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SERUM AND MILK CONCENTRATIONS OF SEVERAL SULPHONAMIDES AND THEIR N^4-ACETYL METABOLITES FOLLOWING ORAL ADMINISTRATION TO COWS

By

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SOBACK, STEFAN, U. LAMMINSIVU and P. TAPIO: *Serum and milk concentrations of several sulphonamides and their N^4-acetyl metabolites following oral administration to cows.* Acta vet. scand. 1983, *24*, 374—383. — Serum and milk concentrations of sulphonamides following oral administration of three commercial sulphonamide products to dairy cows were studied. The fate of the N^4-acetyl derivatives of the compounds was also monitored. Sulfadimidine was found to have the slowest oral absorption rate compared to sulfametoxypyridazine, sulfaphenazole and sulfanilamide. Sulfaphenazole was least absorbed. Excretion of the different sulfonamides into the milk was by passive diffusion and was best for the least ionized sulfanilamide followed by sulfadimidine and sulfamethoxypyridazine. The most ionized sulfaphenazole was not found in milk. Sulfanilamides was found to be readily acetylated (and sulfaphenazole to a lesser degree). N^4-acetylsulfanilamide seemed to be actively secreted from blood into milk in the cow.

sulphonamides; farmacokinetics; acetylation.

Oral administration of sulphonamides to dairy cows is common in treatment of infectious diseases such as mastitis.

The fundamental studies of *Rasmussen* (1958) and *Sisodia & Stowe* (1964) were followed by a multitude of reports on the pharmacokinetics and metabolism of various sulphonamide compounds in cows. Nevertheless, an evaluation of a particular sulphonamide compound as a therapeutic agent or as a source of residues is difficult due to dose, dosage interval and combination differences.

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The present report deals with sulphonamides and their N^4-acetyl metabolite levels in serum and milk of cows following multiple oral treatments of 3 commercial sulphonamide products at recommended dosages. The potential therapeutic value of each product, based on the achieved serum and milk levels, is discussed. The serum levels and the excretion into milk of the N^4-acetyl metabolites are also evaluated.

MATERIALS AND METHODS

Four Ayshire cows, each weighing 500—550 kg, were used in a triple cross over trial. Cows were at the end of their lactation period and produced 10—15 kg of milk daily. The state of udder health was determined before the trials by means of bacteriological tests conducted on aseptically collected milk and by the CMT (California Mastitis Test). The udder of 1 of the cows had 2 quarters shedding Staphylococcus aureus and the cell counts in the milk from the infected quarters were elevated.

Three commercial sulphonamide preparations were used. The first was a combination of two sulphonamides, sulfadimidine (SDD) and sulfanilamide (SA), ("Eutanil" Orion Pharmaceutical Co., Helsinki, Finland), at a 1:2 ratio, presented in a 30 g dosage envelope. The second preparation consisted of sulfamethoxypyridazine (SMP) and sulfanilamide (SA), ("Sulfamax", Lääke Oy, Turku, Finland), at a 1:3 ratio, presented in a 40 g dosage envelope. The third product contained sulfaphenazole (SPZ), ("Eftolon", Pfizer Co., Bruxelles, Belgium), in a 4 g dosage envelope.

The drugs were administered suspended in 1 l of water by ruminal intubation followed by 2—3 l water flush. The dosage of "Eutanil" per cow was 2×30 g, i.e. 60 g, on the first day and 30 g on each of the following 2 days. "Sulfamax" was given at 40 g per cow daily during 3 consecutive days. "Eftolon" was given at 4×4 g, i.e. 16 g per cow on the first day and 3×4 g (12 g) on each of the following 2 days. Dose schedules were according to the manufacturers instructions. A "wash out" period of 12 days was allowed between the administration of each preparation.

Blood and quarter milk samples were collected at 0,1,6,12 h and the at 12 h intervals until 72 h from the initial dosage. Serum and milk samples were kept frozen at —20°C until analyzed.

Microbiological analyses of quarter milk samples were made

according to the modified "Thermocult" method described by *Soback & Lamminsivu* (1979). The sensitivity limit of the method was 1.0 μg/ml.

Chemical analysis of serum and quarter milk samples was made by high performance liquid chromatography (HPLC), using a model M-6000 A pump, (Waters Associates, Inc., Milford, Mass.), 10 μl Wisp injector (Waters Ass.), LC-55 detector (Perkin-Elmer Corp., Norwalk, Conn.) UV_{440}, 254 nm. The column was a μBondapak, C_{18}, 3.9 mm×50 cm (Waters Ass.). The samples were eluted with an acetonitrile - water - acetic acid mixture at ratio of 18:82:0.5 (20:80:0.5 for milk) at pH 3.2 at a flow rate of 1.5 ml/min (1.0 ml/min for milk) for SDD, SMP, SA, N^4-AcSMP and N^4-AcSA and at a ratio of 20:80:0.5 at pH 3.2 at a flow rate of 2.5 ml/min for SPZ and N_4-AcSPZ. For the separation of N^4-AcSDD a ratio 27:73:0.25 was used.

The standard curve was linear in the range of 2—40 μg/ml (1—20 μg/ml in milk) for SA and N^4-AcSA, 2 (4 for SMP) — 100 μg/ml for SDD, SMP, N^4-AcSDD and N^4-AcSPZ. Recoveries of the internal standards were 95 ± 3 % within the given ranges.

Serum samples were prepared for assay by mixing 4 ml (2 ml for SPZ) of acetonitrile with 1 ml of serum in a vortex to denaturate the proteins. This was followed by centrifugation for 10 min (15 min for SPZ) at 1000×g and the supernatant was used for the HPLC analysis.

Milk sample were prepared by adjusting the 2 ml (4 ml for SPZ) sample to pH 5.2—5.5 (5.6—5.8 for SPZ) with 1 mol/l acetic acid followed by extraction with 5.0 ml (4.0 ml for SPZ) ethylacetate for 10 min. The extract was centrifugated for 5 min at 1000×g. Two mls of the extract was evaporated in a N_2 flow at 40°C. The dry extract was dissolved in 2 ml of eluent and centrifugated. The supernatant was used for the HPLC analysis.

RESULTS

Serum concentrations of the parent compounds and their respective N^4-acetyl derivatives measured by HPLC are given in Fig. 1 ("Eutanil"), Fig. 2 ("Sulfamax") and Fig. 3 ("Eftolon"). The values for N^4-AcSDD and N^4-AcSMP are not plotted since they were less than 2 μg/ml and 4 μg/ml respectively, thus below the levels of accurate measurement. Fig. 4 shows the respective concentrations of the compounds in the milk as determined by

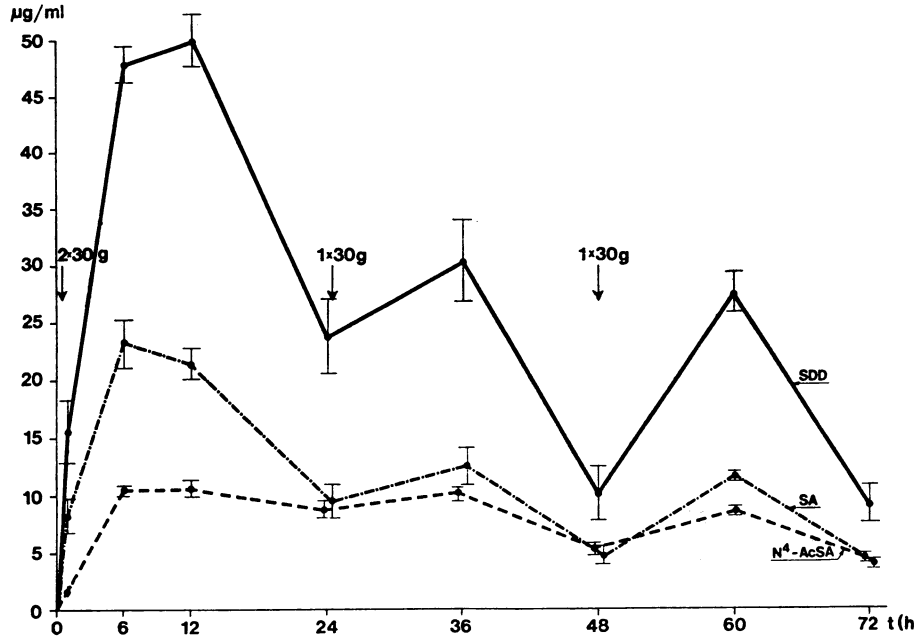

Figure 1. The concentrations of sulfadimidine (SDD), sulfanilamide (SA) and N^4-acetylsulfanilamide (N^4-AcSA) in serum.

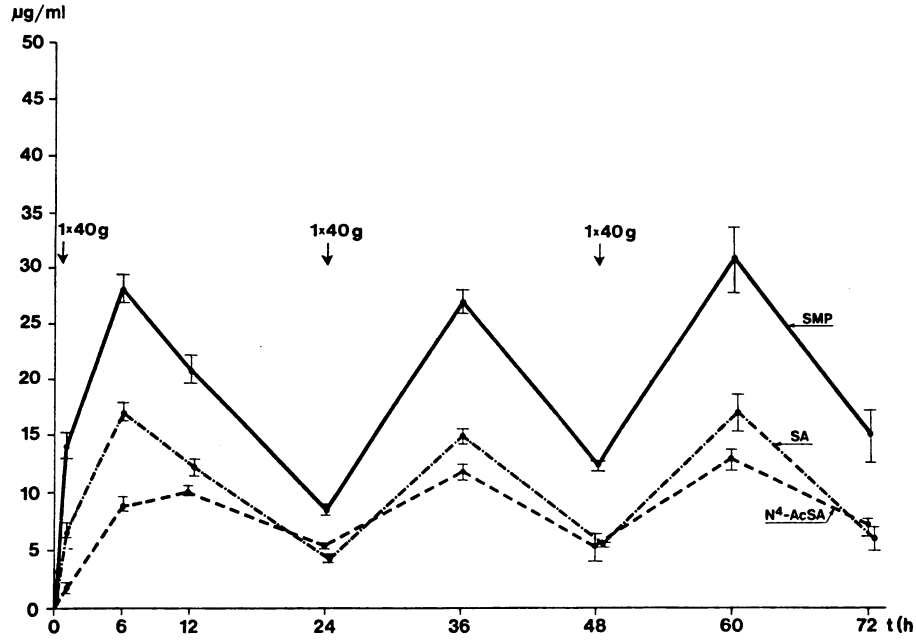

Figure 2. The concentrations of sulfamethoxypyridazine (SMP), sulfanilamide (SA) and N^4-acetylsulfanilamide (N^4-AcSA) in serum.

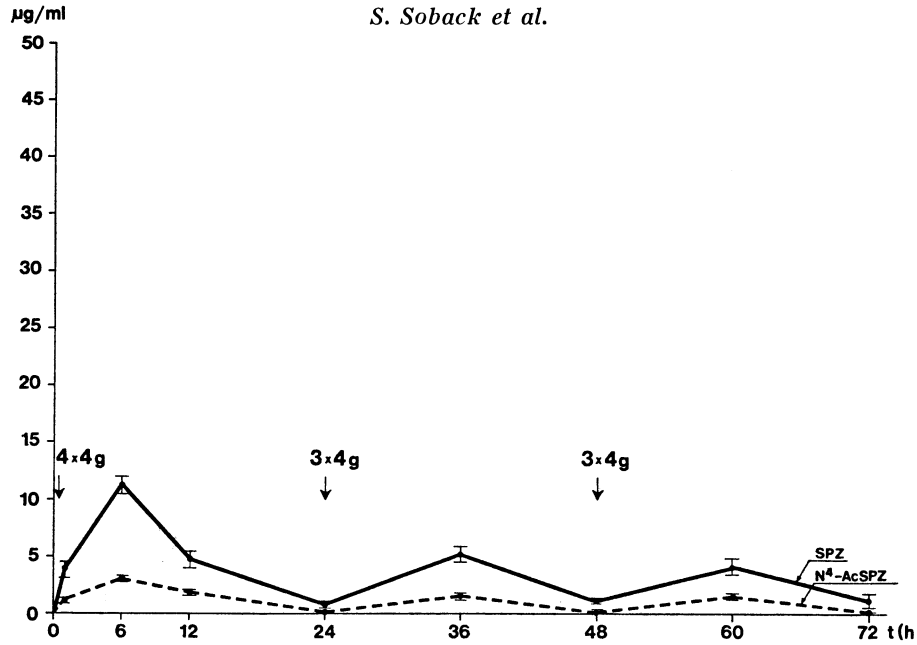

Figure 3. The concentrations of sulfaphenazole (SPZ) and N^4-acetylsulfaphenazole (N^4-AcSPZ) in serum.

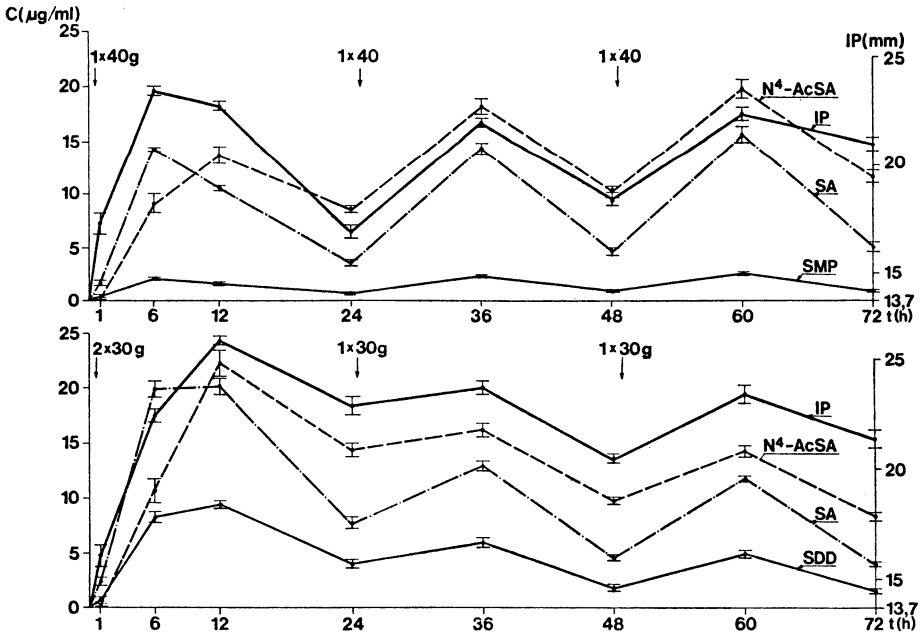

Figure 4. The concentrations of sulfadimidine (SDD), sulfamethoxypyridazine (SMP), sulfanilamide (SA) and N^4-acetylsulfanilamide (N^4-AcSA) and the inhibition potential (IP) in milk. The "Sulfamax" above and the "Eutanil" below.

Table 1. Ionisation and protein binding of sulphonamides and theoretical and observed ratios.

	pKa	% Un-ionized b) serum pH 7.4	% Un-ionized b) milk pH 6.6	Milk ultrafiltr. c) / Serum ultrafiltr. theoretical	% Protein binding	AUC a) milk / AUC serum ×100	AUC a) N^4-Ac deriv. / AUC parent ×100 serum	milk
Sulfadimidine	7.4	50	86	0.58	70	18	n.c.	n.c.
Sulfamethoxypyridazine	6.7	17	56	0.28	76	8	n.c.	n.c.
Sulfaphenazole	6.6	14	50	0.27	82	n.c.	31	n.c.
Sulfanilamide	10.4	100	100	1.00	20	92[1], 87[2]	73[1], 82[2]	127[1], 139[2]
N^4-acetyl sulfanilamide	10.3	100	100	1.00	17—42	159[1], 161[2]	—*	—*

a) AUC = Area Under the Concentration time curve (t = 0 — 72 h)

n.c. = not calculated

*) See sulfanilamide

1) "Eutanil"

2) "Sulfamax"

b) $= 100 \dfrac{100}{1 + \text{antilog } (pK_a - pH)}$

c) $= \dfrac{1 + \text{antilog } (pH_m - pK_a)}{1 + \text{antilog } (pH_s - pK_a)}$

HPLC. The concentrations of SPZ and N^4-AcSPZ did not reach the detection level (0.5 μg/ml) in milk and thus were not plotted. The results of the microbiological assay of drug concentrations in milk are given in terms of IP (inhibition potential) and are expressed as inhibition zone diameters (mm). Concentrations in Figs. 1—4 are given as means ± s.e.m. (standard error of mean). The proportions of sulphonamides and their N^4-acetyl derivatives in serum and milk are given in Table 1.

DISCUSSION

A comparison of in vitro test results obtained by agar plate method with the expected in vivo efficacy is particularly difficult when sulphonamides are concerned because of the presence of sulphonamide antagonists in agars (*Neipp et al.* 1961, *Gudding* 1974, *Then* 1977). Therefore, a safety factor of 5—10 (*Otten & Plempel* 1975) has been suggested for the interpretation of therapeutic sulphonamide concentrations.

For the clinical interpretation of the results of the present study the use of the safety factor is questionable since 2 of the preparations studied were combinations of 2 compounds of different antimicrobial activity. Secondly the use of safety factor should be reconsidered since biasing factors in the agar result in higher MIC (Minimal Inhibitory Concentration) values than achieved in an optimal test.

All the drugs were detected in the serum within 1 h after their oral administration (Fig. 1, 2, 3) indicating fast absorption from the gastro-intestinal tract. Sulfadimidine reaches peak serum concentration more slowly than the other compounds having its peak at 12 h after administration, whereas the other compounds peaked at 6 h after the initial dose. *Luthman & Jacobson* (1979) found sulfadimidine to be more rapidly absorbed (peaks at 4 h) than sulfanilamide or sulfaphenazole (peak at 6 and 8 h), respectively). The disagreement might be due to experimental conditions especially considering the dosage.

The initial double dose of "Eutanil" seems to give this preparation an advantage because, compared to "Sulfamax", higher serum drug levels were produced (Fig. 1 and 2) although the total amount of sulfonamide given to the cows was the same. The concentrations of sulphonamide in serum after treatment with "Eftolon" (Fig. 3) were far lower than those reached by the 2

other preparations. If the amount of SPZ (16 g + 12 g + 12 g) is compared to the respective amounts of SDD (20 g + 10 g + 10 g) and SMP (10 g + 10 g + 10 g) then the concentration of SPZ in serum is only 1/4—1/5 of the concentrations of SDD and SMP at the same given dose. This indicate considerably less absorption of SPZ than SDD or SMP from the gastro-intestinal tract of the cow.

The amounts of N^4-AcSA in the serum as measured by means of AUC (Area under the concentration-time curve) (Table 1) were 73 %—82 % of the amount of parent drug. This is much higher than the values (15 % ± 7) reported by *Nielsen* (1973). The experimental conditions in *Nielsen*'s study and the present study were different. It is suggested that the time (2 h) after i.v. injection, when the acetyl derivative was measured by *Nielsen*, was not sufficiently long for measuring acetylation of this compound. *White & Evans* (1968) measured the "acetylator" phenotypes after oral ingestion of a sulphonamide from blood samples taken eight hours after drug administration. In a similar study, *Whelpton et al.* (1981) collected the samples at 6 h after oral dosage. From the other N^4-acetyl derivatives measured, we found 31 % N^4-AcSPZ from it's parent compound and only trace amounts of N^4-AcSDD or N^4-AcSMP in the serum.

The excretion of the compounds into milk varied greatly as could be expected on the basis of their different pKa values (Table 1); passage into milk was better at the higher pKa value (*Rasmussen* 1958). Concentrations of SA in the milk were 87—92 % of the drug concentrations in the serum (Table 1). The theoretical concentration ratio of milk: serum for SA in ultrafiltrates is 1.0 (*Rasmussen* 1958). Since we measured the total (bound and un-bound) sulphonamide concentration, protein binding may be the reason for the lower values in the milk. The same is apparently true for milk: serum ratio of SDD which was 0.18 instead of the theoretical ratio 0.58, and the respective ratio for SMP which was 0.09 instead of the theoretical ratio 0.28. We could find only traces of SPZ in milk as this drug was also least un-ionized in serum (Table 1).

The concentration curve for SDD (Fig. 4) seems to be dominant for the IP ("Inhibition Potential") curve. The same is true in the case of SMP. This indicates that the antimicrobial effect of SA, though readily excreted to milk, is weak.

According to the results presented in Table 1, N^4-acetyl sul-

fanilamide is present in milk at 1.6 times the concentration of the compound in serum. This is in agreement with the results of *Rasmussen* (1969) in goats and complement the results of *Nielsen* (1973) in cows suggesting active transport of N^4AcSA from blood into milk.

The use of the products studied in this paper in veterinary antimicrobial therapy is, therefore, justified. Among the products tested "Eutanil" seemed to reach the highest while "Eftolon" reached the lowest concentrations of sulphonamides in milk when used according to the manufacturer's recommendations. These findings are of importance for treatment of acute mastitis when good drug excretion into the milk is desirable (*Ziv* 1975).

The high concentrations of SA and especially its N^4-acetyl derivative may form a considerable residue problem since the derivative is not likely to be detected by microbiological tests routinely conducted for the presence of antimicrobial drug residues.

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SAMMANFATTNING

Serum- och mjölkkoncentrationer av flera sulfonamider samt deras N^4-acetyl metaboliter efter oral giva åt kor.

Serum och mjölkkoncentrationer av sulfonamider efter oralt bruk av tre kommerciella sulfonamidprodukter på mjölkkor undersöktes. Likaledes kartlades händelseförloppet hos komponenternas N^4-acetylderivativer. Sulfadimidin befanns uppvisa det långsammaste orala absorptionsförloppet jämfört med sulfametoxypyridazin, sulfafenazol och sulfanilamid. Sulfafenazol absorberades minst.

Avsöndring av de olika sulfonamiderna i mjölken var genom passiv diffusion högst hos det lägst-ioniserade sulfanilamid, därnäst sulfadimidin och sulfamethoxypyridazin. Det högst ioniserade sulfafenazol förekom inte i mjölken. Endast sulfanilamid (sulfafenazol i mindre grad) visade sig vara lätt acetylerat. N^4-acetylsulfanilamid tycks utsöndras aktivt från blodet till mjölken hos kon.

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