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Circadian Changes in Pharmacokinetics of Sulfamethoxazole Administered Orally to Rabbits

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Circadian variations of sulfamethoxazole pharmacokinetics were studied after a single oral administration of sulfamethoxazole, 50 mg/kg, to rabbits at 09:00 (a.m.) and 22:00 (p.m.). The profiles of plasma sulfamethoxazole concentration showed from 6h to 24 h significant statistical difference ($p<0.05$) between 09:00 and 22:00. The half-life ($t_{1/2}$) was significantly shorter in the morning (11.2 ± 3.2 h) when compared to the nighttime (15.4 ± 3.5 h) ($p< 0.05$). The AUC was significantly decreased in the morning (1325 ± 264 μg/ml·h) than that in the nighttime (2059 ± 379 μg/ml·h) ($p<0.05$). Total body clearance (CL_t) was significantly higher when sulfamethoxazole was given in the morning (6.65 ± 0.23 ml/min) versus in the nighttime (4.28 ± 0.20 ml/min) ($p<0.05$).

Key words: Circadian rhythm, Pharmacokinetics, Sulfamethoxazole, Total body clearance, AUC

INTRODUCTION

After Radzialowski and Bousguet demonstrated circadian changes in the oxidative metabolism of drugs in 1968, various physiological processes that govern kinetics of drug in the body appear to be affected by the 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 (Markiewicz, Semenowicz *et al.*, 1980), vancomycin (Choi, You *et al.*, 1996), aspirin (Markiewicz and Semenowicz, 1982), midazolam (Klotz, and Ziegler, 1982), nortriptyline (Nakano and Hollister, 1978), indometacin (Clench, Reinberg *et al.*, 1981), antipyrine (Vessel, Shively *et al.*, 1977), and cyclosporine (Choi and Park, 1999) were increased, and urinary excretions of the drugs were decreased in the morning. In addition, the times to reach a peak concentration and half-lives of clorazepate dipotassium (Hrushesky, W., Levi, F. *et al.*, 1980), theophylline (Kyle, Smolensky *et al.*, 1980), valproic acid (Loiseau, Cenraud *et al.*, 1982) and gentamycin (Choi *et al.*,1999) were shortened with administration of the drugs in the morning, and the urinary excretions of cisplastin (Hrushesky, Borch *et al.*, 1982) and acetaminophen (Mattok. and Mcgilveray, 1973) were increased in the morning.

Sulfamethoxazole is a widely used antibiotics in the clinical setting. The drug is categorized as an intermediate-acting sulfonamide, and is a component of Co-trimoxazole®. It is almost completely absorbed from the gastrointestinal tract and widely distributed into most body tissues (Mandel & Petri 1996). In human, N^4-acetylation, mediated by the polymorphic *N*-acetyltransferase, appears to be the primary route of elimination for sulfamethoxazole (Vree *et al.*, 1979; Levy *et al.*, 1998). Secondary to the *N*-acetylation, N^4-glucuronide conjugation in the liver (Vree *et al.*, 1979; Levy *et al.*, 1998) is also involved in the elimination. In contrast, only 14% of unchanged sulfamethoxazole is excreted via the kidney (Mandel & Petri 1996). Consistent with the minor role of urinary excretion in sulfamethoxazole elimination, it has been reported (Vree *et al.*, 1981) that the plasma half-life of sulfamethoxazole was not apparently affected in patients with impaired kidney function. Since the sulfamethoxazole is primarily excreted via drug metabolism and, in general, circadian change may affect the kinetics of drug metabolism, pharmacokinetics of sulfamethoxazole

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may be affected by the physiological fluctuation. Unfortunately, it is not known whether a temporal dependency exists for sulfamethoxazole pharmacokinetics. Therefore, the purpose of this study is to study the circadian changes in the pharmacokinetics of sulfamethoxazole after a single oral administration of the drug to rabbits.

MATERIALS AND METHODS

Chemicals and animals

Sulfamethoxazole and phenacetin (the internal standard of high performance liquid chromatographic, HPLC assay) were obtained from Sigma Chemical Company (St. Louis, MO., USA). HPLC-grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). Urethane was a product from Junsei Chemical Company (Tokyo, Japan). Other chemicals were of reagent grade or better, and used without further purification.

Male New Zealand white rabbits (Dae Han Laboratory of Aminal Research Center, Eumsung South Korea), weighing 2.0-2.4 kg, were used. The animal was fasted (with free access to water) at least 24 h before the commencement of the study.

Oral administration

Rabbits were anesthetized with a subcutaneous injection of 25% urethane, 4 ml/kg. The animal was then subjected to a surgical procedure involving catheterization of polyethylene tubing (Clay Adams, Parsippany, NJ, USA) into the right femoral artery and the urethra.

Sulfamethoxazole (sulfamethoxazole powder was suspended in 0.2% carboxymethyl cellulose and mixed for 30 min with a magnetic stirrer), was administered orally (dose, 50 mg/kg; total volume of administration, 20 mL) to a.m. (*i.e.*, 9:00 am; n=7) and p.m. (*i.e.*, 22:00 pm; n =7) groups by intubation. Blood samples (approximately 1.5 mL) were collected via the right femoral artery at 0 (to serve as a control), 0.25, 0.5, 1, 1.5, 2, 4, 6, 9, 12 and 24 h. Heparinized normal saline injectable solution (0.25 mL; 75 units per mL), was used to flush the cannula after the blood sampling to prevent blood clotting. Blood samples were centrifuged immediately at 10000 rpm for 5 min, and plasma samples collected and stored at -30°C until the analysis of sulfamethoxazole (see below). Urine samples were collected via the urethra between 0-2, 2-4, 4-6, 6-12 and 12-24 h after administration of the drug. After the measurement of the volume of urine collection, an aliquot of urine sample was stored at -30°C until the analysis. To compensate the loss of blood, normal saline was infused via the ear vein at the rate of 1.5 ml/h using infusion pump (Model 341A, Sage Instrument, Cambridge, MA, USA). Each rabbit was kept in supine position during the entire experimental period.

HPLC analysis of sulfamethoxazole

Sulfamethoxazole concentration in the biological samples was analyzed by the reported HPLC method (Vree *et al.*, 1994) with a slight modification. Briefly, to an aliquot (0.2 ml) of plasma or urine samples, equal volume of acetonitirile containing 10% of phenacetin (i.e., internal standard) was added. After vortex-mixing, the mixture was centrifuged at 10000 rpm for 5 min. and an aliquot (0.2 mL) of the 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 infinity (AUC) was calculated by the trapezoidal rule-extrapolation method (Kim *et al.* 1993); this method employed the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase (Chiou 1978) 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 sulfamethoxazole (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the experimental data. The mean of terminal half-lives was calculated by the harmonic mean method (Eatman *et al.*, 1977).

Statistical analysis

When it was necessary to compare mean values, the student's t-test was used. A P value of less than 0.05 was considered denoting statistical significance. All data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Temporal dependency in plasma concentration-time curves of sulfamethoxazole

Mean plasma concentration-time profiles of sulfamethoxazole in rabbits are shown in Fig. 1. After oral administration, the plasma concentrations of sulfamethoxazole increased; the C_{max} was reached its peak at 3-4 h, then declined thereafter in a monoexponential fashion for all rabbits studied (Fig. 1).

The time of sulfamethoxazole administration appears to affect the kinetics of the drug significantly. Sulfamethoxazole concentration in the plasma was lower for pm administration that that for am (Fig. 1). The difference reached statistically significance ($p<0.05$) for all plasma samples collected after 6 h of administration. As a result, the AUC was significantly decreased for am administration (1325 ± 264 μg/ml·h) than that for pm administration (2059 ± 379 μg/ml·h) ($p<0.05$, Table I).

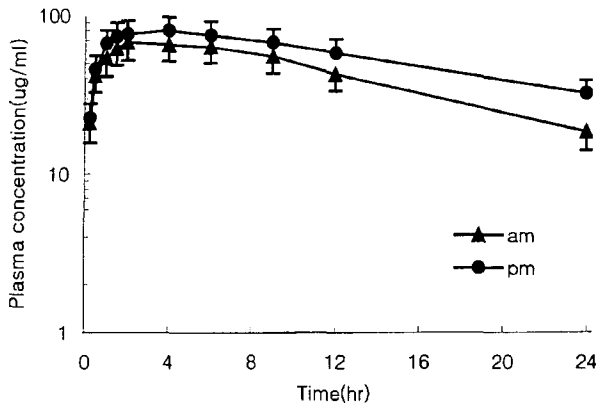

Fig. 1. The plot of mean plasma concentration (μg/ml) of sulfamethoxazole in rabbits administered orally at 09:00 (am) and 22:00 (pm).

Cumulative amount of urinary excretion for orally administered sulfmethoxazole

Cumulative amount of sulfmethoxazole urinary excretion (in mg) was shown in Fig. 2 for am and pm oral administration (dose, 50 mg/kg). The amount of excretion was slightly larger (42.4 ± 15.4 mg) for am than that (38.9 ± 11.7 mg) for pm; however, the difference was not statistically significant. Since it has been shown that the renal elimination is not the primary route of elimination for sulfmethoxazole (Mandel & Petri 1996) and the time of administration does not affect the extent of urinary excretion for the drug, non-renal elimination, most likely hepatic metabolism, may be affected by the time of sulfmethoxazole administration.

Pharmacokinetic parameter

The list of calculated pharmacokinetic parameters are summarized in Table I. In addition to AUC, mean half-life

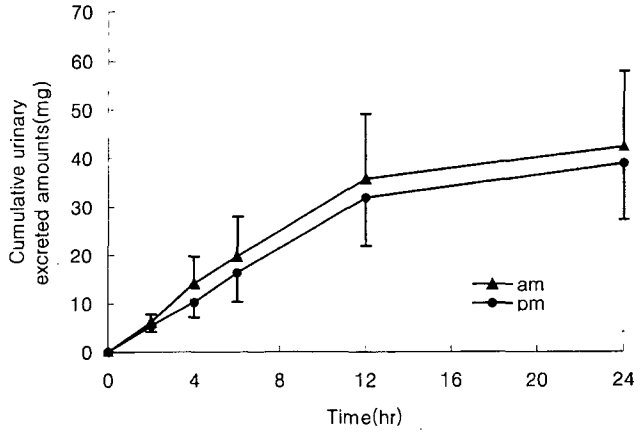

Fig. 2. The Plot of Mean Cumulative Urinary Excreted Amounts (mg) of Sulfamethoxazole in Rabbits Administred Orally at 09:00 and 22:00.

Table I. Summary of pharmacokinetic parameters of sulfamethoxazole with oral administeration in am (9:00) and pm (22:00) in rabbits.

Parameters	a.m.	p.m.
C_{max} (μg/ml)	70.8 ± 16.4#	82.4 ± 19.6
T_{max} (h)	2.62 ± 0.32	2.82 ± 0.42
$K_a(h^{-1})$	1.19 ± 0.21	1.22 ± 0.20
$K_e(h^{-1})$	0.062 ± 0.02	0.044 ± 0.01*
$t_{1/2}$(h)	11.2 ± 3.01	15.4 ± 3.53*
CL_t (ml/min)	6.65 ± 1.23	4.28 ± 0.86*
AUC (μg/ml·hr)	1325 ± 269	2059 ± 388*
AUC ratio (% of am)	100	155.4

#: Mean ± S.D. (n=6) *: $p<0.05$
C_{max}: peak concentration
T_{max}: time to reach peak concentration
CL_t: total body clearance
$t_{1/2}$: terminal half-life
K_a: absorption rate constant
AUC: area under plasma concentration time-curve
K_e: elimination rate constant

of sulfmethoxazole was significantly longer (11.2 ± 3.2 h) for am than that (15.4 ± 3.5 h) for pm ($p<0.05$). As expected from AUC, the total body clearance was significantly larger (47.9 ± 7.8 ml/min) for am administration than that (59.8 ± 8.5 ml/min) for pm administration (35.6% smaller for pm administration, $p<0.05$) (Table I).

In summary, the time of administration affected the pharmacokinetics of sulfmethoxazole; the total body clearance was smaller and the AUC higher for pm administration. Based on the estimation of extent of urinary excretion of sulfamethoxazole, the difference in total body clearance is likely to be related to the difference in hepatic metabolism of sulfamethoxazole at the time of drug administration. In this study, we did not attempt to measure the toxic effect of sulfamethoxazole in the pm administration. Since sulfamethoxazole is a relatively safe drug, the pharmacokinetic difference may not be directly related to sulfamethoxazole toxicity in the clinical setting. However, the primary pathway for sulfamethoxazole (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 indice, the circadian changes in drug metabolism found in this study may be clinically relevant.

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