Alterations in Cisplatin Pharmacokinetics and Its Acute/Sub-chronic Kidney Injury over Multiple Cycles of Cisplatin Treatment in Rats

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Cisplatin (CDDP)-induced acute kidney injury (AKI) is a major clinical concern. CDDP treatment is generally conducted with multiple cycles; the magnitude of the CDDP-induced AKI may be altered by these cycles. Moreover, sub-chronic kidney injury (sCKI) induced by repeated CDDP treatment is often associated with renal interstitial fibrosis, potentially leading to chronic kidney disease. Therefore, it is suggested that the management of not only AKI but also sCKI induced by CDDP in multiple cycles plays an important role in the outcome of CDDP-based chemotherapy. This study investigated the alteration in pharmacokinetics and toxicodynamics of CDDP that was repeatedly administered for three cycles in rats; a cycle consisted of CDDP (5.0 mg/kg, bolus injection) followed by a 21-d washout period. AKI and sCKI were evaluated by plasma creatinine concentration. In repeated multiple administration of CDDP, renal clearance was decreased and the amounts of accumulated Pt in kidneys increased by the cycle. AKI and sCKI were similarly exacerbated by the cycle, whereas the degree of AKI showed a large inter- and intra-individual variation in each cycle. However, the degree of sCKI constantly increased (creatinine increasing ratio in any cycle is about 150%), suggesting that the degree of sCKI in any given cycle was predictable by monitoring the initial creatinine baseline. In this study, therefore, it is suggested that the evaluation of sCKI by monitoring creatinine concentration at base is important for the estimation of CDDP-induced nephrotoxicity. These results may provide useful information for more effective and safe CDDP-based chemotherapy with evidence-based dose adjustment.

Key words cisplatin; repeated administration; pharmacokinetics; creatinine; kidney injury

Cisplatin [cis-diaminedichloroplatinum (II), CDDP] is widely used as a key antitumor agent. However, despite its clinical usefulness, CDDP-induced nephrotoxicity is one of the dose-limiting side effects and a major clinical concern; recent retrospective studies have reported that approximately one-third of patients treated with CDDP-based chemotherapy experience acute kidney injury (AKI), even with plausible renoprotective strategies such as co-administration with hydration. Therefore, the prediction and prevention of CDDP-induced AKI still remain a challenge.

The pharmacokinetics (PK) of CDDP and the pathophysiology of CDDP-induced AKI have been intensively investigated. A unique feature of CDDP PK is the non-specific and irreversible binding of CDDP to proteins; after administration, CDDP rapidly and irreversibly binds to plasma proteins to form inactive CDDP complexes. Therefore, irreversible binding of CDDP to plasma proteins should be considered as metabolic inactivation. Unbound CDDP is excreted mainly by glomerular filtration and partially by active transport mediated via organic cation transporter (OCT)-2 and multi-drug and toxin extrusion transporter 2. During distribution and excretion processes, CDDP accumulates in proximal tubular epithelial cells and contributes to nephrotoxicity. The pathophysiology of CDDP-induced AKI is highly complicated, but several underlying mechanisms involved have been revealed by recent studies; generation of reactive oxygen species, activation of apoptosis signaling pathways, and stimulation of inflammation have been demonstrated as one of the leading causes of nephrotoxicity. On the basis of these findings, novel approaches to prevent AKI, such as co-administration of an OCT-2 inhibitor or antioxidants, have recently been investigated, whereas further clinical evaluations should be warranted.

CDDP treatment is conducted in multiple cycles but not in a single cycle. One cycle generally consists of a single administration or 5-d consecutive administrations of CDDP followed by a 2- or 3-week washout period. It has been reported that the magnitude of CDDP-induced AKI is altered by the cycle; after the washout period, CDDP-induced AKI in the 2nd cycle showed less toxicity than that in the 1st cycle, whereas the toxicity in the 3rd and 4th cycles was higher in an accumulative manner. In addition, although CDDP-induced AKI is generally considered to be reversible, long-term CDDP treatment may induce a cumulative nephrotoxicity [sub-chronic kidney injury (sCKI)]. The creatinine (Cr) clearance in cancer patients treated with multiple CDDP cycles decreased by cycle, even with washout periods. sCKI induced by repeated CDDP administration is often associated with renal interstitial fibrosis and may be irreversible, potentially leading to chronic kidney disease. Therefore, the management of not only AKI but also sCKI induced by CDDP in multiple cycles plays an important role in the outcome of CDDP-based chemotherapy. However, little information is available on the relationship between PK and nephrotoxicity of CDDP under repeated administration. The present study investigated PK and toxicodynamics (TD) of CDDP with multiple administra-
tion cycles in rats.

**MATERIALS AND METHODS**

**Animals and Materials** Male Wistar rats (weight, 240–260 g; 11 weeks old) were purchased from Nippon SLC Co., Ltd. (Hamamatsu, Japan). Nitric acid (HNO₃), sodium fasted overnight on the day before CDDP administration in a dark cycle (light phase, 8:00–20:00) for 3 d, and then fasted overnight on the day before CDDP administration in 1st CDDP cycle. CDDP was obtained by Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Nitric acid (HNO₃), sodium N,N-diethylthiocarbamate (DCC) trihydrate, isoamylalcohol (IAA), and other regents in analytical grades were used without further purification.

**PK Study** A cycle of CDDP administration consisted of the bolus injection of CDDP, followed by a 21-d washout period; this cycle was repeated maximum 3 times. Rats were allocated to the 3 groups based on the number of CDDP cycles: the 1st, 2nd, and 3rd cycle groups (n=6/group). On the day of the last CDDP administration (e.g., day 21 in 2nd cycle group) we ligated their urethra and cannulated the bladder using a polyethylene catheter (0.5 mm i.d., 0.8 mm o.d.). After confirming a stable urine flow rate (approximately 5 µL/min), the bolus injection of CDDP (5.0 mg/kg, 1 mg/mL CDDP in saline) was performed via the femoral vein at 14:00. Blood samples (200 µL) were collected via the jugular vein at 5, 15, 30, 45, 60, 90, and 120 min after CDDP administration. Simultaneously, urine samples were also collected via cannulation of the bladder at 15-min intervals after the administration. To separate the unbound CDDP from blood components, blood samples were immediately centrifuged at 2840×g for 3 min, and the obtained plasma fraction (50 µL) was added to 2-time volume of acetonitrile, vortexed vigorously, and centrifuged again at 15460×g for 5 min. Likewise, after 10-time dilution with saline, urine samples were also conducted with the same procedure as blood samples. The resultant supernatants were collected as the unbound CDDP plasmas and urine samples. This separation procedure of unbound CDDP from sample matrices was performed within 15 min. At the end of the PK study (120 min after bolus injection of CDDP), rats were sacrificed by bleeding from the inferior vena cava and immediately perfused with ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove blood from the kidneys. Subsequently, both kidneys were harvested, weighed, and frozen at −80°C. Unbound CDDP plasma and urine samples were also stored at −80°C until further analysis.

**Assay for Unbound CDDP in Plasma and Urine Samples and Platinum (Pt) in Kidney Samples** After thawing at the room temperature (25°C), kidneys were homogenized with 4-time volume of PBS on ice. The homogenate kidney samples (100 µL) were added to 200 µL of concentrated HNO₃ and wet-ashed at 85°C for 20 h. The following extraction procedure and determination of CDDP using liquid chromatography-tandem mass spectrometry (LC-MS/MS) were conducted with a previously reported method with some modifications. A 100-µL wet-ashed kidney sample as well as unbound CDDP plasma and urine samples were adjusted to a pH of 3–4 with 10-mol/L sodium hydroxide and 1-mol/L HNO₃. To form chelates with Pt⁺, the pH-adjusted samples were then added to 10 µL of 1-mol/L DCC and vortexed. The mixtures were added to 100 µL of IAA to extract Pt⁺ chelates and DDCs. After vortexing and centrifuging (15460×g for 15 min), the IAA layer was obtained, acidified with 100 µL of 1-mol/L oxalic acid, and vortexed and centrifuged again. A 1-µL aliquot of the obtained IAA layer was injected into the LC-MS/MS system, which consisted of the Agilent 1200 Infinity LC (Agilent Technologies Inc., Santa Clara, CA, U.S.A.) and QTRAP 4500 tandem mass spectrometry systems (AB SCIEX, Warrington, U.K.). Methanol was used as the mobile phase and was pumped at a flow rate of 0.2 mL/min without a separation column. Mass spectrometry was conducted with electrospray ionization in the positive mode and multiple reaction monitoring under a source temperature of 400°C and collision energy of 39 V. The multiple reaction monitoring was performed on the basis of transition from the precursor ion Pt(DCC)₃⁺ to the product ion Pt(DCC)⁺; Δm/z 639–491. The ions were monitored using EI in the positive mode. Calibration curves were obtained by spiking a known quantity of CDDP into the corresponding CDDP-free matrix. The Pt⁺ concentration derivatives in samples were quantified by calculation from each peak area and converted to unbound CDDP concentration in plasma and urine samples and to Pt⁺ amounts in kidney homogenate samples. The relative standard error of inter- and intra-assay reproducibility of CDDP was 5–10% according to the International Proficiency Testing Scheme (DW Holt, Analytical Services International, Ltd., London, England); the lower limits of quantification in unbound CDDP in plasma and urine and Pt⁺ concentration of kidneys were <0.01 and <0.1 µg/mL and <0.1 µg Pt/g tissue, respectively.

**PK Analysis of Unbound CDDP** Non-compartmental PK analysis was applied to the plasma concentration–time data and using Phoenix® WinNonlin® 6.4 software (Certara LP, Princeton, NJ, U.S.A.). The terminal slope (λz) was determined using the linear regression of at least 3 data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve from 0 to 120 min (area under the curve (AUC)[plasma,0–120]) was determined using the linear trapezoidal rule up to the last measured plasma concentration ([C][plasma,0–120]) was extrapolated to infinity using the trapezoidal rule. The total body clearance ([CL][tot]) was calculated using the linear trapezoidal rule. The terminal elimination half-life (t½z) was calculated as ln(2)/λz. The area under the first-moment curve from 0 to infinity (AUMC[0–∞]) was also calculated using the linear trapezoidal rule and the addition of the correction term after the last measured point (λz). The mean residence time ([MRT]) was calculated as AUMC[0–∞]/CL[plasma]. The mean residence time ([MRT]) was also determined using the linear trapezoidal rule and the addition of the correction term after the last measured point (λz). The total body clearance ([CL][plasma]) was calculated as dose/AUC[plasma]. The volume of distribution at steady state ([Vd][plasma]) was calculated as CL[plasma]×MRT. The terminal slope of urinary excretion rate curve ([K][ur]) was determined using the linear regression of at least 3 data points from the terminal portion of the urinary excretion rate–time plots. The area under the urinary excretion rate curve from 0 to 120 min ([AUC[0–120]) was calculated using the linear trapezoidal rule up to the last measured urinary excretion rate ([R][ur]) and AURC from 0 to infinity ([AUC[0–∞]) was extrapolated to infinity using a correction term, [R][ur]/K[ur]. The cumulative amount of CDDP urinary excretion ratio ([A]) was calculated as AUC[0–∞]/
dose. Plasma unbound CDDP has a dual-elimination pathway, such as renal excretion (renal clearance, $CL_r$), and irreversibly binding to plasma proteins (metabolic inactivation clearance, $CL_m$). Here, $CL_r$ is secondarily obtained by multiplying $CL_{tot}$ with $A_e$, which is the actual renal excretion ratio. $CL_m$ is the non-renal clearance, $CL_{tot}−CL_r$.

**TD Study** The TD study was performed separately from the PK study. A cycle of CDDP administration was the same as that in the PK study, but CDDP administration was repeated 3 times to all rats in the TD study. At 14:00 on the first day of the 1st cycle (day 0), rats (n=12) received CDDP (5.0 mg/kg, 1 mg/mL CDDP in saline) via the left femoral vein and were housed in individual cages with ad libitum access to food and water during the washout period. On the last day of the 1st cycle (day 20), rats were fasted overnight, and on the next day (day 21), they received the 2nd CDDP administration in the same manner as that in the 1st cycle. The CDDP administration was performed for 3 cycles in the TD study. The plasma Cr concentration was monitored during a cycle as an index of renal function; blood samplings (200 µL, at 14:00) were taken transdermally via the right jugular vein immediately before CDDP administration, every day up to day 7, and every other day from day 8 till the end of each cycle. Plasma fraction (100 µL) was separated from the blood and stored at −80°C until analysis.

**Evaluation of CDDP-Induced AKI and sCKI** The 2012 Kidney Disease: Improving Global Outcomes defined AKI as an increase in serum Cr to ≥1.5-fold from the baseline within until analysis. The previous 7 d or an increase in serum Cr by ≥0.3 mg/dL and chronic kidney disease as abnormalities of kidney structure or function present for greater than 3 months with implications for health.20,21 It has been reported that the Cr concentration greatly increased 2 or 3 d after CDDP administration, reached a peak at approximately 5 d, and subsided within 10 d13,14; nevertheless, Cr concentration did not return to the value before administration.22,23 Based on this information, AKI and sCKI, as an original index in this study, were defined as the maximum Cr concentration (Cr$_{max}$) within the first 8 d (day 0–7) and the arithmetic mean Cr concentration on day 11–19 (Cr$_{mean}$) of each cycle, respectively (AKI and sCKI in this study do not indicate a pathological condition).

**Assay for Cr in Plasma Samples** Plasma Cr concentrations were determined using LC-MS/MS conducted as per a previously reported method with some modifications.24 A 90-µL sample of plasma was added to 10 µL of 1 µmol/L HCl and mildly vortexed. Then, 40 µL of each mixture was added to 360 µL of 0.1% formic acid–acetonitrile (1:9, v/v), vortexed, and centrifuged (4°C, 15460 × g, 15 min). A 3-µL aliquot was injected into the LC-MS/MS system. The 0.1% formic acid–acetonitrile (1:9, v/v) was used as a mobile phase and was pumped at a flow rate of 0.2 mL/min with an insertSustein™ C18 column (2.1 mm i.d.×100 mm, 3 µm size, GL Sciences Inc., CA, U.S.A.). Mass spectrometry was conducted with electrospray ionization in the positive mode and multiple reaction monitoring under a source temperature of 300°C and collision energy of 43 V. The multiple reaction monitoring was performed with transition m/z 639→491. Calibration curves were obtained by spiking a known quantity of Cr in plasma. The Cr concentration in samples was quantified by calculation from each peak area and converted to Cr concentrations in plasma samples. The relative standard error of inter- and intra-assay reproducibility of Cr was <5%; the lower limit of quantification in Cr in the plasma was <0.01 mg/dL.

**Statistical Analysis** All values were expressed as the mean±standard error (S.E.). Statistical analysis of the PK study was performed using one-way factorial ANOVA with Scheffe’s post-hoc test; that of the TD study was performed using Friedman’s test with Steel–Dwass post-hoc test. Differences were considered significant for p<0.05. Statistical analyses were performed using the SPSS software package, version 22.0 (SPSS Inc., Chicago, IL, U.S.A.).

**RESULTS**

**PK Study** Figure 1 depicts the unbound CDDP concentration–time profiles in plasma and cumulative urinary unbound CDDP excretion in each cycle and PK parameters are summarized in Table 1. After CDDP administration, plasma unbound CDDP concentration–time profiles, were very similar in any cycles (Fig. 1A); there was no significant difference in any parameters obtained using non-compartmental PK analysis, except $CL_r$, $CL_m$ in the 3rd cycle was about 2.5-times lower than

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Fig. 1. The Unbound CDDP Concentration Profiles in Plasma (A) and the Cumulative Amounts of Urinary Excreted CDDP (B) after Intravenous Administration of CDDP (5.0 mg/kg) to Rat in Each Cycle

The symbols indicates those in the 1st cycle (○), in the 2nd cycle (△), and in the 3rd cycle (□), respectively. Each data with a bar represents the mean±S.E. of 6 rats in each cycle.
that in the 1st cycle. In addition, $AUC_{\text{plasma,0–120}}$ and $A_e$ were significantly decreased by cycle; and the amounts of accumulated Pt in kidneys was extremely increased by cycle (Table 1).

**TD Study** During the entire study period, body weights at each administration were not significantly altered (1st cycle: 248±5 g, 2nd cycle: 233±7 g, and 3rd cycle: 237±9 g). Plasma Cr concentration–time profiles and TD parameters in each cycle after CDDP administration to rats are shown in Fig. 2 and Table 2, respectively. In any cycle, the mean Cr concentration profiles were very similar; Cr concentrations

### Table 1. Pharmacokinetic Parameters of CDDP over Three Cycles of CDDP Treatment in Rats

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>1st cycle</th>
<th>2nd cycle</th>
<th>3rd cycle</th>
</tr>
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<tbody>
<tr>
<td><strong>Non-compartmental PK parameters</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>$AUC_{\text{plasma,0–120}}$ (µg·min/mL)</td>
<td>182±16</td>
<td>213±38</td>
<td>213±14</td>
</tr>
<tr>
<td>$t_1/2$ (min)</td>
<td>42.9±7.9</td>
<td>28.7±1.5</td>
<td>49.9±7.1</td>
</tr>
<tr>
<td>$V_dss$ (mL)</td>
<td>196±23</td>
<td>153±21</td>
<td>195±25</td>
</tr>
<tr>
<td>$CL_{tot}$ (mL/min)</td>
<td>7.07±0.65</td>
<td>6.37±1.17</td>
<td>5.67±0.49</td>
</tr>
<tr>
<td>$CL_r$ (mL/min)</td>
<td>2.79±0.45</td>
<td>1.96±0.38*</td>
<td>1.10±0.09**</td>
</tr>
<tr>
<td>$CL_m$ (mL/min)</td>
<td>4.28±0.51</td>
<td>4.41±0.84</td>
<td>4.60±0.41</td>
</tr>
<tr>
<td><strong>The urinary parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AUC_{\text{urine,0–120}}$ (µg·min/mL)</td>
<td>72954±8186</td>
<td>46707±3319*</td>
<td>22761±3871**†</td>
</tr>
<tr>
<td>$A_e$ (% of dose)</td>
<td>36.6±5.1</td>
<td>30.7±2.4</td>
<td>19.8±2.1**††</td>
</tr>
<tr>
<td>Amount of renal Pt (µg/g tissue)</td>
<td>13.4±1.1</td>
<td>26.0±4.1*</td>
<td>56.4±4.2**††</td>
</tr>
</tbody>
</table>

$AUC_{\text{plasma,0–120}}$ and $AUC_{\text{urine,0–120}}$: area under the plasma and urine concentration–time curve from 0 to 120 min after CDDP administration, $t_1/2$: elimination half-life, $V_dss$: steady-state volume of distribution, $CL_{tot}$: total body clearance, $CL_r$: renal clearance, $CL_m$: metabolic clearance, $A_e$: cumulative amount of CDDP urinary excretion ratio. *$p<0.05$, **$p<0.01$ compared with 1st cycle, †$p<0.05$, ††$p<0.01$ compared with 2nd cycle, one-way factorial analysis of variance and Scheffe’s test ($n = 6$).
dramatically increased on approximately day 3 after CDDP administration, and reached a peak on day 4–6. Following this, Cr concentrations almost returned to the baseline before each CDDP administration, and Cr base in each cycle gradually increased (Fig. 2B). Cr base in any cycle showed an approximate 150% increase compared with the Cr concentration before administration (Table 2). Figure 2C shows the overlay relative Cr level profiles in each cycle; Cr concentration profiles closely overlapped in all cycles. The TD alterations in each individual rat are depicted in Fig. 3; the individual Cr max showed a large cycle-by-cycle variation, whereas the mean values were significantly increased by cycle (Table 2). In contrast, the mean Cr base is also increased by cycle; however, there is a small variation in any cycle.

**PK–TD Relationship Study** Figure 4 shows the relationship between Cl, and the amounts of accumulated Pt in kidneys, and TD parameters. Previous Cr base (Pre-Cr base) corresponds to Cl. A significant negative linear correlation be-

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**Table 2. Toxicodynamic Parameters over Three Cycles of CDDP Treatment to Rats**

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Pre-dose</th>
<th>1st cycle</th>
<th>2nd cycle</th>
<th>3rd cycle</th>
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<tbody>
<tr>
<td></td>
<td>Mean±S.E.</td>
<td>Mean±S.E.</td>
<td>Mean±S.E.</td>
<td>Mean±S.E.</td>
</tr>
<tr>
<td>Cr max (mg/dL)</td>
<td>N.D.</td>
<td>0.906±0.078</td>
<td>1.46±0.27†</td>
<td>2.17±0.48††</td>
</tr>
<tr>
<td>Cr base (mg/dL)</td>
<td>0.208±0.007</td>
<td>0.329±0.016</td>
<td>0.483±0.027**</td>
<td>0.805±0.067**††</td>
</tr>
<tr>
<td>Cr increasing ratio (%)</td>
<td>N.D.</td>
<td>153±4</td>
<td>144±9</td>
<td>143±5</td>
</tr>
</tbody>
</table>

Cr max: maximum Cr concentration in 0–7 d after CDDP administration, Cr base: arithmetic mean Cr concentration in 11–19 d after CDDP administration, Cr increase ratio; % difference in Cr concentration between day 0 and day 19, N.D.: no data, **p<0.01 compared with pre-dose, †p<0.05, ††p<0.01 compared with 1st cycle, #p<0.05, compared with 2nd cycle, Friedman’s test and Steel–Dwass test (n=12).
tween \( CL_t \) and Pre-Cr\(_{\text{base}}\) was recognized (Fig. 4A, \( r = 0.998 \)); in addition, a significant positive linear correlation was found between the amounts of accumulated Pt in kidneys and \( C_{\text{base}} \) (Fig. 4B, \( r = 0.999 \)).

**DISCUSSION**

The aim of the present study was to investigate the alterations in PK and nephrotoxicity of CDDP treated with multiple cycles in rats. After CDDP administration in each cycle, unbound CDDP plasma concentration–time profiles and PK parameters obtained using non-compartmental PK analysis were not significantly different in any cycles, whereas \( CL_t \) significantly decreased by cycle (Fig. 1A and Table 1). It has been reported that approximately one-third of the intact CDDP administered is excreted from the kidneys by glomerular filtration and tubular secretion, and the remaining approximately two-thirds are eliminated by irreversible binding to proteins (namely, are metabolized into inactive complexes).\(^{25} \) The alteration of \( CL_t \) is assumed to have a minor impact on CDDP PK and to not cause a significant alteration in the systemic exposure because \( CL_t \) is relatively lower than \( CL_m \). On the contrary, the amounts of accumulated Pt in kidneys significantly increased by cycle. Some reports have demonstrated that the turnover rate in the proximal tubular epithelium is slow.\(^{26,27} \)

Devarajan et al.\(^{28} \) reported that the amount of accumulated renal Pt reached a steady state at 1 h after CDDP administration; thereafter, Pt was hardly eliminated from the kidney. Moreover, in our previous study, we reported that Pt in kidney has a long elimination half-life (100–300h).\(^{25} \) The distributed Pt in renal epithelial cells is retained by a covalent bond to proteins in the somata without being eliminated. Although the detailed mechanism remains to be elucidated, it may be conjectured that the main turnover pathway of impaired renal cells is autophagy.\(^{29} \) Based on these reports, Pt accumulation in kidneys is considered too short-term compared to Pt elimination at this dosage and dosing interval. In line with this statement, the increase in urine volume and the decreases in urinary CDDP concentration and \( A_d \) were monitored at each cycle; the amounts of accumulated Pt in kidneys increased with decreasing \( A_d \) and were accelerated by cycle (Fig. 1B and Table 1). In addition, \( CL_t \) most strongly correlated with Pre-Cr\(_{\text{base}}\) (Fig. 4A). These results suggest that the CDDP administration induce sCKI, and subsequently, sCKI decreases the CDDP renal excretion in the next cycle, leading to the acceleration of Pt accumulation in kidneys by cycle.

The mean \( C_{\text{max}} \), an indicator of AKI, increased by cycle (Fig. 2A, B and Table 2). The time to reach the \( C_{\text{max}} \) (on the day 4–6) were similar with previous studies,\(^{3,25,30} \) and were observed in all cycles in the present study, indicating CDDP-induced AKI. However, AKI of each individual rats showed a large inter- and intra-individual variation (Fig. 3A and Table 2); some rats showed lower toxicity when compared with that of the previous cycle. Mizushima et al.\(^{14} \) have reported that when CDDP (4mg/kg) was injected repeatedly at intervals of 3 weeks, a decrease in nephrotoxicity was observed after the 2nd injection; however, after the 3rd injection, nephrotoxicity increased. Miyaji et al.\(^{13} \) also reported that a rechallenge with the same dose of CDDP 14d after the first dose of CDDP induced significantly less injury, and that the overexpression of p21 and proliferating cell nuclear antigen in rechallenge injury may contribute to the acquired resistance in CDDP-induced nephrotoxicity. On the basis of these reports, the reason for the large inter- and intra-variability of AKI in this study may be an acquisition of transient resistance to CDDP. Interestingly, the rats with mild AKI tended to show more severe AKI in the next cycle, and vice versa (Fig. 3A). Consequently, the CDDP-induced AKI in each cycle should be responsible not only for PK alterations but also for host defense responses. Further studies are necessary to evaluate their contributions.

The mean \( C_{\text{base}} \), as an indicator of sCKI, also increased by cycle (Fig. 2 and Table 2). In contrast to AKI, sCKI showed a small inter- and intra-individual variation and significantly increased by cycle (Fig. 3B); it was noted that sCKI consistently increased in all rats over three cycles. There is only limited information available about CDDP-induced chronic nephrotoxicity. It has been reported that the pathophysiological mechanisms leading to renal interstitial fibrosis are considered to be related not directly to the primary effect of CDDP, but to the activation of pro-inflammatory and pro-fibrotic factors\(^{22} \) and imbalance between extracellular matrix synthesis and degradation.\(^{46} \) Although the detail mechanism of chronic nephrotoxicity remains uncertain, there is a lot of information about the mechanism of AKI as described in the introduction. Therefore, sCKI and AKI may be caused by different pathways. In this study, regardless of the level of AKI in any cycle the following sCKI is worsened, supporting this consideration. In addition, these results suggest that the acquisition of resistance by AKI does not affect the following sCKI. In addition, the degree of sCKI strongly correlated with the amounts of accumulated Pt in kidneys in the present study (Fig. 4B), i.e., the amounts of accumulated Pt in kidneys reflect the degree of sCKI. Because the impaired kidney function represented by Cr\(_{\text{base}}\) accelerates the renal accumulation of CDDP in the next cycle as discussed above, sCKI should increase exponentially by cycle. As shown in Table 2, Cr increase ratio in any cycle is about 150%. Therefore, the degree of sCKI in any given cycle may be predictable by the following equation:

\[
C_{\text{base}, n} = 1.50^n \times \text{Pre-dose at } C_{\text{base}} \quad (n = 1–3)
\]

where \( n \) is the number of cycles; \( C_{\text{base}, n} \) is the Cr concentration at the end of \( n \)th cycle of CDDP (viz., sCKI); and the coefficient indicates the Cr increase ratio (Table 2). The value of coefficient should be inherent to the protocol of CDDP treatment (5mg/kg, bolus injection to rats in this study). Therefore, there is a possibility of predicting the sCKI by monitoring of Pre-dose at \( C_{\text{base}} \).

The dosage of CDDP (5.0mg/kg) has been reported to induce detectable nephrotoxicity and low mortality.\(^{13,25} \) This dosage is approximately 2-fold larger than the clinical dosing (highest recommended dosage: 100mg/m\(^2\)); moreover, the relationship between the total dose of CDDP and renal function remains to be clarified.\(^{31} \) Thus, in our laboratory, the nephrotoxicity in multi-cycle CDDP at various dosage is now in progress with the aim to reflect actual clinical settings.

**CONCLUSION**

In repeated multiple administration of CDDP, \( CL_{clp} \), which plays an important role in CDDP elimination, did not change by cycle; however, \( CL_t \) was decreased and the amounts of ac-
cumulated Pt in kidneys was increased by cycle. Additionally, AKI and sCKI were similarly exacerbated by cycle, whereas the degree of AKI showed a large inter- and intra-individual variation in each cycle, which may be an acquisition of resistance to CDDP. However, the degree of sCKI constantly increased (Cr increase ratio in any cycle is about 150%), suggesting that the degree of sCKI in any given cycle was predictable by monitoring the Cr base at Pre-dose. In this study, therefore, it is suggested that the evaluation of sCKI by monitoring Cr base is important for the estimation of CDDP-induced nephrotoxicity, whereas more investigation of the individual factor of increasing Cr base by cycle is necessary. The results of this study may provide useful information for more effective and safe CDDP-based chemotherapy with evidence-based dose adjustment.

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Conflict of Interest The authors declare no conflict of interest.

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