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건강 지원자에서 세프라딘의 약동학적 생체리듬 변화

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Circadian Changes in Pharmacokinetics of Cephradine Administered Orally to Healthy Human Volunteers

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건강한지원자에서 세프라딘 250 mg 캡슐을 오전 09:00시와 오후22:00시에 경구투여하여 세프라딘의 약물동태학적 주기변화 (생체리듬)를 검토하였다. 혈장중 세프라딘의 농도는 두 투여시간에서 유의성 있는 차이를 보여주었다. 혈장농도곡선하면적 (AUC)은 오후 때 보다 오전 투여시에 유의성 있게 증가되었으며, 생물학적반감기($t_{1/2}$)는 오후 때 보다 오전 투여시에 더 연장되었다. 전신청소률 (CL_t) 은 오후 때 보다 오전 투여시 유의성 있게 감소되었다.

☐ Key words - Circadian changes, Pharmacokinetics, Cephradine, Total body clearance, AUC

After Radzialowski and Bousguet demonstrated circadian changes in the oxidative metabolism of drugs in 1968,[1)] various physiological processes that govern kinetics of drug in the body appear to be affected by the time change. For example, temporal variations have been indicated for drug absorption from the gastrointestinal tract, drug distribution, drug metabolism (temporal variations in enzyme activity and hepatic blood flow), and renal drug excretion. The plasma concentrations and the area under the plasma concentration-time curve from time zero to time infinity(AUC) of propranolol, aspirin, midazolam, nortriptyline, indometacin, antipyrine and cyclosporine were increased, and urinary excretions of the drugs were decreased in the morning.[2-8)] In addition, the times to reach a peak concentration and half-lives of clorazepate depotassoum, theophylline, valproic acid and gentamycin were shortened with administration of the drugs in the morning,[9-12)] and the urinary excretions of cisplastin and acetaminophen were increased in the morning.[13,14)]

Cephradine is an orally administered cephalosporin with a broad spectrum of activity against gram-positive and gram-negative bacteria and is highly resistant to beta-lactamase degradation.[15,16)] It is acid-stable and rapidly and almost completely absorbed from the gastrointestinal tract. The serum half-life of cephradine is 0.7-2 h in adults with normal renal function. Cephradine is excreted unchanged in the urine. About 60-90% of a single oral dose is excreted within 6 h in patients with normal renal function.[17)] Since cephradine is primarily excreted via kidney, circadian change may affect the pharmacokinetics of its renal excretion, pharmacokinetics of cephradine may be affected by the circadian change of physiological fluctuation. Unfortunately, it is not known whether circadian change exists for cephradine pharmacokinetics. Therefore, the purpose of this study was to investigate the circadian changes in the pharmacokinetics of cephradine after a single oral administration to healthy male volunteers.

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Materials and Methods

Chemicals and subjects

Cephradine and cephalexin (the internal standard of high performance liquid chromatographic, HPLC assay) were obtained from Donggu Pharmaceutical Cop. (Seoul, Korea). HPLC grades of methanol were purchased from Merck Company (Darmstadt, Germany). Other chemicals were of reagent grade or HPLC grade, used without further purification.

A crossover design of the 9 subjects (male volunteer, aged 22-38 years) was used. Informed consent was obtained from each subject. The subjects were advised to follow routine of diurnal activity from 07:00 to 22:00 for 1 week, before and during the study period. They were asked to perform a common physical exercise plan and to report particular situations when needed. Subjects were also required not to ingest alcohol, coffee, and medications or smoke for 1 week before and during the study period. The administration time of cephradine capsule was 09:00 (daytime) and 22:00 (nighttime) based on normal day and night activity, respectively.

Strict experimental schedules were constructed as follows. For the daytime experiment, the subjects were fed a light carbonhydrate meal excluding food with fat for dinner and were fasted overnight. The drug was administered to subjects in a fasting state at 09:00 under the inspection of a medical doctor. The subjects were allowed to move or rest freely during the experiment. They were provided a light carbonhydrate meal at 13:00 during the experiment. For the nighttime experiment, the subjects were fed a light carbonhydrate meal for lunch, but not dinner. The drug was thereafter administered to the subjects at 22:00 of the same day. Consecutively, one piece of bread with potable water was served at 02:00. The subjects were allowed to move freely or to sleep or rest during experiment. However, they were awakened at the blood sampling times.

Dosing Schedule

Capsules containing 250 mg cephradine were obtained from Donggu Pharmaceutical Co. (Seoul, Korea). The blank blood samples were collected before starting the experiment to measure the serum creatinine concentrations S_{er} to ensure the subjects without renal failure. Cephradine was administered orally at 09:00 (daytime). After one week washout period, all subjects were again administered the same dose at 22:00 (nighttime) by a crossover design. An aliquot (2 ml) of blood samples were drown at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6 and 8 h from the left forearm vein and them centrifuged at 3,000 rpm for 10minutes. The collected serum were harvested and stored in a freezer at -20°C for 1 week until analysis.

HPLC Analysis of Cephradine

The cephradine concentration in the biological samples was analyzed using the reported HPLC method [18,19] with a slight modification. Briefly, to an aliquot (0.5 ml) of serum or urine samples, 50 μl 4 μg/ml cephalexin solution and 50 μl 20% perchloric acid solution were added. After vertex-mixing, the mixture was centrifuged at 10,000 rpm for 10 min. and an aliquot (50 μl) of supernatant was injected directly onto the HPLC column. HPLC condition was identical to the reported method.

Pharmacokinetic Analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method,[20] this method employed the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase[21] and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

The maximum plasma concentration of cephradine (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the experimental data. The mean of terminal half-life was calculated by the harmonic mean method.[22]

Statistical Analysis

All the means are presented with their standard deviation (mean±S.D.). An unpaired student's t-test was used to determine any significant difference between the control and cephradine administered orally at healthy volunteers. The differences to be significant at $p<0.05$.

Results and Discussion

The detailed vital statistics and S_{er} of 10 human subjects are given in Table 1. There were 10 male subjects, mean age of 27 (range 22-41) years old. Measuring serum creatinine concentration before starting the experiment validated renal function. In this study, creatinine clearance was measured based on serum creatinine concentration (0.89 mg/dL) using a common formula of Cockcraft and Gault.[23] It was about 103.9±21.9 ml/minute, a value within the normal range. The serum creatinine methods are used to estimate the creatinine clearance when the stabilized renal function and the steady-state creatinine concentration have to be assumed.

Circadian changes of cephradine pharmacokinetics

Mean plasma concentration-time profiles of cephradine in subjects following oral administration of cephradine in the morning and evenging are shown in Fig. 1. The time of cephradine administration appears to affect the kinetics of the drug significantly. Cephradine concentration in the plasma was higher in the night than in the daytime (Fig. 1). The difference reached statistically significance ($p < 0.05$) for all plasma samples collected after 6h of administration. As a result, the AUC was significantly increased daytime (15.2 ± 3.04 ug/ml· hr) than that nighttime (12.2 ± 2.44 ug/ml· hr) ($p < 0.05$, Table 2).

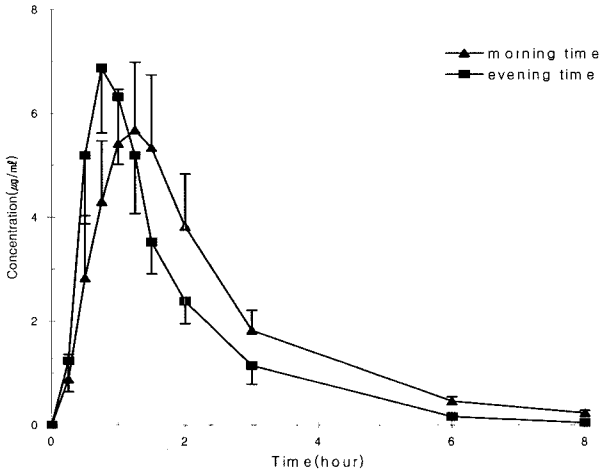

Fig. 1. The plot of mean plasma concentration (ug/ml) of cephradine in human healthy volunteers subjects administered orally at 09:00 and 22:00.

Following oral administration of cephradine (250 mg/capsule) at day and nighttime, cumulative amount of cephradine urinary excretion (mg) was shown in Fig. 2. The amount of excretion was slightly larger (42.4 ±

Table 1. Vital statistics and serum creatinine concentration S_{er} in healthy volunteers

subject	Age (years)	Weight (kg)	Height (cm)	S_{er} (mg/dL)	Cl_{er} (ml/min)
IS Park	22	60	174	0.81	121.4
DH Cho	25	63	172	0.85	118.4
SH Park	24	55	168	0.98	90.4
GH No	41	67	173	0.82	112.3
IS Kim	38	86	174	0.89	134.2
CJ Park	24	55	170	0.9	98.5
JB Pyo	25	65	170	0.93	87.4
JS Park	24	55	172	0.83	97.1
MS Song	25	60	172	0.9	106.5
Mean±SD	27.6±6.9	62.8±11.5	171.7±6.0	0.9±0.2	108.4±17.7

Cl_{er}= serum creatinine clearance by Cockcroft and Gault method (Shargal and Yu 1993).

$$Cl_{er} = \frac{(140 - age) \times body\ weight(kg)}{72 \times S_{cr}}$$

Table 2. Pharmacokinetic parameters of cephradine administered orally at 09:00(a.m.) and 22:00(p.m) in healthy volunteers (n=9)

Parameter	a.m.	p.m.
C_{max} (μg/ml)	5.88±1.18	6.97±1.40
T_{max} (hr)	1.25±0.25	0.75±0.15
$K_a(hr^{-1})$	0.89±0.22	1.28±0.30
$K_e(hr^{-1})$	0.062±0.02	0.044±0.01
$t_{1/2}$(hr)	1.54±0.31	1.07±0.21
CL_t (ml/min)	0.25±0.05	0.33±0.06
AUC (μg/ml · hr)	15.2±3.04	12.1±2.44
AUC (%)	100	79.5

: Mean±S.D. *: $p<0.05$ significantly different from pm
C_{max} : peak concentration. T_{max} : time to reach peak concentration,
CL_t: total body clearance, $t_{1/2}$: half life
K_a : absorption rate constant
AUC : area under plasma concentration time curve
K_e : elimination rate constant
AUC(%): AUC p.m. /AUC a.m.×100

15.4 mg) for daytime than that (38.9 ± 11.7 mg) for nighttime; however, the difference was not statistically significant. After it has been shown that the renal elimination is the primary route of elimination of cephradine The time of administration was not affected to the extent of urinary excretion. Hepatic metabolism may be affected by the time of cephradine administration.

The list of calculated pharmacokinetic parameters are summerized in Table 2. The time to reach the peak concentration (T_{max}) and the terminal half-life ($t_{1/2}$) of cephradine were significantly longer for the day than

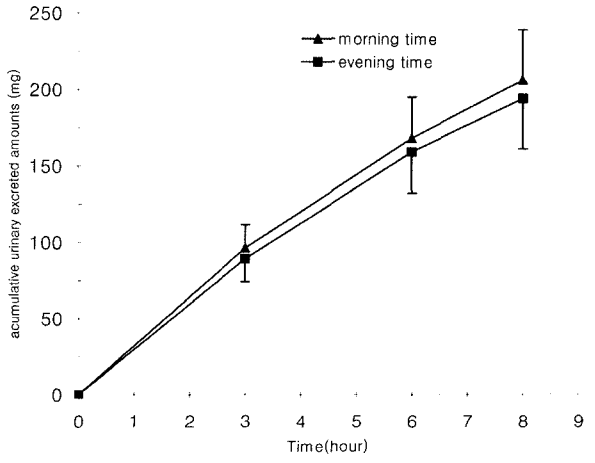

Fig. 2. The plot of mean cumulative urinary excreted amounts (mg) of cephradine in subjects administred orally at 09:00 and 22:00.

the nighttime ($p<0.05$). As expected from AUC, the total body clearance (CL_t) was significantly smaller (0.25 ± 0.05 ml/min) for the day than the nighttime (0.33 ± 0.06 ml/min, $p<0.05$).

In summary, the time of administration affected the pharmacokinetics of cephradine; the total body clearance was smaller and the AUC was higher for the daytime. Based on the estimation of extent of urinary excretion of cephradine, the difference in total body clearance is likely to be related to the difference in renal excretion of cephradine by the time of administration. In this study, we did not attempt to measure the toxic effect of cephradine in the time of administration. Since cephradine is a relatively safe drug, the pharmacokinetic difference may not be directly related to cephradine toxicity in the clinical setting. However, the primary pathway for cephradine (i.e., N-acetylation and N-glucuronidation) metabolism also represents major route of elimination for other drugs. Therefore, for drugs that are metabolized by the phase II metabolism and possess narrow therapeutic indices, the circadian changes in drug metabolism found in this study may be clinically relevant.

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