Pharmacokinetics of an oral extended-release formulation of doxycycline hyclate containing acrylic acid and polymethacrylate in dogs

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OBJECTIVE
To determine the pharmacokinetics of doxycycline hyclate administered orally in the form of experimental formulations with different proportions of acrylic acid–polymethacrylate-based matrices.

ANIMALS
30 healthy adult dogs.

PROCEDURES
In a crossover study, dogs were randomly assigned (in groups of 10) to receive a single oral dose (20 mg/kg) of doxycycline hyclate without excipients (control) or extended-release formulations (ERFs) containing doxycycline, acrylic acid polymer, and polymethacrylate in the following proportions: 1:0.5:0.0075 (ERF1) or 1:1:0.015 (ERF2). Serum concentrations of doxycycline were determined for pharmacokinetic analysis before and at several intervals after each treatment.

RESULTS
Following oral administration to the study dogs, each ERF resulted in therapeutic serum doxycycline concentrations for 48 hours, whereas the control treatment resulted in therapeutic serum doxycycline concentrations for only 24 hours. All pharmacokinetic parameters for ERF1 and ERF2 were significantly different; however, findings for ERF1 did not differ significantly from those for the control treatment.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that both ERFs containing doxycycline, acrylic acid polymer, and polymethacrylate had an adequate pharmacokinetic-pharmacodynamic relationship for a time-dependent drug and a longer release time than doxycycline alone following oral administration in dogs. Given the minimum effective serum doxycycline concentration of 0.26 µg/mL, a dose interval of 48 hours can be achieved for each tested ERF. This minimum inhibitory concentration has the potential to be effective against several susceptible bacteria involved in important infections in dogs. Treatment of dogs with either ERF may have several benefits over treatment with doxycycline alone. (Am J Vet Res 2015;76:367–372)

Doxycycline is a second-generation tetracycline that has been used since 1967 in humans and in domestic species for prophylaxis and treatment of disease caused by several biological agents. Doxycycline possesses a broad-spectrum antibacterial action; recently, anti-inflammatory and antineoplastic roles of doxycycline (by inhibition of matrix metalloproteinases produced by inflammatory cells) have been discovered. Doxycycline has better clinical efficacy at low concentrations, such as at 2 to 4 times the MIC for susceptible microorganisms. Therefore, the inhibition of microorganisms by the drug occurs in a time-dependent manner. However, the use of doxycycline has been limited to some extent because of adverse reactions.

Oral administration can result in adverse reactions, such as irritation of the esophagus and stomach, with risk of ulcerations and vomiting. Tissue irritation after SC or IM injection can also occur, as with other drugs of the tetracycline group. These potential adverse reactions are limiting factors for treatments for which this tetracycline is the only option. Such as during the carrier phases of Leptospira spp, Ehrlichia canis, Brucella canis, and Haemobartonella canis, which require twice daily administration of
the drug for prolonged periods ranging from 21 days to several years.\textsuperscript{8,10}

Controlled-release formulations of doxycycline may reduce adverse effects and may improve the efficacy during lengthy periods of treatment. Because the frequency of administration is decreased, compared with administration of doxycycline alone, ERFs would generate less irritation in the gastrointestinal tract, thereby improving owners' compliance with treatment of their dogs. In veterinary medicine, a long-acting injectable formulation of doxycycline has been used in cattle,\textsuperscript{11} small ruminants,\textsuperscript{12} and dogs\textsuperscript{13}; an oral formulation has been used in horses\textsuperscript{14}; and a subgingival system (for a localized effect) has been used for treatment of periodontitis in Beagles.\textsuperscript{15,16} Following injection of the long-acting formulation, the mean ± SE serum half-life of doxycycline in dogs was 133.61 ± 6.32 hours, but inflammation and signs of pain at the injection site developed during a 30-day period,\textsuperscript{13} which may cause dog owners to decline treatment of their animals.

Since 2004, the pharmaceutical industry has shown increasing interest in the development of ERFs, with focus on the selection of excipients to improve or modify drug delivery. Use of acrylic acid polymer and polymethacrylate as excipients provides advantages for achieving extended release. Acrylic acid is an insoluble polymer with mucoadhesive properties that prolongs the residence time of formulations at the absorption site of the drug and reduces contact and irritation on absorption surfaces.\textsuperscript{17,18} Polymethacrylate is a polymer used for film-coating tablets, pellets, capsules, and granules; it also creates an inert matrix structure, allowing for the diffusion of the drug through pores, and can be used as an extended-release and binding agent.\textsuperscript{19-21} The objectives of the study reported here were to determine the pharmacokinetics of doxycycline hyclate administered orally in experimental formulations with different proportions of acrylic acid and polymethacrylate-based matrices. The intent was to formulate 2 nonirritating, extended-release preparations of doxycycline with acrylic acid polymer and polymethacrylate, to establish the pharmacokinetics after oral administration of a single dose (20 mg of doxycycline/kg) of each ERF in dogs, and to compare results with the pharmacokinetics of a single dose of a preparation of doxycycline (20 mg/kg) administered orally. We proposed that effective ERFs of doxycycline would have the potential to increase the duration of therapeutic blood concentrations of the drug and reduce the frequency of administration, compared with immediate-release products.

**Materials and Methods**

**Animals**

Thirty healthy dogs (2 to 8 years old) of various breeds and both sexes were included in the study. Body weights were recorded for each dog on the day before treatment administration; mean weight of the dogs was 17.75 kg (range, 15 to 30 kg). The dogs were determined to be healthy on the basis of physical examination findings. Dogs had not been medicated with any antibacterial medication for at least 30 days. During the study, all dogs received water ad libitum and were fed a commercial diet twice daily. The same dogs were used throughout.

The study was approved by the Institutional Subcommittee of Research, Care and Use of Experimental Animals according to the Mexican Official Regulation NOM-062-ZOO-1999. The study was conducted at the Facultad de Medicina Veterinaria of the Universidad Nacional Autónoma de México, Mexico City. The owners of the dogs included in this research project gave written consent for their dogs' participation in the study.

**Preformation stage**

The physical and chemical characteristics of the doxycycline hyclate powder\textsuperscript{a} were obtained by means of scanning electron microscopy, particle size distribution, infrared spectroscopy,\textsuperscript{b} x-ray diffraction,\textsuperscript{c} and differential scanning calorimetry.\textsuperscript{2} Furthermore, the rheological properties included the bulk density, tapped density, true density, Carr compressibility index, Hausner ratio, porosity percentage, angle of repose, and flow velocity. The wet percentage of the powder was measured with a thermodifferential\textsuperscript{5} All techniques were performed according to the US Pharmacopeia.\textsuperscript{22} Doxycycline powder was subsequently granulated and inserted in conventional gelatin capsules for administration as the control treatment; to achieve the required dose, 1 capsule was administered by hand into the mouth of each dog.

**Extended-release drug preparation**

For the ERFs, doxycycline hyclate,\textsuperscript{4} acrylic acid polymer,\textsuperscript{f} and polymethacrylate\textsuperscript{g} were mixed in 2 ratios (1:0.5:0.0075 [ERF1] and 1:1:0.015 [ERF2]). After mixing, the preparations were granulated manually by the wet granulation process in ethanol.\textsuperscript{23} Excipient proportions were based on past research,\textsuperscript{19,24} manufacturing recommendations,\textsuperscript{25} and the handbook of pharmaceutical excipients.\textsuperscript{23} For administration, granules of each preparation were inserted in conventional gelatin capsules according to body weight of each animal; to achieve the required dose of each ERF, 1 capsule was administered by hand into the mouth of each dog.

**Study design**

In a crossover study, the 30 healthy adult dogs were randomly assigned (in groups of 10) to receive a single oral dose (20 mg/kg) of doxycycline hyclate\textsuperscript{a} without excipients (control) or each of 2 ERFs containing doxycycline hyclate,\textsuperscript{4} acrylic acid polymer,\textsuperscript{f} and polymethacrylate\textsuperscript{g} in the following proportions: 1:0.5:0.0075 (ERF1) or 1:1:0.015 (ERF2). Each dog received all 3 treatments with a washout period of 30 days between treatments. An experiment was carried

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\textsuperscript{a} DCPH, Alcon Laboratories, Fort Worth, TX.

\textsuperscript{b} Philips XL 30, AMR Instruments, Almelo, the Netherlands.

\textsuperscript{c} Scintag XDS-2000, Scintag, Inc., San Diego, CA.

\textsuperscript{d} TA Instruments, New Castle, DE.

\textsuperscript{e} TA Discovery TTR, TA Instruments, New Castle, DE.

\textsuperscript{f} CP 802, Dowsil, Montpellier, France.

\textsuperscript{g} Etern, Polymet, Schering-Plough Animal Health, Kenilworth, NJ.

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out for 1 group of 10 dogs in 1 day. To evaluate acute toxic effects, the dogs were monitored for signs of discomfort, diarrhea, or vomiting and for 3 days after each experiment and were rechecked at 30 days after each experiment ended.

The dose of 20 mg of doxycycline/kg represents the cumulative dose for 2 days of treatment according to the recommended dosage of 5 mg of doxycycline/kg twice a day. For each experiment, the time of drug administration was designated as 0 hours. A blood sample (3 mL) was obtained by venipuncture from each dog at 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96, and 120 hours after drug administration. The total volume of blood collected from each dog during each experiment was 36 mL. Serum was immediately separated from each sample by centrifugation and was stored at \(-20^\circ\text{C}\) until analyzed.

**Serum doxycycline concentration determination**

Serum doxycycline concentrations were determined by modified agar diffusion analysis with *Bacillus cereus* as a test organism on a Mueller-Hinton dehydrated growth medium. Drug concentrations were determined via linear regression analysis by a comparison of the diameters of inhibition halos with the standard curve (200, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.1562 µg/mL) prepared in pooled antibacterial-free canine serum. The intra-assay coefficient of variance was < 4.9, and the interassay error was < 4.8. The analytic assay was linear over a range of concentrations from 0.1 to 10 µg/mL, with a percentage recovery of 95 ± 1% and a coefficient of determination \(r^2\) of 0.99% ± 0.1. The limit of detection was 0.014 µg/mL, and the limit of quantification was 0.1 µg/mL. The concentrations were determined with the aid of software, and these values were used to determine the pharmacokinetic parameters.

**Pharmacokinetics analysis**

A computerized curve-stripping program was used to analyze serum doxycycline concentration versus time curve for each individual dog after the oral administration of the ERF1, ERF2, and control treatment. The Akaike information criterion and graphical analysis of weighted residuals were used to determine the optimal pharmacokinetic model. For oral administration, fitted curves of doxycycline expressed the decrease in serum drug concentration as a function of time and were approximated to 1 compartment with first-order input and first-order output with the following equation \((r > 0.95)\):

\[
C(t) = \frac{(\text{Dose} \cdot K_a)}{(\text{volume} K_e - K_d)} \cdot \left[ e^{-K_e t} - (e^{-K_d}) \right]
\]

where \(C(t)\) is concentration as a function of time, \(V_d\) is the volume of distribution, \(e\) is the base of the natural logarithm, \(K_e\) is the absorption rate constant, \(K_d\) is the elimination rate constant, and \(t\) is the time since the drug was administered.

The following pharmacokinetic parameters were obtained with a computerized curve-stripping program: elimination half-life, \(C_{max}\), \(AUC\), \(AUC_t\), \(AUMC\), retention time, and elimination rate constant. The time of \(C_{max}\) was determined by inspecting the individual serum drug concentration-time profiles.

Apparent volume of distribution at steady state was determined mathematically with the following equation:

\[
V_d = \frac{(\text{Dose} \cdot AUMC)}{AUC^2}
\]

Elimination half-life \(t_{1/2}\) was calculated as follows:

\[
t_{1/2} = \frac{0.693}{K_{el}}
\]

The total body clearance (Cl) of oral doxycycline was calculated as follows:

\[
Cl = \frac{\text{Dose}}{AUC}
\]

The \(AUC_{0-\infty}\) was calculated as follows:

\[
AUC_{0-\infty} = AUC + \left( C_{last} / K_d \right)
\]

where \(C_{last}\) is the last measurable concentration.

The relative bioavailability (\(F\)) was calculated as the percentage of the AUC in the experimental group relative to the control group:

\[
\text{Relative } F = \left( \frac{AUC \text{ for ERF1/AUC for control}}{} \right) \cdot 100
\]

The pharmacokinetic flip-flop condition was demonstrated by the following equation:

\[
\text{Rate of absorption} = V_z (KC + |\Delta C/\Delta t|)
\]

where \(V_z\) is the terminal exponential volume of distribution, \(K\) is the terminal disposition rate constant once drug absorption is complete, \(C\) is the serum concentration at time \(t\), and \(\Delta C\) is the change in serum concentration over the time interval \(\Delta t\).

**Statistical analysis**

Serum doxycycline concentrations were reported as mean ± SD, and pharmacokinetics parameters of the ERF1, ERF2, and control treatment were calculated for each dog; data were reported as mean ± SE. Normality and uniformity of the data were determined by Shapiro-Wilk tests; if data were not normally distributed, they were corrected by the exponential fitting method. Equality between means was evaluated by an ANOVA and Tukey test to obtain comparison of means. A value of \(P = 0.05\) was considered significant.

**Results**

Mean ± SE serum concentrations-time profiles of doxycycline for the 3 treatments were determined (Figure 1). Data regarding the pharmacokinetics parameters for each of the treatments followed a normal distribution (Table 1). A pharmacokinetic flip-flop condition was demonstrated by use of the equation for rate of absorption: \(V_z (KC + |\Delta C/\Delta t|)\). For the ERF1 (doxycycline hyclate, acrylic acid polymer, and...
polymer, and polymethacrylate in the following proportions: ERF1, 1:0.5:0.0075, or ERF2, hyclate without excipients (control treatment) or ERFs containing doxycycline, acrylic acid groups of 10) in a crossover study to receive a single oral dose (20 mg/kg) of doxycycline —Pharmacokinetic variables derived for 30 healthy adult dogs randomly assigned (in Table 1

Figure 1—Mean ± SD serum doxycycline concentrations in 30 healthy adult dogs randomly assigned (in groups of 10) in a crossover study to receive a single oral dose (20 mg/kg) of doxycycline hyclate without excipients (control; squares) or 2 ERFs containing doxycycline, acrylic acid polymer, and polymethacrylate in proportions as follows: ERF1, 1:0.5:0.0075 (circles) or ERF2, 1:1:0.015 (triangles). Each dog received all 3 treatments with a washout period of 30 days between treatments. The time of each treatment administration was 0 hours.

Table I—Pharmacokinetic variables derived for 30 healthy adult dogs randomly assigned (in groups of 10) in a crossover study to receive a single oral dose (20 mg/kg) of doxycycline hyclate without excipients (control treatment) or ERFs containing doxycycline, acrylic acid polymer, and polymethacrylate in the following proportions: ERF1, 1:0.5:0.0075, or ERF2, 1:1:0.015.

Parameter | Control treatment | ERF1 | ERF2
---|---|---|---
Elimination half-life (h) | 7.54 ± 0.17a | 12.02 ± 0.92a | 17.36 ± 0.42a
Cmax (µg/mL) | 2.03 ± 0.28b | 2.41 ± 0.68b | 4.11 ± 0.20b
Time of Cmax (h) | 2 | 4 | 4.5 ± 1.02
AUC (µg•h/mL) | 22.1 ± 2.52a | 41.57 ± 3.08b | 106.35 ± 4.49b
AUC0–∞ (µg•h/mL) | 24.18 ± 2.47a | 45.11 ± 3.42b | 116.68 ± 4.41b
AUC0–∞ /dose | 18.65 ± 2.06a | 36.73 ± 2.47b | 95.54 ± 3.85b
AUMC (µg•h2/mL) | 239.92 ± 21.79a | 747.42 ± 100.97b | 2710.18 ± 156.63b
Retention time (h–1) | 10.82 ± 0.25a | 17.34 ± 1.33b | 25.26 ± 0.62b
Elimination rate constant (h–1) | 0.09 ± 0.002a | 0.06 ± 0.006b | 0.04 ± 0.001b
Vdss (L/kg) | 10.003 ± 1.37a | 8.34 ± 0.29b | 4.84 ± 0.16b
Total body clearance | 0.91 ± 0.1 | 0.501 ± 0.04 | 0.19 ± 0.008
Relative bioavailability (%) | — | 178.91 ± 15.32a | 481.69 ± 19.16b

Data are reported as mean ± SE. Each dog received all 3 treatments with a washout period of 30 days between treatments. A blood sample of 3 mL was obtained by venipuncture from each animal at 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96, and 120 hours after drug administration (0 hours). Serum was immediately separated from each sample by centrifugation and stored at −20°C until analyzed. Serum doxycycline concentrations were determined via linear regression analysis by a comparison of the diameters of inhibition halos with the standard curve (200, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.1562 µg/mL) prepared in pooled antibacterial-free dog serum. — = Not applicable.

*Within a row, values without a common superscript letter differ significantly (P < 0.05).
to have a much greater plasma AUC than would a non-formulated form of the same active principle ingredient.\textsuperscript{33,34} Additionally, a list of various factors leading to bioavailability > 100% has been provided by Toutain and Bousquet-Méou\textsuperscript{55} and includes chemical-related reasons, sampling and handling errors or experimen-tal design flaws, and analytic deficiencies or miscalcu-lations. Moreover, a recycling phenomenon (redistribu-tion) owing to the noticeably high lipid solubility of doxycycline could explain the large values for bioavailability.\textsuperscript{36} The properties of the excipients (acrylic acid polymer and polymethacrylate) may prolong the residence time of ERFs at the site of drug absorption, which could also explain this result.

For ERFs, it is predictable that the absorption rate is lower than the elimination rate.\textsuperscript{37} For both ERFs evaluated in the present study, the elimination rate was very slow but greater than the absorption rate. This finding was usual for long-acting preparations that follow flip-flop kinetics and may also explain the relative bioavailability data. In turn, to demonstrate flip-flop pharmacokinetics, the overall appearance of the serum concentration-time profile of the drug must be taken into account. A much longer apparent elimina-tion half-life following extravascular dosing, com-pared with findings following administration via the IV route, suggests that flip-flop pharmacokinetics is occurring.\textsuperscript{37} However, it is not possible to directly test this hypothesis with doxycycline because IV adminis-tration of this drug is not recommended.\textsuperscript{4,38,39}

According to Boxenbaum,\textsuperscript{30} a flip-flop model can be recognized when the plasma concentration-time profile tends to closely parallel the rate of absorption. This model provides a simple and effective way to visualize the shape of the rate of the absorption profile. A flip-flop model can be recognized when the plasma concentration-time profile tends to closely parallel either rate of absorption or the overall appearance of the serum concentration vs time profile of the drug, considering that the rate of absorption is slower than the rate of elimination. A flip-flop condition was demonstrated in the preparations used in the present study, which can be therefore considered as ERFs.

The quantitative and qualitative microbiological agar diffusion technique used in this trial to determine the serum concentrations of doxycycline has been re-garded as sufficiently reliable to replace the analytic conclusions derived from high-performance liquid chromatography.\textsuperscript{40} Furthermore, because it determines the active fractions of the drug, the technique offers more clinically meaningful data than concentra-tions derived from purely chemical methods.

Doxycycline is the recommended drug for treatment of various infections in dogs. The MICs of doxy-cycline for important microorganisms are as follows: 0.03 \( \mu \text{g/mL} \) for \textit{Ehrlichia} spp.,\textsuperscript{41} 0.1 to 0.39 \( \mu \text{g/mL} \) for \textit{Leptospiira interrogans} (serovars Australis, Autumnalis, Autumnalis, Bratislava, Canicola, Copenhageni, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohemorrhagi-ae, Pyrogenes, and Wolfi [excluding Pomon and Man-karo-so]),\textsuperscript{42} 0.008 to 0.031 \( \mu \text{g/mL} \) for \textit{Oblunymia} spp.,\textsuperscript{43} 0.06 to 1 \( \mu \text{g/mL} \) for \textit{Brucella} spp.,\textsuperscript{44} and 0.06 to 0.125 \( \mu \text{g/mL} \) for \textit{Rickettsia} spp.\textsuperscript{4,2} Doxycycline is a treatment option but is not necessarily the recommended drug of choice for other microorganismal infections; the MICs of doxycycline for such microorganisms are as follows: 0.052 to 0.125 \( \mu \text{g/mL} \) for \textit{Staphylococcus intermedius},\textsuperscript{45} 0.12 to 0.25 \( \mu \text{g/mL} \) for \textit{Staphylococcus aureus},\textsuperscript{45} 0.03 to 0.06 \( \mu \text{g/mL} \) for \textit{Streptococcus pneumoniae},\textsuperscript{45} 0.106 to 0.51 \( \mu \text{g/mL} \) for \textit{Pasteurella multocida},\textsuperscript{46} 0.19 to 0.75 \( \mu \text{g/mL} \) for \textit{Bordetella bronchiseptica},\textsuperscript{47} and 0.06 to 0.25 \( \mu \text{g/mL} \) for \textit{Mycoplasma} spp.\textsuperscript{4,2}

Considering doxycycline’s MICs and the fact that it is a time-dependent antibacterial drug, the best pharma-cokinetic-pharmacodynamic profile would be achieved when serum concentrations of the drug are never less than the MIC for the infecting microorganism at any time during the dose interval.\textsuperscript{48,49} On that basis, for susceptible bacteria, doxycycline alone (without excipients) should be administered every 24 hours, whereas the dosing interval for the 2 ERFs evaluated in the present study could be increased to every 48 hours. Less frequent dosing should improve prescription compliance among owners of dogs requiring treatment and decrease patient stress level. The determination to use one formulation or the other would depend on the microorganism targeted for treatment.

Undoubtedly, further studies may optimize the formulation of doxycycline, and it is necessary to state that the use of these ERFs cannot be recommended until tissue concentration studies and toxicological assessments have been performed. However, on the basis of manufacturing reports,\textsuperscript{21,25,50} the amounts of excipients (acrylic acid polymer and polymethacrylate) used in this study are less than known toxic doses. Assessment of the safety of these preparations in the species of interest is warranted.

### Footnotes

a. Indükern de México S.A. de CV, Zapopan, Jalisco, Mexico.
b. Spectrometer FTIR RX.I model, Perkin-Elmer, Waltham, Mass.
c. D5000 powder diffractometer with copper anticathode, Sie-mens, Munich, Germany.
d. DSC 521, Mettler-Toledo, Chicago, Ill.
e. MB 2000, Ohaus Corp, Parsippany, NJ.
f. Lubrizol Advanced Materials Inc, Countryside, Ill.
g. EUDRAGIT RL 100, Evonik Industries AG, Darmstadt, Germany.
h. ATCC 11778, American Type Culture Collection, Manassas, Va.
i. BIOXON, Becton Dickinson, Mexico City, Mexico.
j. ORIGIN PRO, version 8.6, OriginLab Corp, Northampton, Mass.
k. PK Analyst, Micromath Scientific Software, Salt Lake City, Utah.

### References


