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Pharmacokinetics and milk distribution characteristics of orbifloxacin following intravenous and intramuscular injection in lactating ewes

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The purpose of the current investigation is to elucidate the pharmacokinetic profiles of orbifloxacin (OBFX) in lactating ewes (n = 6) following intravenous (i.v.) and intramuscular (i.m.) administrations of 2.5 mg/kg W. In a crossover study, frequent blood, milk, and urine samples were drawn for up to 48 h after the end of administration, and were then assayed to determine their respective drug concentrations through microbiological assay using *Klebsiella pneumoniae* as the test micro-organism. Plasma pharmacokinetic parameters were derived from plasma concentration–time data using a compartmental and noncompartmental analysis, and validated a relatively rapid elimination from the blood compartment, with a slope of the terminal phase of 0.21 ± 0.02 and 0.19 ± 0.06 per hour and a half-life of 3.16 ± 0.43 and 3.84 ± 0.59 h, for i.v. and i.m. dosing, respectively. OBFX was widely distributed with a volume of distribution $V_{(d(ss))}$ of 1.31 ± 0.12 L/kg, as suggested by the low percentage of protein binding (22.5%). The systemic body clearance (Cl_B) was 0.32 ± 0.12 L/h·kg. Following i.m. administration, the maximum plasma concentration (C_{max}) of 1.53 ± 0.34 μg/mL was reached at t_{max} 1.25 ± 0.21 h. The drug was completely absorbed after i.m. administration, with a bioavailability of 114.63 ± 11.39%. The kinetic milk AUC_{milk}/AUC_{plasma} ratio indicated a wide penetration of orbifloxacin from the bloodstream to the mammary gland. OBFX urine concentrations were higher than the concurrent plasma concentrations, and were detected up to 30 h postinjection by both routes. Taken together, these findings indicate that systemic administration of orbifloxacin could be efficacious against susceptible mammary and urinary pathogens in lactating ewes.

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INTRODUCTION

Orbifloxacin, a new synthetic third-generation fluoroquinolone antimicrobial drug, was developed exclusively for use in veterinary medicine (Ihrke *et al.*, 1999). Fluoroquinolones (FQs) exhibit bactericidal activity against numerous Gram-negative bacteria, Gram-positive bacteria, and Mycoplasma spp. (Hannan *et al.*, 1997; Papich & Riviere, 2001). FQs are considered to have a concentration-dependent effect although a time-dependent bactericidal effect against certain Gram-positive bacteria has also been described (Spreng *et al.*, 1995; Cester *et al.*, 1996). They also possess other characteristics, including a wide spectrum of bactericidal activity, relatively low *MIC*s against susceptible target micro-organisms, a large volume of distribution, and low plasma protein binding (Spreng *et al.*, 1995; Brown, 1996). The extent of plasma protein binding (in humans) was in the range of 60–93% for the gyrase inhibitors of the first-generation and newer agents such as rosoxacin, trovafloxacin and rufloxacin, and 20–40% for all other third-generation fluoroquinolones (Zlotos *et al.*, 1998). There has been limited published research on orbifloxacin pharmacokinetics in animals, including horses, (Haines *et al.*, 2001; Davis *et al.*, 2006), dogs (Heinen, 2002), and lactating goats (Marín *et al.*, 2007). However, information on the

pharmacokinetics of orbifloxacin in lactating ewes does not appear to exist. This is due to the fact that orbifloxacin and other fluoroquinolones are not approved for use in small ruminants.

The use of antibacterial drugs that could penetrate into the udder parenchyma at therapeutic levels is of considerable importance, particularly in severe and/or life-threatening infectious conditions of the udder (Goudah *et al.*, 2006). Research on the penetration of orbifloxacin antibiotics from blood into the milk produced by normal and/or mastitic mammary glands is scarce (Marín *et al.*, 2007). The presence of drug residues in milk may have public health implications, and is perceived as undesirable by many consumers (Goudah *et al.*, 2006). Therefore, knowledge about the penetration of orbifloxacin from blood to milk is thus worthy of consideration. This report describes the disposition kinetics, absolute bioavailability, urinary concentrations, and protein binding of orbifloxacin in the plasma of lactating ewes as well as its penetration from the blood to the milk of lactating ewes following a single i.v. or i.m. dose of 2.5 mg/kg W.

MATERIAL AND METHODS

Drugs and chemicals

Both the orbifloxacin standard and the commercial product were a generous gift from Prof M. Shimoda, Tokyo University of Agriculture and Technology, Tokyo, Japan. Mueller-Hinton agar was supplied by Mast Group Ltd. (Merseyside, UK).

Experimental animals

Six adult, lactating native Barky breed ewes weighing 45–55 kg and aged 4- to 6-years-old were used in the current study. The ewes were kept at optimal nutritional conditions, fed on barley, high-quality hay, and wheat straw *ad libitum*, and had free access to water. The health of all ewes was monitored prior to and throughout the experimental period. The Advisory Committee composed of faculty members approved the experimental protocol.

Drug administration

The study was performed over a randomized two periods; two treatments crossover design with a 15-day washout interval between periods. Three ewes were given a single i.v. injection of orbifloxacin (2.5 mg/kg W) in the left jugular vein, and the other three received an intramuscular injection (2.5 mg/kg W) in the semimembranous muscle. Five ml whole blood samples were withdrawn by venipuncture of the right jugular into 10 mL heparinized Vacutainer tubes (Becton Dickinson, NJ, USA). The sampling times were 0 (blank sample), 0.166, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, and 48 h postinjection. All of the blood samples were centrifuged at 3000 ***g*** for 15 min in order to separate the plasma. The plasma samples were frozen at −20 °C until analysis.

Urine samples were collected simultaneously from the same animals at various predetermined time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, and 48 h postadministration. The bladder was emptied completely before administration of the drug and at each sampling time as well, via a rubber balloon catheter. On the other hand, milk samples were obtained at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, and 48 h following both routes. At each sampling point, both halves of the udder were completely milked, the milk obtained from each half was homogenized and a 5 mL aliquot was taken for determination of orbifloxacin concentration.

Serum, milk, and urine samples were analyzed within a week of sampling.

Analytical method

The antibacterial activities of orbifloxacin in plasma, urine, and milk were measured by an agar diffusion assay (Klassen & Edberg, 1996; Heinen, 2002) using *Klebsiella pneumoniae* (ATCC 10031) as the reference micro-organism. Urine samples were diluted with phosphate buffer before assaying, as they might have high concentrations at various sampling points, and the dilution factor was subsequently recorded. Each sample was measured in triplicate. Inhibition zones around the sample wells were measured and compared with the inhibition zones produced by the standards. Serial two-fold dilutions (in blank plasma, milk, and urine) from 10 to 0.04 μg/mL were used for preparation of the standard curves. The method was linear, with regression coefficient (r^2) > 0.996 for any given calibration curve and a lower limit of quantification of 0.04 μg/mL in plasma, milk and urine. The mean intra-assay coefficient of variation was <5% in plasma, milk, and urine. Negative control samples (blank) showed no bacterial inhibition, indicating no intrinsic antibacterial activity of the samples.

A mean recovery was evaluated with spiked blank materials (plasma, milk, and urine) at three concentration levels (1.25, 2.5, and 5 μg/mL), each in four replicates. The percentage recovery was determined by comparing the zone of inhibition of fortified blank samples and treated as any samples, with the zone of inhibition of the same standards prepared in phosphate buffer. The mean percentage recoveries of orbifloxacin from plasma, milk, and urine were 90.4 ± 4.25%, 88.22 ± 7.6%, and 93.78 ± 5.7%, respectively. The assay precision expressed as relative standard deviation (RSD) was calculated as follow: RSD (%) = [standard deviation (SD)/Mean] × 100. The intra-day precision was performed with three concentration levels (the same as for recovery determination) in five replicates and the value was <5.2%. The inter-day precision was estimated from three separate occasions with three concentration levels (same as for recovery determination) in five replicates on each occasion and the value was ≤ 8.6%.

Protein binding

The extent of protein binding of various orbifloxacin concentrations (0.625, 1.25, 2.5 and 5 μg/mL) in triplicate was determined *in vitro* according to the method described previously by Craig and Suh (1991). This method was based on the diffusion of free antibiotic into the agar medium. To estimate the protein binding of orbifloxacin, the drug was dissolved in phosphate buffer (pH 6.2) and antibiotic-naive sheep plasma at different concentrations. The differences in the diameter of the inhibition zone between the solutions of the drugs in the buffer and plasma samples were then calculated. The protein bound fraction (as percentage) was calculated according to the following equation:

$$\text{Protein binding}\,\% = \frac{\text{Zone of inhibition in buffer-Zone of inhibition in plasma}}{\text{Zone of inhibition in buffer}}$$

Pharmacokinetic analysis

A computerized curve-stripping program (R Strip version 5.0; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration–time curves for each individual ewe following the administration of orbifloxacin. Ordinary least-squares sum was used as a criterion function in the fitting process. Goodness of fit was estimated by visual inspection of the fitted curve and correlation coefficient, which exceeded 0.95. The number of exponents needed for both i.v. and i.m. administration data were automatically selected by the software according to *F* test and Akaike criterions (Yamaoka *et al.*, 1978). For the i.v. data, the plasma concentration–time relationship was best fitted to a two-compartment open model:

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$$

where C_p is the plasma drug concentration at any time t; C_1, C_2 coefficients of biexponential equation describing disposition curve expressed as μg/mL; λ_1, λ_2 are the first-order rate constants associated with the initial (distribution) and terminal (elimination) phases, respectively, expressed in units of reciprocal time (h^{-1}); and e is the natural logarithm base. The initial and terminal half-lives ($t_{1/2\lambda_1}$ and $t_{1/2\lambda_2}$) and the volume of distribution at steady-state ($V_{(d(ss))}$) were calculated according to standard equations (Gibaldi & Perrier, 1982).

Orbifloxacin plasma concentration curves after i.m. administration were analyzed following the same procedure as used for the i.v. analysis. Each individual curve was analyzed to determine the peak plasma concentration (C_{max}) and the time to peak concentration (t_{max}). The program also calculated the noncompartmental parameters using the statistical moment theory (Yamaoka *et al.*, 1978). The apparent half-life ($t_{½(d)}$) and absorption half-life ($t_{½(a)}$) were calculated as $\ln 2/k_D$ or $\ln 2/k_a$, respectively. The area under the concentration–time curve from 0 to 24 h (AUC_{0-24}) was determined by concentration of the areas obtained with the linear trapezoidal method (ascending portion of curve up to t_{max}) and the log trapezoidal method (descending portion of the curve). The area term was extrapolated to infinity ($AUC_{0-\infty}$) by adding AUC_{0-24} to the portion of area obtained by dividing the final measured plasma concentration by the slope of the terminal phase as follows: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/\lambda_2$. The mean residence time (*MRT*) was calculated as *AUMC*/AUC, where *AUMC* is the area under the first moment curve (Gibaldi & Perrier, 1982). The systemic body clearance was calculated as $Cl_B = D/AUC$. The absolute bioavailability (*F*%) was calculated as $AUC_{i.m.}/AUC_{i.v} \times 100$.

The milk time-concentration data was analyzed with the R Strip program using the drug concentration at each sampling time interval. AUC_{milk} was calculated using the linear trapezoidal rule with extrapolation to infinity. The extent of the penetration of the drug from the blood into the milk was expressed by AUC_{milk}/AUC_{plasma} and $C_{max\text{-}milk}/C_{max\text{-}plasma}$ ratios (Ziv *et al.*, 1995).

Statistical analysis

Descriptive statistical parameters as mean, standard deviation, and coefficient of variation were calculated. The Mann-Whitney test and InStat version 3.0 (GraphPad Software, San Diego, CA, USA) were used to test parameters for significant differences between i.v. and i.m. administration.

RESULTS

Clinical examinations of all ewes before and after each trial, revealed no abnormalities. No local or systemic adverse reactions to orbifloxacin were found to have occurred following i.v. or i.m. administration.

Akaike's Information Criterion test indicated that a two-compartment open model best represented the plasma concentration vs. time data following i.v. or i.m. injection of orbifloxacin in lactating ewes. The mean plasma concentration–time profiles of orbifloxacin following a single i.v. or i.m. administration are presented graphically in Fig. 1. Mean ± SD values of

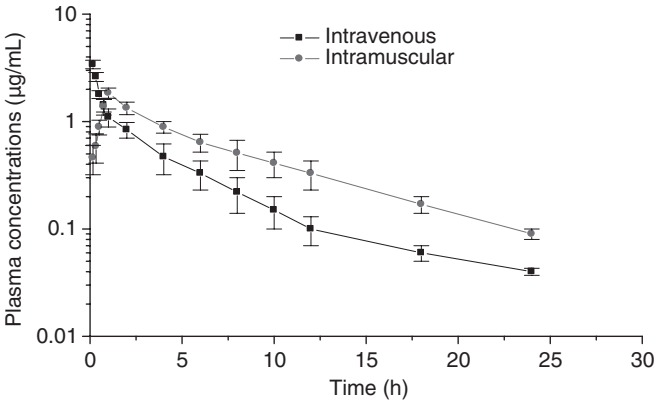

Fig. 1. Mean ± SD plasma concentrations of orbifloxacin in lactating ewes following i.v. and i.m. administration of 2.5 mg/kg W (n = 6).

pharmacokinetic parameters estimated from the fitting of the curve are shown in Table 1. In terms of administration for the terminal phase half-lives, total areas under the plasma concentration–time curve (*AUC*), total area under the first moment curve (*AUMC*), and *MRT*, significant differences were observed between the i.v. and i.m. routes. Following i.v. injection, the $t_{1/2\lambda1}$ and $t_{1/2\lambda2}$ were 0.25 and 3.16 h, respectively.

Table 1. Mean ± SD plasma and milk pharmacokinetic parameters of orbifloxacin in lactating ewes following i.v. and i.m. administration of 2.5 mg/kg W (n = 6)

Parameters	Unit	i.v.	i.m.
Plasma			
λ_1	h^{-1}	2.94 ± 0.42	NA
$k_{(a)}$	h^{-1}	NA	2.16 ± 0.15
$t_{1/2\lambda1}$	h	0.25 ± 0.07	NA
$t_{1/2(a)}$	h	NA	0.34 ± 0.06
$\lambda_2(k_D)$	h^{-1}	0.21 ± 0.02	0.19 ± 0.06
$t_{1/2\lambda2}$ ($t_{1/2(d)}$)	h	3.16 ± 0.43	3.84 ± 0.59*
k_{21}	h^{-1}	0.83 ± 0.07	NA
k_{12}	h^{-1}	1.69 ± 0.14	NA
$V_{(d(ss))}$	L/kg	1.31 ± 0.12	NA
Cl_B	L/h·kg	0.32 ± 0.12	NA
$AUC_{0\text{-}24}$	μg·h/mL	8.51 ± 1.26	10.02 ± 1.46
$AUC_{0\text{-}\infty}$	μg·h/mL	8.73 ± 1.41	10.65 ± 1.56*
AUMC	μg·h^2/mL	27.65 ± 3.25	59.43 ± 6.37**
MRT	h	4.12 ± 0.91	5.88 ± 1.21*
C_{max}	μg/mL	NA	1.53 ± 0.34
t_{max}	h	NA	1.25 ± 0.21
F	%	NA	114.63 ± 11.39
Milk			
C_{max}	μg/mL	NA	1.10 ± 0.33
t_{max}	h	NA	1.34 ± 0.17
$t_{1/2\Lambda}$ ($t_{1/2(d)}$)	h	5.71 ± 0.62	7.31 ± 1.39*
AUC	μg·h/mL	9.41 ± 1.23	11.16 ± 1.43*
$C_{max\text{-}milk}/C_{max\text{-}plasma}$	Ratio	NA	0.74 ± 0.03
AUC_{milk}/AUC_{plasma}	Ratio	1.18 ± 0.11	1.14 ± 0.18

λ_1 and λ_2, first-order rate constants associated with the initial (distribution) and terminal (elimination) phases, respectively; $t_{1/2}$, half-life, which may be qualified by the process to which it refers; k_a, apparent first-order absorption rate constant; k_D, apparent first-order elimination rate constant when drug is administered orally or by any other extravascular route; $t_{1/2(a)}$, absorption half-life; $t_{1/2(d)}$, apparent half-life following extravascular administration of a drug; k_{12} and k_{21}, the first-order rate constants associated with transfer of unbound drug between the central (1) and peripheral (2) compartments, respectively, of the two-compartment pharmacokinetic model; $V_{(d(ss))}$, apparent volume of distribution at steady-state; Cl_B, body (systemic) clearance; *MRT*, mean residence time, which may be qualified by the route of administration; *AUC*, total area under the plasma drug concentration–time curve (from time zero to infinity); *AUMC*, total area under the first moment curve; C_{max}, maximum (peak) concentration of drug in blood plasma (applied to extravascular drug administration); t_{max}, time after drug administration at which peak plasma concentration occurs; *F* (%), fraction of the administered dose which reaches the systemic circulation unchanged; W, body weight of animal (expressed in kg). Symbols and units were cited in accordance to Baggot (2001).

Values after i.m. administration were significantly different from corresponding values following i.v. administration. *$P < 0.05$, **$P < 0.01$.

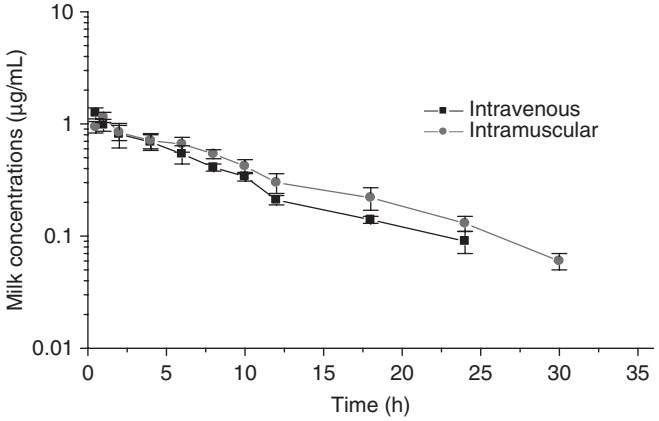

Fig. 2. Mean ± SD milk concentrations of orbifloxacin in lactating ewes following i.v. and i.m. administration of 2.5 mg/kg W (n = 6).

The corresponding pharmacokinetic variables following i.m. administration are shown in Table 1. OBFX was rapidly absorbed with a $t_{1/2(a)}$ of 0.34 h and a maximum plasma concentration (C_{max}) of 1.53 μg/mL attained at 1.25 h (time to peak concentration, t_{max}). The $t_{1/2(d)}$ was 3.84 h, *MRT* was 5.88 h, and the bioavailability was 114.63%. The *in vitro* protein binding percent of orbifloxacin in plasma of lactating ewes ranged from 21.3% to 24.5% with an average of 22.5%.

The concentration and kinetic values of milk following i.v. and i.m. administration are presented in Fig. 2 and Table 1. The AUC_{milk}/AUC_{plasma} ratio indicated a wide penetration of orbifloxacin from the bloodstream to the mammary glands of lactating ewes following administration of orbifloxacin through both routes. Statistical analysis of the milk pharmacokinetic parameters revealed significant differences in the terminal phase half-life and the areas under the concentration–time curves.

The mean concentrations of orbifloxacin in the urine of lactating ewes following i.v. and i.m. dosing are incorporated in Table 2. Concentrations of orbifloxacin in urine were much

Table 2. Mean ± SD urine concentrations of orbifloxacin (μg/mL) in lactating ewes following i.v. and i.m. administration of 2.5 mg/kg W (n = 6)

Time (h)	i.v.	i.m.
0.5	10.46 ± 2.15	3.87 ± 0.87
1	9.17 ± 1.69	6.79 ± 1.24
2	6.45 ± 1.13	9.34 ± 1.12
4	3.49 ± 0.94	4.87 ± 1.23
6	2.63 ± 0.72	2.97 ± 0.69
8	1.61 ± 0.54	1.91 ± 0.51
10	1.20 ± 0.31	1.38 ± 0.42
12	0.67 ± 0.18	0.81 ± 0.23
18	0.31 ± 0.11	0.45 ± 0.20
24	0.16 ± 0.04	0.23 ± 0.07
30	0.06 ± 0.01	0.11 ± 0.01
48	ND	ND

ND, not detected; W, body weight of animal expressed in kg (Baggot, 2001).

higher than those in plasma, and could be detected for up to 30 h postinjection by both routes.

DISCUSSION

This study used a microbiological assay to determine orbifloxacin concentrations. The reasons that we selected the bioassay are: (a) the total antimicrobial activity, as measured by a microbiological assay, is adequate for the determination of a dosage regimen (Dowling *et al.*, 1995); (b) most investigators evaluating the pharmacodynamic variables, which are correlated to the outcome of infection, also used a microbiological assay to determine serum concentrations of various fluoroquinolones (Ingerman *et al.*, 1986; Drusano *et al.*, 1993; Sullivan *et al.*, 1993; Kang *et al.*, 1994; McKellar *et al.*, 1999); (c) orbifloxacin undergoes a limited metabolic process and is primarily excreted intact into the urine. For instance, in pig following an i.m. administration of orbifloxacin 97% of the dose appears in the urine as orbifloxacin and 3% as the glucuronide. In calves 94% of the dose appears in the urine as the parent compound and 5% as oxidative metabolites (Matsumoto *et al.*, 1999); and (d) the bioassay method is precise, reproducible, and does not require specialized equipment or toxic solvents (Ev Lda & Schapoval, 2002).

The study revealed that plasma orbifloxacin concentrations decreased over in a bi-exponential manner following i.v. injection, thus demonstrating the presence of distribution and elimination phases and justifying the use of a two-compartment open model. This finding is in agreement with that reported for orbifloxacin in horses (Davis *et al.*, 2006) and lactating goats (Marín *et al.*, 2007).

Plasma concentration profiles showed a rapid initial distributive phase, followed by a slower terminal λ_2-phase with an estimated mean terminal phase half-life of 3.16 h. This finding was somewhat shorter than those recorded in horses (5.08 h, Davis *et al.*, 2006) and lactating goats (4.12 h, Marín *et al.*, 2007).

Orbifloxacin exhibits a relatively high volume of distribution at steady-state (1.31 L/kg), which suggests that the drug would be distributed well into the tissues. The $V_{(d(ss))}$ was consistent with those reported for orbifloxacin in horses and lactating goats (1.58 and 1.13 L/kg, Davis *et al.*, 2006 and Marín *et al.*, 2007; respectively). The $V_{(d(ss))}$ is a clearance-independent volume of distribution, which is used to calculate the amount of drug in the body under equilibrium conditions (Toutan & Bousquet-Melou, 2004). Good tissue diffusion may be related to the low molecular weight of the drug's affinity for lipid-bearing tissues.

Orbifloxacin clearance in ewes (0.32 L/h·kg) was similar to the value reported by Davis *et al.* (2006) in horses (0.28 L/h·kg) and was somewhat slower than that reported in goats (0.4 L/h·kg, Marín *et al.*, 2007).

Following i.m. injection, the data was best represented by a two-compartment model; this finding is in agreement with that reported for orbifloxacin in lactating goats (*Marín et al.*, 2007). The estimated C_{max} (1.53 μg/mL) was similar to that recorded in lactating goats (1.66 μg/mL, Marín *et al.*, 2007). The time of maximum concentration (t_{max}) in ewes, 1.25 h, was higher than that recorded in goats (0.87 h, Marín *et al.*, 2007). The *MRT* of orbifloxacin (5.88 h) differs from the value recorded in goats (2.82 h, Marín *et al.*, 2007). The mean elimination half-life of orbifloxacin (3.84 h) was similar to those recorded in horses (3.42 h, Davis *et al.*, 2006) and goats (3.43 h, Marín *et al.*, 2007), indicating similar elimination in those species.

The absolute bioavailability of orbifloxacin in lactating ewes after i.m. administration was 114.63%. This higher value was similar to that reported in lactating goats (105%) (Marín *et al.*, 2007). This value indicates an excellent absorption of the drug from the injection site. As the drug is homogenously dissolved with distilled water without any precipitation, the observed findings may be attributable to our choice of pharmacokinetic model (using a mixture of compartmental and noncompartmental analysis) or some other unknown factors, such as nonlinear metabolism. The process of absorption was also quite rapid, with an absorption half-life ($t_{1/2(a)}$) of 0.34 h.

The penetration of orbifloxacin from the blood into the milk was rapid and showed high concentrations in milk secretion. Fluoroquinolones exhibit concentration-dependent killing; therefore, it is not necessary to maintain their concentrations above the *MIC* throughout the entire dosing period (Walker, 2000). Consequently, the peak milk concentration is a very important parameter for consideration. In this study, the milk C_{max} following i.m. dosing was 1.10 μg/mL, a value which is lower than that estimated in lactating goats (1.77 μg/mL) (Marín *et al.*, 2007). Additionally, the AUC_{milk}/AUC_{plasma} ratio indicated a wide penetration of orbifloxacin from the bloodstream to the mammary glands of lactating ewes following administration by both routes. The findings of Fernandez-Varon *et al.* (2006), Carceles *et al.* (2007), and Goudah (2008) for moxifloxacin in lactating goats and ewes support the current observation. Similar trends were also reported for other fluoroquinolones in lactating animals (Soback *et al.*, 1994; Shem-Tov *et al.*, 1997; Abd El-Aty & Goudah, 2002).

As with a number of the fluoroquinolones, orbifloxacin is amphoteric with a pKa range of 5.95–9.01 (Martinez *et al.*, 2007). Passive diffusion across biological membranes is a function of a fluoroquinolone's lipophilicity relative to the pKa values of the two ionizable moieties. The good penetration of orbifloxacin from the blood into the ewe's milk at pH 6.5–6.7 was, therefore, predictable on the basis of the ion trap mechanism (Marín *et al.*, 2007). Owing to the greater persistence of the drug in the milk relative to its persistence in the plasma, the drug is trapped in the milk and demonstrated an AUC_{milk}/AUC_{plasma} ratio that is greater than one. These findings are in agreement with several reports on other fluoroquinolones in lactating animals (Abd El-Aty & Goudah, 2002; Fernandez-Varon *et al.*, 2006; Carceles *et al.*, 2007; Marín *et al.*, 2007; Goudah, 2008). Moreover, orbifloxacin could be a substrate of efflux proteins belonging to the superfamily of ATP-binding cassette transporters such as breast cancer resistance protein (BCRP): the superfamily of ATP-binding cassette transporters is composed of specialized drug transporters that are involved in the passage of drugs from the plasma into the milk. In fact, it has

been established that the expression of BCRP is highly up-regulated in the lactating mammary glands of ruminants (Jonker *et al.*, 2005). Furthermore, it is strongly expected that BCRP plays an important role in the active secretion of orbifloxacin into the milk, as was shown for enrofloxacin in ewes (Pulido *et al.*, 2006); however, this mechanism should be evaluated further. The currently obtained data indicate that orbifloxacin might be effective against susceptible mastitic pathogens in ewes after parenteral administration.

Urine orbifloxacin concentrations following i.v. and i.m. administration were detected for up to 30 h postadministration, attaining higher concentrations than those measured in plasma. These concentrations exceeded the *MIC* (0.1 μg/mL). Therefore, it is suggested that OBFX could be an efficacious drug for use in the treatment of urinary tract infections in ewes.

Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in their distribution, excretion and therapeutic effectiveness (Turnidge, 1999). In this study, orbifloxacin displayed a low level of binding to plasma proteins in lactating ewes. A similar trend was reported for pefloxacin in lactating goats (Abd El-Aty & Goudah, 2002).

Overall, these data concluded that orbifloxacin administered at dosage of 2.5 mg/kg, could be used for treatment of mastitis caused by *Streptococcus* spp. and *P. haemolytica,* but not *S. aureus.* Additionally, it might be useful in the treatment of systemic urinary infections in ewes after specific assessment of susceptible micro-organisms.

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