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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 1 (4): 274-276, 1998 Research Article

Analysis of Metabolites of 4-methyl 2-sulfanilamido-oxazole (Sulfamethoxazole) Excreted in the Urine of Goats

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Abstract
The urinary excretion and metabolism of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) were determined in goats following intravenous injection of a single dose 50 mg/kg body weight. The percentage of intravenous dose of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) excreted in the urine until 24 hour as total amine was 61.0 ± 5.2 per cent, free amine 43.2 ± 4.6 per cent and acetyl amine was 17.8 ± 1.2 per cent. The mean ± SE values for relative percentage of Metabolite I identified as unchanged amine was 42.3 % Metabolite II acetyl amine was 15.67 per cent, Metabolite III hydroxy derivative 18.33 and as conjugated Metabolite were 23.33 per cent. In goats, the urinary excretion and Metabolism of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) revealed differences appprising as adjustment dosage regimen in the indigenous goats.

Key words: Metabolism, sulfamethoxazole, goats

Introduction
In Pakistan drugs are imported either in raw or finished form for the national health programmes in the human and animals although these are proven drugs. Such studies help in describing the drug action, toxicities, development of newer compounds and to understand molecular interactions *in vivo*. The passing of drug or its metabolites in the edible foods of animal origin like butter, milk, cheese and meat may pose serious health hazards like cancer, allergies and bacterial resisatance problems. But studies under indigenous conditions have revealed biochemical, physiological and pharmacological differences between foreign and local species, which have led to the use of an original term "geonetics"- the georaphical influences on genetics. (Nawaz and Shah, 1985; Nawaz, 1988). Earlier investigations on the metabolism of sulfadimidine revealed the quantitative and qualitative differences amongst the local and foreign species of animals. Therefore, the analysis of the drug metabolites is of both the academic and practical consequences.

The sulfonamides hold an important place in therapeutics, because of stability and their use even under adverse environment. These are used either alone or in combination with Trimethoprim against the sensitive organisms. In combination therapy, 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) is one of the commonly employed drug in clinics. Therefore, the present study, describes the urinary excretion and metabolism of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) in goats.

Materials and Methods
The urinary excretion and analysis of the Metabolites of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) were investigated in 08 goats.

Experimental Animals: Clinically healthy goats of Teddy breed mostly in their third or fourth lactation were used for this study. The mean ± SE value for the body weight of the animal was kg, maintained at the Livestock and Nutrition stall of University of Agriculture, Faisalabad. The animals were fed green fodder of the season twice daily and concentration were given to meet their production requirements. The animals milked twice daily at early dawn and dust. The experiments were conducted during the summer months of June and July.

Drug Administration: The powdered drug 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) was made into a 5 per cent solution in sterilized distilled water. To increase the solubility of drug in water, a few drops of IM sodium hydroxide solution were added during a constant stirring on the magnetic stirrer. The drug was injected into the left jugular vein of animals at the dosage rate of 50 mg/kg body weight.

Sampling Procedure: After proper restrain in the stall a balloon catheter (Foly No. 18, 30.5 ml) was introduced aspectically into the urinary bladder through urethra. The urine samples were collected before and at 06, 09, 12, 18 and 24 hour after the administration of sulfamethoxazole. Volume of each urine sample was measured and after through mixing, an aliqupt was kept in the sampling bottles in the freezer at -12°C until analysis.

Assay of Drug and its Metabolites: The analysis of free (unchanged and total drug in the urine sample was donely (Bratton and Marshall, 1939). The concentration of total hydrolysis of the urine with 4M Hydrochloric acid. The difference between the total and free drug was taken as the concentration of acetylated amine. The intensity of colour was measured by spectrophotometer (Spectronic-21) at 545 nm wavelength.

The metabolites of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) excreted in urine of the ruminants

include acetyl derivative, which was synthesized by refluxing the drug with equivolumes of acetic acid and acetic anhydride. The hydroxyl derivative was obtained from the urine of goats.

The metabolites of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) excreted in the urine of goats were measured by determining the relative concentration of each metabolite by thin layer chromatographic procedure (Nawaz *et al*., 1992).

Results

The mean ± SE values for the comulative percentage of intravenous dose excreted in urine of goats at 06, 09, 12, 18 and 24 hour after injection as total, tree and acetylated 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) have been presented in and the mean ± SE values are shown in Table 1. The comulative percentage of dose of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) excreted in the urine goats at 06 hours after injection as total amine free amine and acetylated amine was 52.8 ± 3.9, 37.7 ± 3.8, 15.5 ± 0.83 per cent respectively. At 09 hours, the excretion as total amine free amine and acetylated amine was 56.4 ± 4.7, 40.3 ± 4.3 and 15.9 ± 1.1 per cent respectively. The excretion 12 hours as total amine free amine and acetylated amine 59.8 ± 5.1, 42.4 ± 4.6, 17.4 ± 1.2 per cent. Until 24 hours, the percentage excreted as total amine free amine and acetylated amine was 61.0 ± 5.2, 43.2 ± 4.6, 17.8 ± 1.2 per cent respectively.

Table 1: Urinary Excretion of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) in 08 goats (mean ± SE) following intravenous injection of 50 mg/kg dose

	Percentage of dose excreted		
Time (hours)	Total amine	Free amine	Acetylamine
6	52.8 ± 3.9	37.7 ± 3.8	15.5 ± 0.83
9	56.4 ± 4.7	40.3 ± 4.3	15.9 ± 1.1
12	58.2 ± 4.9	41.5 ± 4.5	16.7 ± 1.2
18	59.8 ± 5.1	42.4 ± 4.6	17.4 ± 1.2
24	61.0 ± 5.2	43.2 ± 4.6	17.8 ± 1.2

The results derived from thin layer chromatographic analysis of the distribution of unchanged Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) and its metabolites in urine of goats collect at 24 hours (pooled samples) after intravenous injection of the drug revealed the presence of three spots in the ethyl acetate extract. In descending order, these spots were designated as Metabolite I, II and III. A comparison of the Rf values of these spots with the standards revealed that the Rf value of Metabolite I was comparable with that of unchanged Methyl 2-sulfanilamido-oxazole (sulfamethoxazole), Metabolite II was acetyl derivative, while Rf value of the Metabolite III was comparable with the Rf value of hydroxy derivative. Compareable Rf values were obtained for the observed metabolites and the standards in both the solvent systems, which supports the tentative identification of the metabolites.

In total amine 601 mcg/ml (mean ±SE) in 24 hours pooled urine the concentration of Metabolite I was 266 mcg/ml, Metabolites II 98 mcg/ml, Metabolite III 115 mcg/ml and the conjugated metabolites in the residual urine was 133 mcg/ml. The relative percentage of Metabolite I identified as unchanged amine was 42.33 per cent, Metabolite II as acetylated amine was 15.67 per cent, Metabolite III as hydroxyl derivative 18.33 per cent and the conjugated metabolites were 23.33 per cent. Analysis of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) by colorimetric and thin layer chromatographic methods did not show any significant differences.

Discussion

Sulfonamides undergo metabolism by Phase I and Phase II reactions in the body in presence of enzymes. These processes include acetylation-de-acetyl-action, hydroxylation, glucuronidation de-glucuronidation, sulfation and de-amination, etc. These extent of concentrations of all these compounds depends upon the concentration of enzymes available inside bio-system. Although the environmental conditions can influence the extent, but still we can estimate the possible concentrations of these compounds. The urinary excretion and Metabolism of sulfamethoxazole established that the major metabolities of sulfamethoxazole in urine was unchanged, N^4-acetyl and 5-hydroxy sulfamethoxazole. In addition small amounts of conjugated metabolites were also identified.

In goats, the percentage of dose of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) excreted in the urine until 24 hours after intravenous injection revealed mean ± SE values for total amine 61.0 ± 5.2 per cent, the free amine 43.2 ± 4.6 per cent and acetyl amine 17.8 ± 1.2 per cent. These values are almost comparable with the dose of sulfadiazine excreted in the urine of cows as total amine 60.4 per cent, free amine 52.4 per cent and acetyl amine 9.0 per cent (Nawaz *et al*., 1992). However, these values are a bit lesser than the dose of sulfamethoxazole excreted in the urine of cows as total amine 62.5 ± 4.3 per cent, free amine 52.1 ± 3.8 per cent and acetyl amine 10.4 ± 1.1 per cent. In earlier studies, the urinary excretion of similar dose of sulfadiazine in goats revealed the mean total amine 58.3 per cent, free amine 46.1 per cent and acetyl amine 12.1 per cent (Nawaz *et al*., 1985). In sheep, these values were 51.4, 43.5 and 7.9 (Nawaz *et al*., 1986) and in Buffaloes the values of sulfamethoxazole were total amine 40.2 ± 1.21 per cent, free amine 27.1 ± 1.6 and acetyl amine 13.6 ± 0.84 per cent. These results indicate that the percentage of intravenous dose excreted in the urine of goats as total and free amine is almost of comparable as that in cows and sheep, but the amount of acetyl derivative is comparable in these species. The excretion of acetyl derivative is comparable in these species. The excretion of acetyl compound is also in agreement with the excretion of acetylated sulfadimidine in sheep and goats (Nawaz and Khan, 1979). The urinary excretion of sulfamethoxazole is higher than that of sulfadimethoxine in men, monkeys, dogs, higher first 24 hours (Bridges *et al*., 1969). The basic urine in goats facilitates the excretion of weakly acidic sulfonamides than in the species with the less basic or acidic urine, the urinary excretion of the drug is comparable to the excretion of sulfamoxazole in buffaloes which excreted 33 to 65 per cent of the drug as total amine (Sharan and Banerjee, 1982).

The metabolic transformation of sulfamethoxazole in goats revealed the presence of three sots. Comparable Rf value for spot I with that of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) indicated that two third fo the drug contents in the urine was unchanged amine of the remaining one third, half was acetyl derivative and the remaining half was constituted by nearly equal quantities of hydroxy derivative and the conjugated metabolites. The amount of N^4 - acetyl 4-Methyl 2-sulfanilamido-oxazole was 22.9 per cent in the urine of sheep as found by thin layer chromatographic analysis of the urine which is higher than the values recorded in the present study. Atef and Nielsen (1975) studied the biotransformation of sulfadiazine in goats and established that the major metabolite was 2 (p-aminobenzene sulfonamide 4-hydroxy pyrimidine, which amounted to be about 15 per cent of the drug content in the urine, while the hydroxy metabolite of sulfamethoxazole in goats was 11.0 per cent.

In the present study, only two metabolites other than the unextractable conjugates including 4-Methyl 2-sulfanilamido 5-hydroxy oxazole and N^4 - acetyl 4-Methyl 2-sulfanilamido-5 hydroxyl oxazole were observed. A distinction between the conjugates can only be made the use of glucuronidase and sulfatase enzymes. This shows the qualitative and quantitative differences in the metabolism of 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) in the same species and also between different species. These studies clearly show that for the metabolism of organic molecules, the local species have distinct biochemical mechanisms.

References

Atef, M. and P. Nielsen, 1975. Metabolism of sulphadiazine in goats. Xenobiotica, 5: 167-172.

Bratton, A.C. and E.K. Marshall, 1939. A new coupling component for sulfanilamide determination. J. Biol. Chem., 128: 537-550.

Bridges, J.W., S.R. Walker and R.T. Williams, 1969. Species difference in the metabolism and excretion of sulfadimine in sheep and goat. Biochem. J., 111: 173-179.

Nawaz, M. and B.H. Shah, 1985. Geonetical considerations in the quality assurance of pharmaceuticals. Proceedings of the International Seminar on Polices, Management and Quality Assurance of Pharmaceuticals, April 21-25, 1985, WHO and Ministry of Health, Govt. of Pakistan.

Nawaz, M. and F.H. Khan, 1979. Pharamacokinetics and urinary excretion of sulfadimidine in sheep and goat. J. Vet. Pharamacol. Therap., 2: 129-132.

Nawaz, M., 1988. Geonetical considerations in therapeutics. Proceedings of the Meetings of the Counterpart scientists on Livestock Under SAARC., May 22-25, 1988, Islamabad, Pakistan.

Nawaz, R., A. Ilahi and M.Z. Iqbal, 1992. Analysis of metabolites of 2-(p-amino-benzene sulfonamide) pyrimidine (sulfdiazine) excreted in the urine of cows. Pak. Vet. J., 12: 116-120.

Nawaz, R., N. Zahara, M. Nawaz and B.H. Shah, 1986. Urinary excretion and metabolism of sulfadiazine in sheep. Pak. Vet. J., 6: 32-36.

Nawaz, R., S. Parveen, B. Saleem and M. Nawaz, 1985. Studies on the metabolism of sulfadiazine in goats. J. Pharm. Pb. Univ., 6: 25-32.

Sharan, K.K. and N.C. Banerjee, 1982. Pharmaco kinetic studies on sulfamoxole in buffalo calves. Indian J. Anim. Sci., 52: 228-231.