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Time-dependent Nephrotoxicity Associated with Daily Administration of Cisplatin in Mice

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Abstract

The chronopharmacokinetics and chronopharmacodynamics of cisplatin were studied in a mouse model to reveal the mechanisms of dosing time-dependent nephrotoxicity induced by daily administration. Chronotoxicity was tested by daily intraperitoneal injections of cisplatin ($6\,mg\,kg^{-1}$) for 5 days at four time points (04 00, 10 00, 16 00 and 22 00 h) in BALB/c mice (n = 6 in each group). After following the changes in body weight, serum concentrations of blood urea nitrogen (BUN) and creatinine obtained on day 6 were compared.

The results showed diurnal variations in cisplatin toxicity, with the 04 00 and 16 00 h time points the best and the worst, respectively. We then measured platinum concentrations in blood, liver and kidney and compared the results of the 04 00 and 16 00 h groups (n = 4 in each group). Kidney sensitivity to cisplatin alone, lipopolysaccharide (LPS) alone, cisplatin with LPS and saline (control) were also measured using a tissue culture system (a measurement system of interleukin-6 (IL-6) production) between the 04 00 and the 16 00 h groups (n = 4 in each group). These results showed no significant difference in platinum accumulation between the two groups. IL-6 production was higher in the 16 00 h group than in the 04 00 h group after saline injection alone ($P < 0{\cdot}05$). Cisplatin treatment alone did not increase IL-6 production. However, IL-6 levels were markedly augmented by cisplatin with LPS.

In conclusion, chrononephrotoxicity induced by daily cisplatin administration does not only depend on cisplatin accumulation, but might also depend on kidney sensitivity to diurnal variations in inflammatory reaction without direct cisplatin toxicity.

Cisplatin is widely used to treat patients with testicular, ovarian, cervical and non-small-cell lung cancer (Clerici et al 1998; Kumar et al 1998; Onda et al 1998; Shimizu et al 1998). However, nephrotoxicity and myelosuppression limit its use (Prestayko et al 1979). Cisplatin also has circadian variations in animals and man depending on the time of administration (Hrushesky et al 1982; Hrushesky 1985; Lévi et al 1990). The mechanism of the nephrotoxicity that occurs at the proximal tubules has been well studied pathophysiologically (Weiner & Jacobs 1983). However, the dosing time-dependent nephrotoxicity of CDDP (chronopharmacokinetics and chronopharmacodynamics of cisplatin) in various dosing protocols is not fully understood. According to a recent clinical protocol of CDDP administration, we have focused on repeated administration of low-dose CDDP. In a previous study, we compared chrononephrotoxicity in rats after repeated administration of weekly high doses and daily low doses, and found that the dosing time-dependent nephrotoxicity of cisplatin was significantly augmented by daily low-dose administration (Kobayashi et al 2000).

In this study, we examined the mechanism of the dosing time-dependent nephrotoxicity induced by repeated administration of cisplatin in a mouse

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model, using pharmacokinetic and pharmacodynamic data. The cisplatin concentration was measured and products of interleukin-6 (IL-6) in the kidney were studied using a tissue slice system.

Materials and Methods

Animals

Six-week-old male BALB/c mice were purchased from Japan SLC Ltd, Sizuoka, Japan. The mice were maintained, 5 per cage, under standardized 12-h light–dark cycle conditions in a specific pathogen-free room for at least 2 weeks. During the acclimatization period, food and water were freely available.

Preparation of dosing drug

Cisplatin was supplied by Nippon Kayaku Co. Ltd (Tokyo, Japan). The compound was dissolved in saline and at a concentration of 0·6 mg mL^{-1}. Cisplatin, 6 mg kg^{-1} daily, was administered intraperitoneally in a single dose or once daily for 5 days at arbitrary times. *Escherichia coli* lipopolysaccharide (LPS) was purchased from Sigma (0111: B4 phenol extract; St Louis, MO). LPS was dissolved in saline and administered intraperitoneally at a dose of 2 mg kg^{-1} to burst inflammatory response as previously described (Kayama et al 1995a).

Administration protocol

Experiment 1. Cisplatin was administered intraperitoneally once daily at 04 00, 10 00, 16 00 or 22 00 h for 5 days (n = 6 in each group). The mice were weighed daily; body-weight gain was calculated as the percentage of weight change of each mouse from the initial treatment (day 0). Under ether anaesthesia, blood samples were collected from the heart 24 h after the final cisplatin dose (day 6). Serum concentrations of urea nitrogen (BUN) and creatinine were determined.

Experiment 2. Mice were divided into the following two groups. Cisplatin was administered intraperitoneally once daily at 04 00 or 16 00 h for 5 days (n = 4 in each group). The mice were killed 24 h after the final cisplatin dose (day 6). Blood samples were collected from the heart of each mouse, and the livers and bilateral kidneys were removed. Instead of cisplatin itself, the concentrations of platinum contained in cisplatin were determined in the kidney, liver and blood. The liver and kidney were weighed and treated with a mixture of nitric acid and sulphuric acid until completely digested. The sample solutions were brought to 10 mL with 0·6 N hydrochloric acid in 0·1 N nitric acid and then diluted with 0·6 N hydrochloric acid in 0·1 M nitric acid (1 : 5) to determine the platinum concentration in the organs. Blood samples were diluted 1 : 10 with 0·6 N hydrochloric acid in 0·1 N nitric acid without digestion. The platinum determination was carried out on a 10-μL sample by flameless atomic absorption spectrophotometry using Zeeman effect correction (Hitachi Polarized Zeeman AAS Model Z-8200) under the following conditions: lamp current, 12·5 mA; wave length, 265·9 nm; slit width, 0·40 nm.

Experiment 3. The mice were divided into four groups according to treatment: saline (control); cisplatin; LPS; and cisplatin with LPS. Cisplatin or saline was administered intraperitoneally at 04 00 or 16 00 h (n = 4 in each group). In the LPS group and the cisplatin with LPS group, LPS was administered 1 h before killing. The mice were killed 24 h after cisplatin administration and the right kidney was removed. Tissue slices from the kidneys and quantitation of IL-6 were carried out by a method previously described (Kayama et al 1995a). Briefly, a cylindrical core (6 mm diameter) was used to obtain punch biopsies from the kidneys and the tissue samples were placed into a tissue slicer. The sections were cut, and individual sliced tissues were cultured in trans-well culture dishes containing RPMI1640 with 10% foetal bovine serum. After 30 min incubation, the culture medium was replaced with fresh medium and incubated for another 6 h. Supernatant was collected and frozen at −70°C until assay. IL-6 levels were quantitated by ELISA.

Experiment 4. The mice were divided into cisplatin- or saline-treated groups (n = 4, each group). The agents were given once a day for 5 days at 04 00 or 16 00 h. Following the above method, the mice were killed 24 h after the final cisplatin dose (day 6), and the right kidneys were removed. IL-6 levels were determined by ELISA.

Statistical analysis

Data are shown as the mean ± s.d. Circadian variations in body weights and the concentrations of BUN and creatinine were analysed by a cosinor method. The statistical significance of other data

was validated by analysis of variance. $P < 0{\cdot}05$ was considered significant.

Results

Circadian variations in body weight and BUN and Creatinine levels after repeated cisplatin injections

The body weights in all cisplatin-treated groups of mice significantly decreased at day 6 compared with day 0 (Figure 1). However, the degree of change did not show a significant circadian variation. BUN concentrations on day 5 showed a significant circadian rhythm with a peak at 16 00 h ($P < 0{\cdot}01$). The BUN levels at 16 00 h differed significantly from those at 04 00 and 22 00 h ($P < 0{\cdot}05$ and 0·01, respectively). There was no significant circadian variation in creatinine, although this variable was significantly greater at 16 00 h than at 10 00 h and 22 00 h ($P < 0{\cdot}05$ and 0·01, respectively).

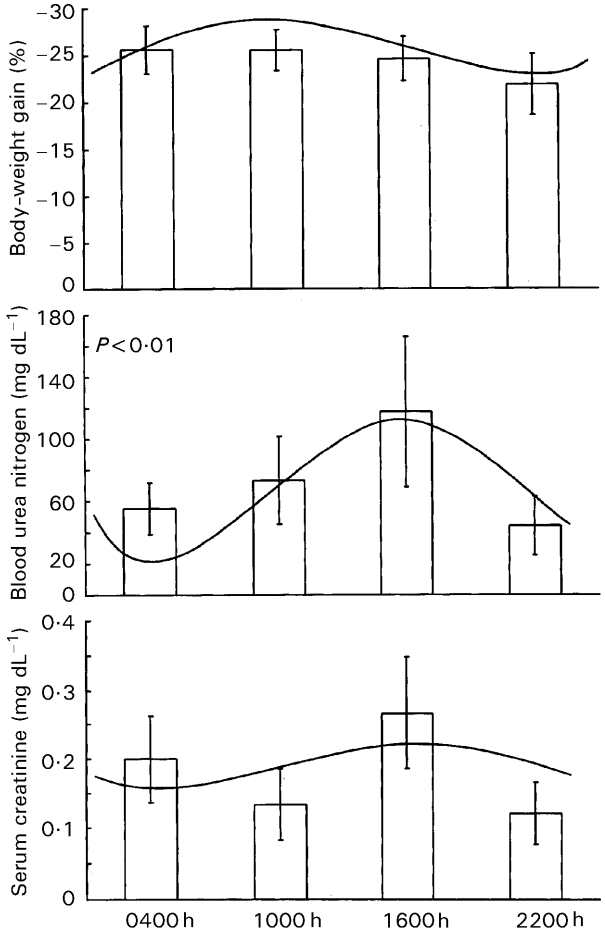

Figure 1. Parameters after 5 days of administration of cisplatin at four time points in mice. Each value is the mean ± s.d. Body-weight gain is shown on day 5 and calculated as the percentage change in each mouse from the initial treatment (day 0). The curved lines indicate the curve of cosinor analysis.

These chronotoxicity data obtained from repeated administrations of cisplatin in mice were well correlated with those of others (Hrushesky et al 1982; Lévi et al 1982a) and with our own (unpublished data) in rats. The 04 00 h and the 16 00 h trials were studied further as the time points with the lowest and the highest toxicity levels, respectively.

Platinum concentrations in kidney, liver and blood after repeated cisplatin

Platinum concentrations at 04 00 and 16 00 h were compared on day 6 in the kidney, liver and blood of the mice that had received five doses of cisplatin (Table 1). There were no significant differences between the two groups.

Local production of IL-6 in the kidney after a single cisplatin dose with or without LPS injection

IL-6 concentrations in sliced kidney specimens after a single injection of saline, cisplatin, LPS or cisplatin with LPS are shown in Figure 2. In the 04 00 h group, LPS tended to increase IL-6 production, but no significant difference was found compared with the saline and the cisplatin groups. However, production was markedly augmented in the cisplatin with LPS group ($P < 0{\cdot}01$). In the 16 00 h trial group, no difference in IL-6 production was found between the controls and the cisplatin groups, but IL-6 production in the LPS and the cisplatin with LPS groups was considerably increased compared with the control group. When IL-6 production in sliced kidney specimens from the 04 00 h group were compared with production in those from the 16 00 h group, the former showed significantly higher production than the latter. This was augmented by the LPS injection.

IL-6 production also was measured in the kidney specimens obtained from mice that received five doses of cisplatin or saline (Table 2). The cisplatin group had augmented IL-6 production compared with the control group, but no significant difference was found.

Table 1. Cisplatin concentrations in kidney, liver and blood after 5-day administration at 04 00 h or 16 00 h in mice.

Dosing time	n	Cisplatin concn		
		Kidney ($\mu g\,g^{-1}$)	Liver ($\mu g\,g^{-1}$)	Blood ($\mu g\,mL^{-1}$)
04 00 h	4	10·768 ± 2·552	15·060 ± 3·169	0·573 ± 0·157
16 00 h	4	10·977 ± 2·639	13·555 ± 5·508	0·573 ± 0·185

Each value represents the mean ± s.d.

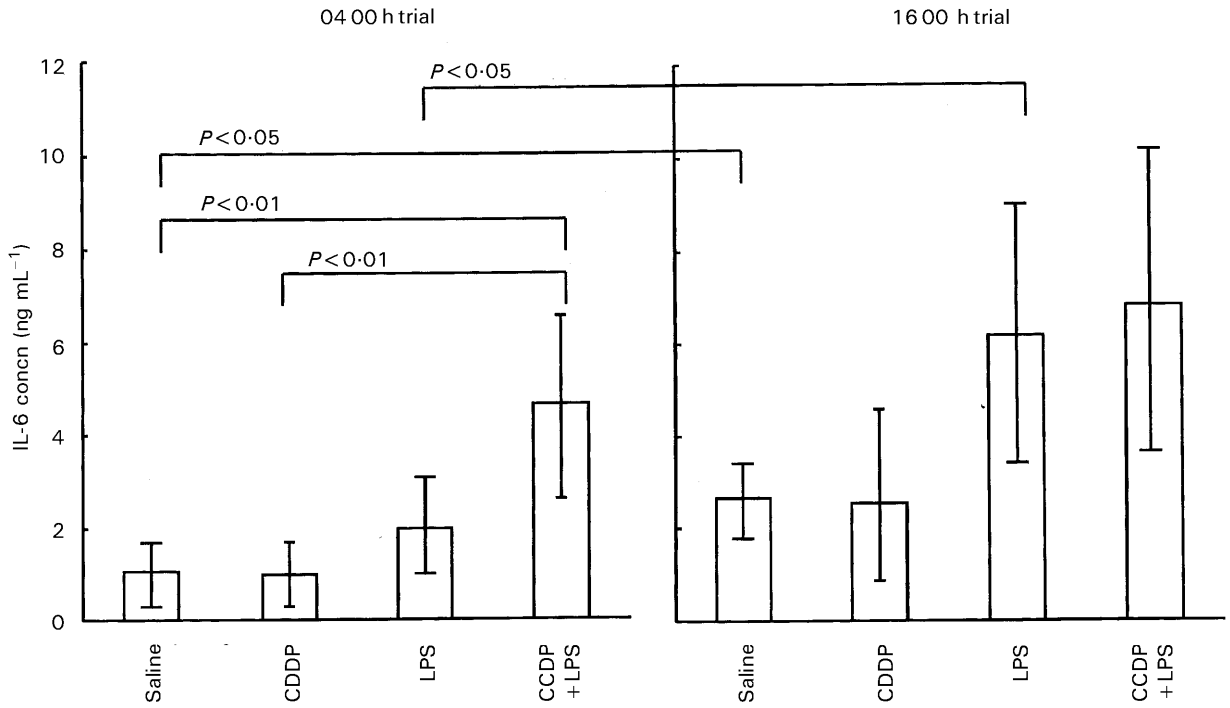

Figure 2. Local interleukin-6 (IL-6) production in kidney after single cisplatin (CDDP) injection at 04 00 or 16 00 h in mice. Each value is the mean ± s.d. LPS, lipopolysaccharide.

Table 2. IL-6 production in the medium cultured with sliced kidney obtained from mice after receiving 5 doses of cisplatin or saline.

Treatment	n	IL-6 concn (ng mL^{-1})		
		04 00 h trial	16 00 h trial	*P* value
Saline	4	2·55 ± 0·93	3·41 ± 1·63	$P = 0.391$
Cisplatin	4	4·95 ± 1·87	5·67 ± 3·09	$P = 0.703$

Each value represents the mean ± s.d.

Discussion

Nephrotoxicity induced by cisplatin administration has been shown pathophysiologically to occur at the proximal tubules initially then at the distal tubules and collecting ducts (Walker & Gale 1981). This harmful effect is thought to be mediated by cisplatin metabolites during transport from the tubular lumen into the tubular cells (Miura et al 1987). Cisplatin accumulation in the kidney might also be a risk factor, because concentrations in the kidney are several times higher than those in plasma (Litterst et al 1977).

Chronotherapy is a novel approach to decrease the frequency and degree of adverse events. Single-dosing studies using rodents showed that cisplatin toxicity was least when the drug was given at 17 hours after lights on (near the mid-activity period) and that nephrotoxicity was minimal when rats received it around the time of peak urinary volume (Hrushesky et al 1982; Lévi et al 1982a). However, the chronopharmacological effect of cisplatin is still unknown when the drug is administered repeatedly. In a previous report, we confirmed that nephrotoxicity was severe in rats during the inactive phase and that daily low-dose cisplatin treatment tended to augment dosing time-dependent nephrotoxicity, compared with weekly high-dose cisplatin treatment (Kobayashi et al 2000).

In this study, we used a mouse model to examine whether the chrononephrotoxicity of repeated cisplatin administration might be caused by dosing time-dependent accumulation of cisplatin. Chronopharmacodynamics were also studied using a system that measured inflammatory cytokine (IL-6) production in the culture medium obtained from sliced kidney specimens. The results showed that the BUN level had a significant circadian variation ($P < 0.01$), with the peak at 16 00 h after daily cisplatin administration for 5 days in mice. However, body-weight gain and creatinine levels did not exhibit significant diurnal variations. In the rat, the dosing time-dependent toxicity of the BUN levels had been reported after single (Hrushesky et al 1982; Lévi et al 1982a) and, recently, daily multiple administrations of cisplatin (Kobayashi et al

2000). The BUN levels were highest when the drug was administered near the mid-inactivity phase in mice, as in rats.

The dosing time-dependent urinary cisplatin pharmacokinetics has been reported after a single injection of cisplatin in rats (Lévi et al 1982b). Urinary cisplatin excretion and concentrations were significantly different between the dosing time of the maximum (acrophase) and minimum (bathyphase) according to a circadian rhythm of urine volume. However, urinary excretion of cisplatin was faster in the acrophase than in the bathyphase, while the urinary concentrations of cisplatin in the bathyphase were greater than those in the acrophase. It is still unknown whether cisplatin accumulates in the kidney when the drug is administered daily. To study whether there is dosing-time difference in the accumulation of cisplatin during repeated administration, cisplatin was administered intraperitoneally once daily at 04 00 or 16 00 h for 5 days. However, platinum concentrations in the kidney, liver and blood after daily cisplatin injections were not significantly different between those two trials. Therefore, it might be considered that the nephrotoxicity caused by cisplatin did not depend on dosing time-dependent accumulation.

In an earlier report, we established a tissue culture system that directly detects organ damage; the level of an inflammatory cytokine such as IL-6 increases in the medium obtained from cultured sliced tissue specimens which suffer damage caused by exogenous agents such as LPS (Kayama et al 1995a). Cytokine-mediated toxicant interactions occur in the liver following LPS priming and exposure to either lead (Honchel et al 1991) or cadmium (Kayama et al 1995b). We reported that LPS increased IL-6 levels in sliced kidney, and enhanced or synergized the effects of cadmium (Kayama et al 1995a). Because cisplatin, as well as cadmium, induces nephrotoxicity at the proximal tubules we studied cisplatin chronotoxicity from the point of chronopharmacodynamics, using this method. The results showed that a single injection of cisplatin did not increase IL-6 production, but repeated administration did. LPS alone, however, significantly induced production of IL-6 in the 1600 h group but not in the 0400 h group. The combination of cisplatin with LPS augmented production of IL-6. These data suggested that cisplatin itself does not induce an inflammatory response but rather an inflammatory reaction that varies during the day in the kidney. It is known that an inflammatory response has a diurnal variation in animals (Banks et al 1998) and man (Lemmer et at 1992; Suzuki et al 1997). Those studies and our data suggest the possibility of an inflammatory response that varies daily which might cause renal damage.

In conclusion, cisplatin nephrotoxicity was enhanced when cisplatin was administered during the inactive phase in rats and mice. Dosing-time-dependent nephrotoxicity may be caused by a delay in urinary cisplatin pharmacokinetics and the augmentation of sensitivity in the inactive phase. Delaying recovery of renal damage in the daily trial may cause more severe chrononephrotoxicity. Clinical studies are needed to evaluate the merit of these findings.

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