BLOOD KINETICS OF SULFAMONOMETHOXINE AND OXYTETRACYCLINE FOLLOWING INTRAUTERINE SPRAY INJECTION IN DAIRY COWS

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SUMMARY

Intrauterine administration was performed in six Friesan cows with a disposable spray preparation containing 3 g of sulfamonomethoxine and 3 g of oxytetracycline, in order to investigate their serum kinetics. Sulfamonomethoxine levels were determined by a reversed-phase HPLC method, whilst oxytetracycline quantities were detected by a microbiological method.

The sulphonamide had a peak 1·17 h after the administration, the tetracycline reached its highest concentration after 8 h. The bioavailability of both drugs was low and detectable drug amounts were no longer recovered after 24 h.

KEY WORDS: sulfamonomethoxine, oxytetracycline, dairy cows, intrauterine administration, pharmacokinetics.

INTRODUCTION

In recent years several antibacterial formulations have found wide applications in the intrauterine therapy of bacterial metritis in cows [1–3]. In veterinary practice, the spray preparations are nowadays considered the most suitable because of their easy handling and their good permeation of the uterine mucosa. Intrauterine administrations of antibacterials proved to be satisfactory, but despite this fact, they are considered as local treatments, although the absorption of many drugs from the uterus has been demonstrated [4–12].

The aim of the present report is to investigate the absorption and the related serum kinetics of a new combination of sulfamonomethoxine (SMM) and oxytetracycline (OTC) for intrauterine spray administration, since there are no data available in the literature referring to the passage of SMM from the uterus into the

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blood. A modified high-performance liquid chromatographic method [13] and a modified microbiological method [14] were used for the determination of SMM and OTC, respectively. The results of the kinetics of OTC are compared with those which we had obtained during previous experiments [15].

MATERIALS AND METHODS

Six Friesian dairy cows, each weighing 450–500 kg, were used. During the trial, they were affected by endometritis clinically judged of moderate gravity. The cows were kept indoors and were fed with hay and tap water *ad libitum*.

Each cow received a dose of 3 g of sulfamonomethoxine and 3 g of oxytetracycline as a single intrauterine spray injection (Endospray preparations kindly supplied by IZO S.p.A. Brescia Italy). Blood samples were collected from the jugular vein 0, 0·5, 1, 2, 4, 8, 12, 24, 48 and 72 h after treatment. Samples were allowed to clot and then centrifuged to obtain sera which were stored at −20°C until the analysis.

Sulfamonomethoxine determination

A Spectra Physics 8100 high-performance liquid chromatograph, equipped with a SP 8840 UV/VIS detector and a SP 4290 printer/plotter integrator was used. A Merck stainless steel column (240 × 4 mm) packed with LiChrosorb RP 8, particle size 5 μm, was connected with the chromatograph. An injection loop of 10 μl was used.

The sulphonamide peaks, presenting a retention time of 3·5 min, were monitored at 260 nm; the detection limit was 0·02 μg/ml. The method of external standard was employed.

The mobile phase was a mixture of phosphate buffer 0·067 M pH 6·7 and methanol (5:1). Samples were eluted isocratically at a flow rate of 1·6 ml/min and at a pressure of 115 atm. The sulfamonomethoxine sodium salt 99% pure was obtained from IZO S.p.A. Brescia, Italy (lot GL008 produced by Daiichi Seiyaku Co. Ltd. Tokyo Japan).

In order to make a calibration curve, five standard solutions were prepared at concentrations of 200, 20, 2, 0·2 and 0·02 μg SMM/ml respectively (Fig. 1). The relation between SMM concentration and peak-height ratio (versus external standard) was linear ($r = 0·99$).

Sample preparation

One ml of bovine serum was added to 4 ml of perchloric acid (0·33 M). The solution was mixed on a Vortex mixer and subsequently allowed to stand for 10 min. After centrifugation at 3000 rpm for 10 min and filtration through paper filter, the supernatant was ready for the injection into the column.

The precision and accuracy of the analytical method were evaluated by processing as replicates on different days, serum samples ($n = 4$) added with known standard amounts (0·2–200 μg SMM/ml), obtaining a recovery of 80 ± 7% and a coefficient of variation ranging from 3·2% to 7·1%.

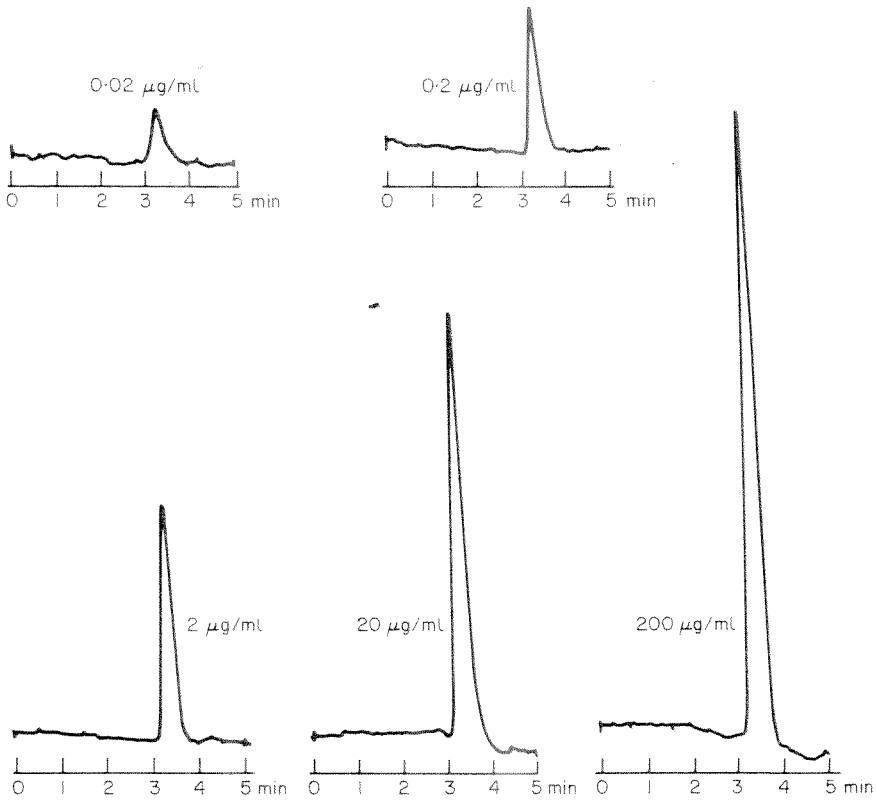

Fig. 1. Chromatograms of sulfamonomethoxine standard solutions.

Oxytetracycline determination

A microbiological hole-plated agar method, using *Bacillus cereus* ATCC 9634 as the assay organism, was performed. The sensitivity of this microbiological system was established to be 0·1 μg/ml. Standard solutions (0·1–5 μg/ml) were prepared in purified water using oxytetracycline 99% pure (lot MP511470 Farmitalia Milan, Italy).

The calibration curve was obtained by plotting on semilogarithmic paper the concentration of the standard solutions on the *y*-axis and the diameter of the zone of inhibition on the *x*-axis and was linear ($r > 0{\cdot}99$). The concentrations of the unknown specimens were determined from the standard curve after measuring the zones of inhibition.

Cross-tests were performed on each method in order to reveal possible interferences during the assays.

Pharmacokinetic parameters

Kinetic parameters shown in Tables I and II were calculated by standard methods [16]. As regards each drug concentration against time curve, the

Table I
Pharmacokinetic parameters of sulfamonomethoxine

Parameter	*Cow*						
	A	*B*	*C*	*D*	*E*	*F*	$\bar{x} \pm SD$
C_{max} (μg/ml)	1·42	1·14	2·17	1·71	1·60	1·26	1·55 ± 0·37
T_{max} (h)	1·0	1·0	2·0	2·0	0·5	0·5	1·17 ± 0·68
K (h^{-1})	0·36	0·28	0·31	0·18	0·22	0·32	0·28 ± 0·07
$t_{1/2\,K}$ (h)	1·90	2·50	2·25	3·75	3·20	2·15	2·63 ± 0·71
AUC (μg/ml/g) 0–8 h	6·83	7·18	9·70	8·81	6·04	3·76	7·05 ± 2·10
AUC (μg/ml/h) 0–∞	7·12	8·39	10·76	11·86	7·95	4·33	8·40 ± 2·68

C_{max}, highest plasma concentration; T_{max}, time at which C_{max} occurs (hours); K, first-order elimination rate constants; $t_{1/2\,K}$, half-time of the first-order elimination rate constant; AUC, total area under the plasma drug concentration versus time curve.

Table II
Pharmacokinetic parameters of oxytetracycline

Parameter	*Cow*						
	A	*B*	*C*	*D*	*E*	*F*	$\bar{x} \pm SD$
C_{max} (μg/ml)	0·97	1·35	1·59	0·70	0·97	1·59	1·20 ± 0·37
T_{max} (h)	8·0	8·0	8·0	8·0	8·0	8·0	8·0 ± 0·0
K (h^{-1})	0·058	0·058	0·029	0·58	0·058	0·058	0·05 ± 0·01
$t_{1/2\,K}$ (h)	12·0	12·0	24·0	12·0	12·0	12·0	14·0 ± 4·69
AUC (μg/ml/h) 0–8 h	7·67	9·12	11·15	5·65	7·15	8·15	8·15 ± 1·87
AUC (μg/ml/h) 0–∞	11·12	12·57	22·87	9·1	10·60	11·60	12·98 ± 4·98

C_{max}, highest plasma concentration; T_{max}, time at which C_{max} occurs (h); K, first-order elimination rate constant; $t_{1/2\,K}$, half-time of the first-order elimination rate constant; AUC, total area under the plasma drug concentration versus time curve.

independent-model parameters K, $t_{1/2}$, AUC 0–8 h, AUC 0–∞, C_{max} and T_{max}, were evaluated. The K values were calculated on the tail of every single curve using a least squares regression analysis [16]. The areas under the curve were estimated by means of the trapezium method from T_0 to T_n h and by the C_n/K integral from T_0 to infinity.

RESULTS AND DISCUSSION

Absorption of the drugs through the uterine mucosa, albeit in small amounts, occurred in all treated cows. The serum concentrations found during the experimental time are summarized in Table III and IV. The extent of absorption was calculated from areas under the curve.

Table III
Sulfamonomethoxine serum levels (μg/ml)

	0	*30 min*	*1 h*	*2 h*	*4 h*	*8 h*	*12 h*	*24 h*	*48 h*	*72 h*
Cow A	—	1·24	1·42	1·38	0·96	0·10	—	—	—	—
Cow B	—	1·04	1·14	1·14	1·14	0·34	—	—	—	—
Cow C	—	1·27	1·94	2·17	0·88	0·33	—	—	—	—
Cow D	—	1·48	1·55	1·71	1·08	0·55	—	—	—	—
Cow E	—	1·60	1·47	1·26	0·75	0·28	—	—	—	—
Cow F	—	1·26	0·90	0·62	0·51	0·32	—	—	—	—
$\bar{x} \pm$ SD		1·32 ±0·20	1·40 ±0·36	1·38 ±0·53	0·89 ±0·29	0·32 ±0·14				

—, <0·02 μg/ml.

Table IV
Oxytetracycline serum levels (μg/ml)

	0	*30 min*	*1 h*	*2 h*	*4 h*	*8 h*	*12 h*	*24 h*	*48 h*	*72 h*
Cow A	—	—	—	0·70	0·78	0·97	0·20	0·10	—	—
Cow B	—	—	—	0·55	0·83	1·35	0·20	0·10	—	—
Cow C	—	—	0·20	0·70	0·97	1·59	0·34	0·24	—	—
Cow D	—	—	0·20	0·51	0·51	0·70	0·20	0·10	—	—
Cow E	—	—	—	0·55	0·68	0·97	0·20	0·10	—	—
Cow F	—	—	0·20	0·36	0·70	1·59	0·20	0·10	—	—
$\bar{x} \pm$ SD			0·10 ±0·11	0·56 ±0·13	0·75 ±0·16	1·20 ±0·37	0·22 ±0·06	0·12 ±0·06		

—, <0·1 μg/ml.

The average concentrations in serum of SMM and OTC, plotted logarithmically against time are illustrated in Fig. 2. The results pointed out a rapid absorption of SMM from the uterine lumen, as detectable concentrations in serum were already present in the 0·30-h sample. The T_{max} was 1·17 h. In spite of this, OTC levels rose in the serum after 2 h presenting a T_{max} of 8 h, and confirming data reported in literature [10]. Moreover SMM presented a more rapid elimination phase ($t_{1/2\,k}$ 2·63 h) than OTC ($t_{1/2\,k}$ 14 h).

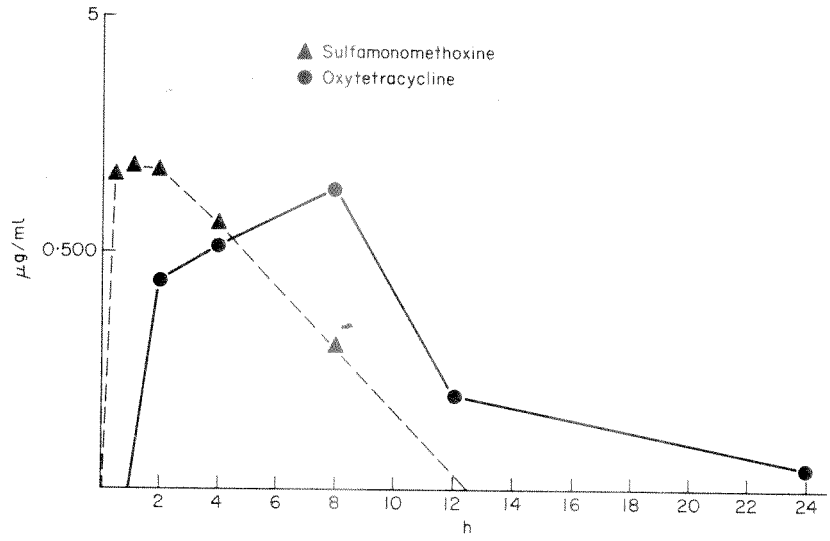

Fig. 2. SMM and OTC serum concentrations (mean values).

A metabolite peak, probably the N_4-acetylated sulphonamide, appeared close to the SMM peak showing a retention time of about 3 min (Fig. 3).

A comparison of the present results with those reported by other authors after i.v. and i.m. administration of SMM and OTC [7–20] emphasizes that the systemic

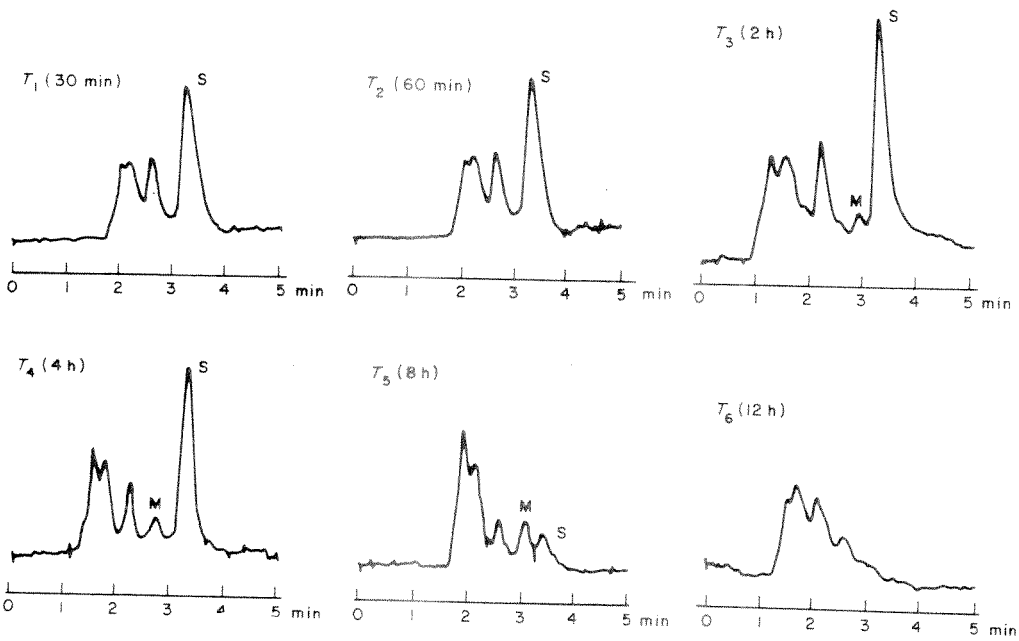

Fig. 3. Chromatograms of a cow treated with SMM: S, sulfamonomethoxine peak; M, metabolite peak.

bioavailability was low, as demonstrated by the values of C_{max} and AUC (Tables I and II). Our results are in close agreement with those already published [8, 10] upon the intrauterine administration of other antibacterials. As far as OTC is concerned, it is interesting to compare data resulting from the present study with those obtained by us in a previous investigation, during which dairy cows of the same herd were treated by intrauterine infusion with an equivalent dose of this drug, but using different kinds of vehicle [15].

In fact, no detectable serum levels of OTC were found when we used polyethylene glycol as the only vehicle for the infusion. By contrast, the uterine infusion of an aqueous solution of tetracycline gave serum levels almost resembling those achieved with OTC spray injection (Fig. 4).

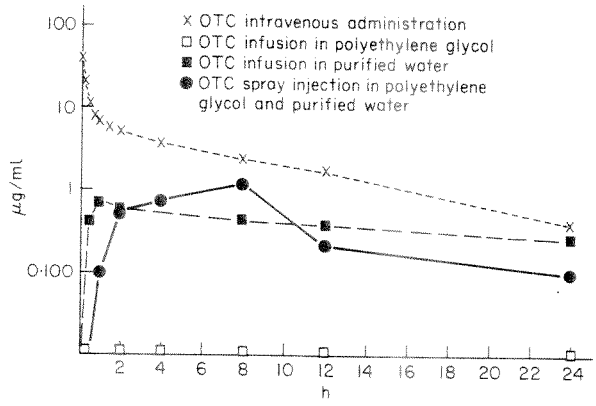

Fig. 4. Oxytetracycline, comparison between OTC spray injection and OTC infusion using different kinds of vehicle.

The slower absorption of sprayed OTC was probably due to the presence of glycol in the vehicle of the pharmaceutical preparation [21–23].

Finally, we suggest that the higher peak of OTC in serum could be ascribed to the spray form used and able to improve the endoperitoneal absorption through the uterine tubes [24] and the crossing of the uterine mucosa.

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