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Disposition kinetics and bioavailability of doxycycline after parenteral administrations in ewes

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ABSTRACT

Doxycycline is a tetracycline, which have been marketed in different species for treating infections caused by susceptible bacteria. There is limited information on the disposition kinetics of this drug in ewes and this antimicrobial may be useful against several sheep pathogens that are common causes of morbidity and economic loss. Therefore, the aim of this work was to establish the pharmacokinetics of doxycycline after intravenous (IV) and extravascular (subcutaneous (SC) and intramuscular (IM)) administrations in this species. A cross-over model was designed ($n = 6$). Doxycycline was dosed at 5 mg/kg for IV administration and 20 mg/kg for extravascular administrations. Non-compartmental pharmacokinetic methods were used to calculate plasma concentration-time data. The value of apparent volume of distribution (V_z) suggests a moderate distribution of this antibiotic in sheep, with a value of 0.84 L/kg. The maximum concentrations achieved after extravascular administrations (C_{max}) were similar, with no significant differences between the two routes of administration (IM and SC). However, doxycycline absorption was slower after SC administration than after IM administration, taking twice as long to reach maximum plasma concentration (t_{max}). Bioavailabilities after extravascular routes of administration were low and after IM administration doxycycline caused lameness in all animals. Therefore, the SC administration showed a better profile with respect to pharmacokinetic properties and safety. Future studies on the susceptibility of isolated sheep pathogens to doxycycline are needed to establish appropriate dosing regimens.

1. Introduction

Doxycycline is a second-generation tetracycline antibiotic derived from oxytetracycline, characterized by higher lipid solubility compared to first-generation tetracyclines. Currently, this antibiotic is available commercially as the calcium salt, the hyclate salt, and the monohydrate salt. In veterinary medicine, the hyclate salt is the most commonly used form due to its better solubility in water compared to the monohydrate form (Mileva and Milanova, 2020; Mitić et al., 2008). Doxycycline hyclate is available in various formulations, including injectable solutions, water-soluble or lactodispersable powders, and tablets and capsules. The European Medicine Agency (EMA) has authorized doxycycline for use in various animal species to treat infections of the respiratory tract, urinary tract, and intestines caused by susceptible microorganisms (CVMP, 1996). However, to date, there is no EMA-approved doxycycline drug specifically for use in sheep.

Doxycycline exerts its antibacterial effects by reversibly binding to the 30S subunit of bacterial ribosomes, thereby inhibiting protein synthesis. This antimicrobial agent has a broad spectrum of activity, being effective against a wide range of microorganisms, including anaerobic and aerobic Gram-negative and Gram-positive bacteria, as well as intracellular pathogens such as Rickettsia spp., Chlamydia spp., and some Mycoplasma spp. (Branger et al., 2004; Bommana and Polkinghorne, 2019; Prats et al., 2005; Rolain et al., 1998; Woldehiwet, 2010). Additionally, doxycycline has been attributed with various anti-inflammatory and antineoplastic properties, including the inhibition of metalloproteinases produced by inflammatory cells. Due to its broad antibacterial spectrum and anti-inflammatory properties, doxycycline is widely used in numerous domestic animal species. This extensive use has facilitated the emergence of resistant bacteria. Therefore, to minimize the development of resistance—which occurs less frequently with doxycycline compared to other tetracyclines—the pharmacodynamic

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and pharmacokinetic properties of the drug should be considered when applying the principles of prudent use (Del Castillo, 2013; Mileva and Milanova, 2020).

The pharmacokinetic properties of doxycycline have been extensively studied in various livestock species, including pigs, horses, donkeys, calves, goats, and sheep (Abd El-Aty et al., 2004; Castro et al., 2009; Castro et al., 2012; Chapuis et al., 2021; Gutiérrez et al., 2014; Meijer et al., 1993; Vargas et al., 2008; Vargas-Estrada et al., 2008; Turk et al., 2020; Zozaya et al., 2013). In sheep, two studies have analysed the pharmacokinetics of doxycycline: one following intravenous (IV) and oral administration at a dose of 20 mg/kg, and the other following intramuscular (IM) administration of the same dose. The pharmacokinetics of doxycycline are characterized by low bioavailability after oral administration, good distribution volumes, long half-lives after parenteral administration, and a low adverse effect profile (CVMP, 1996; Castro et al., 2009; Castro et al., 2012). Oral antibiotics have the potential to cause significant disruption to the gut microbiota, particularly in ruminant species. However, this can also occur when drugs are administered parenterally if biliary elimination and enterohepatic circulation are important for the drug in question, as is the case here.

Doxycycline could be useful against several sheep pathogens that are common causes of morbidity and economic loss, including Pasteurella spp., *Mycoplasma agalactiae*, *E. coli*, and *S. aureus* (Castro et al., 2012). However, neither the bioavailability of doxycycline after IM administration nor the disposition kinetics of this antibiotic after subcutaneous (SC) administration in ewes have been studied, despite the latter being a common route of administration in livestock. Conducting these studies is important for evaluating the doses and dosage regimens that will ensure clinical success and prevent the emergence of bacterial resistance.

Another critical aspect of drug use is its safety profile. Doxycycline has a broad therapeutic index and is relatively well tolerated by most animal species, although it is considered an irritant. IV administration of doxycycline has caused hypertension, tachycardia, and even death in horses, while in sheep and goats it has caused sialism, tachypnea, tremors, and limb weakness. Additionally, symptoms of pain such as screaming, restlessness, lying down, and swelling at the injection site have been reported in horses and goats after IM administration of this antibiotic (Castro et al., 2009; Riond et al., 1992; Turk et al., 2020).

Based on the limited information on doxycycline pharmacokinetics in ewes, the objectives of this investigation were to establish the disposition kinetics of doxycycline following IV and extravascular (SC and IM) administrations, to investigate bioavailability after parenteral administrations, and to select the best route of administration from a pharmacokinetic and safety perspective.

2. Materials and methods

2.1. Animals

The trial involved six healthy *Montesina* sheep (Veterinary Teaching Farm, University of Murcia, Spain) with an average weight of 59.08 kg and an age range of 2–4 years. For a period of at least 21 days before starting the study, animals were fed a diet that was free of any drug substance. Animal health was assessed by physical examination. Before and after doxycycline injection, general health parameters of the animals were assessed at different times (1, 10, 24, 24, 48 and 72 h). The experimental protocol was approved by the University of Murcia Bioethics Committee (CEEA 758/2021).

2.2. Experimental design

A cross-over study (2 × 2 × 2) has been developed in 3 periods of time, with a wash-out period of at least 15 days between each period. Each sheep randomly received a single IV, SC and IM injection of doxycycline at a single dose of 5 mg/kg for IV administrations (Vibravenosa 100 mg solution for injection, HOSPIRA INVICTA, Madrid, Spain) or 20 mg/kg for SC and IM administrations (DFV Doxivet Injectable, DIVASA-FARMAVIC, Barcelona, Spain). Doses were different (after IV and extravascular routes of administration) due to adverse effects observed in other domestic species when this antimicrobial was administered via IV (Castro et al., 2009; Riond et al., 1992; Turk et al., 2020). For IV administration, the solution was administered slowly (over 1 min) as a bolus into the left jugular vein, and IM injections into the semimembranosus muscle. SC administrations were performed in a single site on the thoracolumbar region lateral to the midline, under the skin of the back. Blood was collected from the right jugular vein into heparinized tubes at 0 (pre-treatment), 0.083 (only after IV administration), 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after drug administration, and centrifuged at 1500*g* for 10 min. Plasma was taken and stored at −40 °C until analysis. To assess damage at the site of administration, changes in skin temperature, lameness and inflammatory responses were observed.

2.3. Analytical methods

Doxycycline were quantified using an HPLC assay with a fluorescence detector. The HPLC equipment used was the same as that described previously (Hernandis et al., 2022). Doxycycline and danofloxacin (internal standard (IS)) were bought from Cymit Química (Barcelona, Spain).

Briefly, to 200 μL of plasma, 10 μL of IS solution (10 μg/mL) was added. Plasma proteins were precipitated by adding a mixture of 100 μL of methanol and 100 μL of a 1:2 solution of trifluoroacetic acid and methanol. This sample was then vortexed for 10 s and sonicated for 5 min. The sample was centrifuged for 10 min at 14000 rpm. The supernatant was injected into the HPLC system at a rate of 50 μL per sample. An XBRIDGE, C18 column (100 mm, 4.6 mm, 3.5 μm) supplied by WATERS CROMATOGRAFÍA (Barcelona, Spain), was used for chromatographic separation. The mobile phase was composed of: (A) an aqueous phase containing 50 mM ammonium acetate, 50 mM magnesium chloride and 1 mM Na_2EDTA, buffered to pH 7.5 with ammonium hydroxide. Finally, 1 ml of triethylamine was added to each 500 ml of mobile phase A; (B) acetonitrile. A 15:85 volume ratio of aqueous phase A and acetonitrile *B* was used in this isocratic method. Flow rate was 1 mL/min. Detection was made at a $\lambda_{exitation} = 380$ nm and $\lambda_{emission} = 520$ nm at 20 °C. The total duration of the analysis was 12 min.

2.4. Method validation

The method was validated according to the FDA Guidance for the Validation of Bioanalytical Methods (FDA, 2018). The parameters assessed were as follows: accuracy, precision, linearity, lower limit of quantification (LOQ), lower limit of detection (LOD), recovery, selectivity and carryover. The complete protocols followed to validate each of the above parameters, as well as the coefficients of variation that were considered acceptable, are described in a previous publication of our group (Hernandis et al., 2022). Seven concentrations of doxycycline plus IS in plasma samples were analysed to determine the linearity of the proposed chromatographic method. Three replicates of each level were analysed. The concentration giving a signal-to-noise ratio ≥ 3 was used as the lower limit of detection for doxycycline. The lowest concentration of the calibration curve with a % CV accuracy of less than 20 % was selected as the limit of quantitation. Five replicates of samples from four quality controls spiked with IS were analysed to calculate the precision and accuracy of the method (Intraday: five replicates of each concentration were analysed on the same day; Interday: five replicates of each concentration were analysed on three consecutive days). Three concentrations were analysed in the recovery tests (at each concentration level five samples were analysed). The selectivity of the method was evaluated by analysing six samples of drug-free plasma. Blank samples ($n = 6$) were analysed immediately after injection of a set of samples containing a high concentration of doxycycline to exclude possible

injection carry-over effects.

2.5. Pharmacokinetic analysis

Non-compartmental parameters were determined using the WinNonlinTM software package (WinNonlin; Pharsight Corporation; Mountain View, CA, USA). The abbreviations and descriptions of each pharmacokinetic parameter can be found in the footnote of Table 1. The equation for calculating bioavailability is F (%) = ($AUC_{0–24\ IM\ or\ SC}$ /$AUC_{0–24\ IV}$) × (Dose $_{IV}$ /Dose $_{IM\ or\ SC}$) × 100.

2.6. Statistical analysis

Statistical analysis was performed using Solid version 2.3.28 of the Jamovi software. With the exception of the half-lives, which were expressed as the harmonic mean, the pharmacokinetic data were determined using the arithmetic mean and standard deviation. The test for normality was the Shapiro-Wilk test. A paired *t*-test was used to assess differences between data sets when they were normally distributed; a Wilcoxon signed rank sum test was used if they were not normally distributed. Differences were considered significant if $P < 0.05$.

3. Results

3.1. Animals

After administrations of doxycycline at different doses, sheep did not show any systemic adverse effects such as diarrhea or high fever. Following IM administration, all ewes developed lameness which was resolved 24–48 h after antibiotic administration. No signs of discomfort or inflammation were observed at the injection sites after IV and SC administration. This was assessed by swelling in the loin or vein, changes in skin temperature, or pain on palpation at the injection sites. Therefore, these results suggest that the SC route is safer than the IM route.

3.2. Analytical method

Peaks were obtained at 8.0 min and 5.5 min corresponding to doxycycline and IS, respectively. Doxycycline and danofloxacin peaks were well resolved. Six blank plasma samples were analysed, showing no endogenous interference with doxycycline and danofloxacin retention times. Spiked samples were used to compare these chromatograms. The high selectivity of the method was demonstrated by satisfactory results. Linear regression equation was $Y = 6.0{\cdot}10^{-7}\times$. LOD was 0.065 μg/mL and LOQ was 0.1 μg/mL. The CV precision values for plasma samples were < 5.1 % and < 6.3 % for within-day and between-day precision, respectively. Accuracy ranged from −5.8 % to 10.4 %. The average recoveries of the three concentrations analysed ranged from 89.6 ± 5.6 % to 72.6 ± 2.9 %. Finally, there were no carry-over effects. These results suggest that our method may be suitable for the quantification of doxycycline in plasma of sheep by HPLC.

3.3. Pharmacokinetics

Doxycycline concentrations after IV administration decreased rapidly and were detected in all animals up to 12 h post-administration. After IM and SC administration, concentrations were detected up to 96 h post-administration. In Fig. 1, a second absorption peak can be observed after extravascular administrations (especially after IM administration) which may be due to enterohepatic circulation processes. Fig. 1 shows the plasma concentrations (values are arithmetic mean ± SD) of doxycycline after the three routes of administration.

Table 1 shows non-compartmental pharmacokinetic parameters. Significant differences ($p < 0.05$) between the IV administration and the extravascular administrations were found in λ_z, $t_{½\lambda z}$, MRT, $AUC_{0\text{-}\infty}$ (only between IV and SC administrations) and $AUC_{0–24}$ (only between IV and IM administrations). Significant differences were observed in $AUC_{0\text{-}\infty}$, $AUC_{0–24}$ and t_{max} between both extravascular routes of administration, suggesting a slower absorption of doxycycline after SC administration.

4. Discussion

Doxycycline presents a great lipophilicity in comparison with other tetracyclines, which gives wide distribution in tissues and prolonged half-lives (Mileva and Milanova, 2020). These pharmacokinetic properties allow the drug to be administered in a single dose on a daily basis, which is very useful in the veterinary field. Moreover, its penetration

Table 1
Pharmacokinetic parameters (mean ± SD) of doxycycline in sheep ($n = 6$) after IV, IM and SC administration of a single dose of 5, 20 and 20 mg/kg respectively.

Parameters (units)	Intravenous			Intramuscular			Subcutaneous		
C_0 (μg/mL)	14.89	±	4.03						
λ_z (h^{-1})	0.247	±	0.074	0.018	±	0.003[a]	0.017	±	0.005[a]
$t_{½\lambda z}$ (h)*		2.81			38.40[a]			41.02[a]	
V_Z (L/kg)	0.84	±	0.32						
V_{ss} (L/kg)	0.63	±	0.19						
Cl (L/h/kg)	0.19	±	0.05						
$AUC_{0–24}$ (μg·h/mL)	26.82	±	8.50	14.44	±	2.02[a]	22.56	±	5.20[b]
AUC_{last} (μg·h/mL)	26.82	±	8.50	28.87	±	8.43	48.35	±	11.86
$\%AUC_{extrap.}$	3.25	±	1.53	28.24	±	7.58	25.71	±	10.77
$AUC_{0\text{-}\infty}$ (μg·h/mL)	27.82	±	9.27	40.04	±	9.71	64.39	±	9.27[a,b]
MRT (h)	3.44	±	1.37	55.07	±	8.88[a]	63.26	±	16.50[a]
MAT (h)				51.61	±	8.22	59.80	±	15.65
C_{max} (μg/mL)				1.32	±	0.16	1.81	±	0.42
t_{max} (h)				1.30	±	0.78	2.80	±	1.60[b]
F (%)				31.00	±	10.38	53.66	±	19.65

C_0: concentration of the drug in the serum immediately after intravenous administration, λ_z: the slowest elimination rate constant; $t_{½\lambda z}$: half-life associated with the terminal slope (λ_z) of a semilogarithmic concentration versus time curve; V_z: apparent volume of distribution calculated according to the method of the area; V_{ss}: apparent volume of distribution in the steady state; Cl: total clearance of the drug from the plasma in the body; $AUC_{0–24}$: the area under the plasma concentration versus time curve from zero to 24 h; $\%\ AUC_{extrap.}$: % AUC extrapolated; $AUC_{0\text{-}last}$: the area under the curve up to the last quantifiable point in time; $AUC_{0\text{-}\infty}$: the area under the plasma concentration versus time curve from zero to infinity; MRT: the mean residence time; MAT: the mean absorption time; C_{max}: the peak or maximum plasma concentration after extravascular administration of the drug; t_{max}: the time after extravascular administration to peak or maximum plasma concentration; F: The proportion of the administered dose that is available systemically (bioavailability).

[a] There are significant differences with the IV administration ($p < 0.05$).

[b] There are significant differences with the IM administration ($p < 0.05$).

* Harmonic mean.

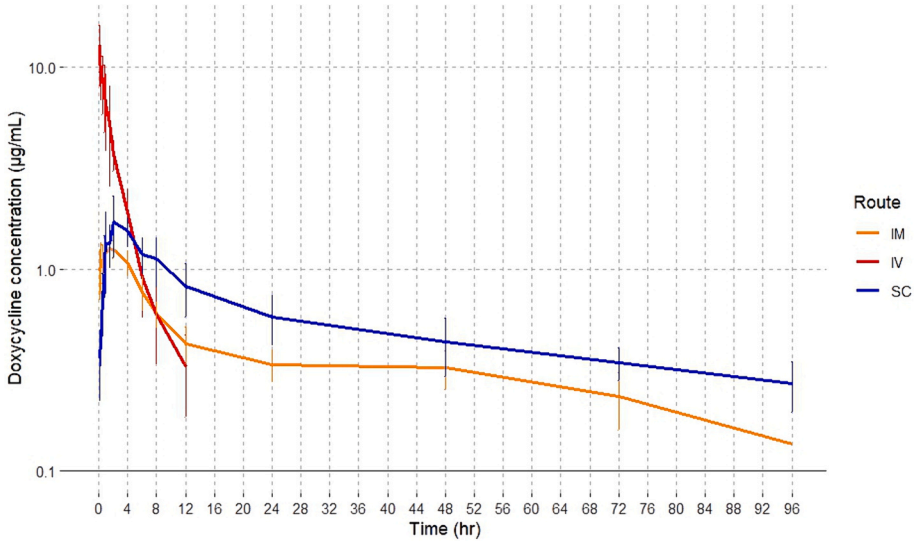

Fig. 1. Semi-log graphs of IV, IM and SC concentrations of doxycycline in sheep at a single dose of 5, 20 and 20 mg/kg, respectively ($n = 6$). Values are arithmetic mean ± SD.

into tissues is excellent, finding levels in therapeutic ranges in most organs and tissues such as the lungs, kidneys, prostate, intestinal tract, myocardium, tonsils, etc. (Jha et al., 1989; Mileva and Milanova, 2020; Saivin and Houin, 1988). However, prior to use in different animal species, it is very important to quantify it in different fluids and tissues, in order to carry out pharmacokinetic studies to optimise the dose and dosing regimen for each species and to avoid the occurrence of drug residues in the food chain. To date, limited information exists on the disposition kinetics of doxycycline in ewes. Compared to cattle and pigs, small ruminants are relatively less commercialised, and only a limited number of drugs are labelled for these species. Consequently, a large proportion of antimicrobials used in sheep are classified as off-label, with posology, withdrawal periods and indications often extrapolated from data calculated for other species (Clark, 2013; Fajt, 2001). Quantification of doxycycline levels in plasma or other biological fluids is therefore essential for optimising the dose of this antibiotic.

After IV administration of doxycycline, the half-life was 2.81 h. This value is lower than that obtained in another study with doxycycline hydrochloride ($t_{½\lambda z} = 7.03$ h) (Castro et al., 2009) and similar to that obtained with tetracycline ($t_{½\lambda z} = 3.3$ h) (Rajaian and Soleimani, 2007). The fact that the doxycycline hyclate used in this study is more water soluble than the hydrochloride may explain these differences in elimination half-lives. Moreover, the pharmacokinetic analysis (compartmental or non-compartmental) of plasma concentrations in the two studies was different. After extravascular administration, the half-lives of doxycycline were longer than after IV administration due to the time of the absorption phase. Half-life after SC administration was apparently longer than that after IM administration, although there was no significant difference. MRT values consistently follow the same scheme. MAT values after extravascular administration were much longer than MRT after IV treatment. This fact suggests that doxycycline follows a flip-flop model where absorption is the limiting step for plasma elimination. Several studies using long-acting doxycycline formulations in calves (Vargas-Estrada et al., 2008) and goats (Vargas et al., 2008) or a commercial oxytetracycline formulation in sheep (Moreno et al., 1998) show that absorption is often the rate-limiting step in overall tetracycline disposition and elimination.

The V_{ss} value was 0.63 L/kg, suggesting a moderate distribution of this antibiotic in sheep. The value obtained in another study with doxycycline hydrochloride was higher ($V_{ss} = 1.76$ L/kg) (Castro et al., 2009) as well as with other tetracyclines such as minocycline (Wilson and Green, 1986). The reason for this lower tissue distribution may be due to the fact that doxycycline has a higher binding to plasma proteins than other tetracyclines (Mileva and Milanova, 2020) and in the case of doxycycline hyclate, being more water soluble, it has a lower capacity to cross biological membranes and a lower affinity for adipose tissue.

The maximum concentrations achieved after extravascular administration (C_{max}) were similar, with no significant differences between the two routes of administration (IM and SC). However, doxycycline absorption was slower after SC administration than after IM administration, taking twice as long to reach maximum plasma concentration (t_{max}), with significant differences between the two routes of administration. The AUC after IV administration from time 0 to the last quantifiable point in time was approximately 96.41 % of the AUC from time zero to infinity; therefore, the $AUC_{0\text{-}\infty}$ value is valid. However, after IM and SC administration, the extrapolated AUC % represented 28.24 % and 25.71 %, respectively, therefore, blood samples should be taken more than 96 h after doxycycline administration to determine the complete elimination fate from the body, although the concentrations in the latter times were close to the LOQ. It would also have been desirable for the method of quantification to have a lower limit of quantification. The bioavailabilities of doxycycline after IM and SC injections were relatively low, with mean values, which did not differ significantly, of 31.00 and 53.66 %, respectively. Low bioavailabilities for this antibiotic has also been reported after oral administration in ewes (36 %) (Castro et al., 2009), goats (31 %) (Turk et al., 2020) and pigs (21 %) (Baert et al., 2000), and after IM administration in goats (51 %) (Turk et al., 2020). In this study, following IM injection, lameness due to swelling has been reported in all animals. Differences in the degree of irritation due to dose differences or different formulations may explain the low extravascular bioavailability. A possible exception may be found in the long-acting doxycycline formulation with poloxamer β-cyclodextrin matrix which has been the subject of proposals for use in veterinary medicine. With these formulations, bioavailabilities were much higher after SC administration of this antibiotic in dogs (199 %) (Gutiérrez et al., 2012), calves (545 %) (Vargas-Estrada et al., 2008) and pigs (70 %) (Gutiérrez et al., 2014).

Doxycycline is a bacteriostatic antibiotic. Therefore, its effectiveness depends on the time between doses during which its concentration at the site of action is higher than the MIC ($T > MIC$) (Castro et al., 2009). However, a number of publications have highlighted the AUC/MIC index as the most important predictor of the effect of tetracycline therapy (Andes and Craig, 2002; Craig, 2002; Toutain et al., 2002). The $AUC_{0\text{–}24}$/MIC ratios for the achievement of bacteriostatic and

bactericidal activity of doxycycline against *Haemophilus parasuis* in swine were determined to be 59 and 98, respectively (Zhang et al., 2018). Minimal inhibitory concentrations of doxycycline against sheep bacterial pathogens are very scarce. The MIC for L. *monocytogenes* (4 μg/ml) is, to the best of our knowledge, the only MIC determined specifically for doxycycline in sheep (Vela et al., 2001). Taking into account these surrogate markers, and that the SC route is the one with the best AUC_{0-24} values and safety profile; the subcutaneously administered formulation of doxycycline evaluated in this study would have a bacteriostatic or bactericidal effect against bacteria isolated from sheep with MIC less than 0.38 μg/ml or 0.23 μg/ml, respectively. Nevertheless, the clinical implications of these ratios need to be interpreted with caution, because the therapeutic effect of antimicrobials depends on a complex set of variables. Therefore, it will be useful to have the data of studies exploring AUC/MIC ratios specifically in sheep to maximise the therapeutic success when doxycycline is given.

In conclusion, the formulation of doxycycline studied in this study showed prolonged half-lives after IM and SC administrations, and moderate tissue distribution. However, the bioavailabilities obtained were low and after IM administration caused lameness in all animals. Therefore, the SC route showed a better profile with respect to pharmacokinetic properties and safety matters. However, to confirm the best drug dosage regimen, it is also necessary to determine the MICs of doxycycline on susceptible microorganisms isolated from sheep.

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Ethical animal research

The University of Murcia Ethics Committee (CEEA 758/2021) approved the animal study protocol.

Informed consent

Explicit owner informed consent for inclusion of animals in this study was stated.

CRediT authorship contribution statement

José Martínez: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Elisa Escudero:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elena Badillo:** Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis. **María Teresa Yuste:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Juan Sebastián Galecio:** Software, Methodology, Data curation, Conceptualization. **Pedro Marín:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors have declared no competing interests.

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References

Abd El-Aty, A.M., Goudah, A., Zhou, H.H., 2004. Pharmacokinetics of doxycycline after administration as a single intravenous bolus and intramuscular doses to non-lactating Egyptian goats. Pharm. Res. 49, 487–491.

Andes, D., Craig, W.A., 2002. Animal model pharmacokinetics and pharmacodynamics: a critical review. Int. J. Antimicrob. Agents 19, 261–268.

Baert, K., Croubels, S., Gasthuys, F., De Busser, J., De Backer, P., 2000. Pharmacokinetics and oral bioavailability of a doxycycline formulation (doxycycline 75%) in nonfasted young pigs. J. Vet. Pharmacol. Ther. 23, 45–48.

Bommana, S., Polkinghorne, A., 2019. Mini review: antimicrobial control of chlamydial infections in animals: current practices and issues. Front. Microbiol. 10, 113.

Branger, S., Rolain, J.M., Raoult, D., 2004. Evaluation of antibiotic susceptibilities of *Ehrlichia canis, Ehrlichia chaffeensis,* and *Anaplasma phagocytophilum* by real-time PCR. Antimicrob. Agents Chemother. 48, 4822–4828.

Castro, L.J., Sahagun Prieto, A.M., Diez, M.J., Fernandez, N., Sierra, M., Garcia, J.J., 2009. Pharmacokinetics of doxycycline in sheep after intravenous and oral administration. Vet. J. 180, 389–395.

Castro, L.J., Sahagún, A.M., Diez, M.J., Fernández, N., Sierra, M., García, J.J., 2012. Pharmacokinetic behavior of doxycycline after intramuscular injection in sheep. Am. J. Vet. Res. 73, 714–718.

Chapuis, R.J.J., Smith, J.S., French, H.M., Toka, F.N., Peterson, E.W., Little, E.L., 2021. Nonlinear mixed-effect pharmacokinetic modeling and distribution of doxycycline in healthy female donkeys after multiple intragastric dosing–preliminary investigation. Animals 11, 2047.

Clark, C.R., 2013. Antimicrobial drug use in sheep and goats. In: Giguére, S., Prescott, J. F., Dowling, P.M. (Eds.), Antimicrobial Therapy in Veterinary Medicine, 5th ed. John Wiley & Sons, Inc, Hoboken, NJ, USA, pp. 529–539.

Craig, W.A., 2002. Pharmacodynamics of antimicrobials: General concepts and applications. In: Nightingale, C.H., Murakawa, T., Ambrose, P.G. (Eds.), Antimicrobial Pharmacodynamics in Theory and Clinical Practice, 2nd ed. Marcel Dekker, Inc, Oxford, England, pp. 1–22.

CVMP, 1996. Committee for Veterinary Medicinal Products. Available online: https://www.ema.europa.eu/en/documents/mrl-report/doxycycline-hyclate-summary-report-committee-veterinary-medicinal-products_en.pdf (accessed on 11 April 2024).

Del Castillo, J.R.E., 2013. Tetracyclines. In: Giguère, S., Prescott, J.F., Dowling, P.M. (Eds.), Antimicrobial Therapy in Veterinary Medicine, 5th ed. John Wiley Black & Sons, Inc, Hoboken, NJ, USA, pp. 257–268.

Fajt, V.R., 2001. Label and extra label drug use in small ruminants. Vet. Clin. North Am. Food Anim. Pract. 17, 403–420.

Food and Drug Administration, 2018. Bioanalytical Method Validation- Guidance for Industry. US Department of Health and Human Services. Retrieved from. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf.

Gutiérrez, L., Velasco, Z., Vázquez, C., Vargas, D., Sumano, H., 2012. Pharmacokinetics of an injectable long-acting formulation of doxycycline hyclate in dogs. Acta Vet. Scand. 54, 35.

Gutiérrez, L., Ocampo, L., Espinosa, F., Sumano, H., 2014. Pharmacokinetics of an injectable long-acting parenteral formulation of doxycycline hyclate in pigs. J. Vet. Pharmacol. Ther. 37, 83–89.

Hernandis, V., Escudero, E., Marín, P., 2022. A novel liquid chromatography-fluorescence method for the determination of delafloxacin in human plasma. J. Sep. Sci. 45, 706–716.

Jha, V.K., Jayachandran, C., Singh, M.K., Singh, S.D., 1989. Pharmacokinetic data on doxycycline and its distribution in different biological fluids in female goats. Vet. Res. Commun. 13, 11–16.

Meijer, L.A., Ceyssens, K.G.F., de Greve, B.I.J.A., de Bruijn, W., 1993. Pharmacokinetics and bioavailability of doxycycline hyclate after oral administration in calves. Vet. Q. 15, 1–5.

Mileva, R., Milanova, A., 2020. Doxycicline pharmacokinetics in mammalian species of veterinary interest – an overview. Bulg. J. Vet. Med. 25, 1311–1477.

Mitić, S.S., Miletić, G.Ž., Kostić, D.A., Nasković-Đokić, D.Č., Arsić, B.B., Rašić, I.D., 2008. A rapid and reliable determination of doxycycline hyclate by HPLC with UV detection in pharmaceutical samples. J. Chem. Soc. 73, 665–671.

Moreno, L., Serrano, J.M., Guimerá, M.E., Cárceles, C.M., 1998. Pharmacokinetics of oxytetracycline after intramuscular administration with lidocaine in sheep, comparison with a conventional formulation. Res. Vet. Sci. 65, 209–213.

Prats, C., El Korchi, G., Giralt, M., Cristòfol, C., Peña, J., Zorrilla, I., Saborit, J., Pérez, B., 2005. PK and PK/PD of doxycycline in drinking water after therapeutic use in pigs. J. Vet. Pharmacol. Ther. 28, 525–530.

Rajaian, H., Soleimani, E., 2007. Pharmacokinetics of tetracycline hydrochloride in fat-tailed sheep. Iran. J. Vet. Res. 8 (2), 138–143.

Riond, J.L., Riviere, J.E., Duckett, W.M., Atkins, C.E., Jernigan, A.D., Rikihisa, Y., Spurlock, S.L., 1992. Cardiovascular effects and fatalities associated with intravenous administration of doxycycline to horses and ponies. Equine Vet. J. 24, 41–45.

Rolain, J.M., Maurin, M., Vestris, G., Raoult, D., 1998. In vitro susceptibilities of 27 rickettsiae to 13 antimicrobials. Antimicrob. Agents Chemother. 42, 1537–1541.

Saivin, S., Houin, G., 1988. Clinical pharmacokinetics of doxycycline and minocycline. Clin. Pharmacokinet. 15, 355–366.

Toutain, P.L., Del Castillo, P.R.L., Bousquet-Mélou, A., 2002. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. Res. Vet. Sci. 73, 105–114.

Turk, E., Corum, O., Tekeli, I.O., Sakin, F., Uney, K., 2020. Effects of single and repeated doses on disposition and kinetics of doxycycline Hyclate in goats. Animals 10, 1088.

Vargas, D., Gutierrez, L., Juárez, I., Gonzalez, F., Sumano, H., 2008. Pharmacokinetics after administration of an injectable experimental long-acting parenteral formulation of doxycycline hyclate in goats. Am. J. Vet. Res. 69, 1085–1090.
Vargas-Estrada, D., Gracia-Mora, J., Sumano, H., 2008. Pharmacokinetic study of an injectable long-acting parenteral formulation of doxycycline hyclate in calves. Res. Vet. Sci. 84, 477–482.
Vela, A.I., Fernández-Garayzábal, J.F., Latre, M.V., Rodríguez, A.A., Domínguez, L., Moreno, M.A., 2001. Antimicrobial susceptibility of *listeria monocytogenes* isolated from meningoencephalitis in sheep. Int. J. Antimicrob. Agents 17, 215–220.
Wilson, R.C., Green, N.K., 1986. Pharmacokinetics of minocycline hydrochloride in clinically normal and hypoproteinemic sheep. Am. J. Vet. Res. 47, 650–652.
Woldehiwet, Z., 2010. In-vitro studies on the susceptibility of ovine strains of *Anaplasma phagocytophilum* to antimicrobial agents and to immune serum. J. Comp. Pathol. 143, 94–100.
Zhang, L., Li, Y., Wang, Y., Sajid, A., Ahmed, S., Li, X., 2018. Integration of pharmacokinetic-pharmacodynamic for dose optimization of doxycycline against *Haemophilus parasuis* in pigs. J. Vet. Pharmacol. Ther. 41, 706–718.
Zozaya, H., Gutierrez, L., Bernad, M.J., Sumano, H., 2013. Pharmacokinetics of a peroral single dose of two long-acting formulations and an aqueous formulation of doxycycline hyclate in horses. Acta Vet. Scand. 55, 21.