Reduction of cis-Diaminedichloroplatinum Nephrotoxicity in Rats by Optimal Circadian Drug Timing


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ABSTRACT

A prominent circadian rhythm in the nephrotoxicity of a therapeutic dose of cis-diaminedichloroplatinum (cisplatin) is demonstrated in female Fischer rats. Rats were randomized to receive two doses of either cisplatin or 0.9% NaCl solution 14 days apart at the times of either high or low values in their circadian rhythm of urinary volume.

Toxicity was assessed by measuring changes in body weight and changes in the 24-hr means of urinary volume, blood urea nitrogen, and urinary β-N-acetylglucosaminidase (NAG) activity.

Toxicity was least in rats which received the drug near the circadian maximum of urinary volume. Conversely, rats which received the same dose of drug near the circadian minimum of urinary volume lost more weight and exhibited a 2-fold increase in the 24-hr mean of urinary volume, a 3-fold rise in the 24-hr mean of blood urea nitrogen, and a 5-fold increase in the 24-hr mean of urinary NAG activity.

A positive correlation between urinary NAG at the time of cisplatin administration and the extent of cisplatin nephrotoxicity was demonstrated (p < 0.02). A correlation also was found between tissue NAG concentration and tissue uptake of cisplatin (p < 0.001). A marked circadian rhythm of NAG activity in proximal tubular cells may contribute to the prominent circadian rhythm in murine renal tolerance for cisplatin.

INTRODUCTION

The purpose of the work reported here is to test the importance of timing as a method of preventing cisplatin toxicity (16, 18). Investigation of possible time dependence of the nephrotoxicity of cisplatin is logical, since renal function exhibits a well-known circadian rhythm in both rodents and human beings (1, 5, 13, 15, 19, 28, 31, 33). This circadian rhythm persists even under conditions of prolonged isolation in caves (13) or on unusual imposed schedules (32).

We have demonstrated a marked circadian rhythm in murine lethal toxicity of 11 mg of cisplatin per kg administered i.p. (12, 22). The highest tolerance for cisplatin occurs at 17 to 19 HALO in rats kept on a standardized regimen of 8 hr of light alternating with 16 hr of darkness. Optimal cisplatin timing occurs approximately 2 hr after the time of daily high values of the circadian rhythm in rectal temperature.

In this study, a longitudinal and transverse design allows a quantification of the benefit which results from optimal cisplatin timing and a definition of the optimal circadian stage for reduction of cisplatin nephrotoxicity. Nephrotoxicity was assessed by measuring the urinary activity of NAG. High concentrations of NAG, a lysosomal enzyme, are found in the brush border of renal proximal tubular cells. The increased concentration of this enzyme in the urine has been shown to be a sensitive indicator of proximal tubular damage (8, 30, 37).

MATERIALS AND METHODS

Animals and Study Design. Fourteen 8-week-old 120- to 140-g female Fischer 344 rats were housed singly and had food and water freely available in a standardized lighting regimen of 8 hr of light alternating with 16 hr of darkness for 2 weeks. Subsequently, food availability was progressively restricted to the first 3 hr of darkness, the usual activity span. After 2 weeks of adjustment to this schedule, all rats were housed singly in metabolic cages. Urine was collected automatically at 4-hr intervals (i.e., 6 samples/day/rat). All rats were weighed daily prior to their feeding time at 8 hr after light onset. At this time, the urine samples were collected, their volumes were measured, recorded, and corrected for evaporation, and the specimens were frozen. The correction for evaporation was calculated experimentally by serial weighing of a known volume. Animals were kept at constant temperature and humidity. This routine was continued throughout the study. After 3 weeks of adjustment to this schedule, the rats received an i.p. injection of either 0.9% NaCl solution or 5 mg cisplatin per kg. Six rats were given cisplatin near their previously determined mean urinary volume daily maximum and 5 rats received cisplatin near the mean urinary volume daily minimum, while 3 control rats received 0.9% NaCl solution injection. This procedure was repeated on the same rats 14 days after the first injection.

Four days after the second injection, blood samples for determinations of BUN were obtained from the lateral tail vein of each rat in 6 consecutive circadian stages at 4-hr intervals. BUN was determined by the urease method. Ten days after the second injection, each rat was killed with ether anesthesia, and both kidneys were removed and fixed in formalin.

Cisplatin. Cisplatin was supplied by the Investigational Drug Branch of the National Cancer Institute in vials containing 10 or 25 mg of the cis-diaminedichloroplatinum with mannitol and NaCl. When reconstituted with sterile water just prior to injection, each ml of solution contained 1 mg of cisplatin, 10 mg of mannitol, and 9 mg of NaCl.

NAG Determination. NAG activity was determined in the urine by the fluorometric method described by Price et al. (29) and expressed in units/mg creatinine. One unit of activity is defined as the hydrolysis of 1 nmol of substrate per hr.

Statistical Methods. Circadian rhythms were validated statistically by the single cosinor method (14). Rhythm characteristics were summarized by the population mean cosinor method (14) using parameter
test (1 4) was used to compare mean rhythm parameter estimates from estimates obtained from each rat with the single cosinor; t tests were also applied to compare the indices of drug toxicity. The Hotelling t test (14) was used to compare mean rhythm parameter estimates from 2 groups.

RESULTS

Before Cisplatin Injection. Circadian rhythms in urinary volume (ml/hr) and urinary NAG activity (units/mg creatinine) were statistically significant for each rat and also for the group as indicated by the single and population mean cosinor methods, respectively. The mean daily maxima of urinary volume and NAG activity occurred in the first half of the dark and light spans, respectively (Table 1). The amplitudes of both rhythms were extremely large, approximately equal to 100% of the 24-hr means.

After Cisplatin Injection. When the cisplatin-injected rats are considered as a group, irrespective of the circadian stage of drug administration, the circadian mean of urinary NAG and that of urinary volume increased. The rats also exhibited body weight loss. These changes were greatest on Days 4, 5, and 7, respectively, after the first cisplatin injection. The role of the circadian stage of drug administration upon the extent of these changes was examined for all 3 variables.

Body Weight Loss. The extent of body weight loss was less in those rats receiving cisplatin near the circadian high point of urinary volume, as compared to those receiving cisplatin near the circadian low point of urinary volume (Chart 1).

24-Hr-Rhythm-qualified Mean of Urinary Volume. The 24-hr-rhythm-adjusted mean of urinary volume increased by 70% in those rats which received cisplatin near the low point of urinary volume. Conversely, cisplatin administered near the high point of urinary volume did not result in polyuria (Chart 2).

24-Hr-Rhythm-qualified Mean of Urinary NAG Activity. When cisplatin was administered near the nadir of urinary volume, a 5-fold increase in the 24-hr-rhythm-adjusted mean of urinary NAG was observed, and the level of urinary NAG activity had not yet returned to usual values by the 14th day postinjection. By contrast, if cisplatin was injected near the high point of urinary volume, the 24-hr mean of urinary NAG activity increased only 2-fold, and it returned close to usual values by the 14th day postinjection (Chart 3).

24-Hr-Rhythm-qualified Mean of BUN. The 24-hr-rhythm-adjusted mean of BUN determined 4 days after the second cisplatin injection did not rise substantially above control values in rats receiving cisplatin near the circadian high point of urinary volume. Rats receiving this drug near the daily low point of urinary volume exhibited almost a 3-fold increase over control values in BUN 24-hr mean (Table 2).

Cisplatin Effect Upon Circadian Rhythm Characteristics of Toxicity End Points: Urinary NAG Activity. When the circadian rhythm characteristics of urinary NAG activity are examined by single cosinor analysis at the time of the maximal extent of change (i.e., on Day 4), cisplatin given at the preinjection nadir of urinary volume results not only in a dramatic increase in 24-hr mean but also in a 3-fold increase in the absolute amplitude of the normal circadian rhythm of this variable (Chart 4). The time of daily high point remains similar to that of the controls. Conversely, minimal alterations were observed in the circadian rhythm characteristics of urinary NAG activity when cisplatin was administered near the timing of the daily maximum of urinary volume. Similar results were observed for the rhythm characteristics of urine volume (not shown).

The circadian rhythm characteristics of urinary NAG activity were also examined on Days 11 to 13 after the first administration of cisplatin. Single and mean cosinor analysis revealed that the mean amplitude of this rhythm relative to its mean

| p from zero- | Mesor A | Amplitude B | Acrophase C (HALO) |
| rejection | ml/hr | units/mg creatinine | |
| Urine volume | 0.007 | 0.20 | 0.17 (0.04, 0.31) | 12.20 (11.38, 14.24) |
| NAG | 0.001 | 66.0 | 7.25 (62.00, 85.0) | 3.56 (3.16, 4.36) |

A Rhythm-adjusted mean.
B Difference between mesor and maximum in cosine function best approximating data.
C Timing of maximum in best-fitting cosine function.
D Numbers in parentheses, 95% confidence limits.
* Note slight overfitting.
decreased from 95 to 55% and the timing of the daily high point advanced by about 3 hr. These changes were not affected by cisplatin timing. This difference in amplitude and timing was statistically significant when tested by a Hotelling t test (14) (F = 11.4, p < 0.001).

**BUN.** A circadian rhythm in BUN on Days 4 to 5 after the second injection was demonstrated by single cosinor analysis in rats receiving cisplatin at the high point (p < 0.01) or at the nadir of urine volume (p < 0.01), as well as in the controls (p ~ 0.09) (Chart 5). The high point of the circadian rhythm in BUN occurred near 13 HALO in all 3 groups. Very small differences in 24-hr mean and amplitude of the BUN rhythm were seen in rats receiving cisplatin near the high point of urine volume as compared to control rats, while a 2-fold and a 3-fold increase characterized these respective rhythm parameters after cisplatin administration near the nadir of daily urinary volume.

**Correlative Renal Toxicity.** The 24-hr mean of urinary NAG activity on Day 4 was positively correlated with weight loss on Day 7 (r = 0.75, p < 0.01) and with the urinary volume mesor on Day 5 (r = 0.65, p < 0.05).

The absolute urine volume during the 4 hr immediately preceding cisplatin injection was not correlated with the maximal NAG 24-hr mean resulting from cisplatin injection in each rat (r = 0.03, p > 0.50). The maximal NAG 24-hr mean following cisplatin was, however, positively correlated with the amount...
Twenty-four-hr mean of BUN on Days 4 to 5 after 5 mg cisplatin per kg
Rats received 5 mg cisplatin per kg i.p. at either the maximum (acrophase) or minimum (bathyphase) of the mean circadian rhythm in urinary volume determined before this injection. Six samples were obtained every 4 hr during 24 hr from each rat.

<table>
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<th>Time of cisplatin</th>
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<td>Acrophase</td>
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*a Results from one-way analysis of variance: F = 24.8, p < 0.001.
*b Mean ± S.E.
*c Comparison with controls: t = 1.5, p = 0.20.
*d Comparison with controls: t = 5.0, p < 0.001; and with acrophase treatment: t = 5.7, p < 0.001.

Circadian Cisplatin Nephrotoxicity

Damaged, these cells release lysosomal enzymes, one of which is NAG. There is a subsequent loss in the ability of the renal tubule to reabsorb water, and polyuria occurs. This results in dehydration, evident by poor skin turgor, dry mucus membranes, and body weight loss. This chain of events is common to other heavy metal-induced nephropathies (9). Histological lesions found within 1 week after cisplatin injection in most animal species investigated are also similar to those induced by other heavy metals (10, 23).

Since hydration has been reported to decrease cisplatin nephrotoxicity (3, 7, 21), it can be argued that the optimal renal tolerance for cisplatin would occur predictably when this drug is administered at the circadian maximum of urinary volume. The absence of any statistically significant correlation (r = 0.04, p > 0.50) between the absolute amount of urine collected during the 4 hr preceding cisplatin injection and the peak of urinary NAG activity following drug injection, however, implies that, within the circadian range, the absolute volume of urine, by itself, is not a determining factor for the extent of cisplatin-induced renal damage. A statistically significant correlation between urinary NAG activity at the time of cisplatin administration (r = 0.85, p < 0.02).

**DISCUSSION**

We have shown previously that the lethal toxicity of a high dose of cisplatin is circadian stage dependent. In the present study, we used a therapeutically effective dose, which resulted in no lethal toxicity. We examined 4 end points of cisplatin toxicity: body weight loss, urinary volume changes, BUN, and urinary NAG activity. The circadian stage of cisplatin administration determined the extent of drug toxicity as measured by each of these end points. We have found that, in Fischer rats given 5 mg of this drug per kg, cisplatin-induced nephrotoxicity can be undetectable by Day 14 posttreatment if this drug is administered at a circadian stage temporally associated with minimal urinary volume excretion.

Cisplatin-induced kidney damage is described by the sequence of peaks in urinary NAG activity, urine volume, and body weight loss following drug administration. The statistically significant correlations between these peaks suggest that the site of primary damage may be the renal tubular cell. When

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*d Comparison with controls: t = 5.0, p < 0.001; and with acrophase treatment: t = 5.7, p < 0.001.

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**Chart 5.** Circadian rhythm in BUN on Days 4 to 5 after second i.p. injection of 5 mg cisplatin per kg or 0.9% NaCl solution to female Fischer 344 rats. Drug (Rx) was given at acrophase (maximum) or bathyphase (minimum) of the circadian rhythm in urine volume of the rats determined before drug injection. Blood was serially sampled for BUN determination every 4 hr for 24 hr.

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**Chart 4.** Circadian rhythm in urinary NAG on Days 3 to 5 after single i.p. injection of 5 mg cisplatin per kg or 0.9% NaCl solution to female Fischer 344 rats. NAG activity is expressed in nm of substrate hydrolyzed within 1 hr. Drug (Rx) was given at acrophase (maximum) or bathyphase (minimum) of the circadian rhythm in urine volume of the rats determined before drug injection.

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**Chart 3.** 1 SE of 3 observations: mean, ± 1 SE, is shown.
enzymes may represent important sites of heavy-metal attachment and resultant cellular entry, leading to cell death by several possible mechanisms (11, 20, 34).

Because of the positive correlation between urinary NAG activity and cisplatin nephrotoxicity, the urinary activity of this enzyme at the time of cisplatin administration may represent a sensitive predictor of subsequent renal damage. Inspection of the effect of the first dose of cisplatin upon the circadian rhythm characteristics of this variable persisted for several possible mechanisms (11, 20, 34).

The effect of the first administration of cisplatin upon the circadian rhythm characteristics of this variable persisted for the full 2-week span of observation preceding the second dose. Just prior to this second dose, the timing of this rhythm had advanced by about 3 hr while the mean relative amplitude was still decreased by 40%. Thus, the circadian nadir of urinary NAG activity (a stage associated with minimal cisplatin nephrotoxicity) remained higher than before the first exposure to cisplatin. This suggests that subsequent cisplatin doses given at the optimal circadian stage may be more nephrotoxic than the initial cisplatin dose, if they are applied before complete maximal shielding of the kidney is that associated with the urine volume daily high point, which occurs about 9 hr after the daily high point of urinary NAG activity.

The optimal circadian stage of cisplatin administration for a maximal shielding of the kidney is that associated with the urine volume daily high point, which occurs about 9 hr after the daily high point of urinary NAG activity.

The clinical relevance of our findings in the rat is suggested by the substantial circadian rhythmicity of the urinary activity of several lysosomal enzymes, including glycosidases (which peak between 6 and 9 a.m.) in healthy human volunteers (27).

If NAG content is mechanistically important, pharmacological manipulation of the circadian rhythm in urinary NAG may be possible in order to conveniently exploit the prominent circadian rhythm in cisplatin nephrotoxicity. We have already demonstrated the feasibility of this approach in mice by manipulat-

ing the circadian rhythm in Adriamycin lethal toxicity by a single injection of adrenocorticotropic (24).

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