Pharmacokinetics of flumequine in sheep after intravenous and intramuscular administration: bioavailability and tissue residue studies

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The pharmacokinetic properties of flumequine and its metabolite 7-hydroxyflumequine were determined in six healthy sheep after single intramuscular (i.m.) and intravenous (i.v) injections at a dose of 6 mg/kg body weight. The tissue residues were determined in 20 healthy sheep after repeated i.m. administration with a first dose of 12 mg/kg and nine doses of 6 mg/kg. The flumequine formulation used was Flumiquil 3% Suspension Injectable®. The mean plasma concentrations of flumequine after i.v. administration were described by a three-compartment open model with a rapid distribution and a relatively slow elimination phase. The low value of volume of distribution at steady state (V_{dss}) (0.52 ± 0.24 L/kg) and high value of volume of distribution ($V_d\lambda_3$) (5.05 ± 3.47 L/kg) emphasized the existence of a small compartment with a slow rate of return to the central compartment. The mean elimination half-life was 11.5 h. The 7-hydroxyflumequine plasma levels represented 2.3% of the total area under the curve. The mean plasma concentrations of flumequine after i.m. administration were characteristic of a two-compartment model with a first order absorption. The mean maximal plasma concentration (1.83 ± 1.15 μg/mL) was obtained rapidly, i.e. 1.39 ± 0.71 h after the i.m. administration. The fraction of dose absorbed from the injection site was 85.00 ± 30.13%. The minimal concentrations of flumequine during repeated treatment were significantly lower in females than in males. Eighteen hours after the last repeated i.m. administration, the highest concentration of flumequine was observed at the injection sites followed by kidney, liver, muscle and fat. The highest concentration of 7-hydroxyflumequine was observed in the kidney and was ten times lower than the flumequine concentration. The longest flumequine elimination half-life was observed in the fat.

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INTRODUCTION

Flumequine, a second generation antibacterial quinolone, is used in veterinary medicine for the treatment of animal diseases caused by a wide-range of gram-negative bacteria (e.g. *Escherichia coli, Salmonella* and *Pasteurella*). The minimal inhibitory concentrations (*MIC*) of flumequine for these targets range from 0.09 μg/mL to 0.78 μg/mL (Ziv *et al.*, 1986). While several studies have described the pharmacokinetics and metabolism of flumequine in calves, there is no such information available for sheep. Two metabolites of flumequine have been detected in calves: the glucuronide conjugate (Mevius *et al.*, 1990) which is observed in the urine, and a minor metabolite, 7-hydroxyflumequine, which is detected in the urine and plasma. 7-Hydroxyflumequine contributes significantly to the overall antimicrobial activity in the urine (Harrison *et al.*, 1984). This metabolite exhibits approximately one-eighth the antimicrobial activity of flumequine (Schuppan *et al.*, 1985) whereas flumequine conjugates are microbiologically inactive.

The major pharmacodynamic effect of flumequine is its antimicrobial activity. From a clinical point of view, microbiologically active metabolites should be assayed in the plasma to compare these plasma concentrations with the *MIC* values of potential pathogens. Knowledge of the bioavailability of flumequine after intramuscular administration and the plasma concentrations obtained during repeated administration is necessary to permit extrapolation of clinical results obtained from a major species (cattle) to a minor species (sheep). For

residue studies, only microbiologically active compounds in the tissue are quantified because the screening methods are based on microbiological inhibition.

The objectives of the present study were: (i) to describe the pharmacokinetic behaviour of flumequine and its 7-hydroxy derivative following intravenous (i.v.) and intramuscular (i.m.) administration; and (ii) to study flumequine and 7-hydroxy-flumequine concentrations in the plasma after repeated intramuscular administration, and the depletion curves of these compounds in edible tissues (muscle, liver, kidney, fat and injection sites) after the final administration.

MATERIALS AND METHODS

Animals

The experiments were performed in 26 sheep (13 males and 13 females weighing 44.9 ± 11 kg, 6–12 months old, 'Rouge de l'Ouest' breed) obtained from a breeding farm. The sheep were placed in pens provided with facilities for drinking (*ad libitum*) and eating (hay, 600 g of pelleted, antibiotic-free food concentrate (Brebis Nourrice, Ucanor, Fougères, France) daily).

The sheep were allocated into two experimental groups. Group A sheep (three males and three females) were used to investigate the pharmacokinetics of flumequine after single i.m. or i.v. administrations. Group B sheep (10 males and 10 females) were used to study plasma concentrations obtained during repeated flumequine administration and tissue residues of flumequine and its hydroxy-metabolite.

Drugs

The flumequine formulation used was Flumiquil 3% Suspension Injectable® (Sanofi Santé Nutrition Animale, Libourne, France). Batch 110A1 was used for the bioavailability study and batch 127B1 for the tissue depletion study. Flumequine and 7-hydroxyflumequine standards were provided by Sanofi Santé nutrition Animale for the analyses.

Experimental design

Pharmacokinetic study (Group A). Flumequine was first administered i.m. in the neck at a dosage of 6 mg/kg. Seven days after the i.m. injection, flumequine was administered i.v. as a rapid bolus through a catheter in the right jugular vein at a dosage of 6 mg/kg. Blood was drawn from a catheter placed in the left jugular vein at 0, 5, 10, 15, 30, 45 min and 1, 2, 4, 8, 12, 24, 26, 30, 48, 54 h after i.v. administration, or 0, 2, 4, 8, 15, 30, 45 min and 1, 2, 4, 8, 12, 24, 26, 30, 48, 54, 72 h after i.m. administration. Blood was collected into heparinized tubes. Plasma was separated by centrifugation (3000 ***g***) and was stored frozen at −20°C until analysis.

Repeated i.m. administration and tissue residue study (Group B). The 20 sheep in group B were allocated into five groups of four animals (two males and two females) according to the time between the last flumequine administration and slaughter: group B1 at 18 h, group B2 at 30 h, group B3 at 48 h, group B4 at 60 h and group B5 at 78 h.

The 20 sheep in group B received an intramuscular injection of flumequine in the *m. gluteus* muscle twice daily for 5 days at different doses: the initial dose was 12 mg/kg and subsequent doses were 6 mg/kg. This is the recommended dosage regimen for this formulation. Injections were made alternatively into the left and right side of the *m. gluteus* at 12 h intervals. The *m. gluteus* muscle was used to ensure true intramuscular administration and easy sampling of the injection sites.

Blood was collected 1, 2, 3, 4, 5 and 7 h after administration in the five groups and 1, 2 and 4 h after each other administration in at least four animals. Blood was collected from the different groups at regular times until slaughter and the plasma obtained as described for group A.

All sheep in group B were slaughtered with a Matador® (Pinot-Cottin, Fougères, France) and bled-out. Edible tissue specimens: 100 g of muscle, 100 g of liver, the two kidneys, 100 g of abdominal fat and right and left injection sites (gluteal muscle; 300 g) were taken after slaughter and frozen at −20°C until analysis. As the drug was administered between the two intramuscular sites (left and right *m. gluteus*) at 12 h intervals, the sampling times for the injection sites after the last administration were 18, 30, 42, 48, 60, 72, 78 and 90 h.

Drug and metabolite assay

The concentrations of flumequine and of the metabolite 7-hydroxyflumequine were determined by high-performance liquid chromatography (HPLC).

Plasma extraction. The plasma samples were acidified with phosphate buffer (pH 6) and shaken with ethyl acetate. After centrifugation, the organic phase was collected and dried under nitrogen. Phosphate buffer (pH 7.8) and hexane were added to the residue. After shaking and centrifugation, the aqueous (lower) phase was injected into the HPLC system.

Tissue extraction. 0.1 mL of water and 0.2 mL of phosphate buffer (pH 7.8) were added to ground tissue samples in Eppendorf tubes. The tissue was pulverised with an ultrasonic disintegrator and 1 mL of ethyl acetate added. After centrifugation, the organic phase was collected and dried under nitrogen. Phosphate buffer (pH 7.8) and hexane were added to the residue. After shaking and centrifugation, the aqueous (lower) phase was injected into the HPLC system.

HPLC analyses. The system was coupled to a fluorimetric detector (excitation length = 320 nm, emission length = 365 nm) and a UV detector (wavelength = 324 nm). A UV detector was used to quantify flumequine and 7-hydroxyflumequine concentrations exceeding 500 µg/kg or µg/L in samples while the fluorimetric detector was used for concentrations below this limit. Fluorimetric detection could not be used for concentrations greater

than 1000 µg/L or 1000 µg/kg because the signal detector became saturated and the limit of quantitation with the UV detector was 100 µg/L or 100 µg/kg. The samples were chromatographed on a LiChrospher Select B column with a mobile phase of water containing 0.25% w/v orthophosphoric acid/mL: dimethylformamide: acetonitrile in the ratio 54:28:18. The analytical methods were validated for recovery rate, linearity, specificity, accuracy and precision as indicated in the recommendations for analytical methods for veterinary drug residues (Anonymous, 1993) in tissue or the international consensus for bioanalytical methods for plasma (Shah *et al.*, 1992). The recovery rates exceeded 75% in the plasma and tissue for flumequine and in the tissue for 7-hydroxyflumequine. The lowest value (60%) was obtained for 7-hydroxyflumequine in plasma. The limit of detection obtained with fluorimetric detection was between 1 and 4 µg/kg or µg/L for both compounds in the plasma and tissue.

Data analyses

The WhinPhar 3® (CNEVA-Fougères), Systat® (Statilogic) and Excel 4® (Microsoft) computer programs were used for the data analyses.

The curves were fitted using the weighted least-square method. The inverse of squared concentration was used as weight for the i.v. curves and the inverse of concentration was used for the i.m. curves. The best adjustment was selected on the basis of Akaike's information criterion (Yamaoka *et al.*, 1978a, b).

The kinetic parameters and bioavailability were calculated according to the advised methods (Gibaldi & Perrier, 1975).

The percentages of flumequine and its metabolite, 7-hydroxyflumequine, in plasma were expressed as a percentage of the sum of the area under the curve (*AUC*) for the parent drug and its metabolite.

The repeated administration of drug enabled the drug level in the therapeutic zone of concentration to be obtained or maintained. The plasma concentration for a repeated i.m. administration of flumequine was fitted to the following equation.

$$C = A_1 \cdot \exp(-\lambda_2 \cdot (n \cdot \tau + t)) + A_2 \cdot \exp(-\lambda_3 \cdot (n \cdot \tau + t))$$

$$-(A_1 + A_2) \cdot \exp(-K_a \cdot (n \cdot \tau + t))$$

$$+A_3 \cdot \frac{(1 - \exp(-(n-1) \cdot \lambda_2 \cdot \tau)}{(1 - \exp(-\lambda_2 \cdot \tau))} \cdot \exp(-\lambda_2 \cdot t)$$

$$+A_4 \cdot \frac{(1 - \exp(-(n-1) \cdot \lambda_3 \cdot \tau)}{(1 - \exp(-\lambda_3 \cdot \tau))} \cdot \exp(-\lambda_3 \cdot t)$$

$$-(A_3 + A_4) \cdot \frac{(1 - \exp(-(n-1) \cdot K_a \cdot \tau)}{(1 - \exp(-K_a \cdot \tau))} \cdot \exp(-K_a \cdot t)$$

in which C is the plasma concentration (µg/mL), A_1, A_2, A_3 and A_4 are mathematical coefficients, λ_2 is the hybrid rate constant for the distribution, λ_3 is the hybrid rate constant for the elimination terminal phase, K_a is the first-order absorption rate constant, n is the number of administrations, τ is the interval between administrations and t is the time after the final administration.

The logarithms of tissue concentrations were fitted by linear regression and the half-lives calculated.

Statistical analysis

The means of the rate constants were harmonic and their standard deviation was calculated using the method proposed by Lam *et al.* (1985). The arithmetic means and standard deviation (SD) were calculated for the other parameters. Differences in pharmacokinetic data between dosage routes were analysed for statistical differences ($P < 0.05$) using the paired Student's *t*-test. For the single intravenous and intramuscular administration, differences between sex were analysed by Student's *t*-test. After repeated intramuscular injection, the effects of sex, time, group and weight as cofactors were tested on minimal concentrations and on other major pharmacokinetic parameters by analysis of variance. Correlations between plasma and tissue concentration were tested by linear regression. All data were tabulated as means ± SD.

RESULTS

The mean plasma concentrations of flumequine and its metabolite 7-hydroxyflumequine for a single intravenous dose of 6 mg/kg flumequine in six sheep are presented in Fig. 1. Flumequine concentrations in plasma were best fitted to a triexponential equation. The mean estimates of the coefficients and exponents of this equation along with the other pharmacokinetic parameters are shown in Table 1. The terminal phase half-life ($t_{½}\lambda_3$) ranged from 6.0 to 26.7 h. The central volume (V_c) was 0.18 ± 0.03 L/kg. The distribution volume at steady state (V_{dss}) was 0.52 ± 0.24 L/kg and the apparent volume of distribution ($V_d\lambda_3$) ranged from 3.1 to 12.0 L/kg. The clearance (Cl) of flumequine was 0.31 ± 0.03 L/h·kg. The 7-hydroxyflumequine concentrations in plasma after intravenous administration are described by a three-exponential equation (Fig. 1) and the pharmacokinetic parameters are summarized in Table 1. The rate of appearance of 7-hydroxyflumequine was rapid ($t_{½}K_a$ = 0.08 ± 0.05 h). The maximal concentration observed 15 min after administration was between 0.11 and 0.29 µg/mL. The area under the curve of 7-hydroxyflumequine represented 2.35 ± 0.66% of the total area under the curves.

The plasma concentrations of flumequine after an intramuscular administration were also best fitted to a triexponential equation (Fig. 2). This equation was described by a bicompartmental model with a first order absorption. The mean pharmacokinetic parameters are shown in Table 1. The mean maximal concentration (1.83 ± 1.15 µg/mL) was obtained rapidly, 1.39 ± 0.71 h after the intramuscular administration. The distribution rate λ_2 obtained after intramuscular administration was close to the second distribution rate λ_2 calculated after intravenous administration. The mean elimination half-life

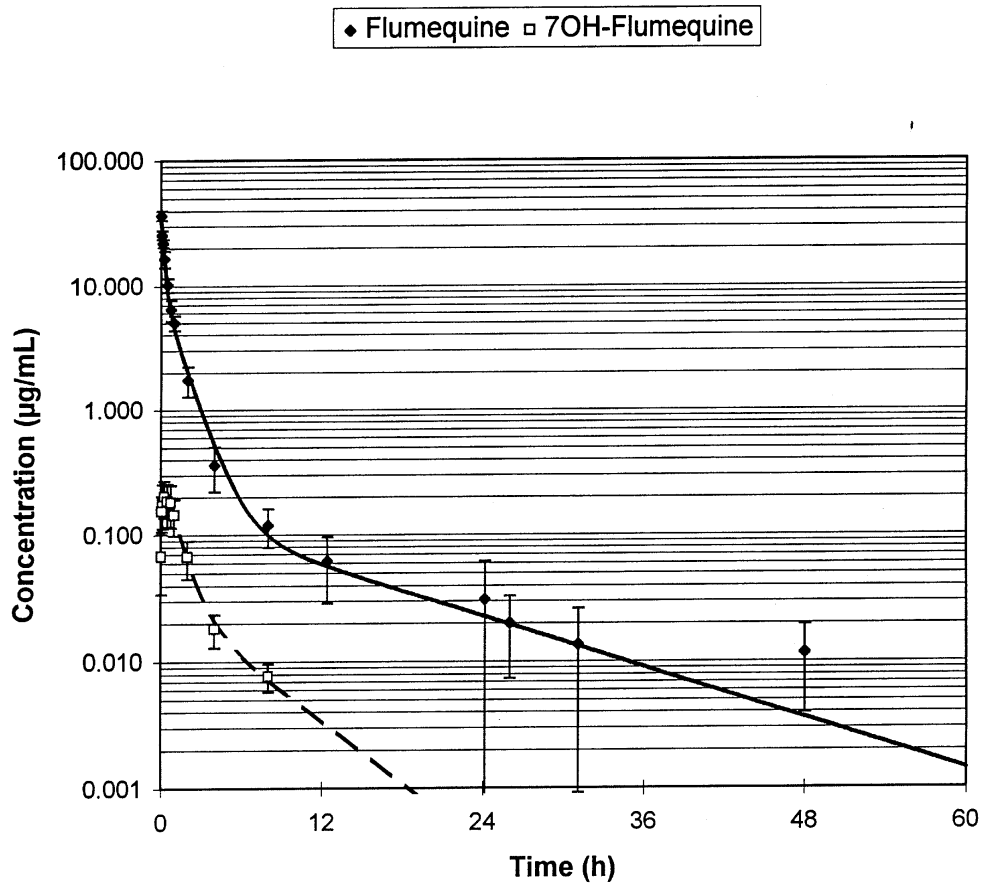

Fig. 1. Mean plasma concentrations (± SD) of flumequine and 7-hydroxyflumequine after intravenous administration of 6 mg/kg to six sheep.

Table 1. Flumequine and 7-hydroxyflumequine pharmacokinetic parameters (mean ± SD) in six sheep after a single intravenous (i.v.) and intramuscular (i.m.) dose of 6 mg/kg and after 10 repeated i.m. administrations (first dose = 12 mg/kg, D2-10 = 6 mg/kg) in 20 sheep

		Flumequine			7-Hydroxyflumequine after flumequine administration
Route		Single i.v.	Single i.m.	Repeat i.m.	i.v
Parameter	Unit				
A_1	µg/mL	25.43 ± 4.64	4.86 ± 3.56	12.46 ± 6.63	0.31 ± 0.18
A_2	µg/mL	8.36 ± 5.96	0.81 ± 0.29	2.10 ± 1.37	0.03 ± 0.01
A_3	µg/mL	0.14 ± 0.08		6.85 ± 3.78	
A_4	µg/mL			0.79 ± 0.46	
$t_{½}$ λ_1	h	0.21 ± 0.13			0.70 ± 0.09
$t_{½}$ λ_2	h	1.35 ± 0.99	1.14 ±0.54	0.89 ±0.28	3.83 ± 0.77
$t_{½}$ λ_3	h	11.47 ± 7.78	9.19 ±2.08	9.09 ±2.60	
$t_{½}$ K_a	h		0.48 ±0.28	0.52 ±0.22	0.08 ± 0.05
V_c	L/kg	0.18 ± 0.03			
$V_d\lambda_3$	L/kg	5.05 ± 3.47			
V_{dss}	L/kg	0.52 ± 0.24			
AUC	mg/h·L	19.58 ± 2.63	16.47 ± 5.71		0.46 ± 0.13
Cl	L/h·kg	0.31 ± 0.03			
MRT	h	2.26 ± 1.02	11.62 ± 4.45		
F	%		85.00 ± 30.13		
C_{max1}	µg/mL		1.83 ± 1.15	3.55 ± 1.38	0.22 ± 0.06
T_{max1}	h		1.39 ± 0.71	1.27 ± 0.53	0.29 ± 0.10

A_i, Mathematical coefficient; λ_i, hybrid rate constant for phase i; K_a, absorption rate for flumequine and appearance rate for 7-hydroxyflumequine; $t_{½}$ λ_i, half-life of phase i; V_c, volume of central compartment; $V_d\lambda_3$, volume of distribution; V_{dss}, volume of distribution at steady state; *AUC*, area under the curve; *Cl*, clearance; *MRT*, mean residence time; *F*, fraction of dose absorbed; T_{max1}, time to obtain first maximal concentration C_{max1}.

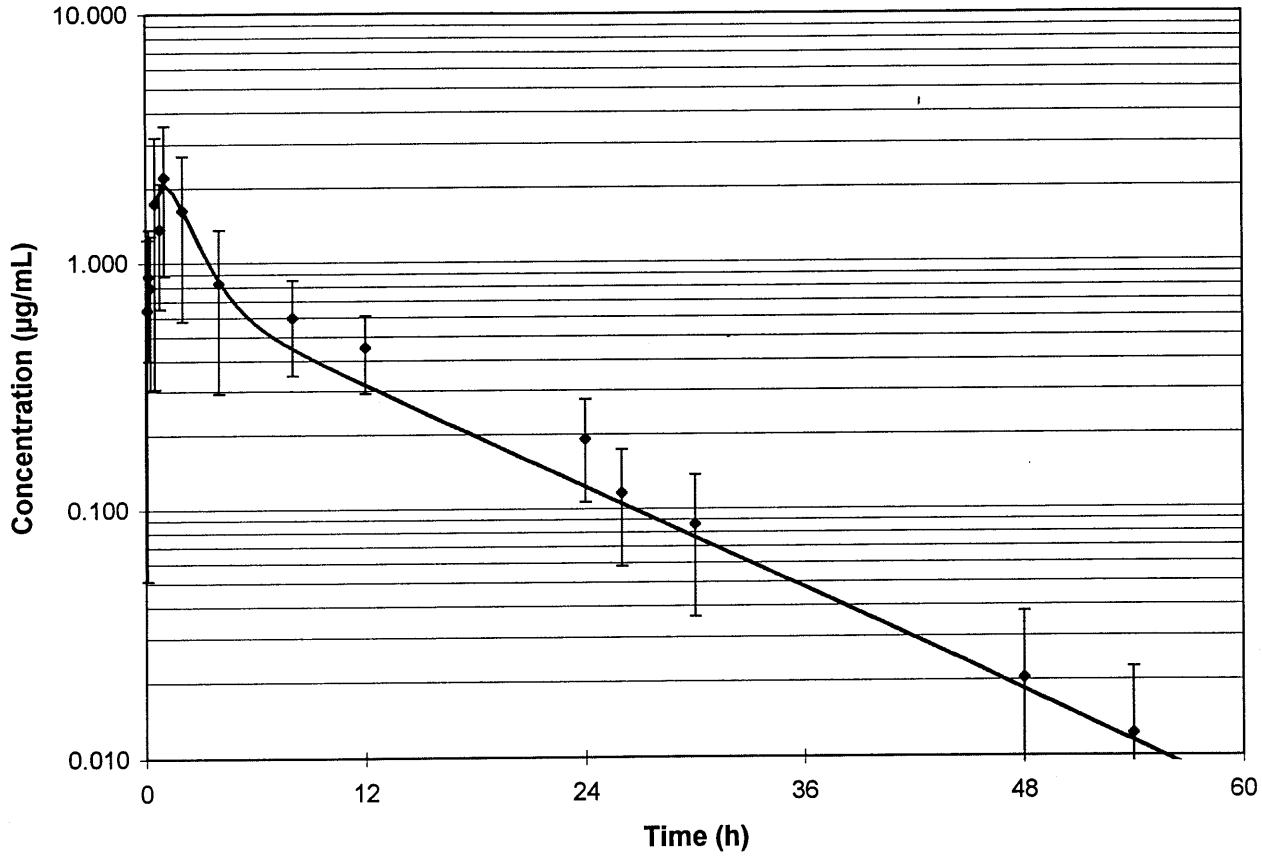

Fig. 2. Mean plasma concentrations (± SD) of flumequine after intramuscular administration of 6 mg/kg to six sheep.

($t_{½}\lambda_3$) ranged from 6.6 to 12.0 h and was not significantly ($P > 0.05$) different from that of the intravenous administration. The mean absorption half-life ($t_{½}K_a$) was 0.48 ± 0.28 h. The fraction of dose absorbed (F) was 85.00 ± 30.13%.

The mean plasma concentrations of flumequine and 7-hydroxyflumequine plotted against the time profiles after repeated i.m. administrations are depicted in Fig. 3. The pharmacokinetic parameters calculated after repeated i.m. administration were close to those obtained after a single i.m. injection. The mean concentrations of flumequine during the treatment ranged from 0.7 µg/mL to 3 µg/mL. Minimal concentrations before each dose in females (0.49 ± 0.07 µg/mL) were significantly ($P < 0.05$) lower than those in males (0.92 ± 0.07 µg/mL) although the peak concentrations did not differ significantly (Fig. 4). Concentrations in the terminal phase were also significantly ($P < 0.05$) lower in females than in males and the terminal half-lives for males (11.18 ± 1.38 h) were higher than in females (7 ± 1.98 h). The relationship between terminal half-life and weight was also statistically significant ($P < 0.05$). The weights of the males (56.7 ± 7.1 kg) were higher than those of the females (35.2 ± 1.5 kg). The plasma concentrations of 7-hydroxyflumequine ranged from 0.01 µg/mL to 0.08 µg/mL and did not differ between sexes. Greater variation in plasma levels was found following single and repeated i.m. administrations and secondary peaks or humps existed in the logarithmic plasma concentration–time profile (Figs 2 & 3).

The tissue concentration–time profiles are presented in Table 2 for the injection sites, muscle, liver, kidney and fat. The half-lives of flumequine in the different tissues were 10.8 h for the injection sites, 16.9 h in muscle, 26.7 h in kidney, 16.5 h in liver and 53.3 h in fat. The flumequine concentrations in the plasma were significantly correlated with the concentrations in muscle (r = 0.996; $P < 0.001$), liver (r = 0.923; $P < 0.001$) and kidney (r = 0.862; $P < 0.01$) but not with fat (r = 0.497; $P = 0.173$).

DISCUSSION

Flumequine kinetics after the intravenous administration could be described by a three-compartment open model with a rapid distribution and a relatively slow elimination phase. The elimination half-life ($t_{½}\lambda_3$) of 11.47 h was similar to the half-lives reported for calves (Mevius *et al.*, 1990, 1991) while the clearance value (0.31 ± 0.03 L/h·kg) was slightly higher than those reported (Mevius *et al.*, 1990, 1991) in healthy (0.24 ± 0.02 L/h·kg) and sick calves (0.15 ± 0.01 L/h·kg). The mean volume of distribution at a steady state of 0.52 L/kg was also similar to that reported for calves. A high apparent volume of distribution ($V_d\lambda_3$) was calculated with high variability between animals because of the dependence of this value on the terminal phase estimate. The assay for flumequine used by Mevius and colleagues was considerably less sensitive than the

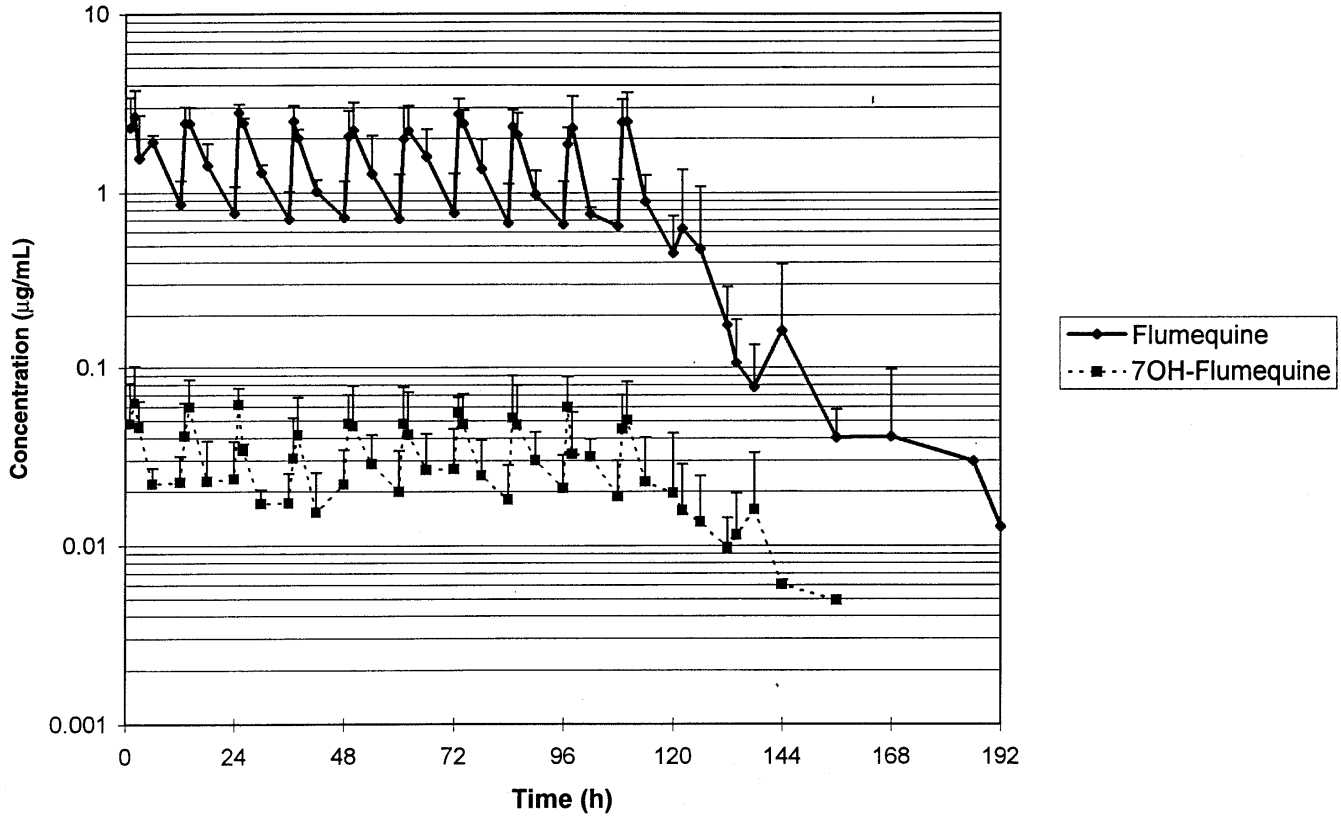

Fig. 3. Mean plasma concentrations (± SD) of flumequine and 7-hydroxyflumequine after repeated intramuscular administrations to 20 sheep with an initial dose of 12 mg/kg and subsequent doses of 6 mg/kg of flumequine at 12 h intervals.

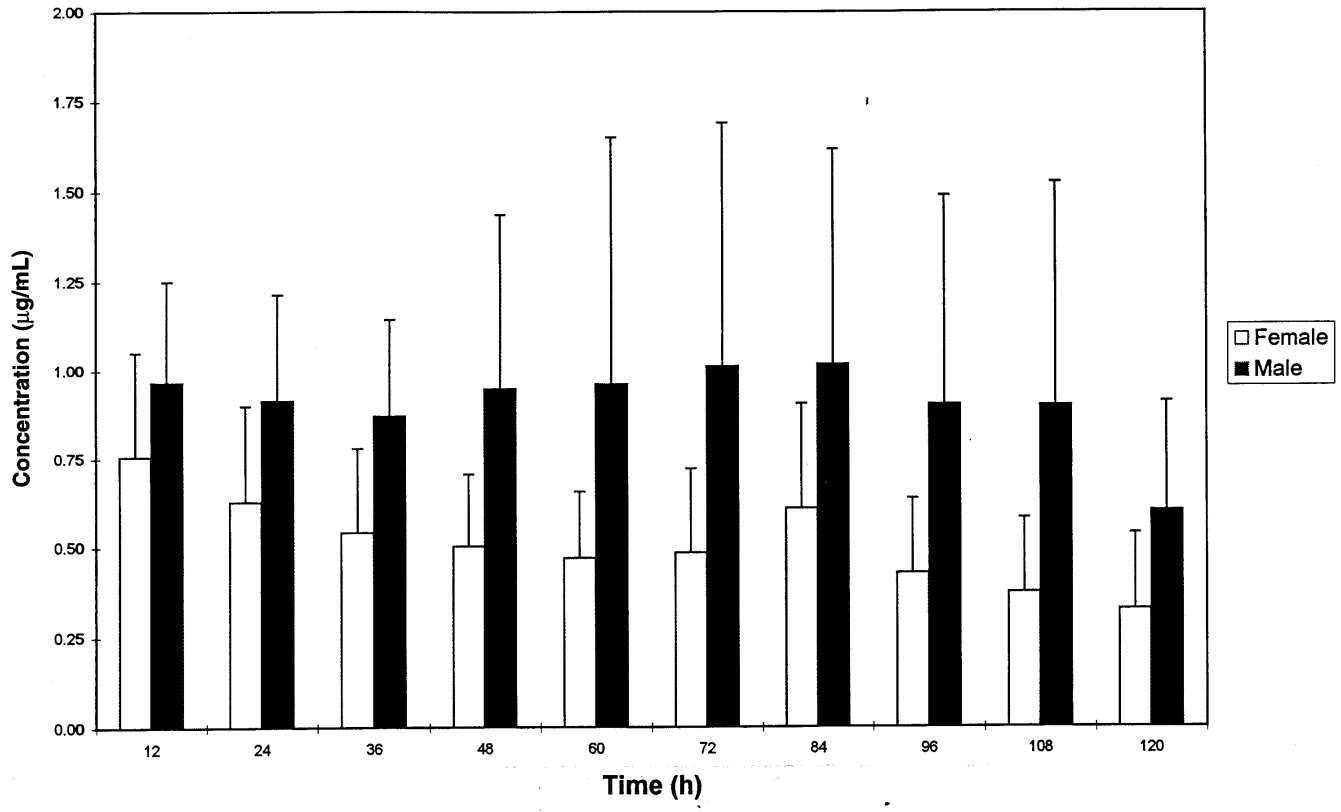

Fig. 4. Mean minimal plasma concentrations (± SD) of flumequine determined by sex (10 males, 10 females), before each new intramuscular administration of the dosage schedule (initial dose of 12 mg/kg and subsequent doses of 6 mg/kg of flumequine at 12 h intervals).

Table 2. Tissue concentrations (μg/kg) of flumequine and 7-hydroxyflumequine in sheep given flumequine intramuscularly (i.m.) (12 mg/kg first dose, 6 mg/kg at 12 h interval for 9 doses). Mean ± SD

Tissue	Time (h)	*n*	Flumequine	*n*	7-hydroxyflumequine
Injection	18	4	2859.3 ± 3427.5	1	13.3
site	30	8	572.6 ± 960.2	1	13.5
	42	4	138.5 ± 130.5		< LOQ
	48	4	38.5 ± 9.7		< LOQ
	60	8	20.9 ± 9.8		< LOQ
	72	4	145.3 ± 258.1		< LOQ
	78	3	8.4 ± 0.6		< LOQ
	90	2	10.0 ± 0.2		< LOQ
Muscle	18	4	179.8 ± 208.4	1	15.3
	30	4	45.3 ± 32.8		< LOQ
	48	4	30.0 ± 7.4		< LOQ
	60	3	13.9 ± 6.2		< LOQ
	78	3	9.0 ± 3.0		< LOQ
Liver	18	4	458.0± 456.8	3	33.3± 32.1
	30	4	80.2± 55.2		< LOQ
	48	4	50.2± 19.3	1	10.24
	60	4	30.6± 19.7		< LOQ
	78	2	13.8± 7.9		< LOQ
Kidney	18	4	1355.5± 1905.5	4	108.7± 122.6
	30	4	514.1± 354.9	4	68.9± 52.3
	48	4	245.9± 84.1	4	26.4± 21.3
	60	4	109.6± 67.3	4	7.8± 5.3
	78	4	38.6± 16.9	1	4.5
Fat	18	4	86.7± 55.0		< LOQ
	30	4	115.1± 167.0		< LOQ
	48	4	74.2± 72.8		< LOQ
	60	4	49.3± 46.3		< LOQ
	78	4	52.5± 79.8		< LOQ

< LOQ, below the limit of quantification.
n, number of values higher than the LOQ.

assay used in this study resulting in a 20-fold decrease in the limit of detection achieved. While the high $V_d\lambda_3$ suggests distribution into an extravascular compartment the V_{dss} is less than 1 L/kg. This difference between the two volumes can be explained by a small compartment with a small rate of return from this compartment to the central compartment. Indeed, flumequine with its p*K*a value of 6.2, is approximately 90% ionized in the plasma at pH 7.35 (Mevius *et al.*, 1990). The undissociated flumequine is lipid soluble and could be distributed into the fat compartment as demonstrated by the slow disappearance from fat observed after repeated i.m. administrations. The variations in fat content between animals might explain the observed differences in terminal half-life in the plasma. This variation in fat content was suggested by Mevius *et al.*, (1990) in cattle to explain the observed age differences in half-lives.

Plasma concentrations of 7-hydroxyflumequine were low after intravenous administration and represented only 2.3% of the total area under the curve. The rates of appearance and elimination of 7-hydroxyflumequine were significantly different ($P<0.05$) to those of flumequine. 7-hydroxyflumequine constituted a small proportion of the total clearance of flumequine in sheep, calves (Mevius *et al.*, 1990) and dogs, whereas it was the major metabolite in rats (Harrison *et al.*, 1986).

Flumequine was rapidly absorbed from the intramuscular injection site. The fraction of dose available was higher than 50% but varied considerably between animals. This good availability from the intramuscular injection site has previously been described for a flumequine suspension (Meijer *et al.*, 1994). High inter-individual variability in bioavailability has been described for other compounds when the neck has been used as the injection site (Houpert *et al.*, 1993). For this reason, the gluteal muscle was used for the residue depletion study to ensure a true intramuscular injection site although this part of the body is not recommended in practice.

The presence of secondary peaks or humps in the concentration–time profile of flumequine in sheep could be attributed to the entero-hepatic cycling of the drug. Indeed flumequine is metabolized as the glucurono-conjugate and part of the drug is eliminated by the liver in the unchanged form or as glucurono-conjugate (Harrison *et al.*, 1986; Mevius *et al.*, 1990, 1991; Vree *et al.*, 1992). Degradative metabolism of conjugates by the intestinal flora would release the parent drug which could then be reabsorbed (Testa & Jenner, 1976) and because of the good oral bioavailability in cattle (Ziv *et al.*, 1986) we suggest that the intestinal reabsorption of flumequine is good in sheep also.

After repeated administration, the mean calculated parameters did not differ significantly from those obtained after a

single administration, thus demonstrating a linear relationship between dosage and concentration. No drug accumulation was observed when the minimal concentrations before the first and last administrations were compared (Fig. 3). The aims of repeated injections are to rapidly obtain therapeutic efficacy and to maintain a continuous active concentration in the plasma (Labaune, 1984). This objective was attained in sheep with the formulation tested. Differences in minimal concentrations between the sexes were statistically significant ($P<0.01$). The significant difference between the sexes in the second group of our study, but not in the first group, was probably detected because of the size of the second group (20 animals) and the repeated doses administered (10) which increased the statistical power of the test in comparison to the first group (6 animals) which was small, and in which the observed variance was considerable. This result could be because of sex differences in tissue proportions (muscle, fat) and/or in liver and kidney clearance. The weight was significantly higher in males than females of the same age. This difference in body weight may result in variations in the pharmacokinetic characteristics of flumequine as described with age in cattle (Mevius *et al*., 1990). In humans, the relative proportion of fat tissue is higher in females than in males (Fiserova-Bergerova, 1995). A larger deep compartment in the female could explain the more rapid decrease in plasma concentration observed after the peak. Differences in clearance could also be related to sex. Sex hormone-dependent cytochrome P-450 activity such as hydroxylation, is described in several species for other compounds such as sulfamethazine (Witkamp *et al*., 1992). In our study however, the plasma 7-hydroxyflumequine concentration did not differ significantly between the sexes after repeated i.m. administration. A specific study of total and renal clearance would be necessary to determine the true effect of sex or weight. This result is important from a clinical point of view if the *MIC* of pathogens are close to the minimal concentration obtained during treatment. Indeed, the interval of time below the *MIC* must be as small as possible to ensure good efficacy.

The 7-hydroxyflumequine concentrations in the plasma were very low in comparison with flumequine concentrations after single or repeated administrations. The 7-hydroxyflumequine concentrations were one-fortieth of the flumequine concentration during repeated administrations. It can be considered that 7-hydroxyflumequine does not contribute significantly to the microbiological activity in the plasma.

The 7-hydroxyflumequine concentrations in the tissues were high in the kidney but low in the liver with detectable levels observed only 18 h after the last dose. 7-Hydroxyflumequine was observed at the injection site and in the muscle 18 h after the cessation of treatment in just one animal, which could represent a difference in metabolic activity. The concentrations observed at both injection sites were close to those obtained in the muscle of this animal. This result could be explained by a distribution of 7-hydroxyflumequine to the muscle. The highest concentrations of 7-hydroxyflumequine in the liver, kidney and plasma were also obtained in this animal. The high concenration of flumequine and 7-hydroxyflumequine in the kidney demonstrates that the kidney could be the primary organ involved in the elimination of both flumequine and its metabolite in sheep. Flumequine is principally eliminated *via* the kidney in rats (90% of the dose) while 60% is renally excreted in dogs (Harrison *et al*., 1986) and $\approx$ 50% in calves (Mevius *et al*., 1990). The true renal clearance should be determined in sheep to confirm this hypothesis. The linear relationship between the flumequine concentrations in muscle, liver, kidney and plasma sampled just before slaughter would explain why no accumulation of flumequine was observed in these three tissues ($T_{½}$ = 16 to 27 h) compared to the fat ($T_{½}$ = 53 h). This may be because of the lipophilic properties of flumequine, which exhibits a high octanol/water ratio, and by the low blood flow in the fat. A similar slow disappearance of flumequine from fat has been described in poultry (Moutafchieva *et al*., 1994; Romvary *et al*., 1994).

The pharmacokinetic parameters obtained in sheep were similar to those obtained in calves. The bioavailability of flumequine after intramuscular injection of the formulation was higher than 50% but varied between animals. Flumequine was distributed in a deep compartment which could be fat, as demonstrated by the results of the residue study. The terminal half-life of flumequine in fat was the highest compared with those observed in tissue and plasma. 7-Hydroxyflumequine was a minor metabolite in sheep, as in calves, with very low concentrations in the plasma and tissue. The minimal plasma concentration could be related to differences in body composition between females and males or related to weight. Studies of renal and non-renal clearances of flumequine in sheep in relation to weight and sex will be necessary for a better interpretation of our results.

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