IN VITRO METABOLISM OF SULFAMETHOXAZOLE BY RUMINAL MICROFLORA OF DOMESTIC RUMINANATS

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ABSTRACT

The metabolism of sulfamethoxazole by ruminal microflora of domestic ruminants (i.e. buffalo, cow, goat and sheep) was investigated by in *vitro* incubation of 40 μg/ml drug with the ruminal fluid. The incubated samples were analysed at different time intervals to measure the amount of free and N^4 acetyl metabolite of the drug. In the initial phase rate of drug metabolism was slow and after 2.5 hours it was maximum in all the species. The average $\pm$ SD percent of the remaining un-metabolized drug until 2.5 hours was 76.37 ± 1.68, 82.16 ± 1.88, 65.29 ± 1.88 and 83.49 ± 0.69 percent in buffalo, cow, goat and sheep, respectively. Average amount of N^4 acetyl metabolite produced by the ruminal microflora until 2.5 hours after incubation of ruminal liquor from buffaloes, cows, goats and sheep was 24, 18, 35 and 17 percent, respectively.

INTRODUCTION

The sulfa drugs are widely used in the treatment of susceptible infections. These are also used in combination with trimethoprim to broaden the spectrum of their activity. Among sulfonamides, sulfamethoxazole is commonly used in combination therapy to have highest activity against most susceptible bacteria. Metabolism, excretion and pharmacokinetics of various sulfa drugs have been investigated in different species of animals (Vree and Hekster, 1985). The presence of microorganisms in rumen and reticulum of compound stomach in ruminanats results in microbial metabolism of drugs. Therefore, the present study was designed to determine the role of ruminal microflora obtained from buffalo, cow, goat and sheep in the metabolism of sulfamethoxazole.

MATERIALS AND METHODS

The metabolism of sulfamethoxazole was investigated by incubating the drug with the ruminal fluid from four species of ruminants i.e. buffalo, cow, goat and sheep.

Sample collection and incubation

Five samples of ruminal fluid from each species were collected at the time of slaughter at the Municipal Slaughter House, Faisalabad. After collection, the samples were transported immediately to the laboratory at low temperature in ice-box and were processed fresh for further studies. The ruminal fluid was filtered through the double layer of muslin cloth. Sulfamethoxazole (40 μg/ml) was added into the ruminal fluid filter. Drug solution 2.4 ml and 27.6 ml of ruminal fluid was incubated in reaction flask in the water bath. Anaerobic conditions were produced by filling nitrogen in incubation flasks. The temperature of the incubated samples was maintained at 37 $\pm$ 2°C an optimum temperature for the ruminal microflora.

Analytical procedure

The concentration of sulfamethoxazole in ruminal fluid at 0.5, 1, 1.5, 2 and 2.5 hours after incubation were determined according to the method of Bratton and Marshall (1939) based on the diazotization of sulfonamides with N-(1-Nephthyl) ethylene diamine dihydrochloride as a coupling reagent.

Determination of metabolites

The samples of sulfamethoxazole, incubated with ruminal fluid for 2 hours were used for the determination of acetyl metabolites. For this pupose samples were hydrolyzed with 4M HCl in simmering water bath (70°C) for one hour. Then samples were analysed for total sulfamethoxazole and the difference of total and unchanged drug gave the amount of N^4-acetyl metabolite. The relative amount of metabolites of sulfamethoxazole was determined by thin layer chromatography. A 50 μl volume of incubated ruminal

liquor was spotted on thin layer plates coated with silica gel (0.2 mm thick). The plates were developed in the chloroform acetone; methanol mixture of 1:1:1 as solvent. The developed plates were sprayed with diazotozing agent and then with the coupling reagent.

RESULTS AND DISCUSSION

The metabolism of sulfamethoxazole by ruminal microflora from four ruminants (i.e. buffalo, cow, goat and sheep) was investigated after incubating sulfamethoxazole (SMZ) (40 μg/ml) with five samples of ruminal fluid in each species and the results are presented in Table 1. There were well- defined species variations in concentration of SMZ at different time intervals after drug incubation ruminal fluid. Nielsen *et al.* (1978) reported that the variations existed even amongst animals of same species because degradation effect of ruminal microflora varied from cow to cow fed on different fodder. They also found that the boiled ruminal fluid was incapable of producing degradation in the samples of trimethoprim and opined that changes in the rate of degradation of trimethoprim in ruminal fluid of different cow were due to the difference in composition of ruminal microflora.

The results of metabolism of SMZ by ruminal microflora expressed as percentage of drug remaining to be metabolized (unchanged) revealed maximum degradation by the ruminal microflora of goats (about 35%), buffaloes (about 24%), cows (about 18%) and sheep (about 17%), after 2.5 hours of incubation. A comparison of SMZ concentration after incubation with the ruminal microflora of buffaloes, cows, goats and sheep has been presented in Fig. 1. Of 40 μg/ml of the drug, amount metabolized was found highest in goat 13.88 ± 0.75 μg/ml, followed by buffalo 9.45 ± 0.67, cows 7.13 ± 0.47 and sheep 6.61 ± 0.28 μg/ml after 2.5 hours incubation. The average concentration of drug that remained unchanged in the body of ruminants showed that during first two hours of incubation the goat showed maximum while sheep showed minimum rate of metabolism

Pharmacokinetics of SMZ in buffalo calves concerning free SMZ and conjugated metabolites in blood and urine were studied after a single oral dose of 200 mg/kg. The maximum level of free drug obtained was 11.60 and 32.83 mg in plasma and urine respectively (Saharan and Banerjee, 1982). The extent of metabolism of SMZ was studied in rats (Kitakaze *et al.*, 1973) where in first 48 hours, 73.8 percent of the dose was excreted in urine.

Table 1: Average ± SD concentration (μg/ml) and percentage of unchanged drug at different times following incubation of sulfamethoxazole 40 μg/ml with the rumen liquor from domestic reminants.

Time In Hours	Concentration of sulfamethoxazole in incubated rumen liquor at 37°C							
	Buffaloes		Cows		Goats		Sheep	
	μg/ml	Percent	μg/ml	Percent	μg/ml	Percent	μg/ml	Percent
0.5	39.38 ±0.29	98.45 ±0.72	39.39 ±0.20	98.48 ±0.51	34.68 ±0.88	86.69 ±2.20	38.91 ±0.26	97.28 ±0.65
1.0	37.55 ±0.37	93.87 ±0.92	37.76 ±0.21	94.40 ±0.53	32.00 ±0.65	80.00 ±1.62	37.78 ±0.66	94.45 ±0.66
1.5	35.16 ±0.34	87.90 ±0.85	35.72 ±0.25	89.29 ±0.61	30.16 ±0.61	75.41 ±1.53	36.40 ±0.39	91.00 ±0.98
2.00	33.14 ±0.27	82.84 ±0.67	34.57 ±0.28	86.42 ±0.69	28.16 ±0.4961	70.39 ±1.53	34.80 ±0.39	87.00 ±0.98
2.5	30.55 ±0.67	76.37 ±1.68	32.87 ±1.68	82.16 ±0.44	26.12 ±0.75	65.29 ±1.88	33.39 ±0.28	83.48 ±0.69

Thin layer chromatographic analysis of the drug and its metabolite in the rumen liquor revealed a single metabolite (N^4-acetyl SMZ) with the unchanged drug in all the four species. The urine of sheep revealed two main metabolites of sulfadimidine along with conjugated metabolites (Bashir, 1979). Tissue, plasma and urine extracts of neonatal calf showed five metabolites of sulfadiazine (Woolley *et al.*, 1980). Urine of adult sheep revealed three metabolites of sulfadiazine including conjugated metabolites (Nawaz *et al.*, 1986) and urine of lambs revealed conjugated metabolites of sulfadimidine (Bevill *et al.*, 1977).

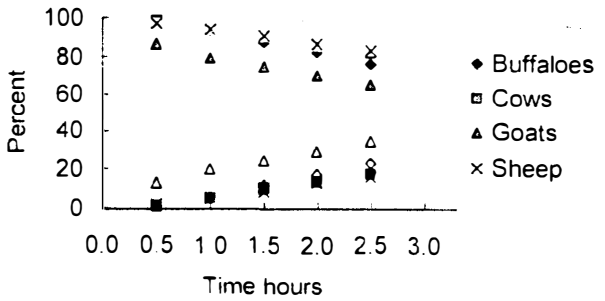

Fig.1: Average amount of unchanged and N_4-acetyl sulfamethaxazole after incubation of 40 μg/mL sulfamethaxazole with rumen liquor from ruminants species at 37°C. Upper curves are for unchanged sulfamethaxazole and the lower curves are for N_4-acetyl metabolites.

These studies indicated that metabolism of sulfamethoxazole takes place in the presence of ruminal microflora of buffaloes, cows, goats and sheep and variations in metabolites of the drug be attributed to the species difference in the populace of ruminal microflora.

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