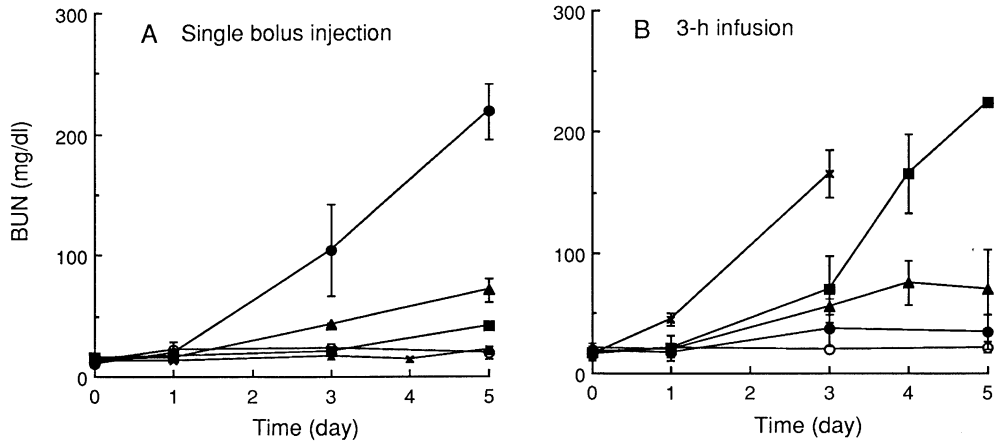

Fig. 3A, B Time-courses of BUN levels during the 5 days after bolus injections (**A**) and 3-h infusions (**B**) of CDDP (**A** × 1 mg/kg, ■ 2.5 mg/kg, ▲ 3.5 mg/kg, ● 5 mg/kg; **B** ● 5 mg/kg, ▲ 6 mg/kg, ■ 10 mg/kg, × 25 mg/kg, ○ 0.9% w/v NaCl solution 5 ml/kg). Values are means ± SD of data obtained from three to four rats

lower with the slower CDDP dosing rate among the dosing schedules.

Effects of dose on pharmacokinetics of unchanged CDDP and nephrotoxicity after single bolus injections and 3-h infusion

The mean BUN levels during the 5 days after single bolus injections (1, 2.5, 3.5, and 5 mg/kg) and 3-h infusions (5, 6, 10 and 25 mg/kg) are shown in Fig. 3. When 1 mg/kg CDDP as a bolus injection and 5 mg/kg as a 3-h infusion were administered, the BUN levels remained almost within the normal range during the 5-day postadministration period. However, the time-courses of BUN were altered depending upon CDDP dose in both dosing schedules. In addition to substantial BUN increases, severe toxicity such as gastrointestinal toxicity (diarrhea) and rapid body weight loss were observed in the rats that received 25 mg/kg CDDP infused over 3 h, and all these rats died within 3 days.

The pharmacokinetic parameters of unchanged CDDP after single bolus injections of CDDP (1, 2.5, 3.5 and 5 mg/kg) were calculated, but no significant differences in Clt or volume of distribution at steady state (Vd_{ss}) between any of the CDDP doses were found (data not shown). The CDDP dose showed significant linear relationships with C_{max} ($C_{max} = 3.27 \times (\text{dose}) - 0.03$, $r = 0.90$, $P < 0.001$), AUC ($AUC = 37.9 \times (\text{dose}) + 4.95$, $r = 0.95$, $P < 0.001$) and kidney platinum level (kidney $Pt = 3.20 \times (\text{dose}) + 1.49$, $r = 0.83$, $P < 0.001$). The pharmacokinetics of unchanged CDDP in rats was found to be linear within the dose ranges used here.

Relationship between pharmacokinetics of unchanged CDDP and nephrotoxicity

The maximum BUN levels showed an exponential-type relationship with C_{max} and AUC after both single bolus injections and 3-h infusions of CDDP (Fig. 4). These pharmacodynamic relationships of the C_{max} or AUC with the BUN levels were different between the groups.

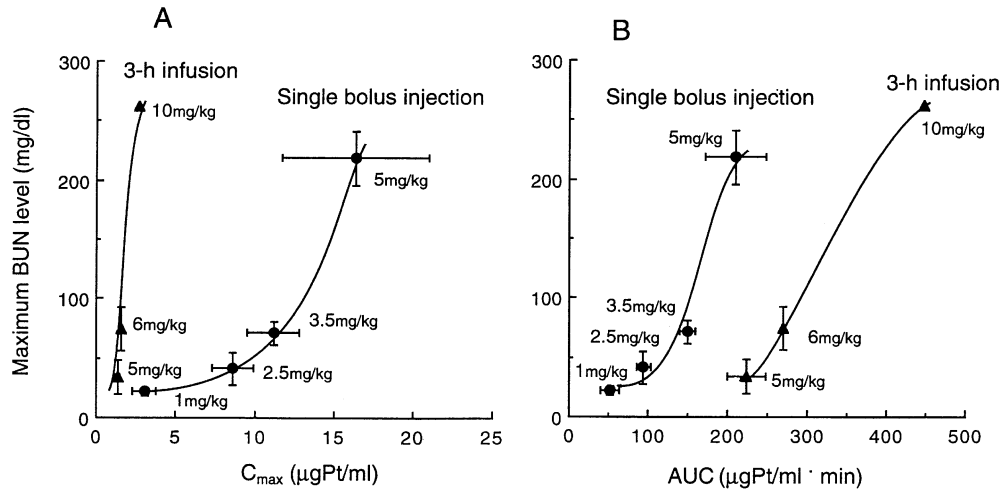

Fig. 4A, B Maximum BUN levels versus C_{max} and AUC after single bolus injections and 3-h infusions of CDDP (● bolus injections, ▲ 3-h infusions). Values are means ± SD

Table 2 Pharmacodynamic analysis for AUC calculated by plasma concentrations of unchanged CDDP greater than the threshold level and maximum BUN level in rats. The pharmacodynamic parameters were estimated using the sigmoid E_{max} model (*CV* coefficient of variation, *AIC* Akaike's information criterion)

C_{min}[a] (μgPt/ml)	BUN_0 (mg/dl)	CV (%)	BUN_{max} (mg/dl)	CV (%)	AUC_{50} (μgPt/ml · min)	CV (%)	γ	CV (%)	AIC
0.5	21.8	358.4	563.3	197.4	452.5	210.1	1.8	253.4	51.6
0.6	26.4	113.8	266.0	56.5	184.5	45.7	3.9	116.2	46.0
0.7	27.8	78.9	255.4	40.4	162.7	33.3	4.2	103.8	42.0
0.8	28.1	55.1	243.4	18.7	141.8	16.9	5.0	71.0	36.7
0.9	27.5	34.6	240.1	9.9	120.3	12.0	5.1	39.8	29.7
1.0	27.5	47.0	242.3	13.2	112.1	15.9	4.8	44.9	32.8
1.1	32.9	35.9	242.4	16.1	106.3	17.5	4.2	55.9	34.1
1.2	39.0	22.8	225.4	10.8	102.8	13.3	7.3	62.0	34.6

[a] Threshold plasma levels of unchanged CDDP

When CDDP was administered by 3-h infusion, the pharmacodynamic relationship was shifted to the left for C_{max}, and to the right for AUC.

Table 2 shows the results of the pharmacodynamic analysis according to the sigmoid E_{max} model in order to describe the relationship between the AUC calculated by plasma concentrations of unchanged CDDP greater than the threshold level ($AUC_{>Cmin}$) and the maximum BUN levels. The estimated plasma threshold level of unchanged CDDP was 0.9 μgPt/ml when the model was most successfully fitted. The estimated pharmacodynamic parameters (BUN_0, BUN_{max}, AUC_{50} and γ) were 27.5 mg/dl, 240.1 mg/dl, 120.3 μgPt/ml · min and 5.1, respectively. Figure 5 shows the relationship between the AUC calculated by plasma concentrations of unchanged CDDP greater than 0.9 μgPt/ml ($AUC_{>0.9}$) and the maximum BUN level in rats. The solid line is the estimated pharmacodynamic model of unchanged CDDP, which was generally described by the following equation, irrespective of dose and schedule:

$$BUN = 27.5 + \frac{240.1\ (AUC_{>0.9})^{5.1}}{(120.3)^{5.1} + (AUC_{>0.9})^{5.1}}$$

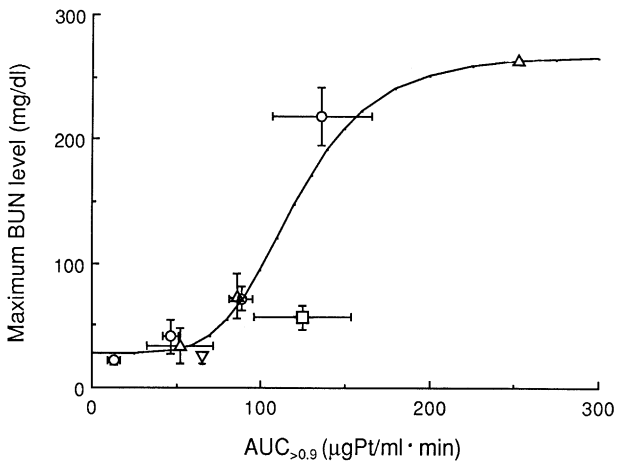

Fig. 5 Relationship between AUC estimated by plasma concentration of unchanged CDDP greater than 0.9 μgPt/ml and maximum BUN level. The simulation curve was obtained using the sigmoid E_{max} model (○ bolus injections, △ 3-h infusion, □ 2-h infusion, ▽ intermittent bolus injections). Values are means ± SD

Discussion

Various dosing schedules have been used to improve the therapeutic index of CDDP in patients undergoing chemotherapy. Of these dosing schedules, continuous infusion has been introduced with considerable success. In patients treated for advanced head and neck, lung and ovarian cancers, nephrotoxicity is well tolerated after infusions of CDDP over 24 h and 5 days, which both show the same level of antitumor efficacy as that observed after shorter infusions [4, 5, 28]. As toxicity is usually the dose-limiting factor in cancer chemotherapy, it has been suggested that pharmacokinetic studies should accompany not only therapeutic response studies but also toxicity studies, and that it may be desirable to link response and toxicity to one or more pharmacokinetic parameters [24].

Although total ultrafilterable platinum is usually monitored as a single species in pharmacokinetic studies of CDDP, it is in fact a mixture of low molecular mass platinum species. We have previously prepared mobile metabolites (MM, comprising ultrafilterable platinum species except for unchanged CDDP) using rat plasma and have compared MM-induced nephrotoxicity with CDDP nephrotoxicity at the same total dose (Pt equivalent), showing negligible renal damage induced by MM compared with CDDP (Hanada and Ogata, unpublished results). It has recently been suggested that monoaqua and monohydroxy species are formed at physiological pH values [3]. However, plasma monoaqua levels are about one-tenth of the levels of unchanged CDDP in patients [2]. Furthermore, unchanged CDDP is easily transported to the rat kidney, which might be linked with its nephrotoxicity [19]. In contrast, monoaqua species in plasma may not be transported to the kidney because it is a charged species. Therefore, although hydrolysis of CDDP has

been considered as an important process in cellular cytotoxicity, the significance of monitoring plasma levels of these species has not been confirmed in vivo. In the present study, the pharmacokinetics of unchanged CDDP were evaluated in rats, and the quantitative relationship between pharmacokinetics of unchanged CDDP and nephrotoxicity were compared after single bolus injections, intermittent bolus injections and continuous infusions of the same total CDDP dose.

After single bolus injections of CDDP doses of 1 to 5 mg/kg, the C_{max} and AUC values of unchanged CDDP and kidney platinum showed linear relationships with the dose, and Clt and Vd_{ss} were constant, suggesting linear pharmacokinetics of unchanged CDDP in rats. There were no differences in AUC, Ae, Clt and Clr values of unchanged CDDP with the same total dose in rats, regardless of the dosing schedule. The Clr of unchanged CDDP exceeded the glomerular filtration rate (GFR) in rats, suggesting that renal tubular secretion of unchanged CDDP occurred. CDDP has been suggested to undergo tubular secretion and reabsorption [6, 9]. The uptake of CDDP into kidney slices [26] and membrane vesicles (Hanada and Ogata, unpublished results) does not show saturation in rats, which suggests that there is a high capacity in the active transport system for CDDP uptake. This may contribute to the linear pharmacokinetics of unchanged CDDP in rats.

The most useful finding of the present study is that nephrotoxicity (BUN increase) could be linked to specific pharmacokinetic parameters of unchanged CDDP, $AUC_{>Cmin}$, and that amelioration of nephrotoxicity by increasing the infusion time or using an intermittent dosing schedule in rats could be explained by the $AUC_{>Cmin}$.

In vitro preclinical studies have indicated that the $C \times T$ value is the best parameter for predicting the efficacy of cell cycle phase-nonspecific anticancer drugs against tumor cells [21, 22]. Therefore, the AUC of unchanged CDDP is suggested to be an important in vivo parameter that reflects the in vitro $C \times T$ value [24]. This type of anticancer drug can be expected to have a similar cell-killing (antitumor) effect, regardless of dosing schedule as long as free AUC is maintained at a constant value [21, 29]. In the present study, considering nephrotoxicity of CDDP in vivo, the AUCs of unchanged CDDP for the same doses were virtually identical in rats (Table 1), even though CDDP was administered according to various dosing schedules. However, nephrotoxicity was dependent on the administration rate. The longer (3-h) infusion ameliorated CDDP-induced nephrotoxicity in rats. The plasma level of CDDP, rather than the AUC is considered to be a determining factor of toxicity [10, 33, 34]. However, severe toxicity was observed after 3-h infusions of CDDP (10 mg/kg), although steady-state plasma levels of unchanged CDDP were maintained less than the C_{max} obtained by 1 mg/kg as a bolus. The pharmacodynamic relationships for C_{max} and AUC apparently differed between the two dosing schedules (Fig. 4). This suggests that neither AUC nor C_{max} are informative pharmacokinetic parameters related to nephrotoxicity.

The concept of threshold plasma level in vivo has been reported in the pharmacodynamic analysis of paclitaxel in humans, in which the duration of plasma concentrations above threshold level ($T > 0.05\ \mu M$) was shown to be an important pharmacokinetic parameter predicting hematologic toxicity [11, 20]. As for the cell cycle phase-nonspecific anticancer drugs, the threshold level has been suggested to describe the anticancer effect of cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). Skipper et al. [29] have reported that a single high-dose administration could be the most effective anticancer schedule for cyclophosphamide, BCNU and CCNU in mice in vivo with the same dose although no quantitative analysis was performed in that study. As a possible reason for such a schedule-dependent anticancer effect, a simulation study [31] used the AUC calculated by plasma concentrations greater than the threshold level in a pharmacodynamic model. In vitro cytotoxicity studies using cultured tumor cell lines have shown that at least 1 μg/ml CDDP needs to be added to the medium to obtain more than two log decades in cell survival after longer exposure times (more than 24 h) [1, 22]. Although the present study was performed on in vivo situation (or condition), the estimated threshold plasma level may provide a reasonable indication of nephrotoxicity.

The pharmacodynamic parameter, BUN_0, that is the BUN level without CDDP treatment, was estimated as 27.5 mg/dl in the final model. This value was close to the normal BUN range in rats. The BUN values of rats that died in the present study were about 250 mg/dl. Therefore, the estimated BUN_{max} in the present pharmacodynamic model means the BUN level resulting with death of individual rats as a side effect of CDDP.

In conclusion, the significance of the $C \times T$ value for cell cycle phase-nonspecific anticancer drugs based on in vitro kinetic analysis could be applied to nephrotoxicity of CDDP in vivo. The AUC calculated from plasma concentrations of unchanged CDDP greater than the threshold plasma level was found to be an important pharmacokinetic parameter related to the toxicodynamics (nephrotoxicity) of CDDP.

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