Daily Variations in Ceftriaxone Pharmacokinetics in Rats

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The aim of this study was to determine whether the time of day ceftriaxone was administered modified its pharmacokinetics. Ceftriaxone was given intraperitoneally at either 0400, 1000, 1600, and 2200 h to Sprague-Dawley rats synchronized under a light-dark cycle of 12 h of light and 12 h of dark. Pharmacokinetic parameters were analyzed for the presence of a 24-h rhythm. Results showed significant daily variations ($P < 0.05$) in ceftriaxone clearance, with the highest values during the dark phase. It is concluded that time-dependent variations in ceftriaxone pharmacokinetics may affect the therapeutic efficacy of current once-daily dosing schedules.

Ceftriaxone is a broad-spectrum parenteral cephalosporin with potent activity against gram-positive and gram-negative bacteria (19). Ceftriaxone is widely used in humans because of its prolonged half-life, which allows its prescription on a once-a-day basis (4, 17, 20, 28, 32).

Chronokinetics examines the influence of the time of day a drug is administered on drug pharmacokinetics. Most pharmacokinetic studies are performed with one dose in the daytime. However, temporal variations in drug absorption, distribution, hepatic metabolism, and renal excretion have been reported for several drugs (5, 24). Chronokinetic studies are clinically relevant to once-daily drug dosing schedules (5, 12, 23).

The aim of this study was to evaluate possible variations in the disposition of ceftriaxone administered to Sprague-Dawley rats by the intraperitoneal route at different times of the day.

(The results of this study were presented in part at the Millennial World Congress of Pharmaceutical Sciences, San Francisco, Calif., April 2000.)

Four groups (50 to 60 animals/group) of 28-day-old female Sprague-Dawley rats weighing 100 ± 18 g were used throughout the study. Rats were purchased from Bioterio Central (University of Buenos Aires, Buenos Aires, Argentina); they had been kept under a light-dark cycle of 12 h of light (from 0700 to 1900 h) and 12 h of dark at a controlled ambient temperature ($22 \pm 2^\circ\text{C}$) from birth. Food and water were freely available. Experiments were conducted in October and November 1999 (spring in the Southern hemisphere). Animal experiments were approved by Secretaría de Ciencia y Técnica, University of Buenos Aires.

Animals were given a single intraperitoneal injection of 100 mg of ceftriaxone (Acantex; Roche, Buenos Aires, Argentina) per kg of body weight either at 0400, 1000, 1600, or 2200 h. At different time intervals after administration of the drug, four to six rats/sampling point were killed by decapitation after cervical dislocation, and blood was collected. Blood samples were allowed to clot, and serum was separated from the blood by

centrifugation and frozen (-20°C) until analyzed. Times of drug injection and sampling were expressed in local time.

Ceftriaxone concentrations in serum were determined by a previously described microbiological assay (2). Standard curves of ceftriaxone were prepared from pooled rat serum samples that were run simultaneously with test samples. The quantification limit was $1.17 \mu\text{g/ml}$. The correlation coefficient for the regression line of the standard solution was 0.997. The within- and between-day coefficients of variation were less than 10% in the range of observed concentrations (600 to $1.17 \mu\text{g/ml}$).

The serum ceftriaxone concentration-time curve for all groups was fitted to a one-compartment model using a nonlinear least-squares regression program (TOPFIT 2.0; Gustav Fischer, Jena, Germany). Pharmacokinetic parameters calculated were as follows: absorption (k_{abs}) and elimination (k_{el}) rate constants, absorption ($t_{1/2\text{abs}}$) and elimination ($t_{1/2\text{el}}$) half-lives, area under the concentration-versus-time curve (AUC), total serum drug clearance (CL_T), volume of distribution (V), lag time (T_{lag}), peak serum concentration (C_{max}), and time to reach C_{max} (T_{max}) (8). Pharmacokinetic parameters were further analyzed by the cosinor method (18) for the presence of a 24-h (circadian) rhythm. This procedure is applicable to longitudinal biologic time series and consists of fitting a cosine curve of constant frequency to sequential data by the least-squares statistical method. Estimated rhythmicity parameters follow: M , mesor (rhythm-adjusted mean); A , amplitude (distance between the mesor and the peak value of the cosine functions); and ϕ , acrophase (peak value of the fitted cosine function, with midnight [local time] the reference time). The corresponding equation model is $Y = (M + A) \times \cos(0.261t + \phi)$. With this method, the goodness of fit of the cosine curve to the data is indicated by minimizing the sum of squares of the residuals from the analysis (probability of rhythm [PR] value). Rhythm detection is determined by the statistical significance of the amplitude, estimated by testing the null hypothesis that $A = 0$ (no rhythm exists) by an F test. A PR value of ≥ 70 and $P \leq 0.05$ were considered indicative of rhythm. Relative amplitude was estimated as percent of the mesor ($A/M \times 100$).

Figure 1 shows the mean concentration-versus-time curve of ceftriaxone in serum after it was administered intraperitoneally at 0400, 1000, 1600, and 2200 h. The pharmacokinetic param-

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FIG. 1. Ceftriaxone concentrations obtained in serum after the intraperitoneal administration (100 mg/kg) to female Sprague-Dawley rats at 0400, 1000, 1600, and 2200 h. Each sampling point represents the mean $\pm$ standard deviation (error bar) of data for four to six rats.

eters for each group are presented in Table 1. Cosinor analysis showed significant daily variations in CL_T (PR = 100, $P = 0.006$). PR values for T_{lag} , AUC, and T_{max} indicated a good fit (98, 98, and 88%, respectively), but the P values were 0.14, 0.12, and 0.34, respectively. Mean estimated rhythm parameters (standard deviations given in parentheses) were as follows: for CL_T , $M = 0.41$ (0.0) ml/min, $A = -0.12$ (0.0) ml/min, and acrophase at 22.08 h; for AUC, $M = 439.7$ (10.2) $\mu\text{g/ml} \cdot \text{h}$, $A = 113.2$ (14.5) $\mu\text{g/ml} \cdot \text{h}$, and acrophase at 9.77 h; for T_{lag} , $M = 0.041$ (0.004) h, $A = 0.03$ (0.005) h, and acrophase at 3.05 h; and for T_{max} , $M = 0.2$ (0.01) h, $A = 0.05$ (0.01) h, and acrophase at 6.11 h. Relative amplitude was higher for T_{lag} (93%) and lower for AUC and T_{max} (25%). Other calculated pharmacokinetic parameters, such as k_{abs} , C_{max} , k_{el} , and V , did not exhibit significant rhythmic variations. Figure 2 shows the daily variations observed for CL_T , T_{lag} , AUC, and T_{max} .

Several studies have reported temporal variations in the pharmacokinetics of antimicrobial drugs (1, 3, 13, 16, 26, 29–31). Our results show that the total clearance of ceftriaxone

varies rhythmically during the day, with its maximum during the dark (activity) period and its minimum during the light (rest) period in rats; however, it must be noted that as intravenous administration was not performed, bioavailability was not included in our CL_T calculation. As ceftriaxone is eliminated by biliary and renal excretion in the rat (9, 15), this temporal variation can be attributed, at least partially, to circadian changes in different steps of the kinetic process, such as the biliary and renal functions. Basal bile flow has been reported to exhibit a circadian rhythm with higher values during the active phase of rats (10). The biliar anion carrier system shows its maximum transport capacity during activity (rather than rest) in rats (16, 30). Urinary water, protein and glycosaminoglycan excretions, and glomerular filtration rate (GFR) and renal plasma flow (RPF) show a circadian rhythmicity in rats, with markedly increased values observed during the dark phase in the animals (6, 21, 22). Similar findings were reported for urinary electrolyte (K, P, Mg, Ca, and Na) excretion (21, 25). Cardiac output changes as a function of the diurnal activity

TABLE 1. Pharmacokinetic parameters calculated after the intraperitoneal administration of ceftriaxone (100 mg/kg) to female Sprague-Dawley rats at 0400, 1000, 1600, and 2200 h

Time (h) of administration	k_{abs} (h^{-1})	$t_{1/2abs}$ (h)	k_{el} (h^{-1})	$t_{1/2el}$ (h)	T_{lag} (h)	V (liter)	CL_T (ml/min)	AUC ($\mu\text{g/ml} \cdot \text{h}$)	T_{max} (h)	C_{max} ($\mu\text{g/ml}$)
0400	19.10	0.036	0.61	1.12	0.073	0.040	0.413	436.00	0.26	240.00
1000	19.70	0.035	0.64	1.08	0.035	0.027	0.285	563.00	0.21	321.00
1600	19.80	0.036	0.74	0.93	0.00001	0.033	0.406	423.00	0.17	276.00
2200	30.60	0.022	1.28	0.54	0.054	0.025	0.532	337.00	0.16	376.00

FIG. 2. Daily profile observed for four pharmacokinetic parameters (CL_T , AUC, T_{lag} , and T_{max}) following intraperitoneal administration of ceftriaxone (100 mg/kg) to Sprague-Dawley rats at different times of day. The observed values (symbols) were plotted, and no linear least-squares fitted sine curves for circadian rhythm (frequency = 1/24) were observed. Dark (activity) and light (rest) periods are indicated by the hatched and white backgrounds, respectively.

and feeding patterns in rats, and renal and biliar blood flow are higher during the activity phase in the rat (7). As GFR, RPF, and renal and biliary excretion follow the same circadian pattern, these factors could be responsible for the time-dependent variation of ceftriaxone clearance.

During the 24 h, CL_T acrophase in the dark phase was 180° out of phase with that of AUC, while T_{max} and T_{lag} profiles were very similar, thus confirming the relationship between these two parameters. No rhythm was seen for k_{el} , as this parameter depends on CL_T and V , and the latter did not exhibit circadian rhythmicity. Circadian changes in the distribution of the cardiac output may account for the acrophase of T_{max} and T_{lag} observed during the end of the dark period, indicating slower absorption at this time. A period of transitory locomotion activity and sleeping behavior with no feeding activity occurs at the beginning of the light phase and may also exist during the end of the dark phase in the rat. During this period, cardiac output is low and skeletal muscle receives the greatest proportion of this output, while visceral blood flow is low (7). Thus, absorption during this transition may be slower than in other times of day.

Our data are in good agreement with the data of other researchers. In humans, the amount of ciprofloxacin eliminated in urine was greater when the drug was administered at 1000 h than when it was given at 2200 h (26). In rats, when tobramycin was administered at 0200 h (dark period), the CL_T was significantly higher and AUC was lower than the values when tobramycin was given at 1400 h (light period) (13). A population pharmacokinetic study of amikacin in humans showed higher values for k_{el} in the morning than in the evening. Ampicillin biliar and renal clearances were significantly higher during the active cycle of rats than during the sleep cycle (16, 30).

Experimental animal models have shown that for antibiotics such as β -lactams that have concentration-independent killing effects in vitro, the time that the antibiotic concentration remains greater than the MIC ($T > MIC$) is the most important factor for determining the in vivo efficacy (11, 14, 27). Therefore, daily variations in pharmacokinetics may account for impairment in the chemotherapeutic effects. Our results

demonstrate that the disposition of ceftriaxone in rats exhibits significant daily changes that could be attributed mainly to enhanced excretion during the active period; thus, $T > MIC$ may be shorter when ceftriaxone is administered during the active phase. This is of great importance when bacteria with low susceptibility are involved in the infectious processes, particularly as ceftriaxone concentrations are lower in tissues than in blood. It can be concluded that the time of administration for ceftriaxone may affect therapeutic efficacy in current once-daily dosing schedules.

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