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Effects of Dosing Time and Schedule on Cisplatin-Induced Nephrotoxicity in Rats

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Abstract

Renal dysfunction induced by a single injection of cisplatin depends on the timing of the dose. However, the effects of repeated administration of cisplatin on time-dependent toxicity have not been evaluated despite the fact that in clinical practice high doses are repeatedly injected at intervals or low doses are administered daily. We studied chrono-nephrotoxicity in rats after weekly or daily cisplatin injections.

Weekly high doses ($5\,mg\,kg^{-1}$) or daily low doses ($1{\cdot}2\,mg\,kg^{-1}$) of cisplatin were injected at four time points (3, 9, 15 and 21 h after the light was turned on (HALO)) for 3 weeks. Changes in body weight after weekly cisplatin administration were independent of the timing of the doses. In the group that received daily cisplatin, the loss in body weight in the 3 HALO group was smaller than in animals receiving injections at 15 and 21 HALO ($P < 0{\cdot}05$ and 0·001, respectively). The blood urea nitrogen and serum creatinine levels in the control rats showed a significant circadian change (peak at 15 HALO and trough at 9 HALO), but these parameters were markedly elevated in both trials and their circadian variations were disturbed. In conclusion, cisplatin-induced nephrotoxicity was the lowest at 3HALO compared with other time points of both dose regimens.

The effects of a wide variety of drugs change daily depending on the time of administration (Fujimura et al 1989; Saito et al 1991; Ohdo et al 1997). These chronopharmacological changes are considered to have a minor impact on regimens of drugs with a wide therapeutic range, but optimal timing of drug administration is recommended for drugs with a narrow therapeutic range, such as anticancer agents (Lemmer & Bruguerolle 1994; Lévi et al 1997).

From an early stage, cisplatin has been shown to cause dose time-dependent nephrotoxicity in animals (Hrushesky et al 1982; Lévi et al 1982a) and humans (Hrushesky 1985). This chrono-pharmacological phenomenon was mainly demonstrated after one or two administrations of cisplatin (Hrushesky et al 1982; Lévi et al 1982a). Recently, low-dose and daily administrations of cisplatin have been prescribed frequently to reduce adverse effects. Therefore, time-dependent variations in cisplatin-induced nephrotoxicity are of great clinical interest when patients receive daily or weekly doses. This study was undertaken to address this issue in rats.

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Materials and Methods

Animals

Six-week-old male Donryu rats were purchased from Charles River Japan Inc. (Yokohama, Japan) and maintained for at least two weeks in two separate rooms under a constant environment with strictly controlled lighting conditions (12-h light–dark) as previously reported (Yamauchi et al 1998). In one room, the light was turned on and off at 07 00 and 19 00 h, respectively and in the second the light was turned on and off at 19 00 and 07 00 h,

respectively. The rats had free access to food and water. The rats then were divided into four groups according to the time of cisplatin administration at 3, 9, 15 or 21 h after the light was turned on (3, 9, 15 or 21 HALO).

Preparation of agents

Cisplatin was supplied by Nippon Kayaku Co. Ltd (Tokyo, Japan). The compound was dissolved in 0·9% NaCl solution to give a concentration of 0·6 mg mL^{-1}

Cisplatin administration protocols

Weekly high-dose regimen. A high dose (5 mg kg^{-1}) of cisplatin was administered intravenously at four time points (3, 9, 15 or 21 HALO) with a one-week interval for three weeks (total dose, 15 mg kg^{-1}; n = 8–11 in each group). A weekly high-dose regimen of 5 mg kg^{-1} was chosen in this study because adult male Donrhy rats often died as the result of more frequently administered doses.

Daily low-dose regimen. A low dose (1·2 mg kg^{-1}) of cisplatin was injected daily at the same four time points for three weeks (total dose, 25·2 mg kg^{-1}; n = 8 in each group). The maximum dose of cisplatin administered daily (1·2 mg kg^{-1}) for adult male Donrhy rats was used because smaller doses had no anticancer effect.

As a control, this variable of untreated rats (n = 8) was also determined.

Assessment of toxicity

The body weights of the rats were measured weekly. Body-weight change was calculated as the percent of weight change of each rat from the initial level (day 0). The rats were randomly selected and approximately 0·5 mL of blood was obtained from a tail vein at 3, 9, 15 and 21 HALO to serve as a control (non-treated groups, n = 8 in each group). On day 21, the rats were killed 24 h after the final cisplatin injection and blood samples were obtained for analysis of serum concentrations of urea nitrogen (BUN) and creatinine.

Statistical analysis

Data are shown as the mean ± s.d. The BUN and serum creatinine levels of each treated group were analysed by a cosinor method to clarify diurnal variation. The statistical significance between the control group and the cisplatin-treated groups at each time point was validated using analysis of variance. $P < 0.05$ was considered to be significant.

Results

Changes in body weight after repeated cisplatin injections

In the groups of rats that received weekly administration of cisplatin, the high dose markedly suppressed increases in body weight compared with the control group (Table 1). There were no significant differences in this variable between the cisplatin groups at any observation point, except for cisplatin-treated groups at 9 and 15 HALO on day 14 ($P < 0.05$).

In the groups that received daily treatment, low-dose cisplatin also suppressed increases in body weight compared with the control group (Table 2). The maximum body-weight gain was observed in the 3 HALO group throughout this study. When single doses of cisplatin were administered, the cisplatin-induced decrease in body weight at 3 HALO was significantly milder than in the other groups (vs 9 HALO, $P < 0.05$; vs 15 HALO, $P < 0.001$; vs 21 HALO, $P < 0.001$). At the end of this experiment, the body weight in the 3 HALO group was significantly greater, or tended to be

Table 1. Changes in body weight during weekly cisplatin injections (5 mg kg^{-1}) at four time points in rats.

Time (h)	n	Body-weight change (%)[a]		
		Day 7	Day 14	Day 21
HALO 3	8	114·6 ± 8·7	101·1 ± 13·4	99·1 ± 8·2
HALO 9	11	114·1 ± 2·5	115·6 ± 7·0	102·2 ± 14·5
HALO 15	8	107·8 ± 7·0	100·4 ± 15·4*	94·8 ± 18·4
HALO 21	10	109·6 ± 5·6	109·0 ± 5·8	103·8 ± 14·9
Control[b]	8	124·9 ± 3·1	147·9 ± 3·9	160·1 ± 6·6

HALO, time after light switched on. [a]The body-weight change was calculated as the percent of weight change in each rat from the initial value (day 0). [b]The body-weight changes of the untreated control rats (n = 8) were also determined. All weekly cisplatin-treated groups had markedly suppressed increases in body weight compared with the control group. Values are expressed as mean ± s.d.; *$P < 0.05$ vs 9 HALO value, using analysis of variance.

Table 2. Changes in body weight during daily cisplatin injections (1·2 mg kg^{-1}) at four time points in rats.

Time (h)	n	Body Weight Change (%)[a]		
		Day 7	Day 14	Day 21
HALO 3	8	113·3 ± 3·8	123·5 ± 6·9	114·5 ± 7·9
HALO 9	8	106·8 ± 3·0*	110·9 ± 8·3*	98·9 ± 10·2
HALO 15	8	102·6 ± 2·7#	109·1 ± 3·1†	97·5 ± 7·6*
HALO 21	8	100·0 ± 5·7**##	113·8 ± 10·5	107·1 ± 5·1
Control[b]	8	124·9 ± 3·1	147·9 ± 3·9	160·1 ± 6·6

HALO, time after light switched on. [a]The body weight change was calculated as the percent of weight change in each rat from the initial value (day 0). [b]The body weight changes of the untreated control rats (n = 8) were also determined. All daily cisplatin-treated groups had markedly suppressed increases in body weight compared with the control group ($P < 0·001$). Values are expressed as mean ± s.d.; *$P < 0·05$ vs HALO 3 values; **$P < 0·05$ vs 9 HALO value; †$P < 0·01$ vs 3HALO value; #$P < 0·001$ vs 3 HALO value; ##$P < 0·001$ vs 9 HALO value, using analysis of variance.

greater, than in the other groups (vs 15 HALO, $P < 0·05$).

Concentrations of BUN and serum creatinine after repeated cisplatin injections

In the control group, both BUN and serum creatinine concentrations showed a clear daily rhythm with a peak at 15 HALO and a trough at 9 HALO ($P < 0·001$; Tables 3 and 4). However, their variations were disturbed by repeated administration of cisplatin.

After high-dose cisplatin administration, concentrations of BUN and serum creatinine were significantly elevated at 3, 9 and 15 HALO, and at 21 HALO these parameters varied widely. In the low-dose cisplatin group, the mean concentrations of BUN and serum creatinine were elevated at each time point with a peak at 9 HALO.

Discussion

Weekly high doses or daily low doses of cisplatin are prescribed to treat malignant diseases (Hunter et al 1991; Kemp et al 1996; Hoshino et al 1997; Yasumoto et al 1998). However, adverse effects, especially nephrotoxicity, limit the drug's usefulness. The daily low-dose regimen is preferred to reduce these effects (Belliveau et al 1986; Shimizu 1997; Ito et al 1998). Chronotherapy is a unique approach to decrease the frequency of adverse events (Lévi et al 1997). Single-dosing studies in rats showed that the toxicity of cisplatin was least when the drug was given at 17 HALO (near the mid-activity span) and that nephrotoxicity was minimal when rats received the drug near the peak of urinary volume (Hrushesky et al 1982; Lévi et al 1982a, b). However, the effects of repeated administrations of cisplatin are still unknown.

In a preliminary study, we evaluated a high-dose bolus injection of cisplatin in rats. One or two administrations of 6 mg kg^{-1} often caused death. Therefore, we chose a dose of 5 mg kg^{-1} for weekly administration, which provided a total dose of 15 mg kg^{-1} of cisplatin at the end of the experiment. Daily administration of 1·0 mg kg^{-1} of cisplatin, however, had only a small therapeutic effect on tumour cell-bearing rats (data not shown). We used a regimen of 1·2 mg kg^{-1} cisplatin daily (total dose 25·2 mg kg^{-1}) for the nephrotoxicity study, although a higher dose was not tolerated for daily administration. Because the anticancer effect is thought to depend on the total dose of anticancer

Table 3. BUN concentrations in rats after cisplatin injections at four time points.

Time (h)	Control	Weekly administration of 5 mg kg^{-1}	Daily administration of 1·2 mg kg^{-1}
HALO 3	20·73 ± 1·06 (8)	62·05 ± 27·95** (8)	74·91 ± 20·14*** (8)
HALO 9	16·86 ± 1·68 (8)	79·61 ± 53·26* (11)	153·36 ± 82·00*** (8)
HALO 15	25·13 ± 3·38 (8)	58·24 ± 18·43* (8)	83·14 ± 39·23** (8)
HALO 21	23·86 ± 2·85 (8)	72·24 ± 88·42 (10)	86·20 ± 70·29 (8)
Cosinor method	$P < 0·001$	$P = 0·94$	$P = 0·104$

HALO, time after lights switched on. Number of rats in each group are given in parentheses. Values are expressed as mean ± s.d.; *$P < 0·05$, **$P < 0·01$ and ***$P < 0·001$, vs control group at each dosing time using analysis of variance.

Table 4. Serum creatinine concentrations in rats after cisplatin injections at four time points.

Time (h)	Control	Weekly administration of $1{\cdot}5\ mg\ kg^{-1}$	Daily administration of $1{\cdot}2\ mg\ kg^{-1}$
HALO 3	$0{\cdot}21 \pm 0{\cdot}04$ (8)	$0{\cdot}51 \pm 0{\cdot}19$*** (8)	$0{\cdot}51 \pm 0{\cdot}11$*** (8)
HALO 9	$0{\cdot}16 \pm 0{\cdot}05$ (8)	$0{\cdot}66 \pm 0{\cdot}33$** (11)	$0{\cdot}86 \pm 0{\cdot}41$*** (8)
HALO 15	$0{\cdot}30 \pm 0{\cdot}05$ (8)	$0{\cdot}66 \pm 0{\cdot}16$** (8)	$0{\cdot}54 \pm 0{\cdot}18$* (8)
HALO 21	$0{\cdot}26 \pm 0{\cdot}05$ (8)	$0{\cdot}76 \pm 0{\cdot}79$ (10)	$0{\cdot}55 \pm 0{\cdot}37$ (8)
Cosinor method	$P < 0{\cdot}001$	$P = 0{\cdot}735$	$P = 0{\cdot}140$

HALO, time after lights switched on. Number of rats in each group are given in parentheses. Values are expressed as mean ± s.d.; *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ and ***$P < 0{\cdot}001$, vs control group at each dosing time using analysis of variance.

drug, daily administration might be more suitable clinically. In the daily low-dose trial, the dose administered was greater than in the weekly high-dose trial.

Although there was no significant difference in body weight among the groups of rats receiving high doses of cisplatin weekly, the 3 HALO group that received a daily low dose had a significant difference in body weight compared with the 15 and 21 HALO groups ($P < 0{\cdot}05$ and $0{\cdot}001$, respectively). The BUN and serum creatinine concentrations were markedly elevated at each time point in the daily and weekly dosing schedules. Dosing-time dependent variations in these parameters were also obvious in the daily low-dose trial. The present findings obtained using a daily low dose of cisplatin were similar to those with a single injection of cisplatin (Hrushesky et al 1982; Lévi et al 1982a).

Cisplatin caused time-dependent nephrotoxicity, especially in the group of rats that received daily injections. These results obtained with repeated cisplatin administration agreed with those induced by a single injection reported by Levi et al (1982a) and Hruskesky et al (1982).

In conclusion, nephrotoxicity in the 9 HALO group was the greatest in both regimens, but this toxicity was accelerated in the low-dose daily trial. Regarding prevention of cisplatin-induced nephrotoxicity, dosing at 3 HALO was considered the best point, but the total doses of cisplatin administered daily were higher than when the drug was administered weekly. The mechanism of chronopharmacological toxicity of cisplatin has also been studied from the standpoint of chronopharmacokinetics in a separate report (To et al 2000).

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References

Belliveau, J. F., Posner, M. R., Ferrari, L., Crabtree, G. W., Cummings, F. J., Wiemann, M. C., O'Leary, G. P., Griffin, H., Phaneuf, M. A., O'Rourke, A., Calabresi, P. (1986) Cisplatin administered as a continuous 5-day infusion: plasma platinum levels and urine platinum excretion. Cancer Treat. Rep. 70: 1215–1217

Fujimura, A., Ohashi, K., Sugimoto, K., Kumagai, Y., Ebihara, A. (1989) Chronopharmacological study of nitrendipine in healthy subjects. J. Clin. Pharmacol. 29: 909–915

Hoshino, K., Ono, K., Kimura, O., Kawaguchi, H., Fukui, H., Ikeda, M. (1997) Usefulness of continuous venous daily chemotherapy of 5-fluorouracil and low dose cisplatin for patients undergoing noncurative surgery. Jpn. J. Cancer Chemother. 24: 2105–2110

Hrushesky, W. J. M. (1985) Circadian timing of cancer chemotherapy. Science 228: 73–75

Hrushesky, W. J. M., Lévi, F. A., Halberg, F. Kennedy, B. J. (1982) Circadian stage dependence of cis-diamminediedichloroplatinum lethal toxicity in rats. Cancer Res. 42: 945–949

Hunter, R. E., Griffin, T. W., Stevens, S. Roman, L. D., Bokhari, F., Reale, F. R., Tak, W. K., Fitzgerald, T. J., Dillon, M. B., Rose, P. G. (1991) High-dose, short-duration cisplatin/doxorubicin combination chemotherapy for advanced ovarian epithelial cancer. Cancer 68: 1890–1894

Ito, T., Yamada, M., Tanemura, H., Oshita, H., Asano, M., Saji, S. (1998) Results of combination therapy of UFT-E with low-dose CDDP for patients with advanced recurrent breast cancer. Jpn. J. Cancer Chemother. 25: 1575–1580

Kemp, G., Rose, P., Lurain, J., Berman, M., Manetta, A., Roullet, B., Homesley, H., Belpomme, D. Glick, J. (1996) Amifostine pretreatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized control trial in patients with advanced ovarian cancer. J. Clin. Oncol. 14: 2101–2112

Lemmer, B., Bruguerolle, B. (1994) Chronopharmacokinetics. Are they clinically relevant? Clin. Pharmacokinet. 26: 419–427

Lévi, F. A., Hrushesky, W. J. M., Blomquist, C. H., Lakatua, D. J., Haus, E., Halberg, F., Kennedy, B. J. (1982a) Reduction of cis-diamminediedichloroplatinum nephrotoxicity in rats by optimal circadian drug timing. Cancer Res. 42: 950–955

Lévi, F., Hrushesky, W. J. M., Borch, R. F., Pleasants, M. E., Kennedy, B. J., Halberg, F. (1982b) Cisplatin urinary pharmacokinetics and nephrotoxicity: a common circadian mechanism. Cancer Treatment Reports 66: 1933–1938

Lévi, F., Zidani, R., Misset, J. (1997) Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folic acid in metastatic colorectal cancer. Lancet 350: 681–686

Ohdo, S., Makinosumi, T., Ishizaki, T., Yukawa, E., Higuchi, S., Nakano, S., Ogawa, N. (1997) Cell cycle-dependent chronotoxicity of irinotecan hydrochloride in mice. J. Pharmacol. Exp. Ther. 283: 1383–1388

Saito, Y., Yoshida, S., Nakaya, N., Hata, Y., Goto, Y. (1991) Comparison between morning and evening doses of simvastatin in hyperlipidemic subjects. A double-blind comparative study. Arteriosclerosis Thrombosis 11: 816–826

Shimizu, Y. (1997) Consecutive low-dose cisplatin-based chemotherapy for gynecologic malignancies. Jpn. J. Cancer Chemother. 24: 431–438

To, H., Kikuchi, A., Turuoka, S., Sugimoto, K., Fujimura, A., Higuchi, S., Kayama F., Hara, K., Matsuno, K., Kobayashi, E. (2000) Time-dependent nephrotoxicity associated with daily administration of cisplatin in mice. J. Pharm. Pharmacol. (In Press)

Yamauchi, H., Kobayashi, E., Sugimoto, K., Tsuruoka, S., Yabana, M., Ishii, M., Fujimura, A. (1998) Time dependent cyclosporine A-induced nephrotoxicity in rats. Clin. Exp. Pharmacol. Physiol. 25: 435–440

Yasumoto, K., Takeda, J., Iwamoto, M., Kawabata, S., Takeuchi, K., Yunoki, T., Miwa, K., Iwakuma, N. Shirouzu, K. (1998) Clinical evaluation as palliative therapy of combination chemotherapy with continuous infusional 5-fluorouracil and consecutive low-dose cisplatin in unresectable gastrointestinal carcinoma. Jpn. J. Cancer Chemother. 25: 1909–1914