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Pharmacokinetics and Renal Excretion of Sulfamethoxazole in Sheep

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Abstract □ Pharmacokinetics and urinary excretion of sulfamethoxazole were investigated in healthy sheep. From the plasma disappearance curves after intravenous bolus injection (50 mg/kg), the half-life and volume of distribution were found to be 76 ± 14 min and 0.41 ± 0.18 lit/kg respectively. Body clearance was 4.06 ± 1.03 ml/kg/min. Very low Concentration of drug was present in plasma after 3 hours of administration and plasma level at 6 hour was only 4.4 ± 2.0 μg/ml. The renal clearance of sulfamethoxazole (22 ± 2.17 ml/min/10 kg) exceeded the creatinine clearance (9.78 ± 1.57 ml/min/10 kg) which may be due to involvement of active tubular secretion and pH dependent back diffusion. Half of the dose of sulfamethoxazole was excreted as unchanged free drug while acetylated amine comprised of 20 percent within the first 6 hours of drug administration.

Keywords □ Pharmacokinetics, Renal excretion, Sulfamethoxazole, Sheep.

Environmental variation can influence the disposition kinetics of drugs in animals.[1,2] Previous study showed that glomerular filtration rate for unit body weight in Pakistani sheep was half to two-thirds that of sheep studied elsewhere.[3] Similarly pH values of blood and urine were found to be higher in local sheep than the values reported in the literature.[4] The variation in pH alongwith environmental influences in our country[1-4] demands extensive investigation in the kinetics of drugs such as sulfonamides which are mainly excreted through kidney and their excretion is largely influenced by the changes in urinary pH.[5] Sulfamethoxazole is one of such drugs that is commonly used either alone or in combination with other antibacterial agents to treat various infections. In view of this and in continuation of our previous studies, the present paper describes the pharmacokinetics and urinary excretion of sulfamethoxazole in sheep.

EXPERIMENTAL METHODS

Experiments were performed during the months of July and August in 7 normal adult sheep of Kajli breed. The average weight of the sheep was 34 kg (32-40 kg). The animals were given free access to food and water and maintained under uniform conditions of management.

Pharmacokinetics

For collection of blood samples, an intravenous cannula was placed in one of the jugular veins. In all experiments, prior to drug administration, control blood and urine samples were collected. Each animal was given a single dose (50 mg/kg) of sulfamethoxazole through the opposite jugular vein. Blood samples were collected in heparinized tubes at 5, 10, 15, 30 and 45 minutes following drug administration and then at 30 minutes interval until 6 hours, and there after at 8 and 12 hours of drug administration. Plasma was separated by centrifugation and stored at 4 °C until the next day when analysis was performed. The concentration of sulfamethoxazole was measured spectrophotometrically by the method of Bratton and Marshall.[6] The plasma concentration time data were analyzed for each animal. For the calculation of various parameters, a programmable calculator (T1-59) was used with the programme for two compartment open model · kinetics and each pharmacokinetic term was expressed as mean ± SD.

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Protein Binding

The extent of binding of sulfamethoxazole to proteins in plasma was determined by ultrafiltration through a celluphane membrane. The celluphane membrane used was dialyzing tube with diameter 32 mm and pore size 20 to 80 Å which should permit molecules with a molecular weight upto 5,000 to pass through.

Renal Clearance

Renal clearance studies were conducted in 6 adult female sheep. At 30 minutes after drug administration, the urinary bladder was emptied and washed with distilled water through a balloon catheter (Foly No. 18, 30 m*l*). First urine sample was collected 30 minutes after washing. Subsequently 3 more urine samples were collected at the interval of 30 minutes. The clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR). Creatinine determination was carried out according to the method described by Bonsness and Taussky.[7] The clearance of total and non-protein bound sulfamethoxazole was determined. Influence of urine pH, rate of urine flow and plasma drug concentration on the renal clearance of drug was examined by regression analysis.

Urinary Excretion

For the study of urinary excretion, urine samples from 6 sheep were collected upto 360 minutes after drug administration. The volume of each urine sample was measured and the pH of the samples was recorded by means of pH meter (Labsco) with glass electrode. The urinary excretion of sulfamethoxazole was expressed as the percentage (Average ± SD) of dose excreted as total, free and acetylated amine.

RESULTS

Pharmacokinetics

The values of kinetic parameters which describe distribution and elimination of sulfamethoxazole in normal sheep are given in Table I. The mean distribution half-time was 7.69 ± 4.6 minutes and the elimination half-life was 76 ± 14 minutes. A semilogarithmic plot of decline in plasma concentration of sulfamethoxazole with time is shown in Fig. 1. It is seen that pseudo-distribution equilibrium was attained within the first half an hour after intravenous injection. The level of drug in blood declined rapidly and the concentration of sulfamethoxa-

Table 1. Disposition kinetics of sulfamethoxazole after a single intravenous dose (50 mg/kg) in sheep (n = 7)

Kinetic parameters	Units	Mean ± S.D.
C_pO	μg/m*l*	262.8 ± 49.2
A	μg/m*l*	141.3 ± 44.8
B	μg/m*l*	121.5 ± 36.9
α	min^{-1}	0.126 ± 0.08
β	min^{-1}	0.0093 ± 0.0016
$t_{1/2}(\alpha)$	min	7.69 ± 4.6
$t_{1/2}(\beta)$	min	76.4 ± 14.0
K_{12}	min^{-1}	0.053 ± 0.038
K_{21}	min^{-1}	0.056 ± 0.044
K_{el}	min^{-1}	0.026 ± 0.097
V_c	lit/kg	0.196 ± 0.044
V_d(area)	lit/kg	0.409 ± 0.178
$V_d(\beta)$	lit/kg	0.457 ± 0.189
^{Cl}B	m*l*/kg/min	4.065 ± 1.035

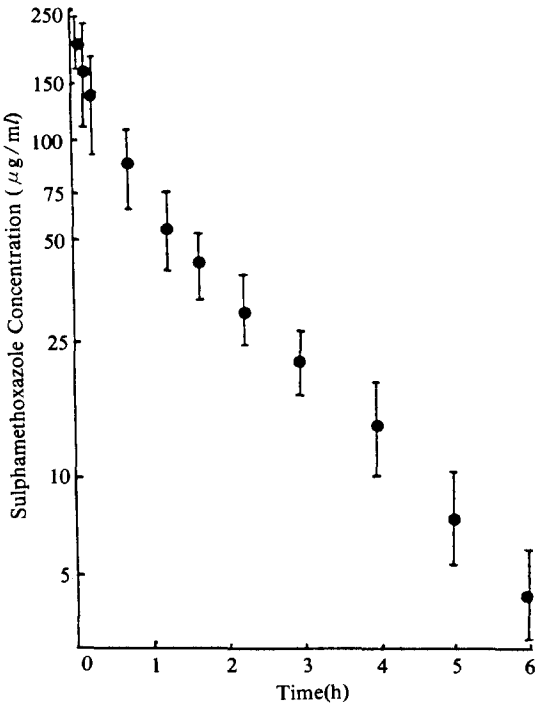

Fig. 1. Plasma concentration (average ± SD) on a semilogarithmic scale versus time after a single intravenous injection of sulfamethoxazole (50 mg/kg) to 7 sheep.

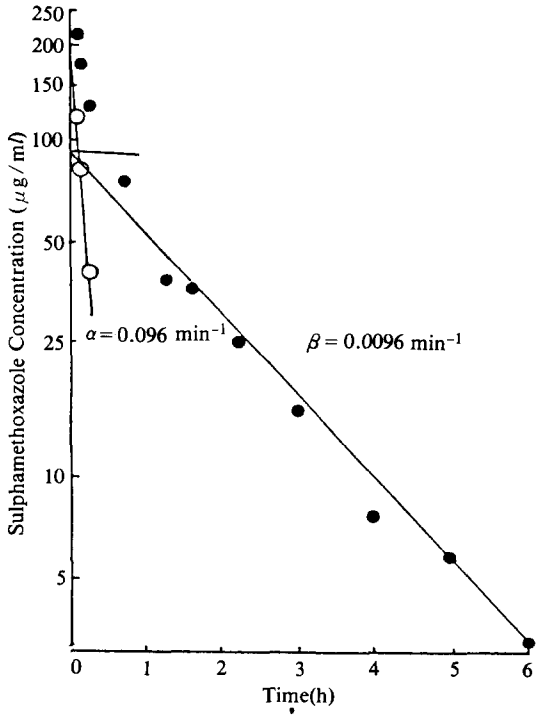

Fig. 2. Semilogarithmic plot of plasma sulfamethoxazole concentration (●) following administration of single dose (50 mg/kg) to sheep 6 weighing 37 kg depicting distribution (α) and elimination phases (β) of the biexponential drug level time profile.

zole in plasma 6 hours after injection was only 4.4 ± 2.0 μg/ml. The therapeutic level (> 25 μg/ml) was maintained only upto 160 minutes. The apparent volume of distribution (V_d) relates drug concentration in plasma to the total amount of drug in the body after the distribution equilibrium has been attained. The value of $V_{d(area)}$ was 0.41 ± 0.18 lit/kg.

Clearance Cl(β) is the volume of blood cleared of the drug by the elimination processes of biotransformation and excretion during unit time and is a product of $\beta \times V_d$ (area) or $K_{el} \times V_d$ in a two compartment open model. The clearance value of sulfamethoxazole in sheep was 4.06 ± 1.03 ml/min/kg.

A semilogarithmic plot of decline in plasma concentration of sulfamethoxazole with time which was obtained in sheep 6 (37 kg body weight) is shown in Fig. 2. The disposition curve is divided into its distribution and elimation components, which are presented as least square regression lines. The distribution phase is based on the calculated points, which were obtained by subtracting the extrapolated portion of the elimination phase from the experimental data points by the method of residual or feathering technique. The co-efficients A and B were found to be 208 and 95 μg/ml respectively and the values of rate constants α and β were 0.096 and 0.0096 per min. The experimental constants are hybrid pharmacokinetic parameters.

Renal Clearance

The renal clearance of endogenous creatinine and sulfamethoxazole determined in sheep after intravenous dose (50 mg/kg) is shown in Table II. The clearance of endogenous creatinine expressing the glomerular filtration rate was on an average 9.78 ± 1.57 ml/min/10 kg b.wt. The sulfamethox-

Table II. Renal clearance of endogenous creatinine and sulfamethoxazole (SMX) in sheep

Sheep No.	Urine flow rate ml/min/10kg	Urine pH mean	Conc. in plasma(μg/ml) sulfamethoxazole		Clearance ml/min/10kg.b.wt sulfamethoxazole		Creatinine	Ratio ^{Cl}SMX / ^{Cl}creat.	Ratio ^{Cl}SMX free / ^{Cl}creat.
			Total	Free	Total	Free			
1	0.56	8.2	43.53	21.09	11.13	22.29	8.07	1.38	2.84
2	0.84	8.5	84.16	44.34	9.72	23.20	7.63	1.27	3.04
3	0.25	7.9	90.93	40.73	9.33	20.83	9.87	0.94	2.11
4	0.36	7.85	99.82	55.35	10.23	18.48	9.43	1.08	1.96
5	0.40	7.81	99.17	40.77	9.04	22.02	11.09	0.81	1.98
6	0.28	7.99	70.78	36.40	12.70	24.73	11.04	1.15	2.24
				Average	10.35	22.04	9.78	1.10	2.36
				±S.D.	1.36	2.17	1.57	0.20	0.46

Each experiment is the average of the 4 experimental periods.

^{Cl}SMX = Sulfamethoxazole clearance
^{Cl}creat. = Creatinine clearance

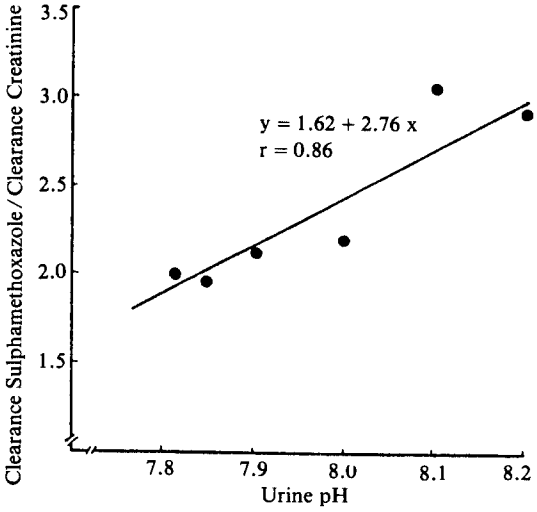

Fig. 3. Regression line showing relationship between ratio of clearance of non-protein bound sulfamethoxazole to endogenous creatinine clearance in relation to urine pH.

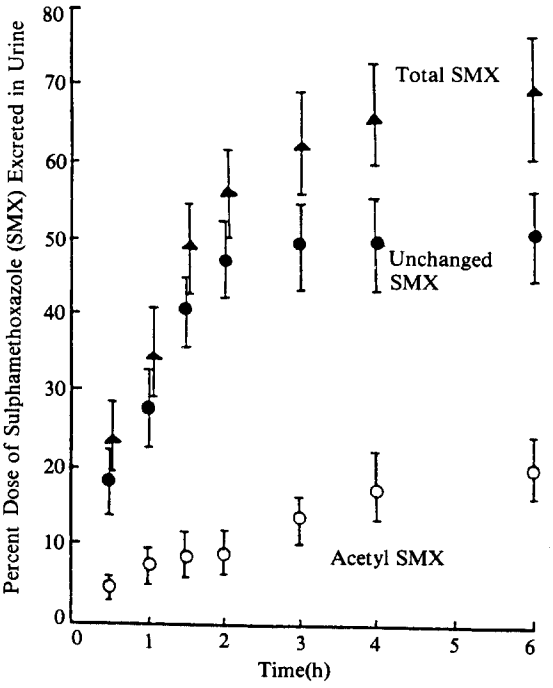

Fig. 4. Urinary excretion of sulfamethoxazole in terms of total (▲), free (●) and acetylated amine (○) expressed as percentage (average ± SD) of dose against time after intravenous injection of a single dose (50 mg/kg).

azole clearance in these experiments was 10.35 ± 1.36 ml/min/10 kg b.wt. and the drug was bound to plasma proteins to an extent of 62 percent. The average renal clearance of non-protein bound (free) sulfamethoxazole was 22.04 ± 2.17 ml/min/10 kg. b.wt.

The ratio between clearance of free drug and clearance of endogenous creatinine ranged between 1.96 to 3.04 with an average of 2.36 ± 0.46. A positive correlation was found between the clearance ratio and urine pH (Table II and Fig. 3). which shows that the ratio between the clearance of free sulfamethoxazole and clearance of creatinine increases with the increase in urine pH and vice versa. Both plasma concentration and rate of urine flow did not affect the clearance of drug.

The urinary excretion of sulfamethoxazole in terms of dose excreted as total, unchanged and acetylated amine is presented in Fig. 4. The average cummulative dose excreted in urine at 2, 4 and 6 hours post-drug administration was 55.4 ± 6.6, 66.1 ± 7.5 and 70.4 ± 10.5 percent respectively. The relative concentration of free and acetylated amine were 50.43 and 20.0 percent respectively of the drug contents in the urine of sheep.

DISCUSSION

The disposition kinetics of sulfamethoxazole (SMX) in sheep has been described by two compartment open model. Half-life of the drug ranged between 58 and 99 minutes (mean 76 min.) whereas quite higher values have been observed in cows (140 min) by Nielsen and Rasmussen[8] and in goats (156 min) by Gogh.[9] The half-life of SMX is quite low when compared with other sulfonamides in sheep, *e.g.*, sulfadimidine 233 min.[10] Sulfadiazine 426 min[11] and Sulfamethylphenazole 2280 min.[12]

The volume of distribution (V_d area) of sulfamethoxazole in the present study was 0.41 lit/kg in sheep which is slightly smaller than that in goats and greater than that in cows[8] in which the values are 0.5 and 0.3 lit/kg, respectively. An apparent volume of distribution which is lower than 1.0 indicates that the concentration of sulfonamide in most tissues is lower than in plasma. The low sulfonamide concentration in tissue extravascular fluids are probably less advantageous than in blood in determining the outcome of therapy in infections localized in tissues other than blood.

Species differences do exist in the rate of absorption, distribution and elimination of sulfamethoxazole and other sulfonamides. The sulfamethox-

azole has shorter elimination half-life in sheep and other ruminants than in non-ruminant species including man. There are several factors responsible for this variation, amongst them the pH and pK_a value, the extent of acetylation, protein binding and lipid solubility of sulfonamide are of more significance. The resurgence of variability particularly in the elimination of sulfamethoxazole may become more pronounced if a change in urine pH is produced.[5]

The renal clearance of sulfamethoxazole (22.04 ± 2.17 ml/min/10 kg) was greater than the creatinine clearance (9.78 ± 1.57 ml/min/10 kg) in sheep (Table II). The ratio between the clearance of free sulfamethoxazole and creatinine clearance was 2.36. The high renal clearance values exceed the creatinine clearance value which alongwith the urine pH dependency may indicate that besides filtration and active tubular secretion, passive tubular reabsorption also participates but to a lesser extent in the renal excretion process of sulfamethoxazole in the sheep. These findings are supported by the results of Vree *et al.* in sheep[13] and by Jorgensen and Rausmussen in goats.[10] According to Vree *et al.* the renal clearance of sulfamethoxazole was greater than creatinine clearance and was 5-10 times higher in ewe than in man.[13]

Sulfonamides in plasma undergo glomerular filtration. Their persistence within the body, however, depends to a great extent on the reabsorption from the renal tubular fluid. It one assumes tubular reabsorption to be a passive diffusion process, it will then be dependent on the extent of ionization of drug. Sulfamethoxazole has pK_a value of 6.0, thus the extent of its ionization within the urine is markedly influenced by variation in the urinary pH. The pH of sheep urine in our studies ranged between 7.8 to 8.3 (Table II), the alkaline urine of sheep resulted in greater ionization of sulfamethoxazole and lesser availability of unionized moiety to undergo reabsorption. Consequently it resulted in rapid excretion of the drug when compared with that in man and dog in which the pH of urine remains mainly on acidic side. The persistence in plasma of sulfadimethoxine and sulfamethylphenazole (both have pK_a 6) and sulfadiazine (pK_a 6.5) have been shown to be markedly influenced by alteration in the urinary pH of dogs, calves and pigs.[15] In man the influence of urine pH on the extent of elimination was observed by Dettli and Spring[16] who showed that the half-life of sulfonamides in humans decreased considerably when urine pH increased from 5 to 8. Vree *et al.* reported that under acidic urine conditions (pH 5.5-6.8) the net sulfamethoxazole excreted unchanged was 10% but when the urine was alkaline (pH 7-8), 30-40% of sulfamethoxazole was excreted unchanged.[15]

In sheep, 50% of the administered drug was excreted as unchanged and 20% as acetyl-derivative over the time period of 6 hrs (Fig. 4). Almost similar pattern of sulfamethoxazole excretion was observed by Vree *et al.* in a pregnant ewe.[13] Sulfamethoxazole is mainly acetylated to N^4-acetyl sulfamethoxazole in blood and the clearance of acetyl metabolite takes place primarily through active tubular secretion as was evidenced by the studies of Vree *et al.* in man who observed that co-medication with probenecid (active tubular secretion blocking agent) reduced the renal clearance of N^4-acetylsulfamethoxazole by four times and prolonged its plasma half-life to almost double.[17] The acetylation of sulfamethoxazole in present studies is of greater magnitude as compared with 7% for sulfadimidine[10] and similar to that for sulphamethylphenazole,[12] the value of which is reported to be 27%. The acetylation of sulfamethoxazole in goats (10-23%) reported by Jorgensen and Rausmussen[14] is comparable to our findings. However, a different pattern of sulfamethoxazole excretion in urine was observed in human beings by Ueda *et al.* who reported that mean fraction of the dose excreted in 72 hours was 87% of which 19% was intact drug, the major metabolite was the N^4-acetyl compound which represented 61% of the total drug excreted.[18]

The shorter elimination half-life of sulfamethoxazole may render its limited use in sheep and other herbivorous animals passing alkaline urine due to the need of repeated drug administrations for maintaining its therapeutic plasma levels.

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