Doxycycline, a second-generation semisynthetic tetracycline, possesses a broad spectrum of antimicrobial activity that includes aerobic and anaerobic gram-positive and gram-negative bacteria as well as other microorganisms, such as chlamydiae, rickettsiae, spirochetes, ehrlichiae, mycoplasmas, and some protozoa. Doxycycline is one of the most commonly prescribed antimicrobials in human and veterinary medicine. In the European Union, doxycycline is used for the treatment of respiratory, urinary, and intestinal infections caused by susceptible microorganisms in cattle, pigs, poultry, and small animals. For sheep, doxycycline might be useful against several pathogens, such as Pasteurella spp, Mycoplasma agalactiae, Escherichia coli, or Staphylococcus aureus, which are common causes of morbidity that often result in substantial economic loss. Domestic sheep with disease experimentally induced by Ehrlichia ruminantium (heartwater disease) have been successfully treated with a single dose of doxycycline administered IM.

The disposition of doxycycline has been established in humans and in various species of veterinary interest. Doxycycline has higher lipid solubility than conventional tetracyclines. As a result, doxycycline has better tissue penetration than conventional tetracyclines as evidenced by its comparatively larger volume of distribution and longer half-life. Doxycycline is absorbed after PO administration, but its bioavailability varies by species, ranging from 36% in sheep to 70% in cattle. When administered IM, doxycycline's bioavailability varies even more, ranging from 17.5% in ostriches to 99.4% in goats. The plasma protein binding of doxycycline also varies by species and ranges from 32.8% in goats to 98.4% in cats. It is eliminated from the body by the kidneys, liver, and digestive tract and excreted via urine and feces. After IV administration in dogs, 25% of the dose is eliminated in the urine within 48 hours.
of the dose was excreted in urine and the remainder in feces (excreted mostly by the gastrointestinal mucosa). Modification of the dose was not required in humans with impaired renal function.\textsuperscript{26,27}

Although pharmacological characteristics of doxycycline may make it a treatment option for several infections in sheep, data on its pharmacokinetics in that species are scarce; only 2 studies on the pharmacokinetics of doxycycline after IV\textsuperscript{24,28} and PO\textsuperscript{24} administration have been conducted in sheep. To our knowledge, information about pharmacokinetics following IM administration in sheep is lacking, even though the IM route is commonly used for drug administration in livestock. The purposes of the study reported here were to investigate the pharmacokinetics of doxycycline in sheep after IM administration of a commercial formulation of doxycycline hyclate at a dose of 20 mg/kg and to evaluate whether the IM route is appropriate for administering doxycycline to sheep. In the study reported here, IM administration of doxycycline in sheep was considered extralabel use because, in Spain, the commercial formulation used is only approved for pigs and poultry via PO administration.

Materials and Methods

Animals—Eleven 4-year-old Spanish Churra ewes that ranged in weight from 33 to 37 kg were used in the study. The sheep were determined to be healthy on the basis of results of physical examination. They were dewormed with netobimin\textsuperscript{5} (10 mg/kg, PO) 45 days before the study. Sheep were housed in a ventilated building and allowed to acclimatize to their environment for 2 weeks before the study was initiated. They were provided an antimicrobial-free diet of alfalfa hay and pelleted feed, with unlimited access to water and a salt lick. The Institutional Animal Care and Use Committee of the University of Leon approved all procedures.

Doxycycline administration and sample collection—A commercial formulation of doxycycline\textsuperscript{5} was administered to animals at a single dose of 20 mg/kg, IM. The dose was selected because it had already been used in sheep for IV\textsuperscript{24,28} and PO\textsuperscript{24} administration. Also, the same dose of doxycycline had been administered IM to goats.\textsuperscript{5,17}

Doxycycline was administered into the deep gluteal muscle of the right hind limb. Serial blood samples (5 mL) were collected into EDTA (K\textsubscript{3}) evacuated tubes\textsuperscript{6} from a jugular vein immediately before (time 0) and at 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes and 4, 6, 10, 16, 24, 32, 40, 48, 60, 72, and 84 hours after doxycycline administration. Samples were immediately centrifuged at 500 \texttimes g, and plasma was harvested and stored at \(-80^\circ\text{C}\) until analyzed. For each sheep, a physical examination was performed daily by the same veterinarian (LJCR), and sheep were visually evaluated at the time of doxycycline administration and when each blood sample was obtained until 3 days after the end of the study.

Analytic procedures—Plasma concentrations of doxycycline were determined via high-performance liquid chromatography with UV detection in accordance with a method previously described,\textsuperscript{28} with minor modifications. Solid-phase extraction was performed with solid-phase extraction cartridges.\textsuperscript{4} Cartridges were conditioned with 1 mL of methanol and 1 mL of water. Then, 1 mL of plasma was added, and the cartridges were washed 3 times with 1 mL of 5% methanol in water. Doxycycline was eluted with 1 mL of a 50:50 (vol/vol) mixture of acetonitrile and water.

The high-performance liquid chromatography system\textsuperscript{6} was equipped with an autosampler\textsuperscript{1} and UV detector\textsuperscript{8} at a wavelength of 350 nm. A reversed-phase column\textsuperscript{5} (4 µm; 3.9 \texttimes 150 mm) was used. The mobile phase consisted of a 50:50 (vol/vol) mixture of acetonitrile and water (pH, 2.5; adjusted with trifluoroacetic acid) at a flow rate of 1.25 mL/min. Oxytetracycline\textsuperscript{1} was used as the internal standard. The minimum limits of detection were 0.007 and 0.02 µg/mL for doxycycline and oxytetracycline, respectively. Interday and intraday coefficients of variation were between 1.95% and 11.2%, and the mean ± SD recovery of doxycycline was 96.5 ± 26.3% for plasma samples fortified with doxycycline to final concentrations ranging from 0.05 to 20 µg/mL.

Pharmacokinetic analysis—Pharmacokinetic analyses were performed for each sheep by use of non-compartmental methods via a computer program.\textsuperscript{1} Expressions based on statistical moments theory\textsuperscript{30} and standard equations were used to calculate the model-independent pharmacokinetic parameters.\textsuperscript{31,32} Slope of the terminal phase was determined by least squares regression of the logarithm of the plasma concentration-time curve divided by the terminal elimination phase.
Table 1—Mean ± SD and range values for pharmacokinetic parameters obtained via noncompartmental analysis after IM administration of a single dose of doxycycline (20 mg/kg) to 11 healthy sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ (1/h)</td>
<td>0.010 ± 0.003</td>
<td>0.005–0.014</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>40.38 ± 8.24</td>
<td>30.72–58.82</td>
</tr>
<tr>
<td>AUC$_{ss}$ (µg·h/mL)</td>
<td>38.10 ± 10.18</td>
<td>27.95–55.60</td>
</tr>
<tr>
<td>AUC$_{in}$ (%)</td>
<td>65.67 ± 9.88</td>
<td>47.65–81.48</td>
</tr>
<tr>
<td>AUMC$_{ss}$ (µg·h$^2$/mL)</td>
<td>11.91 ± 2.34</td>
<td>9.40–16.75</td>
</tr>
<tr>
<td>AUMC$_{in}$ (%)</td>
<td>79.21 ± 8.97</td>
<td>65.68–92.95</td>
</tr>
<tr>
<td>AUMC$_{ss}$ (µg·h$^2$/mL)</td>
<td>64.01 ± 30.10</td>
<td>36.17–133.70</td>
</tr>
<tr>
<td>MRT$_{ss}$ (h)</td>
<td>27.90 ± 2.38</td>
<td>23.23–31.58</td>
</tr>
<tr>
<td>MRT$_{in}$ (%)</td>
<td>62.38 ± 20.94</td>
<td>6.75–84.12</td>
</tr>
<tr>
<td>MRT$_{ss}$ (h)</td>
<td>91.10 ± 40.78</td>
<td>28.13–170.00</td>
</tr>
<tr>
<td>C$_{max}$ (µg/mL)</td>
<td>2.792 ± 0.791</td>
<td>1.765–4.914</td>
</tr>
<tr>
<td>t$_{max}$ (h)</td>
<td>0.856 ± 0.472</td>
<td>0.333–2.000</td>
</tr>
</tbody>
</table>

AUC$_{ss}$ = Area under the plasma concentration–time curve from time 0 to infinity. AUC$_{in}$ = Area under the plasma concentration–time curve from time 0 to the last measured concentration. AUMC$_{ss}$ = Area under the plasma concentration–time curve from the last measured concentration to infinity. AUMC$_{in}$ = Area under the first moment curve from time 0 to infinity. MRT$_{ss}$ = Mean residence time from time 0 to infinity. MRT$_{in}$ = Mean residence time from time 0 to the last measured concentration. MRT$_{max}$ = Mean residence time from the last measured concentration to infinity. $\lambda$ = Slope of the terminal phase. MRT$_{τ}$ = Half-life associated with slope of the terminal phase.

The $C_{max}$ and $t_{max}$ were obtained directly from examination of data points, and the AUC was calculated on the basis of the trapezoidal rule, with extrapolation to infinity.

Results

No adverse effects were observed in any of the sheep during or after the IM injection of doxycycline. Mean plasma concentration of doxycycline as a function of time was plotted (Figure 1). Mean pharmacokinetic parameters calculated for doxycycline on the basis of noncompartmental analysis were determined (Table 1). Doxycycline was detected in plasma 10 minutes after administration, and a mean ± SD $C_{max}$ of 2.79 ± 0.79 µg/mL was achieved at a $t_{max}$ of 0.86 ± 0.47 hours after administration. Mean ± SD half-life associated with the slope of the terminal phase (77.88 ± 28.45 hours), AUC (65.67 ± 9.88 µg·h/mL), and mean residence time (91.1 ± 40.78 hours) was also determined.

Discussion

To our knowledge, the present study is the first conducted to evaluate the pharmacokinetics of doxycycline after IM administration in domestic sheep. Although the pharmacokinetics of doxycycline has been extensively studied in several animal species because of its wide use and efficacy,8–21 in sheep, the pharmacokinetics of doxycycline has only been determined for IV and PO administration.24,25 Prior to the study reported here, the pharmacokinetics of doxycycline after IM administration had only been reported in goats,15,17 calves,23 ostriches,25 and houbara bustards.24

Compared with results in other species, the mean half-life associated with the slope of the terminal phase obtained for sheep in the present study (77.88 ± 28.45 hours) was higher, which suggested doxycycline was slowly eliminated from sheep. The elimination half-life associated with IM administration of doxycycline ranged from 3.7 to 38.3 hours in goats1,20 and was 9.6 hours in calves.21 Slope of the terminal phase represents the absorption process, rather than the elimination process. Thus, the overall disposition of the drug is determined primarily by its absorption rate from the injection site. It is not uncommon for the absorption process to be the rate-limiting step in the overall disposition and elimination of tetracyclines following IM administration as evidenced by results of studies that involved the use of long-acting formulations of doxycycline in goats15,22 and calves23 and a commercial formulation of oxytetracycline in sheep.25

Mean ± SD residence time from time 0 to infinity was 91.10 ± 40.78 hours for IM administration of doxycycline in sheep, which exceeded that for goats15,17,20 (16.4 and 12.9 hours) and calves23 (12 hours). Even if mean residence time from time 0 to the last measured concentration (27.80 ± 2.38 hours) in the present study was considered, it was twice those previously reported.

The IM administration of doxycycline resulted in a mean ± SD $C_{max}$ of 2.79 ± 0.79 µg/mL, which was within the $C_{max}$ range15,17 (1.9 to 5.6 µg/mL) determined for goats following IM administration of doxycycline at the same dose used in the present study. When 10 mg of doxycycline/kg was administered IM, the $C_{max}$ was 3.2 µg/mL in goats and 2.9 µg/mL in calves, whereas 5 mg of doxycycline/kg IM in goats resulted in a $C_{max}$ of 1.6 µg/mL.

The mean ± SD $t_{max}$ (0.856 ± 0.472 hours) in the present study was similar to those (0.83 to 1.17 hours) determined in studies15,17,20 involving goats. Higher $t_{max}$ values have been determined in goats21 (3.15 hours) and in calves23 (4.5 hours).

The mean ± SD AUC from time 0 to infinity (65.67 ± 9.877 µg·h/mL) in the present study was higher than that (39.72 µg·h/mL) determined after IM administration of a long-acting formulation of doxycycline to goats1 at the same dose used in the study reported here. When 10 mg of doxycycline/kg was administered as an aqueous solution to goats22 and calves,23 AUC values were 22.19 and 21.16 µg·h/mL, respectively. The AUC from time 0 to the last measured concentration represented approximately 62% of the AUC from time 0 to infinity; therefore, in the present study, blood samples should have been obtained for a period longer than 84 hours after administration of doxycycline to determine complete elimination from the sheep’s bodies.

The reason the pharmacokinetic parameters in the present study varied from those reported in other studies15,17,20,22,23 was probably the differences in species, age, breed, and health status of the animals studied. Additional factors that may have contributed to the variation in pharmacokinetic parameters are dose, formulation of doxycycline administered, assay method used to determine pharmacokinetic parameters (some investigators used a microbiological method), and interval after doxycycline administration at which blood samples were obtained and assayed. The variation in the pharmacokinetics obtained in the present study and those reported earlier may be due to the differences in the age and health status of the animals studied.
kinetic parameters observed among sheep in the present study may have been caused by differences in local blood perfusion at the injection site. It is important to consider all these variables when pharmacokinetic studies are used to determine an appropriate treatment regimen for a drug in a particular species.

Compared with the pharmacokinetics and pharmacodynamics of β-lactams, fluoroquinolones, and aminoglycosides, those of tetracyclines have been understudied. Thus, information obtained by the use of animals to determine the pharmacokinetics and pharmacodynamics as well as the antimicrobial effects of tetracyclines in species of veterinary interest is limited.

Tetracyclines are regarded as time-dependent antimicrobial drugs, and the best pharmacokinetic and pharmacodynamic indices are achieved when plasma concentrations are maintained above the MIC during treatment or for prophylaxis. Nevertheless, several investigators have recommended that the ratio for AUC_{0-24}:MIC should be used to determine the susceptibility of pathogens to tetracyclines. The efficacy of tetracyclines is related to the time that the plasma concentration exceeds the MIC by 1- to 5-fold for 40% to 100% of the dosing interval, respectively, and a ratio of AUC_{0-24}:MIC ≥ 125 hours; however, these values have not been validated for doxycycline specifically.

Minimum inhibitory concentrations for doxycycline against bacterial pathogens in sheep are lacking because tetracycline and oxytetracycline are the drugs commonly used to represent the tetracycline class during susceptibility testing. To our knowledge, the only MIC determined specifically for doxycycline in sheep is that for Listeria monocytogenes (4 µg/mL). Therefore, in the present study, the MIC values used for comparative purposes were obtained from other animal species or humans.

Mean plasma concentrations of doxycycline detected in sheep after IM administration of a single dose exceeded > 40% of the dosing interval MIC reported for Chlamydia pecorum (0.008 to 0.031 µg/mL), Chlamydia psittaci (0.06 µg/mL), and S aureus (0.12 to 0.25 µg/mL) but did not exceed > 40% of the dosing interval MIC for Pasteurella haemolytica (< 0.5 µg/mL). Given these MIC values and an AUC_{0-24} of 21.80 µg h/mL, the ratio of AUC_{0-24}:MIC after IM administration of doxycycline would be 2,725.2 to 703.3 hours for C pecorum, 363.4 hours for C psittaci, 181.7 to 87.2 hours for S aureus, and > 43.6 hours for P haemolytica. Thus, in the present example, if the recommended ratio of AUC_{0-24}:MIC ≥ 125 hours was used as the cutoff to determine susceptibility of a pathogen to doxycycline, chlamydiae and strains of S aureus with the smaller MIC (0.12 µg/mL) would be susceptible to doxycycline, but strains of S aureus with the higher MIC (0.25 µg/mL) and P haemolytica would be considered resistant to doxycycline. On the basis of these pharmacokinetic and pharmacodynamic indices, administration of 20 mg of doxycycline/kg, IM, once daily to sheep infected with susceptible bacteria would be a viable treatment option. Moreover, the in vivo effectiveness of doxycycline against these pathogens may be improved because it is a highly liposoluble drug that readily penetrates tissues and cells.

A dosing schedule for a specific drug in a particular species should be made on the basis of pharmacokinetic and pharmacodynamic indices obtained from the species that is to be treated. To our knowledge, the present study is the first conducted to evaluate the pharmacokinetics of IM administration of doxycycline in domestic sheep, and the results were characterized by a slow absorption and elimination of doxycycline. Effective plasma concentrations of doxycycline against susceptible pathogens such as Chlamydia spp and S aureus could be achieved with daily administration of doxycycline at 20 mg/kg, IM. These results suggested that IM administration of doxycycline could be an alternative for the treatment of susceptible infections in domestic sheep, although further studies are needed to support the present findings and to optimize the clinical efficacy of doxycycline in sheep.

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