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Pharmacokinetics of tetracycline hydrochloride in fat-tailed sheep

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Summary

Tetracycline may be used to treat several types of bacterial diseases in ruminants. In addition, tetracycline is added to food to promote the growth. There are few reports on the pharmacokinetics of tetracycline in sheep. Therefore, the objective of this study was to examine the pharmacokinetic characteristics of the drug in sheep. Ten apparently healthy mixed-breed sheep were administered 20 mg/kg tetracycline orally and intravenously with a time interval of two weeks. Blood samples were collected before and at various time intervals after the administration of the drug. Sera were separated, kept at -20°C, and analysed using fluorescence spectrophotometry. The volume of distribution (V_d), elimination rate constant (K_{el}), half-life ($t_{1/2}$), and clearance (Cl_B) of tetracycline after intravenous injection were determined to be 0.21 L/kg, 0.21/hr, 3.3 hr, and 0.73 ml/kg/min, respectively. When the drug was given orally, these parameters were found to be 0.37 L/kg, 0.12/hr, 5.8 hr, and 0.73 ml/kg/min, respectively. Moreover, the bioavailability of tetracycline after oral administration was around 55%.

Key words: Tetracycline, Pharmacokinetics, Sheep

Introduction

Tetracycline is a broad-spectrum antibiotic active against aerobic and anaerobic Gram-positive and -negative bacteria, rickettsia, Mycoplasma, and Chlamydia (Riviere and Spoo, 1995). The drug was first introduced for clinical use in 1952 and is still used both in small and food-producing animals. Tetracyclines have been used in ruminants as both prophylactic and therapeutic agents. Rational use of antimicrobial drugs to treat infectious diseases caused by bacteria requires knowledge not only of the bacterial susceptibility to the drug but also of the concentration of the drug that can be achieved in the animal's body fluids. Drug dosage regimens are derived from knowledge of the pharmacodynamics and pharmacokinetics of the drug. Several studies have been reported on the pharmacokinetics of tetracycline in different animal species such as cattle (Pilloud, 1973; Ziv and Sulman, 1974; Bradley *et al*., 1982; Xia *et al*., 1983a; Nouws *et al*., 1985; Riond *et al*., 1989; Meijer *et al*., 1993), sheep (Ziv and Sulman, 1974; Wilson and Green, 1986), goats (Jha *et al*., 1989; Escudero *et al*., 1994; Escudero *et al*., 1996), pigs (Xia *et al*., 1983b; Hall *et al*., 1989), dogs (Baggot *et al*., 1977; Wilson *et al*., 1985) and horses (Pilloud, 1973; Teske *et al*., 1973; Horspool and McKellar, 1990). Relatively little has recently been published on the pharmacokinetics of tetracycline in ruminants. The objective of this study was to determine the basic pharmacokinetic characteristics of tetracycline in sheep after a single oral or intravenous dose of the drug.

Materials and Methods

Animals

Ten apparently healthy mixed breed (Ghezel×Mehraban) fat-tailed sheep weighing 35 to 55 kg were used. Animals had access to water and food ad libitum.

Drug

Tetracycline hydrochloride powder was pooled from the available drug dosage form (250 mg capsules, Daroopakhsh, Tehran, Iran). The volume of antibiotic solution—made by dissolving the drug in water—administered to each animal was calculated based on the body weight and a correction factor for the purity of the drug solution compared with a standard tetracycline hydrochloride solution (Merck Co., Lot No. K21003089).

Experimental design

In a cross-over design, five sheep were given 20 mg/kg of tetracycline hydrochloride intravenously through the right jugular vein. Another five sheep were given the drug at the same dose orally. After a washout period of two weeks post-administration, the groups were switched. Blood samples were collected from the left jugular vein on -5, 5, 15, 30 and 60 min, and 1.5, 2, 3, 4, 6, 12 and 24 hrs after intravenous injection of tetracycline. For the oral route, blood samples were taken on -5, 1, 2.5, 3.5, 4.5, 7, 9, 13, 17 and 24 hrs after administration of the antibiotic. Sera were separated by centrifugation at 1500 g for 15 min and stored at -20°C until drug analysis was performed by fluorescence spectrophotometry.

Tetracycline assay

The concentration of tetracycline in serum was determined by spectrofluorometry (Chang *et al*., 1992). In summary, serum proteins were first precipitated by addition of tricarboxylic acid solution. Then 0.3 M potassium hydroxide (2 ml) solution was added to 1 ml of each deproteinized serum samples or standards. After vortex, tubes were kept in boiling water bath for 30 min. After cooling, the tube volume was adjusted to 10 ml by addition of distilled water. The fluorescence of each sample and standards were determined by fluorescence spectrophotometery (Spectro-Plus, MSE Scientific Instruments) at excitation and emission wavelengths of 333 and 450 nm, respectively. The mean ± SEM recovery of tetracycline was 87.4 ± 3.2 (n=5; CV<10%). The sensitivity of the test was >2 µg/ml.

Pharmacokinetic analysis

1- *Single intravenous dose:* The blood concentration-time data were analysed by non-linear least squares regression analysis (Fig. 1a). A non-compartmental analysis was found to be appropriate to estimate the major pharmacokinetic parameters. Area under the concentration-time curve from time zero to infinity (AUC) was derived by the trapezoidal rule with extrapolation to infinite time by dividing the last available blood concentration value by the terminal disposition rate constant; the apparent first-order rate constant for elimination (K_{el}) was obtained from the slope of the terminal phase of the log concentration-time data. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_{el}$. Drug clearance (Cl_B) was calculated as Dose/AUC and apparent volume of distribution (V_d) as Dose/(AUC×K_{el}).

2- *Single oral dose:* The blood concentration-time data after oral

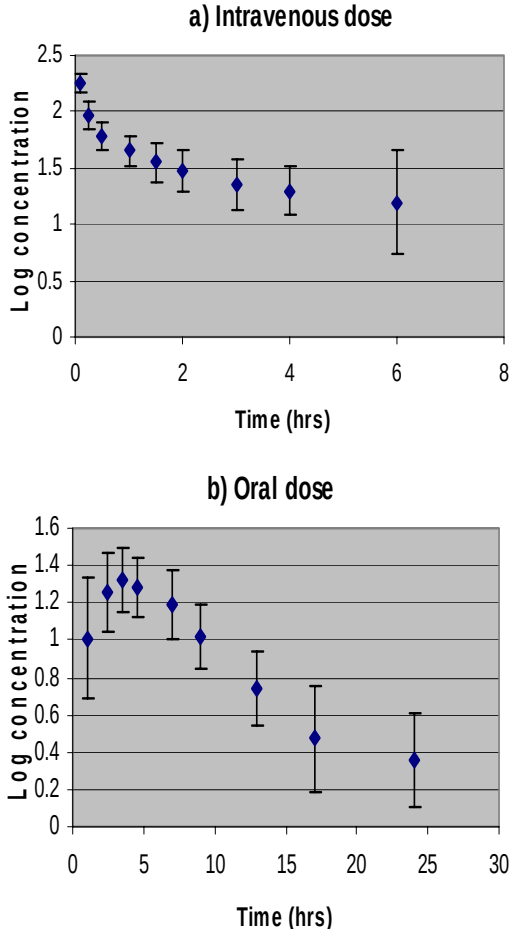

Fig. 1: Log blood concentration-time plot for tetracycline (20 mg/kg) administered to sheep

administration of tetracycline (Fig. 1b) was best described by a one-compartment model with first-order input (absorption). The fraction of dose absorbed (F) was estimated by dividing the $AUC_{(oral)}$ by AUC for intravenous administration of the drug. K_{el} was obtained from the slope of the log-linear portion of the elimination phase of serum concentration vs time curve. Cl_B and V_d were calculated as described earlier and were corrected for F.

Statistical analysis

Results were presented as mean±SEM. Means were compared by Student's t-test for independent samples. The F-test was used to determine which compartmental model best described the kinetics of tetracycline in sheep.

Results

Intravenous dose

After intravenous injection of 20 mg/kg tetracycline hydrochloride, an initial concentration of 200 μg/ml was attained in serum. Six hrs later, the concentration decreased to one-tenth of its initial level, i.e., to almost 20 μg/ml. The mean log blood concentration-time plot (Fig. 1a) for tetracycline was non-linear after intravenous administration of the drug; the disappearance of the drug from sheep blood seemed to be bi-exponential with a rapid distribution phase ($t_{1/2}$ <0.5 hr) followed by a slow elimination phase ($t_{1/2} \approx$ 3 hrs; Table 1). The apparent volume of distribution of tetracycline was 0.21 L/kg. The blood clearance of tetracycline in sheep was found to be 0.73 ml/min/kg (Table 1).

Oral dose

Comparison of AUC for the oral dose (251 μg hr/ml) with that of intravenous dose (456 μg hr/ml) revealed that the bio-availability (F) of the drug was almost 55%. Two hrs following oral administration of the same dose of the drug, a serum concentration of around 20 μg/ml was attained. The maximum blood concentration (C_{max}) was almost 22 μg/ml that was attained almost four hrs after drug administration (T_{max} = 4 hrs). The decline in tetracycline concentration in blood was mono-exponential and the $t_{1/2}$ after oral administration (5.8 hrs) was about 70% greater than its corresponding mean value following intravenous administration ($P \leq 0.05$). The apparent V_d of tetracycline in sheep was found to be 0.37 L/kg; and Cl_B was 0.73 ml/min/kg (Table 1).

Table 1: Disposition kinetics in sheep given a single oral or intravenous dose of tetracycline hydrochloride (20 mg/kg)

Parameters	Oral	Intravenous
F (%)	55.3	-
C_{max} (μg/ml)	22.1	-
C_0 (μg/ml)	-	200
AUC (μg hr/ml)	251	456
V_d (L/kg)	0.37	0.21
K_{el} (1/hr)	0.12	0.21
$t_{1/2}$ (hr)	5.8	3.3
Cl_B (ml/ min/kg)	0.73	0.73

Key: F (bioavailability); C_{max} (maximum blood concentration); C_0 (concentration at time zero); AUC (area under the serum concentration versus time curve); V_d (apparent volume of distribution); K_{el} (elimination rate constant); $t_{1/2}$ (elimination half-life); Cl_B (clearance)

Discussion

The serum concentrations of tetracycline in sheep were proportionally similar to those obtained for doxycycline and minocycline in cows and ewes (Ziv and Sulman, 1974). Moreover, the pharmacokinetics of tetracycline, administered intravenously to sheep, was compatible with the two-compartment open model that previously described for cows and chickens (Ziv and Sulman, 1974; Anadon, 1985), and for oxytetracycline in buffaloes and horses (Pilloud, 1973; Ziv and Sulman, 1974; Varma and Paul, 1983). However, marked differences were observed between the pharmacokinetic parameters for tetracycline in sheep vs other domestic animals.

After oral administration of tetracycline, the drug concentration decreased to less than 3 μg/ml after 24 hrs. Bearing in mind that the antimicrobial activity of tetracycline in biological fluids will be achieved at concentrations >0.5 μg/ml (Pijpers *et al*., 1990), the antimicrobial activity can possibly be attainable with administration of the drug every 24 hrs. Of course, the

problem of change in the rumen flora should be considered.

Relatively, small apparent V_d after intravenous injection, may indicate that diffusion of tetracycline out of blood vessels is poor. The apparent V_d after oral administration of tetracycline was more than that after intravenous route (0.37 vs 0.21 L/kg, Table 1). This may be explained by the distribution of the drug in the gastrointestinal tissues and antibiotic adsorption to the food contents, particularly of the rumen. On the other hand, comparison of tetracycline $t_{1/2}$ and K_{el} indicates that the drug is eliminated at a slower rate when administered orally than intravenously (Table 1). This difference in part can be due to the durable nature of the drug absorption following its oral administration.

Pharmacokinetics of tetracycline is poorly studied in animal species, particularly in ruminants. Only few studies have examined the disposition of tetracycline in some species, though, numerous reports are available on other members of tetracyclines. Faghihi (1980) has reported that the concentration of tetracycline following intravenous administration of the drug to sheep will remain more than 3 μg/ml for at least 12 hrs. The $t_{1/2}$ of tetracycline was also reported to be 11.2 hrs—almost three times greater than the value we found.

Table 2 illustrates the pharmacokinetic characteristics of various tetracycline compounds in different animals. V_d of tetracycline after intravenous injection of the drug in sheep is almost five times less than the value found in rabbits (Percy and Black, 1988). It is, however, similar to the value reported for chickens (Anadon *et al*., 1985). The highest apparent V_d is reported for doxycycline in goats (Jha *et al*., 1989; Table 2).

The drug elimination $t_{1/2}$ for tetracycline in sheep is close to those values reported for oxytetracycline in buffaloes (Varma and Paul, 1983), chlortetracycline in cows and ewes (Ziv and Sulman, 1974), tetracycline in pigs (Kniffen *et al*., 1989) and minocycline in sheep (Wilson and Green, 1986). Furthermore, the longest $t_{1/2}$ (around17 hrs) is reported for doxycycline in goats (Jha *et al*., 1989); the shortest $t_{1/2}$ (2 hrs) belongs to tetracycline in rabbits (Percy and Black, 1988).

Clearance of tetracycline in sheep is similar to that in horses for oxytetracycline (Horspool and McKellar, 1990). The largest clearance is reported for oxytetracycline in cows and ewes (Ziv and Sulman, 1974).

In conclusion, the discrepancies found between the pharmacokinetic properties of

Table 2: Pharmacokinetics of tetracyclines following intravenous administration in different animals

Drugs	Parameters						
	Species	Dosage (mg/kg)	V_d* (L/kg)	K_{el}† (/hr)	$t_{1/2}$‡ (hr)	Cl_B§ (ml/min/kg)	References
Tetracycline	Rabbits	10	1.1	0.35	2.0	6.1	Percy and Black, 1988
Tetracycline	Chickens	65	0.2	0.25	2.8	1.6	Anadon *et al*. 1985
Tetracycline	Cows and ewes	20	3.3	0.12	5.7	6.6	Ziv and Sulman, 1974
Tetracycline	Pigs	11	4.5	0.25	2.8	3.1	Kniffen *et al*. 1989
Oxytetracycline	Horses	10	0.7	0.05	13.0	0.7	Horspool and McKellar, 1990
Oxytetracycline	Cows	5	0.9	0.27	2.6	1.2	Nouws *et al*. 1985
Oxytetracycline	Buffalo	22	0.4	0.22	3.2	1.2	Varma and Paul, 1983
Oxytetracycline	Cows and ewes	20	5.4	0.17	4.1	15.1	Ziv and Sulman, 1974
Oxytetracycline	Horses	4.5	NR**	NR	15.7	NR	Teske *et al*. 1973
Oxytetracycline	Turkeys	1	3.6	1.41	0.7	3.7	Dyer, 1989
Oxytetracycline	Goats	10	1.2	0.34	6.5	2.7	Escudero *et al*. 1994
Chlortetracycline	Calves	11	1.93	0.08	8.3	2.7	Bradley *et al*. 1982
Chlortetracycline	Cows and ewes	20	1.9	0.17	4.2	5.3	Ziv and Sulman, 1974
Doxycycline	Calves	20	1.3	0.05	14.9	1.1	Riond *et al*. 1989
Doxycycline	Goats	5	9.8	0.04	16.6	6.9	Jha *et al*. 1989
Doxycycline	Cows and ewes	20	2.3	0.08	9.2	2.9	Ziv and Sulman, 1974
Doxycycline	Chickens	10	1.4	0.1	6.8	0.14	Laczay *et al*. 2001
Minocycline	Dogs	5	1.95	0.10	6.9	3.4	Wilson *et al*. 1985
Minocycline	Sheep	2.2	1.32	0.27	2.6	5.9	Wilson and Green, 1986
Minocycline	Cows and ewes	20	2.3	0.08	8.8	3.2	Ziv and Sulman, 1974

*V_d (apparent volume of distribution); †K_{el} (elimination rate constant); ‡$t_{1/2}$ (elimination half-life); §Cl_B (clearance); **NR, not reported

tetracyclines in sheep and other domestic animals indicate the importance of pharmacokinetic studies for establishing a correct dosage regimen for an optimal therapy.

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