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Analysis of Metabolites of 4-methyl 2-sulfanilamido-oxazole Sulfamethoxazole Excreted in the Urine of Cows

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Abstract

The urinary excretion and metabolism of 4-Methyl 2-sulfanilamido-oxazole sulfamethoxazole were determined in milking cows 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 hours as total amine was 62.3 ± 4.3 per cent, free amine 52.1 ± 3.8 per cent and acetyl amine was 10.4 ± 1.1 per cent. The mean ± SE values for relative percentage of Metabolite I identified as unchanged amine was 61 per cent. Metabolite II acetyl amine was 19 per cent, Metabolite III hydroxy derivative 8.0 per cent and as conjugated Metabolite were 12.0 per cent. In cows, urinary excretion and Metabolism of 4-Methyl 2-sulfanilarnido-oxazole sulfamethoxazole revealed differences apprising as adjustment dosage regimen in the indigenous cows.

Key words: Metabolism, sulfamethoxazole, cows

Introduction

For the national health programmes in the human and veterinary medicine, the drugs are being imported in Pakistan either in raw or finished form researches on these drugs are conducted in an environment completely different from the local. Studies under indigenous conditions have revealed biochemical, physiological and pharmacological differences between foreign and local species and have led to the use of an original term "geonetics" the geographical influences on geonetics (Nawaz and Shah, 1985; Nawaz, 1988; Nawaz *et al*., 1988). Earlier investigations on the metabolism of sulfadimidine revealed quantitative and qualitative difference amongst the local and foreign animals. Such differences are supposed to exist for the metabolism of other drugs in different species.

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 human health hazards like cancer, allergies and bacterial resistance problems. Therefore, the analysis of the drug metabolites is of both academic and practical consequences.

The sulfonamides hold an important place in therapeutics, because of stability and their use even under adverse environments. Sulfa-drugs have fewer side effects than the antibiotics and are remarkable for their passage across blood brain barrier. Sometimes sulfanamides are preferred either alone or in combination with Trimethoprim against the sensitive organisms. In combination therapy, 4-Methyl 2-sulfanilamdio-oxazole sulfamethoxazole is one of the commonly employed drug in clinics. The present study, describes the urinary excretion and metabolism of sulfamethoxazole in cows.

Materials and Methods

The urinary excretion and analysis of the Metabolites of sulfamethoxazole were investigated in 08 milking cows.

Experimental animals: Clinically healthy milking cows of Sahiwal breed mostly in their third or fourth lactation were used for this study. The mean ± SE value for the body weight f animal was 324 ± 7 kg, maintained at the Livestock Production Research Institute, Bahadur Nagar, OKARA. The animals were fed green fodder of the season twice daily and concentrates were given to meet their production requirements. The animals milked twice daily at early dawn and dusk. The experiments were conducted during the summer months of June and July.

Drug administration: The powdered drug 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 bw.

Sampling procedure: After proper restrain in the stall a balloon Catheter (Foly No.30, 30-50 ml) was introduced aspectically into the urinary bladder through urethra. The urine samples were collected before and at 6, 9, 12, 18 and 24 hours after the administration of sulfamethoxazole. Volume of each urine sample was measured and after through mixing, an aliquot 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 int he urine sample was done by the colorimetric procedure of Bratton and Marshall (1939). The concentration of total 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) was measured after 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

spectrophotometre (Spectronic-21_ at 545 nm wavelength. The metabolites of 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 cows were measured by determining the relative concentration of each metabolite by thin layer chromatographic procedure which has been described earlier (Nawaz *et al*., 1992).

Results

The mean ± SE values for the cumulative percentage of intravenous dose excreted in urine of cows at 6, 9, 12, 18 and 24 hours after injection as total, free and acetylated sulfamethoxazole have been presented and the mean ± and the mean ± SE values are shown in Table 1. The cumulative percentage of dose of sulfamethoxazoel excreted in the urine of 6 hours after injection as total amine free amine and acetylated amine was 51.0 ± 3.95 was 44.2 ± 3.8 and 6.8 ± 0.84 per cent respectively. At 09 hours, the 48.6 ± 3.9 and 8.3 ± 1.2 per cent respectively. The excretion at 12 hours as total amine free amine and acetylated amine was 59.4 ± 4.4, 50.1 ± 3.8 and 9.4 ± 1.2 per cent respectively. At 18 hours, the excretion of total amine was 51.0 ± 3.8 respectively 51.0 ± 3.8 and 9.7 ± 1.2. Until 24 hours, the percentage of the intravenous dose of sulfamethoxazole excreted as total amine free amine and acetylated amine was 62.5 ± 4.3, 52.1 ± 3.8 was 10.4 ± 1.1 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	51.0 ± 3.95	44.2 ± 3.8	6.4 ± 0.84
9	54.7 ± 4.4	48.4 ± 3.9	8.3 ± 1.2
12	59.4 ± 4.4	50.1 ± 3.8	9.4 ± 1.2
18	60.8 ± 4.3	51.0 ± 3.8	9.7 ± 1.2
24	62.5 ± 4.3	52.1 ± 3.8	10.4 ± 1.1

The results derived from thin layer chromatographic analysis of the distribution of unchanged 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) and its metabolites in the urine collected 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 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole), Metabolite II with acetyl derivative, while Rf value of the Metabolite III was comparable with the Rf value of 4-methyl 2-sulfanilamido 5 Hydroxy-oxazole. Comparable 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 958 mcg/ml (mean ± SE) in 24 hours urine the concentration of Metabolite I was 587 mcg/ml. Metabolite II 180 mcg/ml, Metabolite III 79 mcg/ml and the conjugated metabolites in the residual urine were 113 mcg/ml. The relative percentage of Metabolite I identified as unchanged amine was 60.95 ± 2.2 per cent, Metabolite II acetylated amine was 18.63 ± 0.9 per cent, Metabolite III hydroxy derivative 8.33 ± 3.8 per cent and the conjugated metabolites were 10.99 ± 1.1 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 1 and Phase II reactions in the body in presence of enzymes. These processes include acetylation-de-acetyl-ation, hydroxylation, glucuronidation-de-glucuronidation, sulfation and de-amination, etc. The extent of concentrations of all these compounds depends upon the concentration of enzymes available inside the 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 is established that the major metabolites of sulfamethoxazole in urine was unchanged, N^4-acetyl 2-sulfanilamido-oxazole, 5-hydroxy sulfamethoxazole. In addition small amounts of conjugated metabolites were also identified.

In cows, 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 62.5 ± 4.3 per cent free amien 52.1 ± 3.81 per cent and acetyl amine 10.4 ± 1.1 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 amien 52.4 per cent and acetyl 9.0 per cent (Nawaz *et al*., 1992). In earlier studies, urinary excretion of similar dose of sulfadiazine in goats revealed the mean total amine 58.3, free amine 46.1 percent and the acetyl amine 12.1 percent (Nawaz *et al*., 1985). In sheep, these values were 51.4, 43.5 and 7.9 per cent (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 per cent and acetyl amine 13.6 ± 0.84 per cent. These result indicate that the percentage of intravenous dose excreted in the urine of cows as total and free amine is almost comparable as that in goats and sheep, but the amount 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, rabbits and guinea pigs excreting 20 to 40 per cent of the drug during first 24 hours (Bridges *et al.*, 1969). The basic urine in cows 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 4-Methyl 2-sulfanilamido-oxazole (sulfamethoxazole) in cows revealed the presence of three spots. (Comparable Rf value for spot I with that of 4-Methyl 2-sulfanilamido-oxazole (Sulfamethoxazole) indicated that three fourth of the drug contents in the urine was unchanged amine. Of the remaining one fourth, 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 in cows was 8.33 per cent. It was found that 87 per cent of the drug was excreted in the urine of cows until 14 days after the last dose (Nawaz *et al*., 1992).

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-hydroxy oxazole were observed. A distinction between the conjugates can be made by 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.

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