GASTROINTESTINAL ABSORPTION OF SULFAGUANIDINE IN NEONATAL AND ADULT RATS*

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The pharmacokinetics of sulfaguanidine in rats was studied after its intravenous or oral administration. In adult rats, its elimination from plasma, after intravenous administration of 2.5 or 25 mg/kg doses, could be described by a two-compartment open model, while its plasma concentration after oral administration of 25 mg/kg doses, agreed with the one- or two-compartment open model. Neonatal rats displayed a lower elimination of sulfaguanidine than adult rats. Comparison of the drug's gastrointestinal absorption showed that the maximum plasma concentration after oral administration was significantly higher for neonatal than for adult rats. However, there was no significant difference between the times required to reach maximum plasma concentrations. The area under the plasma concentration-time curve at 0– ∞ h was significantly higher for neonatal than for adult rats. The absolute bioavailability (57.86%) in neonatal rats was approximately five times that (12.76%) in adult rats. Thus, sulfaguanidine was poorly absorbed by adult rats, but was efficiently absorbed by immature gastrointestinal tracts of neonatal rats.

Keywords — sulfaguanidine; gastrointestinal absorption; *in vivo*; neonatal rat; pharmacokinetics; bioavailability; plasma level

INTRODUCTION

During the neonatal period, the intestinal mucosa in mammalian species is immature, for various lengths of time. The physiological, morphological and functional characteristics of the gastrointestinal tract differ between neonate and adult, and thus, the gastrointestinal absorption of drugs differs between these age groups.^{1–5)} The oral bioavailability of several acid-labile drugs, *e.g.*, benzylpenicillin,⁶⁾ ampicillin⁷⁾ and nafcillin,⁸⁾ in neonates increases because the stomach pH approaches neutrality. However, delayed oral absorption has been reported for nalidixic acid,⁹⁾ rifampicin¹⁰⁾ and phenytoin.¹¹⁾ Oral absorption in neonates comparable with that observed in older children has been reported for various readily absorbable sulfonamides,^{12–15)} *e.g.*, sulfisoxazole, sulfadiazine, sulfisomidine and sulfamethoxypyrazine, with no differences among these drugs. In this study, we examined rat gastrointestinal absorption of sulfaguanidine, which is a poorly absorbable drug,¹⁶⁾ and determined the pharmacokinetic parameters after its intravenous and oral administration.

EXPERIMENTAL

Materials — Sulfaguanidine was purchased from Tanabe Seiyaku Co., Ltd. All other chemicals were of analytical reagent grade.

Animal Experiments — 1) Adult Rats (8–10 Weeks Old): Male Wistar rats weighing 223 $\pm$ 21 g were used. A jugular vein was cannulated with silicon tubing (Dow Corning Co., inner dia. 0.02 inch, outer dia. 0.037 inch) according to literature.^{17,18)} Rats were housed in individual cages and were fasted overnight after operation.

a) Intravenous Administration: The rats were given 1.0 ml of sulfaguanidine solution (2.5 or 25 mg/kg body weight) by injection into the jugular vein. b) Oral Administration: The rats were given 2.5 or 25 mg/kg of sulfaguanidine (0.1 ml/10 g body weight of the solution) *via* a stomach tube and were not allowed to take water. Blood samples (0.15 ml) were collected from a jugular vein at regular intervals after drug administration. The blood was centrifuged immediately after collection and the plasma was preserved at –20 °C until analysis.

2) Neonatal Rats (1 Week Old): Male and female Wistar rats weighing 10.6 $\pm$ 1.3 g were

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used. The rats were fasted overnight prior to the experiments. a) Intravenous Administration: The rats were anesthetized with pentobarbital Na and kept at 37 °C during the experiments. Parts of the cervicalis of each rat were opened and 0.01 ml of sulfaguanidine solution (2.5 mg/kg body weight) was injected into the jugular vein using a 10- μ l microsyringe. The incised areas were closed using Aronalph[®]. b) Oral Administration: Each rat was given 2.5 mg/kg body weight of sulfaguanidine (0.1 ml/10 g body weight) into the stomach *via* a polyethylene tube (inner dia. 0.28 mm, outer dia. 0.61 mm), which was attached to a 0.5-ml syringe and introduced through the mouth. The rats were then fasted. Blood samples (0.2 ml) were collected by cardiocentesis at various times after drug administration. The blood was centrifuged immediately after collection and the plasma was preserved at -20 °C until analysis.

Determination of Sulfaguanidine in Plasma — To 0.05 ml of the plasma sample, 0.05 ml of distilled water containing 20 μ g/ml of sulfamethizole as an internal standard, 0.05 ml of distilled water, 0.05 ml of phosphate buffer solution (pH 7.5) and 5.0 ml of ethyl acetate were added. After mixing, the mixture was centrifuged at 1500 rpm for 3 min. A 4-ml portion of the ethyl acetate layer was then transferred to a 15-ml glass-stoppered centrifuge tube and evaporated to dryness at 40 °C. The dry extract was reconstituted in 0.25 ml of 0.1 N HCl mixed and then 100 μ l of the solution was injected in a Hitachi Model 655-15 high-performance liquid chromatograph equipped with a 100- μ l loop injector. The wavelength of the ultraviolet (UV) detector was set at 260 nm and sensitivity kept at 0.01 a. u. f. s. The mobile phase of water-methanol-acetic acid (85:15:0.5) was filtered through a 0.45- μ m membrane filter (Toyo Roshi, Tokyo, Japan) and degassed in an ultrasonic bath prior to use. Analyses were performed at room temperature on a stainless steel column (25 cm $\times$ 4.6 mm i.d.) packed with Fine SIL C₁₈ (10- μ m particles) (Japan Spectroscopic Co., Tokyo, Japan). The flow rate was 1.5 ml/min.

Calibration — Peak-height ratios were calibrated by dividing the heights of the sulfaguanidine peak by the height of the internal standard peak and were plotted against the amounts of sulfaguanidine. The calibration graphs were linear over the range of 0.1–10.0

μ g/ml of sulfaguanidine. The correlation coefficient for sulfaguanidine was greater than 0.98 ($n = 10$).

Data Analysis — Plasma concentration-time curves were analyzed according to the least-squares regression analysis program MULTI¹⁹⁾ for mono- or bi-exponential decline. The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were calculated by the standard linear trapezoidal integration with extrapolation to infinite time. The steady state distribution volume per body weight (V_{dss}) and the total body clearance per body weight (CL_t) were estimated as described by Yamaoka *et al.*²⁰⁾ The significant difference in pharmacokinetic parameters of sulfaguanidine was assessed by means of Student's *t*-test.

RESULTS AND DISCUSSION

Adult Rat

Figure 1 shows the mean semilogarithmic concentration-time plots of plasma concentrations of sulfaguanidine after intravenous administration of 2.5 or 25 mg/kg to adult rats. The elimination of many sulfonamides from rat plasma is considered to fit a one-compartment

FIG. 1. Mean Plasma Concentration of Sulfaguanidine after Intravenous Administration to Neonatal (2.5 mg/kg; ▲) and Adult (2.5 mg/kg, ●; 25 mg/kg, ◆) Rats

All points represent mean + S. D. of three rats.

TABLE I. *Pharmacokinetic Parameters after Intravenous Administration of Sulfaguanidine (2.5 mg/kg or 25 mg/kg) to Adult Rats*

Parameter		Dose 2.5 mg/kg	Dose 25 mg/kg
A	($\mu\text{g/ml}$)	6.63 ± 1.74	65.24 ± 6.81
B	($\mu\text{g/ml}$)	2.43 ± 0.59	18.16 ± 2.17
α	(h^{-1})	4.24 ± 0.39	4.73 ± 0.26
β	(h^{-1})	0.42 ± 0.19	0.34 ± 0.02
V_c	(l/kg)	0.29 ± 0.07	0.30 ± 0.03
K_{12}	(h^{-1})	1.99 ± 0.36	2.52 ± 0.14
K_{21}	(h^{-1})	1.45 ± 0.05	1.30 ± 0.20
K_{10}	(h^{-1})	1.22 ± 0.55	1.25 ± 0.07
$AUC_{0-\infty}$	($\mu\text{g}\cdot\text{h/ml}$)	8.18 ± 1.70	77.24 ± 4.52

All data are mean $\pm$ S.D. of three rats.

model.²¹⁾ However, in the case of sulfaguanidine, the data fitted the two-compartment model expressed by the formula.²²⁾

$$(C_p = A e^{-\alpha t} + B e^{-\beta t})$$

C_p is the drug concentration in the plasma at time t . The constants A and B are intercepts on the y axis for each exponential segment of the curve in the equation.

The pharmacokinetic parameters are shown in Table I. The A, B and $AUC_{0-\infty}$ values were proportional to the administered doses. The α , β , K_{12} , K_{21} , K_{10} and V_c values were the same after dosing with 2.5 or 25 mg/kg.

Figure 2 presents the plasma concentration of the sulfaguanidine-time curve at each sampling time after oral administration of 25 mg/kg to adult rats. The pharmacokinetic parameters calculated for these models are shown in Table II. The number of compartments changed with the route of administration. The number of exponential terms depended not only on the drug but

also on the animal used.²⁰⁾ In the present study, three cases of five rats were consistent with a

FIG. 2. *Plasma Concentration after Oral Administration of Sulfaguanidine (25 mg/kg) to Adult Rats*TABLE II. *Pharmacokinetic Parameters after Oral Administration of Sulfaguanidine (25 mg/kg) to Adult Rats*

Parameter		1-Compartment			2-Compartment	
		1	2	3	4	5
Lag time	(h)	0	0.16	0.30	0	0
α	(h^{-1})	—	—	—	0.39	0.86
β	(h^{-1})	—	—	—	0.48	0.39
K_a	(h^{-1})	0.73	1.06	1.50	1.51	0.83
K_{el}	(h^{-1})	0.65	0.49	0.92	—	—
K_{12}	(h^{-1})	—	—	—	0.11	0.01
K_{21}	(h^{-1})	—	—	—	0.72	0.84
K_{10}	(h^{-1})	—	—	—	0.26	0.40
$AUC_{0-\infty}$	($\mu\text{g}\cdot\text{h/ml}$)	7.11	13.60	23.51	31.48	24.82

one-compartment model

$$(C_p = B e^{-K_{el}t} - A e^{-Kat})$$

but the data for the other two rats fitted the two-compartment model expressed by the formula.

$$(C_p = B e^{-\beta t} + A e^{-\alpha t} - (A+B) e^{-Kat})$$

The pharmacokinetic parameters obtained after oral administration were influenced more by the differences among individual rats than the pharmacokinetic parameters obtained after intravenous injection. Thus, comparison of the gastrointestinal absorption between neonatal and adult rats was evaluated by moment analysis, a model-independent method.

Comparison of Neonatal and Adult Rats

1) *Intravenous Administration* — Plasma concentrations of sulfaguanidine after its intravenous injection at 2.5 mg/kg to neonatal rats are shown in Fig. 1. The means of the initial plasma concentrations (C_p^0) in neonatal and adult rats were about the same (9.60 and 9.05 $\mu\text{g/ml}$, respectively). The neonates displayed delayed elimination of other sulfonamides from their bodies because their metabolic and excretive functions are immature.¹²⁻¹⁵ Thus, the mean of the elimination rate (0.25 h^{-1}) in neonatal rats was significantly lower than that (0.42 h^{-1}) in adult rats.

Table III gives the results of moment analysis after intravenous administration of sulfaguanidine (2.5 mg/kg) to neonatal and adult rats. There were significant differences in the total body clearance per body weight (CL_t) and the mean residence time (MRT) between neonatal and adult rats, suggesting that elimination of sulfaguanidine was slower from neonatal than from adult rats. There was also a significant difference in the area under the plasma concentration-time curve ($AUC_{0-\infty}$), although C_p^0 were about the same in both types of rats. Therefore the dif-

ference of $AUC_{0-\infty}$ between neonatal and adult rats was attributable to delay of the elimination rate in neonatal rats, although the steady state distribution volume per body weight ($V_{d_{ss}}$) values were about the same.

2) *Oral Administration* — Figure 3 shows the mean plasma concentrations of sulfaguanidine following its oral administration at 2.5 mg/kg to neonatal and adult rats. Its plasma concentrations in neonatal rats at each time measured were significantly higher than those in adult rats.

Table IV presents the pharmacokinetic parameters. The values of the maximum plasma concentration (C_{max}) and the time required to

FIG. 3. Mean Plasma Concentration after Oral Administration of Sulfaguanidine (2.5 mg/kg) to Adult (●) and Neonatal (▲) Rats
All points represent mean $\pm$ S. D. of three rats.
a) $p < 0.05$, compared to adult rat.

TABLE III. Pharmacokinetic Parameters after Intravenous Administration of Sulfaguanidine (2.5 mg/kg) to Adult and Neonatal Rats

Parameter	Adult	Neonate
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/ml}$)	8.18 ± 1.70	20.46 ± 8.41^b
MRT (h)	2.28 ± 0.78	4.33 ± 0.38^a
$V_{d_{ss}}$ (l/kg)	0.65 ± 0.13	0.59 ± 0.25
CL_t (l/h/kg)	0.29 ± 0.04	0.14 ± 0.07^a

All data are mean $\pm$ S.D. of three rats. a) $p < 0.05$, compared to adult rat. b) $p < 0.10$, compared to adult rat.

TABLE IV. Bioavailability Parameters after Oral Administration of Sulfaguanidine (2.5 mg/kg) to Adult and Neonatal Rats

Parameter		Adult	Neonate
$C_{\max}$	($\mu\text{g/ml}$)	0.41 ± 0.06	3.56 ± 1.70^b
$T_{\max}$	(h)	1.67 ± 0.29	1.50 ± 0
MAT	(h)	0.81 ± 0.34	0.83 ± 0.38
$AUC_{0-\infty}$	($\mu\text{g}\cdot\text{h/ml}$)	1.04 ± 0.25	11.84 ± 2.67^a
Bioavailability	(%)	12.76 ± 3.07	57.86 ± 13.04^a

All data are mean $\pm$ S.D. of three rats. a) $p < 0.01$, compared to adult rat. b) $p < 0.05$, compared to adult rat.

reach the maximum plasma concentration ($T_{\max}$) were read directly from the concentration-time data. The absolute bioavailability (BA) was given by:

$$\text{BA} = AUC_{0-\infty} (p.o.) / AUC_{0-\infty} (i.v.)$$

The mean absorption time (MAT) was calculated by:

$$\text{MAT} = \text{MRT} (p.o.) - \text{MRT} (i.v.)$$

$C_{\max}$ of sulfaguanidine was significantly higher in neonatal than in adult rats. The mean of the $AUC_{0-\infty}$ was significantly higher for neonatal than for adult rats. Therefore, the absolute bioavailability of neonatal rats was about five times that of adult rats. The gastric emptying time in the neonates is commonly prolonged and thus may be expected to delay $T_{\max}$.¹⁻⁴⁾ However, there was no significant difference in $T_{\max}$ and MAT between neonatal and adult rats. This result may be attributable to a difference of absorption sites. The gastric pH value is considered to be higher in neonates.¹⁻⁴⁾ The absorption rate of sulfonamides increases and decreases passing the maximum point with increasing pH.²³⁾ Therefore, neonates may absorb more sulfaguanidine from the stomach than adult rats. Royer *et al.*²⁴⁾ found increased gastrointestinal absorption of sulfaguanidine in patients with gastrointestinal diseases. Nadai *et al.*²⁵⁾ reported that the gastrointestinal absorption of sulfaguanidine, having no interaction with tetracycline in rats, increased to a considerable extent in the presence of tetracycline. This tetracycline effect is attributable to changes of the small intestinal tissue with respect to permeability. Kakemi *et al.*¹⁶⁾ also reported that the gastrointestinal absorption of sulfaguanidine in rats increased in the presence of bile salts, which directly acted on the structure of the absorption surface. The intesti-

nal mucosa in the neonate is immature compared with that in the adult and the gastrointestinal function is abnormal in the neonate.²⁶⁾ Thus, absorption of sulfaguanidine readily occurs through the gastrointestinal in neonatal rats.

REFERENCES

- 1) P. L. Morselli: Clinical pharmacokinetics in neonates, *Clin. Pharmacokinet.*, **1**, 81–98 (1976).
- 2) P. L. Morselli, R. Morselli and L. Bossi: Clinical pharmacokinetics in newborns and infants. Age-related differences and therapeutic implications, *Clin. Pharmacokinet.*, **5**, 485–527 (1980).
- 3) T. P. Green and B. L. Mirkin, "Pharmacokinetic Basis for Drug Treatment," ed. by L. Z. Benet *et al.*, Raven Press, New York, 1984, pp. 269–282.
- 4) D. M. Hilligoss, "Applied Pharmacokinetics," ed. by W. E. Evan, J. J. Schentag and W. J. Jusko, Applied Therapeutics Inc., San Francisco, 1980, pp. 76–94.
- 5) S. J. Henning, "Intestinal Toxicology," ed. by C. M. Shiller, Raven Press, New York, 1984, pp. 17–31.
- 6) N. N. Huang and R. H. High: Comparison of serum levels following the administration of oral and parenteral preparations of penicillin to infants and children of various age groups, *J. Pediatr.*, **42**, 657–668 (1953).
- 7) J. Silverio and J. W. Poole: Serum concentrations of ampicillin in newborn infants after oral administration, *Pediatrics*, **51**, 578–580 (1973).
- 8) W. J. O'Connor, G. H. Warren, L. S. Edrada, P. S. Mandala and S. B. Rosenman: Serum concentrations of sodium nafcillin in infants during the perinatal period, *Antimicrob. Agents. Chemother.*, **1965**, 220–222.
- 9) R. J. Rohwedder, C. Simon, W. Kübler and M. Hohe-nauer: Untersuchungen über die Pharmakokinetik von Nalidixinsäure bei Kindern verschiedenen Alters, *Z. Kinderheilkd.*, **109**, 124–134 (1970).
- 10) G. Acocella: Clinical pharmacokinetics of rifampicin, *Clin. Pharmacokinet.*, **3**, 108–127 (1978).
- 11) B. Jalling, L. O. Boreus, A. Rane and F. Sjoqvist: Plasma concentrations of diphenylhydantoin in young infants, *Pharmacol. Clin.*, **2**, 200–202 (1970).
- 12) F. H. Dost and E. Gladtko: Pharmakokinetik des 2-Sulfanilamido-3-methoxy-pyrazin beim Kind, *Arzneim.-Forsch.*, **19**, 1304–1307 (1970).

- 13) B. Krauer, P. Spring and L. Dettle: Zur Pharmakokinetik der Sulfonamide im ersten Lebensjahr, *Pharmacol. Clin.*, **1**, 47–53 (1968).
- 14) F. Sereni, L. Perletti, E. Marubini and G. Mars: Pharmacokinetic studies with a long-acting sulfonamide in subjects of different ages, *Pediatr. Res.*, **2**, 29–37 (1968).
- 15) W. Brumfitt, J. M. T. Hamilton-Miller and J. Kosmidis: Trimethoprim-sulfamethoxazole: the present position, *J. Infect. Dis.*, **128**, 778–791 (1973).
- 16) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura and A. Okita: Effect of bile salts on the gastrointestinal absorption of drugs. II. Mechanism of the enhancement of the intestinal absorption of sulfaguanidine by bile salts, *Chem. Pharm. Bull.*, **18**, 1034–1039 (1970).
- 17) J. R. Weeks and J. D. Davis: Chronic intravenous cannulas for rats, *J. Appl. Physiol.*, **19**, 540–541 (1964).
- 18) R. A. Upton: Simple and reliable method for serial sampling of blood from rats, *J. Pharm. Sci.*, **64**, 112–124 (1975).
- 19) K. Yamaoka, Y. Tanigawara, T. Nakagawa and T. Uno: A pharmacokinetic analysis program (MULTI) for microcomputer, *J. Pharm. Dyn.*, **4**, 879–885 (1981).
- 20) K. Yamaoka, T. Nakagawa and T. Uno: Moment analysis for disposition kinetics of several cephalosporin antibiotics in rats, *J. Pharm. Pharmacol.*, **35**, 19–22 (1983).
- 21) M. Yamazaki, M. Aoki and A. Kamada: Biological activities of drugs IV. Physicochemical factors affecting the excretion of sulfonamides in rats, *Chem. Pharm. Bull.*, **16**, 721–727 (1968).
- 22) S. Riegelman, J. C. K. Loo and M. Rowland: Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment, *J. Pharm. Sci.*, **57**, 117–123 (1968).
- 23) T. Koizumi, T. Arita and K. Kakemi: Absorption and excretion of drugs XX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (2). absorption from rat small intestine, *Chem. Pharm. Bull.*, **12**, 421–427 (1964).
- 24) R. J. Royer, J. Y. Hesse, M. J. Royer-Morrot, P. Netter and A. Petit: Sulfamides intestinaux et absorption digestive, *Ann. Med. Nancy. Est.*, **20**, 509–512 (1981).
- 25) T. Nadai, K. Nishii and A. Tatematsu: Effect of tetracyclines on the absorption of drugs from the intestine. II. Effect of tetracyclines on the absorption of sulfonamides, *Yakugaku Zasshi*, **92**, 384–387 (1972).
- 26) I. H. Stevenson, S. A. M. Salem, K. O'Malley, B. Cusack and J. G. Kelly, "Drug absorption," ed. by L. F. Prescott and W. S. Nimmo, ADIS Press, Australia, 1979, pp. 253–261.