Pharmacokinetics and milk penetration of difloxacin after a long-acting formulation for subcutaneous administration to lactating goats

P. Marín,¹ E. Escudero, E. Fernández-Varón, M. J. Ramírez, and C. M. Cárceles

Department of Pharmacology, Faculty of Veterinary Medicine, University of Murcia, Campus de Espinardo, 30.071-Murcia, Spain

ABSTRACT

The single-dose disposition kinetics of difloxacin were determined in clinically normal lactating goats ($n = 6$) after intravenous (IV) and subcutaneous (SC) administration and subcutaneous administration of a long-acting poloxamer 407 gel formulation (P407). Difloxacin concentrations were determined by HPLC with fluorescence detection. Minimum inhibitory concentrations of difloxacin against 14 strains of *Staphylococcus aureus* isolated from mastitic goats' milk in Spain were determined to compute pharmacodynamic surrogate markers. The concentration-time data were analyzed by compartmental and noncompartmental pharmacokinetic methods. Following SC and P407 administration, difloxacin achieved maximum milk concentrations of 1.34 ± 0.12 and 2.97 ± 1.18 mg/L, respectively, at 4.00 ± 0.00 h (SC) and 3.60 ± 0.89 h (P407) after administration. The absolute bioavailabilities after SC and P407 administration were $81.74 \pm 15.60\%$ and $72.58 \pm 20.45\%$, respectively. Difloxacin penetration from the blood into the milk was good and high concentrations were found in milk secretions. From these data, a 15 mg/kg dose of difloxacin P407 would appear to be effective against *Staphylococcus aureus* isolated from mastitic goats' milk with minimum inhibitory concentrations ≤ 0.25 mg/L.

Key words: difloxacin, pharmacokinetics, long-acting formulation, lactating goat

INTRODUCTION

Difloxacin is a fluoroquinolone carboxylic acid antimicrobial agent with high in vitro activity against a wide range of gram-positive and gram-negative aerobes and anaerobes (Granneman et al., 1986). Like other fluoroquinolones, difloxacin is a concentration-dependent bactericidal agent and it acts by inhibiting bacterial DNA topoisomerases II and IV (Wolfson and Hooper, 1989; Drlica and Zhao, 1997). It has been shown to

be effective in the treatment of experimentally induced pneumonic pasteurellosis in calves (Olchoway et al., 2000) and experimental *Staphylococcus aureus* endocarditis in rabbits (Boscia et al., 1988).

Poloxamer 407 (P407) is a polyoxyethylene:polyoxypropylene block co-polymer that contains 7% polyoxyethylene units and 30% polyoxypropylene blocks. This polymer can be used in some formulations because of its low toxicity, excellent compatibility with other chemicals, and high-solubilizing capacity for different drugs. Poloxamer 407 exhibits temperature-dependent gelation (gel at 37°C and liquid at 4°C ; Zhang et al., 2002). The P407 gel has been evaluated for the delivery of biologically active proteins such as urease and interleukin-2 (Johnston et al., 1992; Pec et al., 1996) and antibiotics such as ceftiofur (Zhang et al., 2002). Pharmacokinetic characteristics of several drugs have been improved by P407 gel, such as melanotan-I (Bhardwaj and Blanchard, 1996), insulin (Yun et al., 1999), and tacrolimus (Overhoff et al., 2008).

In Europe, the problem of mastitis is a highly relevant issue not only because of the economic losses to producers, but also with regard to the hygienic and safety aspects of dairy products intended for human consumption, particularly with respect to bacteriological quality. Intramammary infections in dairy goats are mainly of bacterial origin. Most of the published papers about mastitis treatments report clinical observations or recommendations adapted from results obtained in cows. Therefore, inappropriately low doses of an antibiotic can lead to therapeutically ineffective blood or milk concentrations and increased risk of development of resistant microorganisms. Equally, toxicity may result if the extrapolated dose is too high.

It is generally accepted that xenobiotics cross the blood–milk barrier in the udder by nonionic passive diffusion. The extent to which a drug passes from the plasma to the milk when it is administered systemically depends on its main pharmacokinetic properties: lipid solubility, degree of ionization, and extent of binding to serum and udder proteins (Ziv, 1980). Drug disposition may change as an outcome of disease state. The milk to serum concentration ratio for drugs is often unknown in goats.

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¹Corresponding author: pmarin@um.es

Thus, the objective of the present investigation was to study the pharmacokinetics of difloxacin in lactating goats following intravenous and subcutaneous administration and after subcutaneous administration as a long-acting P407 gel formulation, and to compute the main surrogate efficacy markers using MIC determined against *Staphylococcus aureus* strains isolated from mastitic goats' milk.

MATERIALS AND METHODS

Animals

Six clinically healthy Murciano-Granadina female goats weighing between 47.5 and 64.5 kg and from 4 to 5 yr of age were obtained from the caprine farm of the University of Murcia (Spain). The animals were housed and fed an antibiotic-free diet for at least 30 d preceding the study. For each treatment period of the crossover, goats were observed daily for general health, and clinical observations were made before injection and at 2, 10, and 24 h postinjection. Alfalfa hay and water were provided ad libitum together with a drug-free concentrate.

Each goat was machine-milked once a day except on sampling days when milk sampling for the study was performed as specified in the experimental design section. The number of actual lactation cycles of the participating goats was 2 or 3 and all animals were in mid lactation. Milk production (mean $\pm$ SD) was 2.00 ± 0.46 L/d.

The animals had been vaccinated against common infectious diseases and last received anthelmintic drugs prophylactically at least 2 mo prior to the start of the studies. Milk samples of all animals, before inclusion in this study, were microbiologically screened. The study was approved by the Bioethical Committee of the University of Murcia (Spain).

Experimental Design

A crossover design ($2 \times 2 \times 2$) was used in 3 phases. In each phase, each animal received a single intravenous (IV) or subcutaneous (SC) injection of difloxacin 5% (Dicural, Fort Dodge Veterinaria, Gerona, Spain) at a dose of 5 mg/kg of BW or a subcutaneous administration of a long-acting P407 gel formulation (P407) of difloxacin at 15 mg/kg of BW with at least a 21-d washout period.

For IV administration, the solution was injected into the left jugular vein, and blood samples (4 mL) were collected from the contralateral jugular vein into heparinized tubes. Subcutaneous injections were administered under the skin of the back at a single location in

the thoraco-lumbar region lateral of the midline. Blood samples were collected at 0 (pretreatment), 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, and 144 h postdosing. Samples were centrifuged at $1,500 \times g$ for 15 min and the plasma taken and stored at -45°C until assayed.

Milk samples for analysis were collected from homogenized milking yields collected immediately before dosing on the day of treatment administration (time 0) and at 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, and 144 h postadministration. Milk samples were collected after complete evacuation of the udder by manual stripping of each gland as completely as possible, to avoid a dilution effect during the normal milking routine (once a day). For each sample, 2 aliquots of 5 mL of milk were stored at -20°C until analysis.

Gel Preparation

Gel was prepared on a weight basis using the cold method (Schmolka, 1972). Concentrations of P407 and difloxacin reported here are expressed as weight percentage (% wt/wt). An amount of P407 sufficient to yield 25% gel was slowly added to cold water (5°C) maintaining constant stirring. The dispersion was kept refrigerated until a clear solution was formed (6–12 h). Difloxacin sufficient to yield a 5% concentration was then dissolved in the cold solution.

Bacterial Isolates

Fourteen goat strains of *Staphylococcus aureus*, all isolated from mastitic goats' milk in Spain, were tested.

Dilution Susceptibility Testing

The MIC were determined using the Clinical and Laboratory Standards Institute microdilution broth method (CLSI, 2009). After overnight incubation at 37°C , the inocula were suspended in 0.9% NaCl solution to a 0.5 McFarland standard and finally diluted again 40-fold before testing. The MIC assays were performed in U-bottomed, 96-well, custom-designed microtiter plates. The plates contained antimicrobial dilutions (difloxacin concentrations ranging from 0.03 to 128 $\mu\text{g/mL}$) in 100 μL /well of cation-adjusted Mueller-Hinton broth inoculated with the different strains. The U-bottomed microtiter plates were incubated at 37°C and observed after 24 h. The MIC was defined as the lowest concentration producing no visible growth. The reference strains *Staph. aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) were used as controls for each plate.

Analytical Method

Plasma and milk concentrations of difloxacin were measured using a modified HPLC method as previously reported (Siefert et al., 1999). The HPLC system was equipped with a model LC-10ASvp pump, an RF-10AXL fluorescence detector, and a model SIL-10ADvp autoinjector (Shimadzu, Kyoto, Japan). The above-mentioned system was connected to a computer with a Shimadzu class-VP Chromatography Data System program (Shimadzu, Washington, DC).

Difloxacin pure substance (Fort Dodge Veterinaria) was used for quality controls, and ciprofloxacin (Vita Pharma, Madrid, Spain) was used as internal standard. After addition of 10 μ L of the internal standard to 200 μ L of plasma or milk, 200 μ L of acetonitrile was added. Plasma and milk proteins were precipitated by shaking in an ultrasonic bath followed by centrifugation for 10 min at $1,600 \times g$. The supernatant was diluted 4-fold with mobile phase and transferred to HPLC autosampler vials. The HPLC separation was performed using a reverse-phase Kromasil C₁₈ column (250 $\times$ 4 mm, 5- μ m particle size; Tecnokroma, Barcelona, Spain) with an injection volume of 50 μ L. Autosampler vials and column temperature were set at 25°C. The mobile phase consisted of acetonitrile (16%) and tetrabutylammonium hydrogen sulfate solution (5 g/L; 84%) for difloxacin determination in plasma, and acetonitrile (20%) and trifluoroacetic acid solution (0.1%; 80%) for difloxacin determination in milk, using an isocratic method with a flow rate of 1.0 mL/min in both methods. Difloxacin eluted at approximately 9.0 min. Fluorescence detection was performed at an excitation wavelength of 280 nm and an emission wavelength of 445 nm.

Method Validation

This method was validated before the start of sample analysis. The selectivity of the method was demonstrated because no interfering peaks from endogenous compounds in the blank goat plasma or milk samples were observed with the same retention times as difloxacin or ciprofloxacin in the chromatograms of blank samples.

Quality controls were prepared from a pool of blank goat plasma or milk spiked with 9 concentrations of difloxacin between 5 and 2,000 μ g/L. Plasma and milk aliquots were stored at -45°C until assay. Aliquots of quality control samples were extracted as above and 50 μ L of each was injected into the chromatographic system. Standard curves were obtained by unweighted linear regression of difloxacin peak areas versus known concentrations. Each point was established from an average of 5 determinations. Correlation coefficients

(*r*) were >0.999 for calibration curves. The percentage recovery was determined by comparison of the peak areas of plasma and milk blank samples spiked with different amounts of drug and treated as any samples, with the peak areas of the same standards prepared in phosphate buffer. Each point was established from an average of 5 determinations. The mean percentage recoveries of difloxacin from plasma and milk were 97.19 ± 4.27 and $98.35 \pm 7.21\%$, respectively. The assay precision (relative standard deviation, **RSD**) was assessed by expressing the standard deviation of repeated measurements as a percentage of the mean value. Intraday precision was estimated from 6 replicates of 3 standard samples (plasma or milk) used for calibration curves (**RSD** $<5.8\%$). Interday precision was estimated from the analysis of standard samples (plasma or milk) on 3 separate days (**RSD** $<6.9\%$). The limit of quantification for plasma and milk was 5 and 10 μ g/L, respectively.

Pharmacokinetic Analysis

The plasma concentration-time data obtained after IV administration in each individual animal were initially fitted to 1-, 2-, and 3-exponential equations by the retroprojection method (Gibaldi and Perrier, 1982). The PKCALC computer program (Shumaker, 1986) was used to obtain the best estimates of the parameters of these equations. The final curve-fitting was carried out using nonlinear regression with the MULTI computer program and the Gauss-Newton damping algorithm (Yamaoka et al., 1981). Akaike's information criterion (Yamaoka et al., 1978) was used to determine the best equation that defined plasma concentration-time data for each animal and the most appropriate weighting of these data. Pharmacokinetic parameters were obtained from the individual fitted equations (Gibaldi and Perrier, 1982). The disposition half-lives ($t_{1/2}$) were calculated as $t_{1/2\lambda_1} = \ln 2/\lambda_1$ and $t_{1/2\lambda_z} = \ln 2/\lambda_z$ where λ_1 = largest (fastest) disposition (= hybrid) rate constant, and λ_z = smallest (slowest) disposition (= hybrid) rate constant.

Noncompartmental pharmacokinetics parameters were estimated using WinNonlin Professional (version 5.1, Pharsight Corp., Mountain View, CA). WinNonlin model 200 was used for extravascular administrations and model 201 for intravenous administration. Noncompartmental parameters calculated were area under the plasma concentration-time curve (**AUC**) using the linear trapezoidal rule with extrapolation to time infinity, mean residence time (**MRT**), plasma clearance (**Cl**), and apparent volume of distribution at steady state (**V_{ss}**). Mean absorption times (**MAT**) were calculated as $\text{MAT} = \text{MRT}_{\text{SC, P407}} - \text{MRT}_{\text{IV}}$.

Figure 1. Semilogarithmic plot of plasma (●) and milk (○) concentrations (mean $\pm$ SD) of difloxacin following a single intravenous dose of 5 mg/kg of BW ($n = 6$).

Bioavailability (**F**) was calculated by the method of corresponding areas:

$$F (\%) = [(AUC_{SC, P407}/D_{SC, P407})/(AUC_{IV}/D_{IV})] \times 100,$$

where D is the administered dose.

The milk concentration-time data were analyzed by noncompartmental methods using WinNonlin Professional program (version 5.2; Pharsight Corp.). Drug concentrations at each sampling time interval and the volume of milk at each time interval were used to calculate the cumulative amounts of difloxacin eliminated in milk and the terminal half-life; AUC_{milk} was calculated using the linear trapezoidal rule with extrapolation to infinity.

Statistical Analysis

Descriptive statistical parameters as mean and standard deviation were calculated. Harmonic means were calculated for the half-lives of disposition. The Wilcoxon rank sum test and Student's *t*-test were used to test parameters for significant differences ($P < 0.05$) between IV, SC, and P407 administration (Powers, 1990). The SPSS (version 11.0; SPSS Inc., Chicago, IL) statistical software was used.

RESULTS

The difloxacin plasma concentration versus time data after IV administration could best be described by a 2-compartment open model, for which the general polyexponential equation is

$$C = \sum_{i=1}^n C_i \cdot e^{-\lambda_i t},$$

Figure 2. Semilogarithmic plot of plasma (●) and milk (○) concentrations (mean $\pm$ SD) of difloxacin following a single subcutaneous dose of 5 mg/kg of BW ($n = 6$).

where C is the plasma concentration of drug; t is the time after drug administration; C_i and λ_i are the intercept and slope, respectively, of the different disposition phases, and e is the base of natural logarithm. The mean ($\pm$ SD) plasma and milk concentrations of difloxacin following IV, SC, and P407 administration are plotted in Figures 1, 2, and 3, respectively. The mean ($\pm$ SD) pharmacokinetic parameters based on compartmental and noncompartmental pharmacokinetic analysis are presented in Table 1. The MIC values and surrogate markers are shown in Tables 2 and 3, respectively.

For plasma, significant differences were found between IV and SC administration for MRT; between IV and P407 administration for λ_z , $t_{1/2\lambda_z}$, AUC, and MRT; and between SC and P407 administration for λ_z , $t_{1/2\lambda_z}$,

Figure 3. Semilogarithmic plot of plasma (●) and milk (○) concentrations (mean $\pm$ SD) of difloxacin following subcutaneous administration of a long-acting poloxamer 407 gel formulation at a dose of 15 mg/kg of BW ($n = 6$).

AUC, MRT, MAT, and maximum concentration following administration ($C_{\max}$). With respect to milk, significant differences were found between IV and SC administration for $C_{\max}$, time to reach maximum concentration following administration ($T_{\max}$), and $t_{1/2\lambda_z}$; between IV and P407 administration for $T_{\max}$, $t_{1/2\lambda_z}$, and AUC_{milk} ; and between SC and P407 administration for $C_{\max}$, $t_{1/2\lambda_z}$, and AUC_{milk} .

Clinical examination of all goats before and after each phase of the trial did not reveal any abnormalities. No local or systemic adverse reactions occurred after IV, SC, or P407 injection of difloxacin.

DISCUSSION

During the last decade, several fluoroquinolones such as difloxacin, enrofloxacin, danofloxacin, sarafloxacin, and marbofloxacin have been carefully investigated for veterinary application in the treatment of bacte-

rial infections. Many papers have been published on the pharmacokinetics of these antimicrobial agents in various animal species, but there are limited published data on long-acting formulations of these antibiotics in animals. The purpose of studying long-acting formulations of difloxacin for lactating goats is to use a lesser quantity of drug (decreasing the collateral effects and accumulation in long-term treatments), increase treatment efficacy (less fluctuation of the stationary concentrations and longer release times), and reduce handling (stress of animals and veterinary costs are decreased).

The disposition of difloxacin after IV administration according to a 2-compartment open model is in agreement with other pharmacokinetic studies with fluoroquinolones in goats (Abd El-Aty and Goudah, 2002; Atef et al., 2002; Marín et al., 2007a,b,c; Goudah and Mouneir, 2008).

Difloxacin showed a wide distribution in lactating goats with a V_{ss} of 1.05 ± 0.19 L/kg, suggesting, as seen

Table 1. Pharmacokinetic parameters (mean $\pm$ SD) of difloxacin in goats ($n = 6$) after intravenous and subcutaneous administration at a dose of 5 mg/kg of BW, and as a long-acting poloxamer 407 (P407) gel formulation for subcutaneous administration at a dose of 15 mg/kg of BW

Parameter ¹	Unit	Mode of administration		
		Intravenous	Subcutaneous	P407
Plasma				
λ ₁	h ⁻¹	1.05 ± 0.31	—	—
λ _z	h ⁻¹	0.13 ± 0.04	0.12 ± 0.04	0.02 ± 0.01 ^{a,b}
t _{1/2λ₁}	h	0.66	—	—
t _{1/2λ_z}	h	5.23	5.69	32.36 ^{a,b}
V _{ss}	L/kg	1.05 ± 0.19	—	—
AUC	mg·h/L	21.93 ± 6.86	17.07 ± 2.74	41.50 ± 2.75 ^{a,b}
AUC ₂₄	mg·h/L	21.20 ± 6.22	16.67 ± 2.61	26.77 ± 1.18 ^{a,b}
MRT	h	4.61 ± 1.64	7.39 ± 0.61 ^a	38.85 ± 6.16 ^{a,b}
Cl	L/h·kg	0.25 ± 0.09	—	—
MAT	h	—	2.78 ± 1.34	33.88 ± 5.10 ^b
C _{max}	mg/L	—	1.97 ± 0.14	3.10 ± 1.29 ^b
T _{max}	h	—	3.33 ± 1.03	2.60 ± 1.23
F (%)	—	—	81.74 ± 15.60	72.58 ± 20.45
Milk				
C _{max}	mg/L	3.09 ± 1.09	1.34 ± 0.12 ^a	2.97 ± 1.18 ^b
T _{max}	h	1.16 ± 0.41	4.00 ± 0.00 ^a	3.60 ± 0.89 ^a
t _{1/2λ_z}	h	6.24	4.02 ^a	26.44 ^{a,b}
AUC _{milk}	mg·h/L	15.10 ± 7.51	11.46 ± 1.61	38.07 ± 8.05 ^{a,b}
AUC _{milk24}	mg·h/L	14.44 ± 6.97	11.20 ± 1.60	26.03 ± 7.70 ^{a,b}
C _{max-milk} /C _{max-plasma}	—	—	0.68 ± 0.08	0.95 ± 0.34
AUC _{milk} /AUC _{plasma}	—	—	0.67 ± 0.04	0.92 ± 0.20 ^b

^aSignificantly different from intravenous administration ($P < 0.05$); ^bsignificantly different from subcutaneous administration ($P < 0.05$).

¹ $t_{1/2\lambda_1}$ = disposition half-life associated with the initial slope (λ_1) of a semilogarithmic concentration-time curve (harmonic mean); $t_{1/2\lambda_z}$ = disposition half-life associated with the terminal slope (λ_z) of a semilogarithmic concentration-time curve (harmonic mean); V_{ss} = apparent volume of distribution at steady state; AUC = area under the plasma concentration-time curve from zero to infinity; MRT = mean residence time; Cl = total body clearance of drug from the plasma; F = fraction of the administered dose systemically available (i.e., bioavailability); $T_{\max}$ = time to reach peak or maximum plasma or milk concentration following extravascular administration; MAT = mean absorption time; $C_{\max}$ = peak or maximum plasma or milk concentration following extravascular administration; AUC_{milk} = area under the milk concentration-time curve from zero to infinity.

²Harmonic mean.

Table 2. Number of *Staphylococcus aureus* strains isolated from mastitic goats' milk (n = 14) by difloxacin MIC

Species	Origin	Difloxacin MIC ($\mu\text{g/mL}$)													
		≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
<i>Staph. aureus</i>	Goats' milk				7	5	1	1							
<i>Staph. aureus</i> ATCC 29213	Control				X										
<i>Escherichia coli</i> ATCC 25922	Control		X												

for other fluoroquinolones, good penetration through biological membranes. This V_{ss} is similar to those determined in goats for other fluoroquinolones (Atef et al., 2002; Marín et al., 2007a,b,c) and for difloxacin in other species (Fernández-Varón et al., 2006; Marín et al., 2007d).

The terminal half-life ($t_{1/2\lambda z}$) after IV dosing was 5.23 h, very close to that reported for several fluoroquinolones in lactating goats (Elmas et al., 2001; Atef et al., 2002; Marín et al., 2007a,c). Elimination (terminal) half-lives ($t_{1/2\lambda z}$) after SC and P407 dosing were 5.69 and 32.36 h, respectively. The value after SC administration was lower than those reported in rabbits (7.53 h; Fernández-Varón et al., 2008) and sheep (12.02 h; Marín et al., 2007d). The drug was eliminated from plasma at a significantly slower rate after P407 treatment than after IV treatment, suggesting the presence of a “flip-flop” effect after P407 administration, because MAT was greater than MRT_{IV} (Toutain and Bousquet-Mélou, 2004). In that model, the last phase of the curve is determined by the absorption rate constant and not by the apparent elimination constant, because the rate of absorption is a limiting factor for the elimination process.

Difloxacin was well absorbed following SC and P407 administration, with absolute bioavailabilities (F) of 81.74 and 72.58%, respectively. Similar high values have been obtained for difloxacin in rabbits, goats, lambs, pigs, and broilers (Fernández-Varón et al., 2006; Marín et al., 2007a; Ding et al., 2008; Goudah and Mouneir, 2008).

Different fluoroquinolones has been studied for allometric scaling. Data indicated that allometric scaling of the species studied was feasible and that the use of clearance and volume of distribution was preferable to half-life (Cox, 2007); that study included sheep but not cattle data. It was concluded that volume of distribution was proportional to body weight, and clearance was allometrically related to body mass for moxifloxacin, difloxacin, marbofloxacin, and danofloxacin (Cox, 2007).

The various solute transport and secretion processes involved in milk production offer pathways for the movement of the drug molecules from plasma to milk (McManaman and Neville, 2003). For the movement of

drugs from plasma to milk, however, passive diffusion seems to be the most likely route of transport.

Most drugs are weak organic acids or bases and exist in solution (in plasma or milk) in both ionized and un-ionized forms. The proportion of the drug that is in the un-ionized state is dependent on the pK_a (acid dissociation constant) value of the drug and the pH of the environment, with the pK_a of the drug being the pH at which 50% of the drug molecules are in the ionized state. Provided the drug molecule itself is sufficiently lipophilic, it is the un-ionized form that is able to diffuse across the lipid membranes. Another important factor that affects distribution of drugs into tissues is the extent of reversible binding to proteins in the solvent (plasma or milk) and tissues. This nonspecific interaction between drug and proteins results in the formation of drug-protein complexes, which are too large to cross tissue membranes (Gehring and Smith, 2006). The extent of serum protein binding of difloxacin in goats was 13.79% (Atef et al., 2002), so its influence in distribution processes will be not clinically significant. Equally, Goudah and Mouneir (2008) reported a low level of binding to plasma proteins of difloxacin for goat kids (10.31%) and lambs (12.98%).

Difloxacin, like other fluoroquinolones, is amphoteric because of the presence of a carboxylic acid and one or more basic amine functional groups. At a pH of 6

Table 3. Surrogate markers of efficacy from milk pharmacokinetics parameters obtained after subcutaneous injection and subcutaneous administration of a long-acting poloxamer 407 gel formulation (P407) of difloxacin at a single dose of 5 and 15 mg/kg of BW (n = 6), respectively

Parameter ¹	Subcutaneous	P407
MIC = 0.25 mg/L		
AUC ₂₄ /MIC	44.8	104.12
C _{max} /MIC	5.36	11.88
MIC = 0.5 mg/L		
AUC ₂₄ /MIC	22.4	52.06
C _{max} /MIC	2.68	5.94
MIC = 1 mg/L		
AUC ₂₄ /MIC	11.2	26.03
C _{max} /MIC	1.34	2.97

¹AUC = area under the plasma concentration-time curve; C_{max} = peak or maximum plasma or milk concentration following extravascular administration. Surrogate markers: C_{max}/MIC₉₀ = 8 (Craig, 1998) and AUC₂₄/MIC₉₀ = 100 (Turnidge, 1999).

to 8, this compound is sufficiently lipid-soluble to be able to penetrate tissues (Brown, 1996). Therefore, the extensive penetration of difloxacin from the blood to goat's milk was predictable based on the ion trap mechanism (Atkinson and Begg, 1990). We used 2 ratios to evaluate penetration of difloxacin into milk: $AUC_{\text{milk}}/AUC_{\text{plasma}}$ and $C_{\text{max-milk}}/C_{\text{max-plasma}}$ ratios. The $AUC_{\text{milk}}/AUC_{\text{plasma}}$ ratios were 0.67 and 0.92 after SC and P407 administration, respectively. These data showed better penetration into the milk of difloxacin after P407 administration than after SC injection. Moreover, the $C_{\text{max-milk}}/C_{\text{max-plasma}}$ ratio (≥ 1) shows that high concentrations of difloxacin are reached in milk after P407 dosing. These findings are in agreement with several reports of other fluoroquinolones in lactating animals (Marín et al., 2007a,c).

Staphylococcus aureus is the most important etiological agent involved in all forms of goat mastitis. The prevalence of *Staph. aureus* IMI ranged from 5 to 6% to 17% (Lerondelle and Poutrel, 1984; Kalogridou-Vassiliadou, 1991; White and Hinckley, 1999) with some researchers reporting identification of *Staph. aureus* in up to 37% of subclinical mastitis isolates (Silva et al., 2004). Efficacy of difloxacin treatment of mastitis in goats has not been tested, but the efficacy of the drug against respiratory infections of lambs (Mavrogianni and Fthenakis, 2005), calves (Olchoway et al., 2000), dogs (van den Hoven et al., 2000), and poultry (Kempf et al., 1998) has been well documented. Low MIC values of difloxacin against *E. coli* have been reported (MIC = 0.065 $\mu\text{g/mL}$; Abd El-Aty et al., 2005), and similar results have been published by Stamm et al. (1986; MIC = 0.03–0.2 $\mu\text{g/mL}$). Moreover, Fernández-Varón et al. (2008) have reported a MIC of difloxacin (0.5 $\mu\text{g/mL}$) against *Staph. aureus* strains isolated from commercial rabbitries with chronic problems of staphylococcosis.

Antimicrobial drugs that act predominantly by concentration-dependent mechanisms generally exert significant postantibiotic sub-MIC effects. Such drugs continue to inhibit bacterial growth for a period of hours after they have been completely removed from the system. Work in disease models has established that optimal outcome of therapy with this type of bactericide requires attainment of high concentrations, and the success of therapy correlates with the AUC/MIC ratio, whereas prevention of the development of resistance correlates with the $C_{\text{max}}/\text{MIC}$ ratio (Shojaee Aliabadi and Lees, 2000).

Consequently, the ratios $C_{\text{max}}/\text{MIC}_{90}$ and AUC_{24}/MIC_{90} are the best parameters for predicting the antimicrobial effect of fluoroquinolones (Lode et al., 1998). Previous investigations have shown that for fluoroquinolones, a $C_{\text{max}}/\text{MIC}_{90} > 3$ produced 99% reduction in bacterial count and a $C_{\text{max}}/\text{MIC}_{90}$ of ≥ 8 prevented the

emergence of resistant organisms (Craig, 1998). Furthermore, $AUC_{24}/\text{MIC}_{90} > 100$ h should be achieved to give maximum clinical and bacteriological efficacy (Turnidge, 1999). The MIC values of difloxacin against *Staph. aureus* strains isolated from mastitic goats' milk have been tested in the present work (Table 2). The $C_{\text{max}}/\text{MIC}_{90}$ and AUC_{24}/MIC_{90} ratios were calculated (Table 3), and from these ratios, difloxacin appeared to present optimum ratios for bacteria with MIC of 0.25 mg/L for P407 administration. However, from these data and according to MIC_{90} of 0.5 mg/L, difloxacin would not be appropriate for treating *Staph. aureus* intramammary infections in goats.

However, it is necessary to note that the numerical values of $C_{\text{max}}/\text{MIC}_{90}$ and AUC_{24}/MIC_{90} used as surrogate markers to predict optimal dosage were generated in experimental infections in laboratory animals or in human clinical trials (Toutain and Lees, 2004); thus, these numerical values may not be applicable to goat infections or to animal infections in general. In fact, Lees and Shojaee Aliabadi (2002) studied, in ex vivo experiments, the ratio of AUC/MIC producing bacteriostasis, bactericidal activity, and elimination of bacteria with different fluoroquinolones. In all these cases, the surrogate markers (AUC/MIC) for cattle, sheep, goat, and camel were lower than 100 to 125. Moreover, we also have to take into account that inflammation of the mammary gland leads to vascular permeability changes and differences in milk composition. Milk pH generally increases, milk casein concentrations decrease, milk albumin concentrations increase, somatic cells increase, and milk fat contents can decrease as a result of IMI. All of these factors could modify the pharmacokinetics of drugs; however, their exact importance is not well understood (Gehring and Smith, 2006). For example, the study of Fang and Pyörälä (1996) showed that milk from an uninfected mammary gland reduced the activity of enrofloxacin against *E. coli* strains only by a factor of 2, but maintained similar activity in mastitic milk.

In conclusion, the systemic difloxacin exposure achieved in goats following P407 administration is consistent with the predicted milk levels needed for a positive therapeutic outcome for *Staph. aureus* strains with a MIC of 0.25 mg/L but not for the MIC_{90} of 0.5 mg/L obtained. Therefore, it would be necessary to assay higher doses of difloxacin to reach optimal ratios of the surrogate markers and to establish repeated dosage regimens and clinical efficacy.

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