Doxycycline, a semisynthetic derivative of oxytetracycline, is a potent bacteriostatic drug commonly used as doxycycline hyclate. It possesses a broad-spectrum antimicrobial action through inhibition of protein synthesis. Additionally, it is highly lipophilic, has a large apparent volume of distribution, and has a prolonged half-life in domestic species. The plasma protein–binding rate of doxycycline hyclate is greater than the binding rate of oxytetracycline and chlortetracycline. In dogs, up to 40% of a dose of doxycycline is metabolized, and it is largely excreted in feces in the bile and intestinal secretions (<5% and 75%, respectively), primarily in a microbiologically inactive form.

Pharmacokinetics after administration of an injectable experimental long-acting parenteral formulation of doxycycline hyclate in goats

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Objective—To determine the pharmacokinetics after SC administration of an experimental, long-acting parenteral formulation of doxycycline hyclate in a poloxamer-based matrix and after IV and IM administration of an aqueous formulation of doxycycline hyclate in goats.

Animals—30 clinically normal adult goats.

Procedures—Goats were allocated to 3 groups (10 goats/group). One group of goats received doxycycline hyclate (10 mg/kg) IM; a second group received the same dosage of doxycycline hyclate IV; and the third group received the long-acting parenteral formulation of doxycycline hyclate SC. Serum concentrations of doxycycline were determined before and at various intervals after administration.

Results—The long-acting parenteral formulation of doxycycline hyclate had the greatest bioavailability (545%); mean ± SD maximum serum concentration was 2.4 ± 0.95 µg/mL, peak time to maximum concentration was 19.23 ± 2.03 hours, and elimination half-life was 40.92 ± 4.25 hours.

Conclusions and Clinical Relevance—Results indicated that the long-acting parenteral formulation of doxycycline hyclate distributed quickly and widely throughout the body after a single dose administered SC, and there was a prolonged half-life. Bioavailability of the long-acting parenteral formulation of doxycycline hyclate after SC administration was excellent, compared with bioavailability after IV and IM administration of an aqueous formulation of doxycycline hyclate. Although no local tissue irritation and adverse effects were detected, clinical assessment of drug-residues and toxicologic evaluations are warranted before this long-acting parenteral formulation of doxycycline hyclate can be considered for use in goats with bacterial infections. (Am J Vet Res 2008;69:1085–1090)
when injected,\(^7\) which explains why parenteral preparations are not commonly available throughout the world. On the basis of PK/PD relationships, it can be postulated that optimal clinical efficacy could be obtained when a drug is regarded as a time-dependent antimicrobial\(^8\); hence, an unelaborated aqueous formulation of doxycycline hyclate would require daily IM administrations to maintain therapeutic concentrations in plasma.\(^10,11\)

Therefore, the objective of the study reported here was to test a novel, nonirritating, long-acting formulation of doxycycline hyclate and determine its pharmacokinetics after SC injection.

### Materials and Methods

**Animals**—Thirty healthy 1-year-old female Alpine goats that weighed (mean ± SD) 50 ± 5.8 kg were included in the study. Goats were considered clinically normal on the basis of results of physical examination. Goats had not been medicated with any antimicrobial agent for at least 30 days before enrollment in the study. Goats were maintained on a diet of alfalfa hay and pelleted feed concentrate, and they had ad libitum access to water. The study was conducted at one of the experimental farms of the Facultad de Medicina Veterinaria at the Universidad Nacional Autónoma de México. The study was approved by the Postgraduate Committee of Research, Care, and Use of Experimental Animals in accordance with the Mexican official regulation NOM-062-ZOO-1999.

**Procedures**—The study was a prospective, longitudinal, and comparative-parallel pharmacokinetic analysis of a long-acting formulation of doxycycline hyclate in goats, compared with results for the aqueous version of doxycycline hyclate. Goats were randomly allocated into 3 groups (10 goats/group). Two groups of goats received a single injection of the aqueous formulation of doxycycline hyclate (10 mg/kg); injections were administered IM in one of these groups and IV in the other group. Immediately before injection, the aqueous formulation of doxycycline (10% solution) was prepared from powdered doxycycline hyclate that was diluted in sterile distilled water. Each goat was weighed, and the dose of doxycycline (10 mg/kg in a volume of approx 5.0 mL) was administered, respectively, IV in a jugular vein or IM in a semitendinosus or semimembranosus muscle of either hind limb.

Goats in the third group received a single SC injection of an experimental long-acting formulation of doxycycline hyclate at the same dosage (ie, 10 mg/kg). A long-acting formulation of doxycycline hyclate (100 mg/mL) was prepared by use of sterile conditions. Doxycycline hyclate was mixed with β-cyclodextrin by use of the kneading method to form a 10% (w/v) doxycycline solution. First, β-cyclodextrin (0.1M) and distilled water were mixed by use of a mortar-and-pestle to obtain a homogeneous paste. Then, doxycycline hyclate (1M) was added slowly. The compound was mixed for 30 minutes, and an appropriate quantity of water was added to maintain suitable consistency of the paste. The paste was dried in an oven at 40° to 50°C for 24 hours, and the dried complex was then pulverized into a fine powder.\(^12\) The resulting powder was diluted with a solution of 15% propylene glycol–10% ethyl alcohol in water. This mixture was then included in a gel copolymer polyoxypropylene–polyoxyethylene (ie, poloxamer),\(^9\) which was adjusted to pH 7.0 by addition of PBS solution with constant stirring at 4°C to achieve a total concentration of 10% doxycycline–15% poloxamer. The formulation was considered ready for use when a microemulsion formed; this point could be determined when the mixture clarified. Finally, 100-mL vials were prepared; vials were stored at 4°C until used during the week after preparation.

To minimize variation in absorption patterns from sites of SC injections, the dose of the long-acting formulation (10 mg/kg) was divided into 2 equal volumes, each of which was injected SC in the scapular region of each goat.

To achieve accurate intervals between administration of the drug and collection of blood samples, an 18-gauge, permanently heparinized catheter\(^6\) was inserted into a jugular vein in each goat. Blood samples (5 mL) were obtained before injection of doxycycline hyclate, at the time of doxycycline injection (time 0), and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours after doxycycline injection for the IV or IM route of administration and 1, 2, 4, 8, 24, 30, 48, 72, and 96 hours after injection for the SC route of administration. Blood samples were obtained by removing 5 mL of heparin-containing blood from the catheter, discarding it, and then collecting an additional 5 mL of blood, which was placed into separate glass tubes. Blood was allowed to clot at 12 ± 1.5°C for 2 hours. Serum was harvested after centrifugation at 1,500 × g for 5 minutes and frozen at −20°C for 1 week until analysis.

**Measurement of doxycycline concentrations**—Serum doxycycline concentrations were determined by use of modified agar-diffusion analysis\(^13\) with Bacillus cereus\(^4\) as a test organism grown on Mueller-Hinton agar.\(^4\) Drug concentrations were determined by comparing the diameter of zones of inhibition for samples with those of various dilutions of a standard prepared in pooled antimicrobial-free goat serum; comparisons were made by the use of linear regression analysis. The intra-assay coefficient of variance was < 1.9, and interassay error was < 1.8. The analytic assay was linear over a range of concentrations from 0.1 to 10 µg/mL. Mean ± SD recovery was 94 ± 2% (r = 0.97). Limit of detection was 0.07 µg/mL, and limit of quantification was 0.1 µg/mL.

**Pharmacokinetic analysis**—A computerized curve-stripping program\(^6\) was used to fit and analyze the concentration-versus-time patterns for each goat and the mean values for each group of goats. Models of best fit (r ≥ 0.99) were chosen after analysis by use of residual sum of squares and the minimal Akaike information criterion. For IM and SC routes, best fit was obtained by use of 2 compartment models with first-order input and first-order output in accordance with the following equations:

\[
\text{Concentration} = (A \times e^{-αt}) + (B \times e^{-βt}) + (C \times e^{-γt})
\]

where concentration is the concentration at a specific time point (t), A is the y-axis intercept of the extrapolated zero-time serum drug concentration of the distri-
bution phase, $e$ is the base of the natural logarithm, $\alpha$ is the slope for the distribution phase, $B$ is the y-axis intercept of the extrapolated zero-time serum drug concentration of the elimination phase, $\beta$ is the slope for the elimination phase, $C$ is the y-axis intercept of the extrapolated zero-time serum drug concentration of the absorption phase, and $K_{\text{AB}}$ is the rate constant for drug absorption.

\[
\text{Concentration} = (\frac{(\text{Dose} \times K_{\text{AB}})}{\text{Volume}} \times (e^{\alpha X t} - e^{\beta X t}))
\]

where dose is the dose of doxycycline hyclate injected, $X_K$ is the serum concentration, and $K_{\text{elim}}$ is the rate constant for drug elimination. Pharmacokinetic variables determined for the extravascular routes were AUC, MRT, $\beta$, $t_{1/2\text{absorb}}$, $t_{1/2\text{absorb}}$, $C_{\text{max}}$, and $T_{\text{max}}$.

The concentration-versus-time curve for IV administration of doxycycline hyclate was best fit by use of a 2-compartment model ($r \geq 0.99$) in accordance with the following equation:

\[
\text{Concentration} = (A \times e^{-\alpha X t}) + (B \times e^{-\beta X t})
\]

Pharmacokinetic variables determined for the IV route of administration were AUC, MRT, $\alpha$, $\beta$, the transfer rate from the first compartment to the second compartment, the transfer rate from the second compartment to the first compartment, $t_{1/2\text{absorb}}$, $K_{\text{AB}}$, the rate constant for drug absorption, $C_{\text{max}}$, $T_{\text{max}}$, and the maximum serum concentration at time 0.

Other variables, such as the apparent volume of distribution based on the trapezoidal method for determining AUC, AUC from time 0 to infinity, and body clearance, were determined by use of standard equations. Absolute bioavailability was calculated by use of the following equation:

\[
\text{Bioavailability} = \left(\frac{[\text{AUC}_{\text{IV}}/\text{AUC}_{\text{EV}}] \times [\text{Dose}_{\text{IV}}/\text{Dose}_{\text{EV}}]}{100}\right)
\]

where AUC$_{\text{EV}}$ is the AUC after an extravascular route of administration (IM or SC), AUC$_{\text{IV}}$ is the AUC after IV administration, Dose$_{\text{IV}}$ is the dose of doxycycline administered IV, and Dose$_{\text{EV}}$ is the dose of doxycycline administered via an extravascular route of administration (IM or SC). Data were reported as the mean ± SD of 10 observations for each variable. An ANOVA and Bonferroni test were used to compare $C_{\text{max}}$, $T_{\text{max}}$, AUC, MRT, and $t_{1/2\text{absorb}}$ among groups.

### Results

Goats did not have signs of pain or discomfort after injection of the long-acting formulation. In contrast, swelling and signs of pain were evident for at least 15 days after IM injection of the aqueous formulation. No inflammatory response was evident at the injection site after administration of the long-acting formulation of doxycycline hyclate. However, a clearly defined bulge was detectable in 3 goats. These bulges had an initial size of 2 × 1 × 1 cm and gradually disappeared by day 7 after injection. The bulges did not appear to cause pain or discomfort to any of the affected goats. The bulges were not believed to be an inflammatory response; instead, they were considered to be space occupied by the poloxamer when it transformed to a gel at body temperature.

Mean ± SD serum concentrations-versus-time patterns of doxycycline for the 3 groups were plotted (Figures 1 and 2). Pharmacokinetic variables determined for the 3 groups were summarized (Table 1). Comparisons of AUC, MRT, and $t_{1/2\text{absorb}}$ revealed that all 3 variables were significantly larger for goats administered the long-acting formulation of doxycycline hyclate SC, compared with results for goats administered the aqueous formulation of doxycycline hyclate IM or IV. Mean ± SD $t_{1/2\text{absorb}}$ after SC administration of the long-acting formulation of doxycycline hyclate was 40.92 ± 4.25 hours, which was significantly longer than the values determined after IV (4.11 ± 0.46 hours) and IM (4.19 ± 0.66 hours) administration of the aqueous formulation of doxycycline hyclate.

![Figure 1](image1.png)

Figure 1—Mean ± SD serum concentrations of doxycycline in goats (n = 10 goats/group) after IV (white squares) or IM (black circles) injection of an aqueous preparation of doxycycline hyclate (10 mg/kg). Time 0 was the time of injection.

![Figure 2](image2.png)

Figure 2—Mean ± SD serum concentrations of doxycycline following SC injection of a dose of 10 mg/kg in goats (n = 10) of a long-acting preparation of doxycycline hyclate in a poloxamer and β-cyclodextrin. Minimum serum concentration for susceptible bacteria (lower horizontal line) and minimum serum concentration for moderately resistant bacteria (upper horizontal line) are indicated.
Bioavailability of doxycycline for the group administered the long-acting formulation was 33%, whereas bioavailability in goats after IM injection of a dose of 5 mg/kg was 99% in another study. Bioavailabilities as high as that for the study reported here are not uncommon for formulations with prolonged absorption, which have flip-flop kinetics. The reason may simply be that the slope used for extrapolation to infinity is not representative of elimination. Therefore, the extrapolated AUC is larger than it would be if concentrations were measured for a longer period (ie, until all of the drug has been absorbed). It has been proposed that this phenomenon is a fact or a flaw in handling of samples, experimental design misconceptions, or analytic deficiencies or miscalculations. A careful review of these and other factors listed by the authors of that study offered no clear explanation as to the large value for bioavailability obtained for the long-acting doxycycline preparation in our study, except for a recycling phenomenon attributable to the noticeably high lipid solubility of doxycycline and the controlled release of doxycycline from the long-acting formulation.

Doxycycline has maximum clinical efficacy when serum concentrations of the drug are 4 times the MIC and remain at that concentration as long as possible during the dosing interval. Values for MIC adopted from the literature for our study were categorized as susceptible at ≤0.4 μg/mL and moderately resistant at ≥1.5 μg/mL. These values agree with reports from human isolates. For example, investigators in 1 study found that the MIC for Escherichia coli was 1.5 μg/mL, the MIC for Staphylococcus aureus was 0.28 μg/mL, the MIC for Pasteurella multocida was 0.09 μg/mL, and the MIC for Streptococcus pneumoniae was 0.16 μg/mL. Additionally, it was suggested in another report that the minimal therapeutic serum concentration for doxycycline is 0.5 to 1 μg/mL. However, no similar data could be found for goats after a thorough review of the literature. On the basis of the aforementioned information, a minimal therapeutic concentration can be defined from 0.4 to 1.5 μg/mL. The amount of time for which a minimal therapeutic concentration can be achieved by IV administration of the aqueous formulation of doxycycline (greater than the susceptibility concentration) was 11.9 hours, but it was only 2.7 hours for concentrations greater than the moderately resistant concentration. The long-acting formulation extended these time periods to 84 hours and 53 hours, respectively. The interval for which serum concentrations were greater than the susceptibility or moderately resistant concentrations was significantly greater for the long-acting formulation administered SC, compared with results for the aqueous formulation administered IV or IM.

Discussion

The primary purpose for designing a long-acting formulation of doxycycline hyclate was to enable use of a single injection to provide serum doxycycline concentrations equivalent to 1 or more injections per day of a conventional doxycycline product. A poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide) block copolymer (ie, poloxamer) was used as the carrier to facilitate absorption, reduce hydrolytic degradation, achieve controlled release, and often results in improved bioavailability. The poloxamer has low viscosity at temperatures of 28°C to 32°C and transforms to a gel at body temperature (37°C to 40°C), thus allowing a long-acting effect. To reduce irritation and promote an initial priming absorption, β-cyclodextrin was included. The complex of doxycycline hyclate and β-cyclodextrin may diminish or prevent tissue irritation by reducing the local concentration of free drug to less than an irritancy threshold. Furthermore, it is known that β-cyclodextrin enhances the absorption rate of various drugs.

The long-acting doxycycline hyclate formulation achieved a Cmax of 2.4 ± 0.95 μg/mL at a Tmax of 19.23 hours. These values exceeded by a wide margin values reported for doxycycline in goats (Cmax, 1.60 μg/mL; Tmax, 0.86 hours) in another study. Administration of 20 mg/kg IM, resulted in a Cmax of 3.87 μg/mL in pneumonia goats and 5.56 μg/mL in healthy goats. However, the same dose reportedly achieved a Cmax of only 1.87 μg/mL with a Tmax of 0.85 hours in African goats in another study. After reaching Cmax, serum concentrations of doxycycline decreased slowly in goats administered the long-acting doxycycline formulation, but the concentration always exceeded the lower limit of 0.4 μg/mL for 84 hours with an MRT of 44.83 hours.

Table 1—Mean ± SD values for pharmacokinetic variables after IV or IM injection of a dose (10 mg/kg) of an aqueous formulation of doxycycline hyclate and after SC injection of a dose (10 mg/kg) of a long-acting parenteral formulation of doxycycline hyclate in a poloxamer and β-cyclodextrin in goats (n = 10 goats/group).

Table 1—Mean ± SD values for pharmacokinetic variables after IV or IM injection of a dose (10 mg/kg) of an aqueous formulation of doxycycline hyclate and after SC injection of a dose (10 mg/kg) of a long-acting parenteral formulation of doxycycline hyclate in a poloxamer and β-cyclodextrin in goats (n = 10 goats/group).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aqueous IV</th>
<th>Aqueous IM</th>
<th>Long-acting SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µg·h/mL)</td>
<td>21.23 ± 1.18</td>
<td>20.99 ± 2.19</td>
<td>115.71 ± 6.17^*</td>
</tr>
<tr>
<td>AUCmax (µg·h/mL)</td>
<td>22.33 ± 3.22</td>
<td>22.19 ± 2.91</td>
<td>123.71 ± 7.23^*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>13.18 ± 2.99</td>
<td>12.88 ± 1.99</td>
<td>48.83 ± 3.75^*</td>
</tr>
<tr>
<td>Kp (h)</td>
<td>0.51 ± 0.01</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>A (µg/L)</td>
<td>2.38 ± 0.02</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B (µg/L)</td>
<td>1.91 ± 0.25</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>α (h)</td>
<td>0.59 ± 0.08</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>β (h)</td>
<td>0.33 ± 0.06</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>T1/2 (µg/L)</td>
<td>4.11 ± 0.46</td>
<td>4.19 ± 0.68</td>
<td>40.92 ± 4.25^*</td>
</tr>
<tr>
<td>Vdss (µL/kg)</td>
<td>3.07 ± 0.03</td>
<td>3.20 ± 0.03</td>
<td>2.40 ± 0.05^*</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>3.40 ± 0.05</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>ND</td>
<td>3.15 ± 0.04</td>
<td>19.23 ± 2.03^*</td>
</tr>
<tr>
<td>Vd (µL/kg)</td>
<td>4.91 ± 0.13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Clearance ([L/h]·kg)</td>
<td>0.69 ± 0.05</td>
<td>0.52 ± 0.05</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>ND</td>
<td>98.99 ± 7.91</td>
<td>548.00 ± 7.91^*</td>
</tr>
</tbody>
</table>

AUCmax = AUC from time 0 to infinity. Kp = Transfer rate from the first compartment to the second compartment. ND = Not determined. Kp = Transfer rate from the second compartment to the first compartment. α = The γ-axis intercept of the extrapolated zero-time serum drug concentration of the distribution phase. B = The γ-axis intercept of the extrapolated zero-time serum drug concentration of the elimination phase. A = Slope for the elimination phase. β = Slope for the elimination phase. Kc = Rate constant for drug elimination. Kc = Rate constant for drug elimination. Vdss = Maximum serum concentration at time 0. Vdss = Apparent volume of distribution based on the trapezoidal method to determine AUC.
reached after a single dose of the long-acting formulation was administered SC, a theoretic 5-time repetition of the concentration-versus-time pattern revealed that administration of the long-acting formulation every 72 hours yielded a predicted AUC of 227 µg/mL/h, as calculated by use of the trapezoidal method. This also yielded AUC-to-MIC ratios of 568 or 151, regardless of whether a susceptible or moderately resistant bacterial strain was considered. In contrast, AUC for the IV and IM administration of the aqueous formulation of doxycycline hydrochloride was 29.98 and 32.2 µg/mL/h, respectively. For the IV and IM administration, AUC-to-MIC ratios were extremely low (ie, 65.2 and 17.4 for susceptible and moderately resistant bacteria, respectively, after the IV administration and 28.5 and 21.5 for susceptible and moderately resistant bacteria, respectively, after the IM administration). An AUC-to-MIC ratio of 568 or 151 for the long-acting formulation of doxycycline hydrochloride, regardless of whether a susceptible or moderately resistant bacterial strain was considered, would be almost 8 times as large as the corresponding ratios derived from IV administration of the aqueous doxycycline formulation and much higher than ratios obtained during an experimental trial conducted in humans with pneumonia. Considering the aforementioned fact and the fact that serum concentrations of doxycycline should never be less than the MIC at any time during the dosing interval, it is safe to regard the long-acting formulation of doxycycline hydrochloride as complying with PK/PD principles. In contrast, the aqueous formulation of doxycycline hydrochloride should be injected IV every 11.8 hours or IM every 12.99 hours. For these 2 routes of administration for the aqueous formulation, such frequent injections appear impractical, whereas a 3-day dosing interval for the long-acting formulation of doxycycline hydrochloride appears to be a reasonable option.

Because doxycycline is a time-dependent antimicrobial, it would be expected that clinical efficacy of the long-acting formulation should be at least equivalent to clinical efficacy obtained with formulations that are not long acting and administered once or twice per day but with a substantial reduction in work load, cost, and animal stress. Hence, among other considerations, clinical trials and residue studies are needed to assess whether this long-acting formulation of doxycycline hydrochloride can be potentially useful in ruminants.

Additional studies may optimize the long-acting formulation. It must be stated that use of this drug cannot be recommended until tissue concentration and residue studies have been performed. Also, despite the evidence that indicates lack of toxic effects for poloxamer and β-cyclodextrin in various species, assessment of the safety of this preparation in goats is warranted.

References


