Rev. sci. tech. Off. int. Epiz., 2009, **28** (3), 1129-1142

Pharmacokinetics of enrofloxacin after single intravenous administration in sheep

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Submitted for publication: 11 November 2008
Accepted for publication: 22 June 2009

Summary

The disposition of enrofloxacin in sheep was investigated after single-dose intravenous administration of 2.5 mg/kg body weight. Blood samples were drawn from the jugular vein at predetermined times after drug administration. Plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin were simultaneously determined by reverse-phase high performance liquid chromatography. The data collected were subjected to non-compartmental and compartmental kinetic analysis. Statistical model theory was used to determine non-compartmental pharmacokinetic parameters. Disposition of enrofloxacin was described by a three-compartment open model with elimination from the central compartment following intravascular administration.

The elimination half-life, the volume of distribution, and the area under the concentration vs time curve (AUC) were 4.31 h, 1.10 l/kg and 9.24 µg·h/ml, respectively.

Enrofloxacin was metabolised to ciprofloxacin and the ratio between the AUCs of ciprofloxacin and enrofloxacin was 0.26 after intravenous administration.

With predictive models of efficacy (maximum plasma concentrations/minimum inhibitory concentrations [C_{max}/MIC] and AUC/MIC ratios in plasma) for most of the sheep pathogen microorganisms, enrofloxacin produced scores higher than 15 and 50, respectively. After intravenous administration at the dose of 2.5 mg/kg, enrofloxacin achieved concentrations several times above the MIC for major pathogen bacteria in plasma, and it may prove useful in the treatment of infectious diseases caused by sensitive pathogens in sheep.

Keywords

Ciprofloxacin – Enrofloxacin – Intravenous – Pharmacokinetic – Sheep.

Introduction

The fluoroquinolones are a class of synthetic antimicrobials for which the pharmacologic properties, clinical uses, and toxicities in animals have been reviewed (10, 61). These drugs differ in their chemical structure from the older quinolone analogues such as nalidixic acid, oxolinic acid, and cinoxacin, in two common features. The first is the presence of a fluorine atom at position 6, and the second is the presence of a piperazinyl or pyrrolidinyl substituent in position 7 of the quinoline nucleus (26).

The fluoroquinolones that have been used in human and veterinary medicine are generally effective at much lower concentrations against a broader spectrum of bacterial pathogens than are their unfluorinated predecessors (42).

The mode of action of fluoroquinolones involves interactions with both DNA gyrase, the originally

recognised drug target, and topoisomerase IV, a related type II topoisomerase (30). These synthetic drugs act as bactericidal antimicrobials at relatively low concentrations, and produce a postantibiotic effect (10, 42).

Enrofloxacin (1-cyclopropyl-7-(ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) is a fluorinated quinolone carboxylic acid derivative which was developed exclusively for use in animals (3, 57). It exhibits a wide spectrum of antimicrobial activity against Gram-negative bacteria and intracellular organisms such as *Rickettsia*, *Chlamydia*, and *Mycoplasma*, and a tighter spectrum of activity against Gram-positive organisms (10, 51).

Extensive clinical studies performed in several species of food animals as well as companion animals documented its efficacy and tolerability. The pharmacokinetic behaviour of enrofloxacin after intravascular administration has been determined in cattle (33, 34, 43), goats (18, 19, 54) and other ruminants (13, 24, 53), horses (32), pigs (5), dogs (37), cats (55), rabbits (9), chickens (4, 35), birds (7), and fish (60). Information on enrofloxacin pharmacokinetic characteristics in sheep (8, 19, 45, 52) and in other small ruminants (13) is also available.

The characteristics of enrofloxacin in most species include good absorption after parenteral and oral applications, large volume of distribution, suggesting wide tissue penetration, and a terminal half-life in the range of 2 h to 6 h. However, the oral absorption of enrofloxacin in adult ruminants is poor, approximately 10% (61).

The drug is partially metabolised in the liver to ciprofloxacin, a primary metabolite which is a potent antimicrobial agent itself (34, 37).

Several bacterial infectious diseases, including colibacillosis in lambs, contagious agalactia and mastitides, cause morbidity, mortality, and significant economic losses in sheep, and the aetiological agents are potentially susceptible to enrofloxacin (11, 23).

The antimicrobial and pharmacokinetic properties of enrofloxacin indicate that it might have marked advantages for use in sheep. However, there is not enough information on the disposition of enrofloxacin in sheep to design rational dosage regimens for clinical use.

Most of the published pharmacokinetic data for enrofloxacin in sheep were based only on results of a microbiological assay, which does not allow differentiation between enrofloxacin and ciprofloxacin pharmacokinetic variables.

The purpose of this study was to determine the pharmacokinetic characteristics of enrofloxacin and its metabolite ciprofloxacin after single intravenous (IV) administration of enrofloxacin in sheep, and to establish pharmacokinetic/pharmacodynamic (PK/PD) parameters.

Materials and methods

Experimental animals

Eight healthy adult female sheep weighing 40 kg to 65 kg were used in the study. The sheep were housed together in an open yard for four weeks prior to the beginning of the study. Prior to the day of drug administration they were weighed and clinically inspected to ensure that they were healthy. No treatments were administered in the two weeks leading up to the start of the study.

All animals were fed once a day with hay and pelleted food, and had *ad libitum* access to water. Prior to the days of drug administration and intensive sampling food was withheld overnight.

The experiment was conducted from September to December when the range of daily shade temperature was 15ºC to 30ºC.

Chemicals

All chemicals were analytical grade. Enrofloxacin (Fluka Chemical Corporation, Milwaukee, USA) was made available as pure (99.5%) reagent-grade chemical. Ciprofloxacin (99%) was obtained from Orofarma (Buenos Aires, Argentina). All solvents were high performance liquid chromatography (HPLC) grade. Acetonitrile, methanol and orthophosphoric acid were from J.T. Baker (Phillipsburg, USA).

Drug administration and sampling procedure

Enrofloxacin was solubilised in deionised, sterile, pyrogen-free water acidified with orthophosphoric acid (85%) to a final pH of 3.0 to reach a final concentration of 2.5 mg/ml.

Enrofloxacin was given as a bolus in the left jugular vein at a dosage of 2.5 mg/kg body weight. Doses were given between 8 a.m. and 10 a.m.

After shaving and cleaning the area, blood samples (10 ml) were collected from the right jugular vein. Samples were drawn at the following post-injection times: 2.5, 5, 10, 15, 20, 30 and 40 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 36 h. Heparinised syringes were used for the extraction of blood samples. Ten millilitre disposable plastic syringes (0.8 × 38 mm [Nissho Co., Osaka, Japan]) were rinsed with two drops of a solution containing 1,000 IU/ml of heparin in distilled water.

Blood samples were centrifuged within 1 h of collection, and the plasma was separated and stored at –20°C until analysis.

Drug assay

Determination of enrofloxacin and ciprofloxacin was performed by means of high performance liquid chromatography in reverse phase (HPLC-RP) with ultraviolet detection. The method was developed from modifications of published methods of Horie *et al.* (31), Stegeman (59), Rose *et al.* (56) and Manceau *et al.* (44), and considering the physical and chemical properties of enrofloxacin (39).

Enrofloxacin and ciprofloxacin were extracted using a solid phase extraction (SPE) method. In order to extract enrofloxacin and ciprofloxacin from plasma, 1 ml of trichloroacetic acid 5%-acetonitrile (70:30 vol/vol) was added to 1 ml of plasma. The mixture was vortex-mixed for 10 min and centrifuged for 30 min at 1500 *g*. The supernatant was separated and transferred into a second tube. Fresh trichloroacetic acid 5%-acetonitrile mixture (1 ml) was added to the first tube and the same extraction procedure was repeated once. All the collected supernatant was diluted in 6 ml of an aqueous solution of potassium dihydrogen phosphate (8.75 mg/l), adjusted to pH 2.1 with orthophosphoric acid. A Bakerbond C_{18} SPE 200 mg solid phase extraction cartridge (J.T. Baker, Phillipsburg, USA) was conditioned with 3 ml acetonitrile and then 9 ml of the same aqueous solution of potassium dihydrogen phosphate adjusted to pH 2.1 with orthophosphoric acid. After application of the diluted extract, the cartridge was washed with 3 ml of potassium dihydrogen phosphate pH 2.1 and dried for 3 min. The elution of analytes was performed with 3 ml of orthophosphoric acid 0.5 M-methanol (30:70 vol/vol) solution. The eluate was dried under a gentle stream of nitrogen at 50°C and the dried extract was dissolved in HPLC mobile phase (0.5 ml). The tube was shaken for 5 min and a 100 µl aliquot was injected into the HPLC system.

The HPLC system consisted of a Rheodyne 7725 injector (Cotati, Redwood, California, USA), a 307 model pump, a UV/VIS-155 ultraviolet detector and UniPoint System Integrator Software (Gilson, Villiers-le-Bel, France). The column was a 5 µm, 125 mm × 4 mm LiChrospher 100 RP-18 (Merck, Darmstadt, Germany). The column was protected with a LiChroCART 4-4 RP-18 5 µm pre-column (Merck, Darmstadt, Germany). The mobile phase consisted of 14% acetonitrile and 86% water containing 0.4% triethylamine and 0.4% phosphoric acid (85%). Elution was isocratic (1.5 ml/min) at room temperature; the detection wavelength was 278 nm, and the sensitivity was set at 0.001 absorbance unit full scale.

The mean retention times for ciprofloxacin and enrofloxacin were 4.9 ± 0.49 min and 7.6 ± 0.81 min, respectively (n > 600). The method used was selective for the analysed compounds and no interferences were observed.

In order to validate the analytical method, detection limits, linearity, reproducibility and drug recovery from the plasma were calculated following the methodologies of the Center for Drug Evaluation and Research (21) and the Center for Veterinary Medicine (22) at the Food and Drug Administration in the USA.

Stock solutions of 1 mg/ml enrofloxacin and ciprofloxacin were prepared in acetonitrile and 0.1 M sodium hydroxide (1:1).

Working solutions (0.05, 0.10, 0.25, 0.5, 1, 2, 3, 5 and 10 µg/ml) were prepared daily by mixing and diluting the stock solutions in water. Solutions for sample spiking (0.5, 1, 2.5, 5, 10, 20, 30, 50 and 100 µg/ml) were prepared by mixing and diluting the stock solutions in water. Standard calibration curves were prepared from blank plasma samples containing known concentrations of enrofloxacin and ciprofloxacin.

For both drugs, limits of detection and quantification were calculated as 3.3 and 10 times the standard deviation (SD) of blank plasma samples, respectively.

The external standards were enrofloxacin (Fluka Chemical Corporation, Milwaukee, USA) and ciprofloxacin (Orofarma, Buenos Aires, Argentina). The calibration curve for enrofloxacin (ranging from 0.05 µg/ml to 10 µg/ml) was characterised by its regression coefficient (r^2= 0.998), slope (5.128E-06) and intercept (0.0858), and was used to determine the analyte concentrations in the sample and the detection limits. The calibration curve for ciprofloxacin (ranging from 0.05 µg/ml to 10 µg/ml) was characterised by its regression coefficient (r^2= 0.996), slope (5.466E-06) and intercept (0.090), and was used to determine the analyte concentrations in the sample and the detection limits.

Enrofloxacin and ciprofloxacin were quantified from their respective peak areas and the concentrations in plasma samples determined by means of calibration curves based on analysis of blank plasma samples spiked with enrofloxacin/ciprofloxacin and analysed as described for the experimental samples.

Recovery was calculated from sheep plasma samples spiked with enrofloxacin and ciprofloxacin, respectively, at final concentrations of 0.1, 0.5 and 1 µg/ml of each drug. The samples spiked were analysed with the same HPLC method used for experimental samples. Chromatography peak area values at the same concentrations were

compared between plasma samples and standard solutions of both enrofloxacin and ciprofloxacin, in order to calculate the percentage of recovery.

Intra-assay and inter-assay method reproducibility was obtained for both enrofloxacin and ciprofloxacin.

Pharmacokinetic analysis

Pharmacokinetic compartmental and non-compartmental modelling of the drug concentration-time data was performed.

Pharmacokinetic parameters were derived from concentration vs time curves obtained from the experimental concentrations obtained individually for each sheep, and reported as mean ± SD, except for half-lives, for which harmonic means were calculated too.

Pharmacokinetic compartmental modelling of enrofloxacin plasma concentrations was performed by weighted least-squares non-linear regression analysis using a program package known as ADAPT II (15) that uses the simplex algorithm (47). Discrimination between the different pharmacokinetic models was performed by using the Akaike Information Criterion and its adaptation defined for model discrimination by Yamaoka *et al.*, i.e. the Minimum Akaike Information Criterion Estimation (MAICE) test (64).

Weightings used for the x^{th} measured drug concentration were 1, $1/\sqrt{x}$, $1/x$ and $1/x^2$. For all the animals and weighting schemes, plots of weighted residuals vs time were inspected and the best weighting scheme was selected based on the scatter and random distribution of residuals about the abscissa axis.

An open tricompartmental pharmacokinetic model was selected, in which the evolution of the drug plasma concentrations changed according to the following triexponential equation:

$$C_p = A_e^{-\alpha t} + P_e^{-\pi t} + B_e^{-\beta t}$$

Where C_p is the plasma concentration at any time, *A, P* and *B* are the intercept terms of the disposition curve, α, π and β are the hybrid rate constants representing the slopes of distribution and elimination, *e* is the base of natural logarithm, and *t* is time.

The model-dependent pharmacokinetic parameters and the micro-constants describing the rates of transfer between compartments were determined using ADAPT II. After IV administration the following pharmacokinetic parameters and micro-constants were determined: zero-time drug concentration intercepts of the triphasic IV disposition curve (*A, P, B*); hybrid rate constants related to the slopes of the distribution and elimination phases (α, π, β); fast distribution ($t_{½\alpha}$,), slow distribution ($t_{½\pi}$) and elimination ($t_{½\beta}$) half-lives; first-order rate constants for drug distribution between the central and peripheral compartments (K_{12}, K_{21}, K_{13}, K_{31}); first-order transfer rate constants for elimination of the drug from the central compartment (K_{10}); apparent volume of the central compartment (*Vc*); apparent volume of the peripheral compartment (*Vp*); apparent volume of distribution calculated using the steady state method (Vd_{ss}); and body clearance of the drug (Cl_B).

Non-compartmental model pharmacokinetic parameters for enrofloxacin were estimated for each individual using the statistical moment theory to obtain values for mean residence time (MRT).

The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the area under the first moment curve were calculated using the trapezoidal rule and extrapolated to infinity by dividing the last plasma measured concentration by the slope of the elimination phase. Classical methods were used for these calculations (25).

The metabolite ratio of enrofloxacin/ciprofloxacin was estimated by dividing ciprofloxacin AUC by enrofloxacin AUC.

To calculate both compartmental and non-compartmental pharmacokinetic parameters, the plasma concentration-time profile for each animal was analysed individually.

Because of variation between plasma levels in the different samples it was not possible to determine PK parameters for ciprofloxacin by non-linear regression. Instead, a non-compartmental analysis was performed (6, 16, 25).

The results are shown as the mean ± SD, with the corresponding coefficient of variation. Harmonic mean was used with data not distributed normally.

Pharmacodynamic analysis

Ratios of maximum plasma concentrations/minimum inhibitory concentrations (C_{max}/MIC) and AUC/MIC were calculated for several microorganisms using the means of C_{max}, AUC and the respective *in vitro* MIC_{90} published values.

Results

Enrofloxacin and ciprofloxacin were quantified from their respective peak areas and the concentration in plasma

samples determined by means of calibration curves based on analysis of blank plasma samples spiked with enrofloxacin/ciprofloxacin and assayed as described for the experimental samples.

The limits of detection and quantification in plasma samples were 0.03 µg/ml to 0.05 µg/ml for ciprofloxacin, and 0.05 µg/ml to 0.1 µg/ml for enrofloxacin, respectively. The relationship was linear from 0.05 µg/ml to 10 µg/ml for both drugs.

Average intra-day and inter-day variability was 6.74% and 12.21% for ciprofloxacin, and 6.89% and 13.32% for enrofloxacin, respectively.

The recovery was better than 80% for both enrofloxacin and ciprofloxacin.

The mean (± SD) enrofloxacin and ciprofloxacin plasma concentration-time profiles after single IV administration of 2.5 mg/kg enrofloxacin are shown in Figure 1. The sum of concentrations of enrofloxacin and ciprofloxacin ≥ 0.1 µg/ml was maintained in plasma up to 10 h after enrofloxacin administration (Fig. 1).

Although it is not appropriate to consider a C_{max} after IV administration, a plasma concentration of 6.83 ± 0.71 µg/ml was measured at 0.042 h, the time when the first sample was taken. If the C_{max} is theoretically considered as the sum of the *Y*-axis intercept terms (*A* + *P* + *B*), its resulting value was 8.85 µg/ml. Mean plasma concentration at 10 h was 0.24 µg/ml.

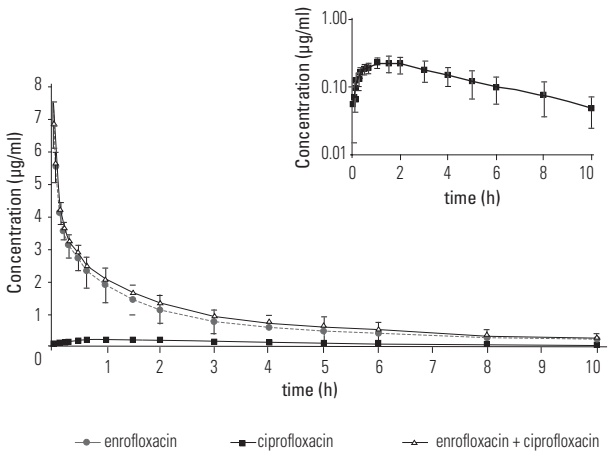

Fig. 1
Mean ± standard deviation (n = 8) of enrofloxacin, ciprofloxacin, and enrofloxacin plus ciprofloxacin plasma concentration-time profiles after single intravenous administration of 2.5 mg/kg enrofloxacin

Plasma concentration-time data of enrofloxacin after IV 2.5 mg/kg administration to sheep were adequately described by a three-compartment open model with first order elimination from the central compartment. The best fit was obtained by the use of the MAICE test. According to the inspection of the graphs of weighted residuals vs time $1/\sqrt{X}$ was selected as the best weighting scheme for the data, based on the scatter and random distribution of residuals about the abscissa axis.

Compartmental parameters describing the disposition of enrofloxacin after the single IV administration of 2.5 mg/kg are presented in Table I. The parameters obtained after fitting the data by non-compartmental methods based on the statistical moment theory are shown in Table II.

Table I
Mean ± SD compartmental pharmacokinetic parameters for enrofloxacin after its intravenous administration to eight sheep at a dose rate of 2.5 mg/kg

Parameter	Mean	SD
A (µg/ml)	5.13	1.26
P (µg/ml)	2.74	0.46
B (µg/ml)	0.98	0.57
α (h^{-1})	11.76	4.28
π (h^{-1})	0.97	0.30
β (h^{-1})	0.16	0.05
$t_{½\alpha}$ (h)	0.07 (0.06*)	0.04
$t_{½\pi}$ (h)	0.76 (0.71*)	0.17
$t_{½\beta}$ (h)	4.57 (4.31*)	1.08
K_{12} (h^{-1})	5.16	2.25
K_{21} (h^{-1})	5.41	2.12
K_{12} / K_{21}	0.97	0.31
K_{13} (h^{-1})	0.67	0.24
K_{31} (h^{-1})	0.35	0.13
K_{13} / K_{31}	1.96	0.64
K_{10} (h^{-1})	1.00	0.37
V_c (l/kg)	0.29	0.04
Vp 2 (l/kg)	0.27	0.06
Vp 3 (l/kg)	0.55	0.12
Vd_{SS} (l/kg)	1.10	0.09
Cl (l/h·kg)	0.29	0.11

*Harmonic mean
A, B, P. zero-time intercepts of the triphasic disposition curve
α, β, π : hybrid rate constants representing the slopes of distribution and elimination
$t_{½\alpha}$: fast distribution half-life
$t_{½\pi}$: slow distribution half-life
$t_{½\beta}$: elimination half-life
K_{21}, K_{12}, K_{31}, K_{13}: first-order rate constants for drug transfer to and from the central compartment
K_{10}: first-order transfer volume rate constants for elimination of the drug from the central compartment
Vc: apparent volume of the central compartment
Vp 2: apparent volume of the superficial compartment
Vp 3: apparent volume of the superficial compartment
Vd_{ss}: apparent volume of distribution at steady state
Cl: total body clearance

Table II
Mean ± SD non-compartmental pharmacokinetic parameters for enrofloxacin after its intravenous administration to eight sheep at a dose rate of 2.5 mg/kg

Parameter	Mean	SD
C_{max}	6.83	0.71
$t_{½\alpha}$ (h)	2.12	0.63
MRT (h)	3.02	1.14
AUC_{0-t} (µg·h/ml)	8.37	3.23
$AUC_{0-\infty}$ (µg·h/ml)	9.24	4.14
$AUMC_{0-t}$ (µg·h.h/ml)	22.67	14.88
$AUMC_{0-\infty}$ (µg·h.h/ml)	30.85	25.71

C_{max}: maximum plasma concentration
$t_{½\beta}$: elimination half-life
MRT: mean residence time
AUC_{0-t}: area under the plasma drug concentration-time curve from 0-time
$AUC_{0-\infty}$: area under the plasma drug concentration-time curve from 0-infinity
$AUMC_{0-t}$: area under the first-moment curve from 0-time
$AUMC_{0-\infty}$: area under the first-moment curve from 0-infinity

After IV administration of enrofloxacin the distribution phase was rapid, with a short distribution half-life ($t_{½\alpha}$) of 0.07 ± 0.04 h to the shallow peripheral compartment and a longer distribution half-life ($t_{½\pi}$) of 0.76 ± 0.17 h to the deep peripheral compartment. The distribution phase was followed by a long elimination phase, with a terminal half-life ($t_{½\beta}$) of 4.57 ± 1.08 h. The distribution kinetic of enrofloxacin was further characterised by a large volume of distribution of the drug in the body (Vd_{ss} 1.10 l/kg).

The mean areas under the plasma drug concentration-time curve from 0 to the time when the last sample was obtained (AUC_{0-t}), and from 0 to infinity ($AUC_{0-\infty}$) were 8.37 ± 3.23 µg·h/ml and 9.24 ± 4.14 µg·h/ml, respectively.

The non-compartmental IV parameters of the apparent elimination half-life ($t_{½\lambda}$) and MRT were 2.12 ± 0.63 h and 3.02 ± 1.14 h, respectively.

Following intravascular administration of enrofloxacin, low concentrations of its metabolite ciprofloxacin were detected in plasma of all animals.

The plasma concentrations of ciprofloxacin, calculated as the ratio between the AUC of ciprofloxacin and enrofloxacin, accounted for 26% of the total antimicrobial concentrations.

Ciprofloxacin maximum plasma concentration of 0.26 µg/ml was determined at 1.57 h. The mean AUC_{0-t} and $AUC_{0-\infty}$ values for ciprofloxacin were 1.37 ± 0.17 and 2.40 ± 0.76 µg·h/ml, respectively.

The PK/PD integration parameters for the *in vivo* PK data and the C_{max}/MIC and AUC/MIC were calculated from the PK parameters using low (0.05 µg/ml), intermedial (0.125 and 0.25 µg/ml) and high (0.50 µg/ml) theoretical MICs (Table III).

By using published *in vitro* MIC values of common infectious agents in sheep, and reported *in vivo* PK parameters, the PK/PD parameters for plasma after IV dosing of enrofloxacin were determined (Table IV).

Table III
Enrofloxacin pharmacokinetic/pharmacodynamic parameters after intravenous administration of 2.5 mg/ml of enrofloxacin, considering minimum inhibitory concentrations of 0.05, 0.125, 0.25 and 0.5 µg/ml and an AUC of 9.24 µg·h/ml

MIC (µg/ml)	AUC/MIC (hours)
0.05	184.8
0.125	73.92
0.25	36.96
0.50	18.48

AUC: area under the concentration vs time curve
MIC: minimum inhibitory concentration

Discussion

The introduction of *in vitro* susceptibility testing into clinical practice a long time ago, has allowed considerable advances to be made in providing relevant data to improve antimicrobial therapy. However, there is often a poor correlation between *in vitro* susceptibility and clinical outcome, principally due to failure to consider the pharmacokinetic characteristics of the drug and the *in vivo* pharmacodynamics of the selected antimicrobial in individual patients (17).

The rational use of antimicrobial drugs must be based on the knowledge of the structures and biochemical characteristics of microorganisms and on the pharmacodynamic and pharmacokinetic properties of antimicrobial drugs (2).

Fluoroquinolones are considered to be among the most effective drugs for the treatment of bacterial infections, particularly for serious infections with bacteria resistant to other antibiotic agents (10, 61). Although enrofloxacin represents a possible therapeutic alternative for the treatment of infectious diseases in sheep, there is little information about the pharmacokinetic characteristics of enrofloxacin and the PK/PD efficacy predictor parameters in sheep, which is necessary in establishing rational therapeutic dosage regimens in these animals.

High performance liquid chromatography assay was used to allow simultaneous detection of both enrofloxacin and

Table IV
Enrofloxacin pharmacokinetic/pharmacodynamic parameters after intravenous administration of 2.5 mg/ml of enrofloxacin, according to several minimum inhibitory concentrations (MIC) reported by other authors

Agents	MIC (µg/ml)	AUC/MIC	References
Corynebacterium pseudotuberculosis	≤ 0.25	36.96	Prescott and Yielding (51)
Staphylococcus aureus	0.12	77	Scheer (57)
Staphylococcus aureus	0.13	71.08	Watts *et al.* (62)
Staphylococcus aureus	0.06	154	Wetzstein and de Jong (63)
Streptococcus agalactiae, S. dysgalactiae	0.5	18.48	Watts *et al.* (62)
Mycoplasma agalactiae	0.125 - 0.5	73.92 – 18.48	Hannan *et al.* (27), Loria *et al.* (40)
Escherichia coli	0.0625	147.84	Orden *et al.* (49)
Haemophilus somnus	0.015 - 0.03	616 - 308	McDermott *et al.* (41)

$AUC_{0-\infty}$: area under the plasma drug concentration-time curve from 0-infinity

ciprofloxacin, because microbiological methods are not useful in quantifying both drugs separately. As enrofloxacin and ciprofloxacin are not equally potent and because the ratio of the two drugs does not remain constant through the dosing interval, it is not correct to use the sum of the plasma concentrations of both drugs when establishing a minimally effective antimicrobial concentration.

Enrofloxacin kinetic behaviour after IV administration was best described by a three-compartment open model, with first order distribution and elimination kinetics, and elimination from the central compartment, according to the MAICE test (64).

Other authors (8, 19, 28, 52) described enrofloxacin kinetics after IV administration in sheep with a bicompartmental model. Elsheikh *et al.* (19) and Haritova *et al.* (28) discriminated the model through Akaike's Information Criterion (64). In our study the MAICE test showed a significant difference in favour of the tricompartmental model. It must be considered that a bicompartmental model is the most commonly used, maybe because of practicality and simplicity of analysis. It must be considered too that the more sensitive the analytical technique the more likely it is that there will be evidence of a new terminal phase of elimination of the drugs.

Plasma concentration of 6.83 ± 0.71 µg/ml was measured at 0.042 h, the time when the first sample was drawn. C_{max} estimated by the sum of the *Y*-axis intercept terms (C_0), resulted 8.85 µg/ml. A mean enrofloxacin plasma concentration of 0.24 ± 0.18 µg/ml was found at 10 h.

The results showed that enrofloxacin is quickly and widely distributed after IV administration. Disappearance of enrofloxacin from plasma of sheep was characterised by an initial rapid distribution phase ($t_{½\alpha}$ 0.07 h) followed by a slower distribution phase ($t_{½\pi}$ 0.76 h). A similar mean distribution half-life for enrofloxacin (0.073 h) was reported in goats after single IV administration of 2.5 mg/kg body weight (54), and in cows (0.085 h) administered with 5 mg/kg enrofloxacin intravenously (43), although in these two cases bicompartmental models were used. Haritova *et al.* (28), using a microbiological method, reported a mean distribution half-life for enrofloxacin of 0.088 h in sheep, and Pugliese *et al.* (52) and Elsheikh *et al.* (19), with the same method, reported values of 0.17 h and 0.24 h, respectively, both of them using bicompartmental models.

Considering that in most of the other studies bicompartmental models were used, it is difficult to compare distribution and elimination half-lives between works. When bicompartmental analysis is used, the second (slower) distribution phase can be at least partially included in the elimination phase, which could explain why the elimination curve slope is more pronounced and elimination half-lives shorter when simpler models are used.

The mean values of AUC_{0-t} and $AUC_{0-\infty}$ for enrofloxacin in sheep were 8.37 µg.h/ml and 9.24 µg·h/ml, respectively. In agreement with these findings, Pozzin *et al.* (50) and Bregante *et al.* (8), using HPLC methods, reported 10.4 µg·h/ml and 8.98 µg·h/ml for AUC in sheep, respectively, at the same enrofloxacin dose. AUC values reported in the present study are comparatively higher than the 1.65 µg·h/ml, 4.19 µg·h/ml and 5.47 µg·h/ml reported in sheep by Pugliese *et al.* (52), Mengozzi *et al.* (45) and Haritova *et al.* (28), respectively. Unexpectedly, microbiological methods, which commonly give higher AUC values (consequence of enrofloxacin and ciprofloxacin summarised concentrations), used by Pugliese *et al.* (52) and Haritova *et al.* (28) at the same doses of 2.5 mg/kg gave rise to lower AUCs. In those cases the clearances reported were higher (1.3 l/h·kg and 0.6 l/h·kg respectively) than our study (0.29 l/h·kg), explaining the differences found.

On the other hand, Gavrielli *et al.* (24) and Harron *et al.* (29), using microbiological methods, reported much higher values for AUC in camels after administering enrofloxacin at a dose of 2.5 mg/kg. Gavrielli *et al.* (24) found an AUC of 18.95 µg·h/ml. Harron *et al.* (29) found AUC values of 41.5 ± 3.06 µg·h/ml and 29.31 ± 6.08 µg·h/ml for young and mature camels, respectively.

The large individual values of K_{12} (5.16 ± 2.25 h^{-1}) and K_{21} (5.41 ± 2.12 h^{-1}) indicate fast distribution into the shallow peripheral compartment. As the K_{12}/K_{21} ratio was nearly 1, the extent of distribution into the shallow peripheral compartment can be defined as intermedial.

In the case of distribution to the deep peripheral compartment, K_{13} and K_{31} values (0.67 and 0.35 h^{-1} respectively) were, logically, lower than K_{12} and K_{21}, indicating a slower transfer rate. The ratio K_{13}/K_{31} of almost 2, however, indicates extensive distribution to the deep compartment, which is coherent with the high volume of distribution and penetration into tissues of these drugs.

Values of mean central compartment volume (0.29 l/kg), mean shallow peripheral compartment volume (0.27 l/kg) and mean deep peripheral compartment volume (0.55 l/kg) seem to demonstrate the same. From the values of transference rates between compartments, and values of compartmental volumes, it is possible to calculate volumes of distribution, that in the present case are good indicators of a wide tissue distribution.

It is well known that in general, fluoroquinolones are extensively distributed with high volumes of distribution. The values of volumes of distribution in this study agree with this general statement, and indicate good penetration of biological membranes and tissue distribution. The mean Vd_{ss} of 1.10 l/kg reported here was similar to 1.20 l/kg and 1.28 l/kg reported for goats (18, 54), 1.13 l/kg for camels (24), and 1.26 l/kg in pigs (5) after intravascular administration of enrofloxacin. Using the same route of administration in sheep, Bregante *et al.* (8) reported a volume of distribution of 1.53 l/kg, calculated using the area method.

In our study, the mean enrofloxacin elimination half-life in sheep, 4.57 ± 1.08 h, resulted from both a low total body clearance and a large volume of distribution. It was similar to the mean half-lives of 4.0 h, 4.87 h and 5.76 h reported for goats (18), calves (33) and camels (29), respectively. This value is slightly higher than the mean values of 3.73 h, 3.8 h, 2.5 h, 3.6 h and 3.3 h obtained in other intravascular studies in sheep (8, 19, 28, 45, 50), 3.60 h in camels (24), and 3.38 h in llamas (13).

On the other hand, the value which we obtained was much higher when compared with the 1.46 h found in sheep (52), and the values of 1.14 h, 0.73 h and 2.39 h reported in goats (20, 53, 54). Remarkably, Pugliese *et al.* (52) and El-Sooud (20) reported a low AUC, in spite of having used a microbiological method and, in the case of the latter author, a higher dose. A much higher mean $t_{1/2\beta}$ value of 11.85 ± 0.88 h was reported in young camels (29). This very slow $t_{1/2\beta}$ of enrofloxacin, together with a very high AUC value in young camels, could reflect an immature metabolic and/or elimination system, but the authors did not present data for this parameter in adult animals.

In considering these differences in half-life of elimination, it is necessary to take into account the PK models used. When bicompartmental models are used, the elimination curve slope is more pronounced, with a larger elimination rate constant and a consequent shorter elimination half-life. In our study, when elimination half-life was calculated by non-compartmental methods, the mean value was 2.12 h, which was similar to values reported by other authors where only statistical moment theory was used.

The MRT (2.75 ± 0.75 h) was in the order of that reported by other authors in intravascular studies. Bregante *et al.* (8) reported an MRT of 3.22 h in sheep and El-Sooud (20) 2.73 h in goats. In goats, the slower MRT values of 1.5 h and 0.97 h reported by Rao *et al.* (53, 54) were in concordance with the slow elimination half-life in this species. Slightly higher values, of 3.05 h (36) and 3.89 h (58) were found in buffaloes.

The mean clearance value obtained in the present study (0.29 l/h·kg) was in the order of the 0.276, 0.277, 0.37 and 0.39 l/h·kg reported in sheep (8), camels (24), pigs (48) and calves (33), respectively. Consequently with the much shorter half-life reported in goats, Rao *et al.* (53, 54) and El-Sooud (20) found faster clearances in these species, with values of 0.81 to 1.33 l/h·kg, and 1.67 l/h·kg, respectively.

After administration of enrofloxacin by the different routes used in our study, a significant fraction of enrofloxacin was metabolised to ciprofloxacin. The metabolite accounted for 26% of the active drug plasma concentration, as calculated by the ratio between the AUC of ciprofloxacin and enrofloxacin after IV administration. This is similar to the percentages of 35% of ciprofloxacin as metabolite reported in sheep by Mengozzi *et al.* (45) after IV administration of enrofloxacin. Other authors found values in the same order of magnitude for goats. They reported that the plasma ratio of ciprofloxacin to enrofloxacin following IV injection of enrofloxacin in goats at doses of 5 mg/kg and 2.5 mg/kg was 28.8% (53) and 24% (54), respectively. Malbe *et al.* (43) found that the serum AUC values for ciprofloxacin relative to those of enrofloxacin were 29% after IV injection of enrofloxacin in cows. Cester and Toutain (12) developed a comprehensive model of enrofloxacin metabolism in dogs, where they demonstrate that enrofloxacin

transformation to ciprofloxacin is slow but occurs in large amounts.

Antimicrobial drugs which act predominantly by concentration-dependent mechanisms generally have significant post-antibiotic effects. Such drugs continue to inhibit bacterial growth for a period of hours after their plasma concentrations are below the MIC (2, 38). Results obtained in disease models have established that optimal outcome of therapy with this type of bactericide agents requires attainment of high concentrations, and the success of therapy correlates with the AUC/MIC (AUIC) ratio, while prevention of the development of resistance correlates with the C_{max}/MIC ratio.

Fluoroquinolones are concentration-dependent antimicrobial agents and exert long post-antibiotic antibacterial effects. It has been demonstrated that C_{max}/MIC and AUIC ratios are highly correlated with the successful outcome of treatment with these antimicrobial agents. Therefore, optimal dosing of quinolones involves administration of large doses with long inter-dose intervals, because the post-antibiotic effect prevents bacterial regrowth even when serum and tissue concentrations decrease below MIC (1, 14, 28).

Peak plasma concentrations/MIC ratios of 8 or higher should be obtained, and AUIC ratios greater than 100 h and in some cases than 250 h are desirable (2, 14). On the other hand, for drugs that act by concentration-dependent mechanism, maintenance of drug concentrations in excess of the MIC (*T*>MIC) for long periods is less important in determining the outcome of therapy (2, 14, 38).

Enrofloxacin and ciprofloxacin have a broad spectrum of antimicrobial activity, with MIC values ranging from 0.008 µg/ml to 0.06 µg/ml for the more sensitive microorganisms, and from 0.125 µg/ml to 0.50 µg/ml for the less sensitive microorganisms (51, 57). Scheer (57) suggested that a MIC value of 1.0 µg/ml can be taken as the breakpoint for sensitivity or resistance. Later, the Clinical and Laboratory Standards Institute, formerly the National Committee for Clinical Laboratory Standards, suggested breakpoints for enrofloxacin of 0.5 µg/ml for susceptible microorganisms, 1 µg/ml for microorganisms of intermediate susceptibility and 2 µg/ml for those that are resistant (46).

In our study, enrofloxacin achieved concentrations in plasma which were above the MIC for common ovine pathogens, including *Corynebacterium pseudotuberculosis* (51), *Staphylococcus aureus* (57), *Streptococcus agalactiae* and *S. dysgalactiae* (62), *Mycoplasma agalactiae* (27, 40), *Escherichia coli* (49) and *Haemophilus somnus* (41). Considering that a C_{max}/MIC of 8 or higher and/or an AUC/MIC of above 125 should be attained in order to accomplish bacteriological cure and avoid emergence of resistant strains, the IV administration route is an interesting possibility.

Considering the MIC for the common bacterial pathogens in sheep (Table IV), the AUC/MIC ratios were below the desirable value of 125 for several of the considered microorganisms after IV enrofloxacin administration of 2.5 mg/kg. However, these derived values do not take into account the contribution made by the active metabolite ciprofloxacin, and therefore underestimate enrofloxacin efficacy. Mean plasma concentrations of the sum of enrofloxacin and ciprofloxacin at 10 h (0.29 µg/kg) following IV administration of enrofloxacin at 2.5 mg/kg exceeded the MIC of many pathogenic bacteria isolated from sheep. It must be taken into account that all these values refer to the plasmatic concentration, and that for drugs like enrofloxacin, which is characterised by an excellent distribution, considerably higher concentrations would be expected in tissues, easily reaching the ideal AUC/MIC ratio. We conclude that enrofloxacin pharmacokinetic parameters after IV administration in sheep at 2.5 mg/kg of body weight are characterised by high bioavailability and a high volume of distribution, implying the likelihood of high tissue concentrations.

■

Pharmacocinétique de l'enrofloxacine après injection unique en intraveineuse chez les ovins

J.L. Otero, N. Mestorino & J.O. Errecalde

Résumé

Une étude a été conduite sur les données d'élimination de l'enrofloxacine chez les ovins après administration par voie intraveineuse d'une dose unique de 2,5 mg/kg de poids corporel. Des prises de sang ont été réalisées à la veine jugulaire après l'injection, à des intervalles prédéfinis. Les concentrations plasmatiques de l'enrofloxacine et de son métabolite actif, la ciprofloxacine, ont été visualisées simultanément, par chromatographie en phase liquide à haute performance à polarité de phase inversée. Les données recueillies ont été soumises à des analyses cinétiques, sans séparation et avec séparation. Les paramètres pharmacocinétiques sans séparation ont été déterminés au moyen de la théorie des modèles statistiques. L'élimination de l'enrofloxacine a été décrite au moyen d'un modèle ouvert à trois compartiments, l'élimination intervenant à partir du compartiment central suite à l'administration intravasculaire.

Les résultats ont été les suivants : demi-vie d'élimination : 4,31 h ; volume d'élimination : 1,10 l/kg ; et aire sous la courbe (AUC) représentant la concentration en fonction du temps : 9,24 µg·h/ml.

L'enrofloxacine a été métabolisée en ciprofloxacine ; le rapport des AUC de la ciprofloxacine et l'enrofloxacine mesurées après l'injection intraveineuse était de 0,26.

Les valeurs prédictives d'efficacité de l'enrofloxacine (ratios plasmatiques concentration maximale/concentration minimale inhibitrice [CMI] et AUC/CMI) ont généré, pour la plupart des agents pathogènes affectant les ovins, des scores supérieurs à 15 et à 50, respectivement. Injectée par voie intraveineuse à une dose de 2,5 mg/kg, l'enrofloxacine atteint des concentrations plasmatiques supérieures à plusieurs fois la CMI des principales bactéries et peut s'avérer utile pour le traitement des maladies infectieuses ovines causées par des agents pathogènes sensibles.

Mots-clés

Ciprofloxacine – Enrofloxacine – Ovin – Pharmacocinétique – Voie intraveineuse.

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Farmacocinética de enrofloxacina en ovinos tras su administración intravenosa

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Resumen

Se evaluó la disponibilidad plasmática de enrofloxacina en ovinos tras su administración en dosis única (2,5 mg/kg de peso corporal) por la vía intravenosa. Posteriormente a la administración del antimicrobiano, se obtuvieron muestras de sangre de la vena yugular a diferentes tiempos preestablecidos. Las concentraciones plasmáticas de enrofloxacina y de su metabolito activo, ciprofloxacina, fueron determinadas simultáneamente por cromatografía líquida de alta resolución. Con los datos obtenidos se realizó un

análisis cinético no compartimental y compartimental. Para determinar los parámetros farmacocinéticos no compartimentales se aplicó la teoría de modelos estadísticos. La disponibilidad plasmática de enrofloxacina luego de su administración intravenosa fue descrita siguiendo un modelo tricompartimental abierto con eliminación desde el compartimento central.
Se obtuvieron los siguientes valores de semivida de eliminación, volumen de distribución y área bajo la curva de concentración en función del tiempo (AUC): 4,31 horas, 1,10 l/kg y 9,24 µg·h/ml, respectivamente.
Enrofloxacina fue metabolizada a ciprofloxacina, dando una relación entre AUC de ciprofloxacina/AUC de enrofloxacina de 0,26 tras su administración intravenosa.
Aplicando la integración farmacocinética/farmacodinámica, los parámetros predictores de eficacia para enrofloxacina frente a la mayoría de los microorganismos patógenos de los ovinos (concentración plasmática máxima/concentración inhibitoria mínima [C_{max}/CIM] y AUC/CIM en plasma), fueron superiores a 15 y 50 respectivamente. Tras la administración intravenosa a razón de 2,5 mg/kg, enrofloxacina alcanzó concentraciones plasmáticas varias veces superiores a la CIM de los principales patógenos, lo que revela que puede ser útil para tratar enfermedades infecciosas de los ovinos causadas por patógenos sensibles al fármaco.

Palabras clave
Ciprofloxacina – Enrofloxacina – Farmacocinética – Intravenosa – Ovinos.

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