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# Pharmacokinetics of doxycycline in sheep after intravenous and oral administration

Luis J. Castro, Ana M. Sahagún *, M. José Diez, Nélida Fernández, Matilde Sierra, Juan J. García

*Pharmacology, Department of Biomedical Sciences, Veterinary Faculty, University of Leon, Spain*

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**Abstract**

The pharmacokinetics of doxycycline were investigated in sheep after oral (PO) and intravenous (IV) administration. The IV data were best described using a 2- ($n = 5$) or 3- ($n = 6$) compartmental open model. Mean pharmacokinetic parameters obtained using a 2-compartmental model included a volume of distribution at steady-state ($V_{ss}$) of 1.759 ± 0.3149 L/kg, a total clearance (Cl) of 3.045 ± 0.5264 mL/kg/min and an elimination half-life ($t_{1/2\beta}$) of 7.027 ± 1.128 h. Comparative values obtained from the 3-compartmental mean values were: $V_{ss}$ of 1.801 ± 0.3429 L/kg, a Cl of 2.634 ± 0.6376 mL/kg/min and a $t_{1/2\beta}$ of 12.11 ± 2.060 h. Mean residence time ($MRT_{0-\infty}$) was 11.18 ± 3.152 h. After PO administration, the data were best described by a 2-compartment open model. The pharmacokinetic parameter mean values were: maximum plasma concentration ($C_{max}$), 2.130 ± 0.950 μg/mL; time to reach $C_{max}$ ($t_{max}$), 3.595 ± 3.348 h, and absorption half-life ($t_{1/2k_{01}}$), 36.28 ± 14.57 h. Non-compartmental parameter values were: $C_{max}$, 2.182 ± 0.9117 μg/mL; $t_{max}$, 3.432 ± 3.307 h; $F$, 35.77 ± 10.20%, and mean absorption time ($MAT_{0-\infty}$), 25.55 ± 15.27 h. These results suggest that PO administration of doxycycline could be useful as an antimicrobial drug in sheep.
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*Keywords:* Pharmacokinetics; Doxycycline; Sheep; Intravenous; Oral

## Introduction

Doxycycline is a tetracycline antibiotic obtained by modification of the oxytetracycline molecule. It has a broad-spectrum of activity against a wide variety of microorganisms, including aerobic and anaerobic Gram-positive and Gram-negative bacteria, chlamydiae, rickettsiae and mycoplasmas, and it exerts a bacteriostatic effect by inhibiting protein synthesis. Doxycycline has been successfully used in man for more than 40 years (Aronson, 1980; Cunha et al., 1982; Riond and Riviere, 1988), and is commonly employed in certain respiratory, skin and soft tissue and genitourinary infections. In veterinary medicine, doxycycline is used to treat infections in several animal species, such as ehrlichiosis or respiratory tract diseases in dogs, pneumonia in cattle and pigs, and colibacillosis and psittacosis in poultry.

A major advantage of doxycycline, compared to other members of the tetracycline family, is a high lipophilicity, which will increase its distribution and tissue penetration, plus prolong its half-life, all of which contribute to its enhanced antimicrobial activity (Aronson, 1980; Shaw and Rubin, 1986; Riviere and Spoo, 2001). In addition, this drug has limited adverse effects and is relatively inexpensive. Consequently, there has been a growing interest in using doxycycline in veterinary clinical practice.

The pharmacokinetics of doxycycline have been fully documented in humans (Schach von Wittenau and Chiaini, 1968; Raghuram and Krishnaswamy, 1982; Riond and Riviere, 1988) and, to a lesser extent, in veterinary species, such as dogs (Wilson et al., 1988; Riond et al., 1990), cats (Riond et al., 1990), pigs (Riond and Riviere, 1990; Baert et al., 2000), horses (Davis et al., 2006), and poultry

* Corresponding author. Tel.: +34 987 29 18 46; fax: +34 87 29 12 56.
*E-mail address:* a.sahagun@unileon.es (A.M. Sahagún).

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(Anadón et al., 1994; Santos et al., 1996; Laczay et al., 2001). Limited information is available on the pharmacokinetics of doxycycline in ruminant species, where it has been studied in calves in mature and immature ruminal function (Riond et al., 1989; Meijer et al., 1993) and in goats (Jha et al., 1989; Abd El-Aty et al., 2004), but to date no studies have been conducted in sheep. Ziv and Sulman (1974) briefly described the intravenous (IV) kinetics of this drug in sheep, although data were reported as a mean of values obtained from the two animal species used in the study, namely cows and sheep.

The goals of the current study were to determine the IV pharmacokinetics of doxycycline in sheep, and to evaluate the oral (PO) bioavailability of a commercial formulation of doxycycline hyclate when given to the same animals at a dose of 20 mg/kg.

## Material and methods

### *Animals*

Eleven healthy 4-year-old female Spanish Churra sheep with a mean bodyweight (BW) of 35.63 ± 1.91 kg were used. Animals were determined to be clinically normal by physical examination, and parasites were killed 45 days before the experiments with netobimin (Hapasil, Schering-Plough, 8 mL/20 kg BW).

Sheep were acclimatised for 15 days before the trial began, and maintained indoors on a diet of hay and pelleted feed concentrate, with water and saltlick available ad libitum. The use of sheep in this study was approved in advance by the Institutional Animal Care and Use Committee of the University of León.

### *Study design*

A randomised 2-period crossover design was carried out. The animals were divided into two groups. Doxycycline was administered at a dose of 20 mg/kg by the IV (group I) and PO (group II) routes. After a suitable washout period (2 weeks), group I received the drug by PO administration, and group II by IV injection.

For the IV administration, doxycycline HCl (Sigma Chemical Company) was dissolved in 20 mL sterile isotonic saline solution and injected into the left jugular vein over a 30 s duration to prevent cardiac shock. Blood samples (5 mL) were collected into EDTA-$K_3$ vacuum tubes (Venoject, Terumo Europe) from the contralateral jugular vein just prior to administration, and at 5, 10, 15, 25, 35 and 45 min, then 1, 2, 4, 6, 10, 16, 24, 32, 40, 48, 60 and 72 h.

For the PO route, a commercial formulation, Syvadox-10 (10% doxycycline hyclate, Lab Syva) was administered using a gavage needle. After administration, the needle was flushed with an additional 5 mL of water for cleaning. Prior to drug delivery, animals were fasted for 12 h, with free access to water. Blood samples were withdrawn from both jugular veins before drug administration and at 30, 60, 90, 105, 120, 135, 150 and 165 min, then 3, 4, 6, 10, 16, 24, 32, 40, 48, 60, and 72 h. Plasma was immediately separated by centrifugation and stored at −80 °C until analysed.

### *Doxycycline determination*

Plasma doxycycline concentrations were analysed by reversed-phase high-performance liquid chromatography (HPLC) with UV detection using a method previously described (Axisa et al., 2000) with minor modifications. Solid-phase extraction with OASIS HLB 1 L30 mg cartridges (Waters Associates) was used to prepare plasma samples. Briefly, cartridges were conditioned with 1 mL methanol and 1 mL water. After having added 1 mL plasma, cartridges were washed three times with 1 mL 5% methanol in water. Doxycycline was eluted with 1 mL acetonitrile:water 50:50 (v/v). The eluant (200 μL) was injected into the chromatograph twice.

Conditions for HPLC analysis were as follows: a Nova-Pack $C_{18}$ 4 μm 3.9 × 150 mm (Waters Corporation) was used for separation; the mobile phase consisted of acetonitrile:water 50:50 (v/v), with pH adjusted to 2.5 with trifluoroacetic acid. The flow rate was 1.25 mL/min, and the wavelength was set at 350 nm. Oxytetracycline was used as an internal standard (10 μg/mL). Under these conditions, the retention times were 2.7 min for oxytetracycline and 3.5 min for doxycycline.

The limits of quantification and detection were 0.02 and 0.007 μg/mL, respectively. The extraction recovery was 96.47 ± 26.29%, whereas inter- and intra-day accuracy was between 1.95% and 11.2%.

### *Pharmacokinetic analysis*

Pharmacokinetic analysis was performed using both a compartmental and a non-compartmental description of the observed data. For compartmental analysis, plasma doxycycline concentration–time profiles were individually fitted to the following experimental equation:

$$C_p = \sum_{i=1}^{n} C_i e^{-\lambda_i t}$$

where $C_p$ is the plasma doxycycline concentration, $C_i$ is the $y$-intercept, $\lambda_i$ is the slope of each of n first-order rate processes, e is the exponential function (base e) and $t$ is time. The pharmacokinetic model best describing the plasma concentration–time curves of doxycycline was determined using WinNonLin 4.0.1 software (Pharsight Corporation). A weighting factor of $1/C$ was used to identify the best fit, where $C$ is the doxycycline experimental concentration.

Akaike's information criterion (Yamaoka et al., 1978a) and graphical analysis of weighted residuals were used to determine the optimal pharmacokinetic model (one, two and three compartments) (Wagner, 1993; Gabrielsson and Weiner, 2000). Other compartmental parameters were calculated by standard methods (Gibaldi and Perrier, 1982; Wagner, 1993).

Model-independent pharmacokinetic parameters were calculated using WinNonLin, with expressions based on statistical moments theory (Yamaoka et al., 1978b) and on formulae mentioned above (Gibaldi and Perrier, 1982; Wagner, 1993). Plasma elimination rate constant ($\lambda$) was estimated by least squares regression of the logarithm of plasma concentration versus time curve over the terminal elimination phase, and maximum plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($t_{max}$) were determined by direct observation of the plasma concentration–time curves. The area under the concentration–time curve (AUC) was calculated by the trapezoidal rule, and extrapolate to infinity by dividing the last experimental concentration by the terminal slope.

The fraction of dose absorbed ($F$) was calculated as $F = AUC_{PO}/AUC_{IV} \times 100$, where $AUC_{IV}$ and $AUC_{PO}$ are the area under the curve after IV and PO administration, respectively.

### *Statistical analysis*

All pharmacokinetic parameters were calculated for each sheep, and data were reported as mean ± standard deviation (mean ± SD). Normality of the data and uniformity of the variance were determined by Skewness and Cochran tests, respectively. If data were normal and uniform, a $t$ test was used to evaluate differences between data sets. When data were not normal or if there was not uniformity in the variance, a Wilcoxon test was used. A value of $P < 0.05$ was used to determine significance.

## Results

One sheep suffered tachypnoea, tremors, sialism and rear-end weakness after IV injection of doxycycline solu-

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tion. These symptoms were transient and resolved without treatment in a few minutes. No signs of discomfort were seen in any animal following PO administration.

Mean plasma concentrations of doxycycline as a function of time after IV and PO administration of 20 mg/kg are shown in Fig. 1A, and initial sample points are presented in full detail in Fig. 1B.

Following IV administration, plasma doxycycline concentration–time curves best fit a 2-compartment open model in five sheep, and a 3-compartment open model in the other six animals. The pharmacokinetic parameters calculated after compartmental and non-compartmental analysis are summarised in Table 1.

Plasma doxycycline concentrations declined rapidly, with a half-life associated with the α-phase ($t_{1/2\alpha}$) of 0.280 ± 0.493 h, whereas the half-life associated with the terminal β-phase ($t_{1/2\beta}$) was about 25-fold higher when a bi-exponential fit was used. Similarly, in sheep best described by a 3-compartment open model, the initial

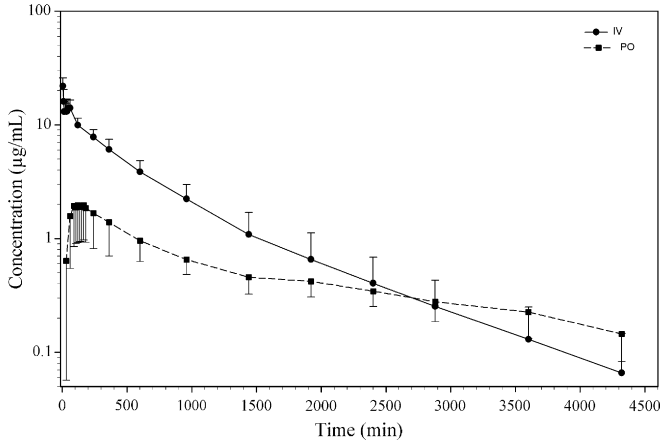


Fig. 1A. Semilogarithmic plot of the mean ± SD plasma doxycycline concentrations in 11 sheep following a single intravenous and oral administration of 20 mg/kg.

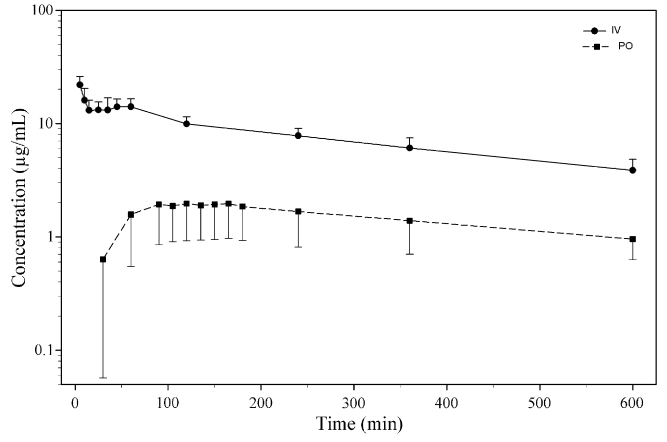


Fig. 1B. Semilogarithmic plot of the mean ± SD plasma doxycycline concentrations in 11 sheep following a single intravenous and oral administration of 20 mg/kg (data points until 600 min).

Table 1
Pharmacokinetic parameters (mean ± SD) obtained in 11 sheep after intravenous administration of 20 mg/kg doxycycline

| Parameters | Compartmental | | Non-compartmental |
|---|---|---|---|
| | 2-compartmental | 3-compartmental | |
| $C_0$ (μg/mL) | 37.71 ± 14.56 | 57.32 ± 22.66 | |
| $D$ (μg/mL) | | 41.76 ± 23.04 | |
| $A$ (μg/mL) | 27.06 ± 15.10 | 11.16 ± 3.941 | |
| $B$ (μg/mL) | 10.65 ± 1.593 | 4.411 ± 1.971 | |
| $t_{1/2k_{10}}$ (h) | 2.447 ± 1.275 | 1.773 ± 0.634 | |
| $t_{1/2\gamma}$ (h) | | 0.040 ± 0.018 | |
| $t_{1/2\alpha}$ (h) | 0.280 ± 0.493 | 3.268 ± 0.661 | |
| $t_{1/2\beta}$ (h) | 7.027 ± 1.128 | 12.11 ± 2.060 | |
| Cl (mL/kg/min) | 3.045 ± 0.5264 | 2.634 ± 0.6376 | 2.708 ± 0.5780[a] |
| $V_1$ (L/kg) | 0.6206 ± 0.2988 | 0.4092 ± 0.1931 | |
| $V_a$ (L/kg) | 1.820 ± 0.2983 | 2.690 ± 0.4124 | 2.408 ± 0.2994 |
| $V_{ss}$ (L/kg) | 1.759 ± 0.3149 | 1.801 ± 0.3429 | |
| $AUC_{0-t}$ (μg min/mL) | | | 7636 ± 1673 |
| $AUC_{t-\infty}$ (μg min/mL) | | | 78.05 ± 87.06 |
| $AUC_{0-\infty}$ (μg min/mL) | | | 7714 ± 1732 |
| $MRT_{0-t}$ (h) | | | 10.52 ± 2.745 |
| $MRT_{0-\infty}$ (h) | | | 11.18 ± 3.152 |

$C_0$, Sum of the α, β and γ zero-time intercepts; $D$, zero-time intercept for the γ-phase; $A$, zero-time intercept for the α-phase; $B$, zero-time intercept for the β-phase; $t_{1/2k_{10}}$, $t_{1/2\gamma}$, $t_{1/2\alpha}$, $t_{1/2\beta}$, half-lives associated with $k_{10}$, γ, α and β, respectively; Cl, total body clearance; $V_1$, apparent volume of distribution in the central compartment; $V_a$, area volume of distribution; $V_{ss}$, volume of distribution at steady-state; AUC, area under the plasma concentration–time curve; MRT, mean residence time.

[a] Significantly different from compartmental parameter (test $t$, $P < 0.05$).

disappearance of doxycycline was fast, with a half-life associated with the γ-phase ($t_{1/2\gamma}$) of 0.040 ± 0.018 h, with a slower decline in the second ($t_{1/2\alpha}$ of 3.268 ± 0.661 h) and third phases ($t_{1/2\beta}$ of 12.11 ± 2.060 h).

This drug was widely distributed, as evidenced by the high volumes of distribution at steady-state ($V_{ss}$) found (1.759 ± 0.3149 and 1.801 ± 0.3429 L/kg, when a 2- or a 3-compartment open model was used, respectively). In the same way, doxycycline showed and extensive and ready access to peripheral compartment/s, with a higher tendency to distribute than to be eliminated, as it is also indicated by the apparent volumes of distribution in the central compartment ($V_1$), lower than those obtained for the peripheral compartments. On the other hand, total clearance (Cl) was 3.045 ± 0.5264 mL/kg/min after a 2-compartmental fit, and 2.634 ± 0.6376 mL/kg/min in a 3-compartment open model.

Regarding the non-compartmental pharmacokinetic parameters, $V_a$ was 2.408 ± 0.2994 L/kg; total mean residence time ($MRT_{0-\infty}$), 11.18 ± 3.152 h; Cl, 2.708 ± 0.5780 mL/kg/min, and $AUC_{0-\infty}$ 7714 ± 1732 μg min/mL. Significant differences were found for Cl between data obtained by compartmental and non-compartmental methods.

After PO administration, compartmental analysis showed that, for all animals, the individual plasma

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Table 2
Pharmacokinetic parameters (mean ± SD) obtained in 11 sheep after oral administration of 20 mg/kg doxycycline

| Parameters | Compartmental | Non-compartmental |
|---|---|---|
| $t_{1/2k_{01}}$ (h) | 36.28 ± 14.57 | |
| $t_{lag}$ (h) | 0.282 ± 0.148 | |
| $AUC_{0-t}$ (μg min/mL) | 2200 ± 515.7 | 2250 ± 539.3[b] |
| $AUC_{t-\infty}$ (μg min/mL) | 566.7 ± 394.7 | 423.2 ± 296.6[a,b] |
| $AUC_{0-\infty}$ (μg min/mL) | 2767 ± 722.9 | 2674 ± 660.3[b] |
| $C_{max}$ (μg/mL) | 2.130 ± 0.950 | 2.182 ± 0.9117 |
| $t_{max}$ (h) | 3.595 ± 3.348 | 3.432 ± 3.307 |
| $MRT_{0-t}$ (h) | | 22.22 ± 4.833[b] |
| $MRT_{0-\infty}$ (h) | | 36.73 ± 13.86[b] |
| $MAT_{0-t}$ (h) | | 11.71 ± 8.405 |
| $MAT_{0-\infty}$ (h) | | 25.55 ± 15.27 |
| $F$ (%) | | 35.77 ± 10.20 |

$t_{1/2k_{01}}$, absorption half-life; $t_{lag}$, time delay; $C_{max}$, maximum plasma concentration; $t_{max}$, time to reach $C_{max}$; MAT, mean absorption time; $F$, fraction of dose absorbed.
[a] Significantly different from compartmental parameter (test $t$, $P < 0.05$).
[b] Significantly different from intravenous parameter (test $t$, $P < 0.05$).

concentration–time profile was best described by a 2-compartment open model. As shown for the intravenous route, compartmental and non-compartmental parameters obtained following PO administration are listed in Table 2. The absorption process was slow, with and absorption half-life ($t_{1/2k_{01}}$) of 36.28 ± 14.57 h, and initiated with a delay ($t_{lag}$) of 0.282 ± 0.148 h. A $C_{max}$ of 2.130 ± 0.950 μg/mL was reached at 3.595 ± 3.348 h ($t_{max}$).

The oral bioavailability ($F$) was moderately low, with a mean value of 35.77 ± 10.20%, and the total mean absorption time ($MAT_{0-\infty}$), 25.55 ± 15.27 h. Significant differences were found between values obtained by compartmental and non-compartmental analysis only for $AUC_{t-\infty}$.

When both routes of administration were considered (PO and IV), significant differences were found for $AUC_{0-t}$, $AUC_{t-\infty}$, $AUC_{0-\infty}$, $MRT_{0-t}$ and $MRT_{0-\infty}$ when non-compartmental data were compared.

## Discussion

Doxycycline pharmacokinetics have been studied in several animal species (Wilson et al., 1988; Jha et al., 1989; Riond et al., 1989; Riond and Riviere, 1990; Riond et al., 1990; Greth et al., 1993; Meijer et al., 1993; Santos et al., 1996; Baert et al., 2000; Laczay et al., 2001; Abd El-Aty et al., 2004; Davis et al., 2006) and in man (Schach von Wittenau and Chiaini, 1968; Raghuram and Krishnaswamy, 1982; Riond and Riviere, 1988), but few studies have been done in sheep (Ziv and Sulman, 1974).

In our study, doxycycline disposition after IV injection is best described by a 2- or 3-compartment open model. Other authors studying IV injection of doxycycline have reported a 2-compartment open model in calves (Riond et al., 1989), dogs and cats (Wilson et al., 1988; Riond et al., 1990), pigs (Riond and Riviere, 1990), goats (Abd El-Aty et al., 2004) and man (Raghuram and Krishnaswamy, 1982). Meijer et al. (1993) obtained a best fit with a 3-compartment open model in calves, but indicated that the 2-compartment model was also a viable fit.

It should be noted that in six animals an increase of plasma concentrations occurred between 35 and 60 min after IV drug administration, which could be indicative of an enterohepatic cycle. Enterohepatic recirculation of doxycycline has been previously documented in humans (Fabre et al., 1966; Gibaldi, 1967; Malmborg, 1984) and pigs (Riond and Riviere, 1990). In our study, doxycycline showed an intermediate disposition between the 2- and 3-compartment models, and the existence of enterohepatic cycling could contribute to this distorted fit. For instance, if only samples taken before 32 h are considered, a bi-exponential equation best describes plasma concentration–time profiles, whereas the inclusion of data points after 32 h favours a tri-exponential fit.

Mean values of $t_{1/2\beta}$ obtained by both 2- (7.03 ± 1.13 h) and 3-compartmental (12.11 ± 2.06 h) fits are shorter than those estimated by Ziv and Sulman (1974) in cows and ewes (24.8 ± 2.7 h). Nevertheless, our values are near to those obtained by Riond et al. (1989) in calves with mature (14.8 ± 0.95 h) and immature (9.88 ± 0.65 h) ruminal function, as well as to the findings of Meijer et al. (1993) in calves with immature ruminal function (9.5 ± 3 h), Riond et al. (1990) in dogs (6.99 ± 1.09 h), and Laczay et al. (2001) in chickens (6.78 ± 0.06 h). Lower values than ours have been calculated by Abd El-Aty et al. (2004) in lactating goats (4.62 ± 0.11 h), Riond et al. (1990) in cats (4.56 ± 0.68 h), and Riond and Riviere (1990) in pigs (4.04 ± 0.58 h).

The $V_{ss}$ values obtained in our study indicate that doxycycline is widely distributed. This drug is highly lipophilic, and would be expected to exhibit extensive tissue distribution, probably with tissue binding and/or intracellular penetration (Riond et al., 1989). Jha et al. (1989) and Abd El-Aty et al. (2004) suggested that doxycycline could be accumulated in fat. Our values are similar to those previously reported in calves with immature (1.81 ± 0.24 L/kg) or mature (1.31 ± 0.11 L/kg) ruminal function (Riond et al., 1989), and higher than those reported in non-ruminant species such as cats (0.34 ± 0.03 L/kg) (Riond et al., 1990), dogs (0.93 ± 0.14 L/kg) (Riond et al., 1990), and pigs (0.53 ± 0.04 L/kg, and 0.89 ± 0.16 L/kg) (Riond and Riviere, 1990 and Baert et al., 2000, respectively). Other authors have reported values of the volume determined by the area method ($V_a$). In our study, $V_a$ was clearly lower than values obtained by Abd El-Aty et al. (2004) in goats (6.48 ± 0.12 L/kg) and by Jha et al. (1989) in lactating goats (9.78 ± 0.86 L/kg).

According to Toutain and Bousquet-Mélou (2004), doxycycline Cl in sheep is low. The extensive binding to plasma proteins (90.2%) (Ziv and Sulman, 1974) and the reservoir effect of the forestomachs may account for this low values (Riond et al., 1989). Data obtained for Cl are higher than those determined in calves with mature

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ruminal function (1.07 ± 0.06 mL/kg/min), and in small animals, dogs (1.72 ± 0.17 mL/kg/min) and cats (1.09 ± 0.21 mL/kg/min) (Riond et al., 1990). Other authors determined values similar to ours in calves with immature ruminal function (2.20 ± 0.21 mL/kg/min) (Riond et al., 1989), and chickens (2.32 ± 0.12 mL/kg/min) (Laczay et al., 2001). Our values were lower than those calculated in goats with mature ruminal function (11.83 ± 0.67 mL/kg/min) (Abd El-Aty et al., 2004).

The discrepancies between values calculated for pharmacokinetic parameters may be attributed to the animal species, the drug formulation employed, the age, size or sex of the animals, to differences in fatty tissue deposits between animal species or breeds, or even to inter-individual variations (Riond et al., 1989; Jha et al., 1989).

Doxycycline pharmacokinetics after PO administration were best described in this animal species by a 2-compartment open model, which is in accordance with previous studies in pigs (Baert et al., 2000), calves with immature ruminal function (van Gool et al., 1986; Meijer et al., 1993), chickens (Anadón et al., 1994; Espigol et al., 1997; Laczay et al., 2001) and turkeys (Santos et al., 1996).

There are no previous data available describing doxycycline PO bioavailability in sheep. In our study, the absorption half-life (36.3 ± 14.6 h) was longer than that obtained in pigs (0.77 ± 0.44 h) at a dose of 10.5 mg/kg (Baert et al., 2000), and pre-ruminant calves (1.54 ± 3.61 h) with a dose of 10 mg/kg (Meijer et al., 1993), showing that the absorption process is slower in sheep than in the above-mentioned species.

The mean values reported in the current study for $C_{max}$ (compartmental and non-compartmental) are proportionally higher than those obtained in pigs by Baert et al. (2000) with a dose of 10.5 mg/kg (1.52 ± 0.62 μg/mL). Meijer et al. (1993) found a higher value (3.32 ± 0.04 μg/mL) with a dose of 10 mg/kg in calves with immature ruminal function.

The lack of ruminal function does not cause important differences in $t_{max}$, as our values (3.6 ± 3.3 h) are similar to those reported in pre-ruminant calves (3.48 ± 0.63 h) (Meijer et al., 1993) and higher than those determined in pigs (2.30 ± 1.22 h) (Baert et al., 2000). In turkeys, however, $t_{max}$ was considerably higher (7.5 ± 4.3 h), possibly due to doxycycline adsorption to the intestinal wall or food particles, or to a slower absorption process in this avian species (Santos et al., 1996).

The PO bioavailability of doxycycline in the commercial formulation has been calculated with AUC data obtained from non-compartmental analysis after IV and PO administration, as they have been determined by the same method and therefore, they are more comparable. As expected in ruminants, the results of our study indicate that doxycycline is partially absorbed when administered orally. Meijer et al. (1993) reported higher bioavailability values in calves with immature ruminal function (69 ± 12%), but most previous studies observed an absorption value similar to ours, including studies performed in chickens (41.3 ± 2%) (Anadón et al., 1994), turkeys (25–64%) (Santos et al., 1996), and pigs (21.2 ± 7.5%) (Baert et al., 2000). Although the loss of doxycycline in the forestomachs may play an important role, these discrepancies are in accordance with those reported for other tetracyclines, and are related to the animal species studied (Nielsen and Gyrd-Hansen, 1996), the pharmacokinetic curve-fitting routines or the analytical techniques used (Meijer et al., 1993), the drug formulation administered, and the health status of the animals (Abd El-Aty et al., 2004).

Regarding the antibacterial activity of doxycycline against sheep bacterial isolates, we have only found one study where a reduced susceptibility of *Listeria monocytogenes* to doxycycline, with a minimum inhibitory concentration (MIC) of 4 μg/mL (Vela et al., 2001). In cattle, MIC values have been categorised as sensitive (⩽0.5 μg/mL) and resistant (⩾1.5 μg/mL) (Yoshimura et al., 2001; Hospenthal and Murray, 2003; Jee et al., 2004). Other values are 0.008–0.031 μg/mL for *Chlamydia pecorum* (Pudjiatmoko et al., 1998); <0.5 μg/mL for *Pasteurella haemolytica* (Ole-Mapenay and Mitema, 1997); 0.06–2 μg/mL for *Mycoplasma mycoides* (Egwu and Aliyu, 1998) and 0.1 μg/mL for *Bacillus anthracis* (Brook et al., 2001). MIC values determined in human isolates were 0.016–0.5 μg/mL for *Streptococcus pneumoniae* (Zhanel et al., 2003, and Ross and Jones, 2004); 0.064 mg/mL for *Chlamydia psittaci* (Suchland et al., 2003); 0.12–0.5 μg/mL for *Staphylococcus aureus* (Firsov et al., 2004; Ross and Jones, 2004); 0.5 μg/mL for *Mycoplasma pneumoniae* (Waites et al., 2003) and 1 μg/mL for *Haemophilus influenzae* (Koeth et al., 2004).

On the other hand, three pharmacokinetic/pharmacodynamic (PK/PD) indices ($T >$ MIC, AUC/MIC and $C_{max}$/MIC) have been proposed to predict the success or failure of therapy: AUC/MIC ratio for quinolones, $C_{max}$/MIC ratio for aminoglycosides, and $T >$ MIC for β-lactams (Toutain et al., 2002; Toutain and Lees, 2004). Although doxycycline is regarded as a time-dependant antibacterial drug, the index most associated with efficacy for tetracyclines is AUC/MIC (Craig, 1998; Andes and Craig, 2002; Toutain et al., 2002). Extrapolating from the MIC mentioned above and our single-dose AUC values, $AUC_{0-\infty}$/MIC ratio for the oral route was 1437.6 h for *C. pecorum*, 89.1 h for *S. pneumoniae*, *P. haemolytica* and *S. aureus*, 44.6 h for *H. influenzae*, and 22.3 h for *M. mycoides*. To our knowledge, only a doxycycline AUC/MIC threshold has been established for *H. influenzae* (Koeth et al., 2004), and ⩾25 for tetracyclines for *Neisseria meningitidis* (Burguess et al., 2007).

Nevertheless, in our study and with an oral single dose of 200 mg/kg doxycycline, concentrations remain above the MIC reported throughout the time sampling for *C. pecorum*, and 16 h for *P. haemolytica*, *S. pneumoniae* and *S. aureus*. Additional multiple dose clinical trials by the oral route would be necessary to predict more accurately its efficacy against the bacterial species indicated by the manufacturer.

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## Conclusions

Despite its moderately low oral bioavailability, the advantageous pharmacokinetic properties of doxycycline, such as its large volume of distribution and the long elimination half-life, indicate that this drug can be a good option for treating some infectious diseases in sheep. Nevertheless, a multiple-dose study by the oral route would be necessary to establish more accurately its efficacy.

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