

Tissue distribution of enrofloxacin after intramammary or simulated systemic administration in isolated perfused sheep udders

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Objective—To determine the tissue distribution of enrofloxacin after intramammary or simulated systemic administration in isolated perfused sheep udders by measuring its concentration at various sample collection sites.

Sample—26 udders (obtained following euthanasia) from 26 healthy lactating sheep.

Procedures—For each isolated udder, 1 mammary gland was perfused with warmed, gassed Tyrode solution. Enrofloxacin (1 g of enrofloxacin/5 g of ointment) was administered into the perfused gland via the intramammary route or systemically via the perfusion fluid (equivalent to a dose of 5 mg/kg). Samples of the perfusate were obtained every 30 minutes for 180 minutes; glandular tissue samples were obtained at 2, 4, 6, and 8 cm from the teat base after 180 minutes. The enrofloxacin content of the perfusate and tissue samples was analyzed via high-performance liquid chromatography with UV detection.

Results—After intramammary administration, maximum perfusate enrofloxacin concentration was detected at 180 minutes and, at this time, mean tissue enrofloxacin concentration was detected and mean tissue enrofloxacin concentration was 123.80, 54.48, 36.72, and 26.42 µg/g of tissue at 2, 4, 6, and 8 cm from the teat base, respectively. Following systemic administration, perfusate enrofloxacin concentration decreased with time and, at 180 minutes, tissue enrofloxacin concentrations ranged from 40.38 to 35.58 µg/g of tissue.

Conclusions and Clinical Relevance—By 180 minutes after administration via the intramammary or systemic route in isolated perfused sheep mammary glands, mean tissue concentration of enrofloxacin was greater than the minimum inhibitory concentration required to inhibit growth of 90% of many common mastitis pathogens in sheep. Use of either route of administration (or in combination) appears suitable for the treatment of acute mastitis in sheep. (*Am J Vet Res* 2012;73:1728–1734)

Fluoroquinolones are synthetic antimicrobials chemically related to nalidixic acid. These compounds have been developed for the treatment of a variety of microbial infections in both veterinary and human medicine.^{1–6} Fluoroquinolones act principally by inhibition of bacterial DNA-gyrase, which is necessary for supercoiling of DNA to provide a suitable spatial arrangement of DNA within bacterial cells.⁷

Enrofloxacin, 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid, is a fluoroquinolone exclusively developed for use in veterinary medicine (cattle, goats, sheep, pigs, horses, poultry, dogs, and cats).⁸ It is characterized by

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ABBREVIATIONS

C _{max}	Peak plasma concentration
HPLC	High-performance liquid chromatography
MIC	Minimum inhibitory concentration
MIC ₉₀	Minimum inhibitory concentration required to inhibit growth of 90% of bacterial isolates tested

a broad antibacterial spectrum and high bactericidal activity against major pathogenic bacteria (both gram positive and gram negative) in animals.^{9,10} Enrofloxacin has an excellent pharmacokinetic behavior pattern, almost complete absorption, and tissue distribution that guarantees the MIC against microorganisms that cause most illnesses in sheep.

Inflammatory diseases of sheep udders are among the most important problems in modern agricultural milk production. Bacterially induced inflammation of sheep mammary glands (mastitis) is mainly treated with intracisternally administered antimicrobials.

When an infection is detected, the effectiveness of the antibacterial drug used as treatment is determined by the susceptibility of the causal organism to that drug. Furthermore, the active principles of the drug must have good distribution within the tissues to reach the causal organism. The absorption and distribution of drugs given systemically are studied in live animals. For ethical and financial reasons, drug availability within mammary glands is generally assessed by collection of milk and blood samples.¹¹ However, the antimicrobial concentration in the various areas of the glandular tissue after intramammary administration cannot be estimated via these methods. Therefore, it is important to develop *ex vivo* models to determine drug concentrations at various regions of the mammary gland and to establish pharmacokinetic-pharmacodynamic correlations in effectiveness studies. The purpose of the study reported here was to determine the tissue distribution of enrofloxacin after intramammary or simulated systemic administration in isolated perfused sheep udders by measuring drug concentrations at different sample collection sites.

Materials and Methods

Animals—Twenty-six female lactating Spanish Asaf sheep were used in the study. The sheep ranged in body weight from 50 to 56 kg. Sheep were allowed to acclimatize to their environment for 2 weeks before the experiment, and were maintained indoors in an adequately ventilated building. They were provided an antimicrobial-free diet of alfalfa hay and pelleted feed concentrate, with unlimited access to water and salt lick. All animal procedures were approved in advance by the Institutional Animal Care and Use Committee of the University of León.

Test formulations of enrofloxacin—To simulate systemic administration, enrofloxacin^a (5 mg/kg) was dissolved in the fluid used to perfuse the isolated sheep mammary glands. For intramammary administration, injectors containing an enrofloxacin preparation (1 g of enrofloxacin/5 g of ointment) were used.

Isolation and perfusion of sheep udders—The method of perfusion of sheep udders was developed on the basis of caprine and bovine udder perfusion procedures described by other authors.^{12–19} The udder (considered medium sized among sheep breeds) of each lactating sheep was examined via palpation, and each mammary gland was milked. The milk pH was measured as an indicator of lesions to the blood-milk barrier. All udders had no skin lesions, and no pathological changes of the milk or glandular tissue were detected. Each sheep was sedated with propofol^b (3 mg/kg, IV) prior to administration of embutramide^c (100 mg/kg, IV). Within 15 to 30 minutes after euthanasia and after surgical dissection of the mammary gland with all its skin, blood clots in the vessels of the mammary glands were cleared via injection of 50 mL of Tyrode solution containing heparin. The large vein (mammary vein) was cannulated to allow sample collection, whereas the smaller arteries and veins were closed with suture material or artery forceps. By use of the skin in the area

of body wall attachment and suspensory ligament, the udder was fixed in a natural position to a metal frame. After a few minutes, perfusion fluid was delivered into the large artery (mammary artery) of the right mammary gland via silicone tubes with use of a peristaltic pump to provide perfusion pressure between 50 and 60 mm Hg.

One mammary gland of each udder was perfused with Tyrode solution (NaCl, 8 g/L; KCl, 200 mg/L; CaCl₂-2H₂O, 265 mg/L; MgCl₂-6H₂O, 213 mg/L; NaHCO₃, 1 g/L; NaH₂PO₄, 65 mg/L; and glucose, 1.1 mg/L) with heparin^d (1,000 U/L), which was gassed with carbogen (95% O₂ and 5% CO₂) and maintained at 39°C. To inhibit the development of tissue edema, mannitol^e (60 g/L) was added to the solution. After an initial 30-minute equilibration phase, the perfusate flow was adjusted to 60 to 70 mL/min to provide a basal perfusion pressure of 50 to 60 mm Hg. This perfusion pressure is lower than the physiologic pressure of sheep (80 mm Hg) but is sufficient for the perfusion fluid to reach all the blood vessels, as confirmed by trypan blue testing by other researchers.^{14,15,17,19} The relationship between flow rate and perfusion pressure was examined in a previous study (unpublished data). At the end of the 30-minute equilibration phase, the mammary gland was milked. The perfusion was then continued for a further 180 minutes.

Viability of the udders—The viability of the 26 perfused mammary glands was evaluated by measuring several biochemical variables in samples of the perfusion fluid obtained from each of the glands every 30 minutes during the 180-minute perfusion period to determine glucose consumption^f and lactate production^g; the pH of the perfusion solution was also determined.^h All udders were weighed before and after the perfusion period (180 minutes) to minimize any problems that might be associated with the onset of edema. A weight increase > 11% was arbitrarily set as a criterion for exclusion of an udder from the study. During the period of perfusion, a macroscopic evaluation of the perfused glandular tissue was performed to detect development of tissue edema. At the end of perfusion, histologic examination of the perfused glandular tissue was performed to assess edema development. For histologic examination, 2 samples of the udder tissue were taken from each perfused gland next to the planned teat base sample collection sites. These tissue samples were fixed in buffered 10% formol solution and then embedded in paraffin. Histologic preparations (thickness, 3 µm) were stained with H&E stain and compared with published data for histologic preparations of udder tissue from freshly slaughtered sheep.²⁰

Furthermore, trypan blue solution was infused via the mammary artery at the end of the perfusion period to assess whether dermal perfusion was sufficient. Lack of stained dermal blood vessels was a criterion for exclusion of an udder from the study.

Experimental treatments—Of the 26 isolated sheep udders, 14 received administration of enrofloxacin via the intramammary route and 12 received administration of enrofloxacin via the simulated systemic route. The intramammary treatment was administered

into the perfused mammary gland of each of the 14 udders after the 30-minute equilibration phase (designated as 0 minutes of perfusion). A preparation of enrofloxacin (1 g of enrofloxacin/5 g of ointment) was applied by syringe through the teat canal and massaged into the gland cistern of the perfused mammary gland. During the following 180-minute period of perfusion, perfusate samples (used for determination of enrofloxacin concentrations and assessment of udder viability) were obtained from each perfused gland every 30 minutes. After 180 minutes, samples of glandular tissue (5 g each) were obtained with a scalpel at distances of 2, 4, 6, and 8 cm (4 samples/site) from the teat in a vertical direction. All tissues samples were stored at -80°C until analysis.

For systemic treatment, a plasma concentration of enrofloxacin was simulated by the addition of enrofloxacin^a to the perfusion fluid after the 30-minute equilibration phase. The amount of drug used simulated the *in vivo* concentration in the udder of a sheep after a single IV injection of enrofloxacin at a dose of 5 mg of enrofloxacin/kg of body weight. Samples of perfusate fluid and tissue were obtained in a similar manner as that described following intramammary administration of enrofloxacin.

Extraction and purification of the perfusate and tissue samples—Extraction and purification of the perfusate samples were performed according to a reported method²¹ with minor modifications. Perfusate samples were analyzed following solid-phase extraction. Cartridges were previously activated with 2 mL of methanol and then with 2 mL of purified water before use; 2 mL of perfusate sample was placed into the cartridge.¹ After washing with 0.75 mL of purified water and then 0.5 mL of acetonitrile, the cartridge was properly dried and eluted with 1 mL of mobile phase.

Extraction and purification of the tissue samples were performed according to a reported method²¹ with minor modifications. One gram of glandular tissue from each collected sample was finely minced, and 4 mL of dichloromethane was added. The tissue mixture was homogenized^l at 13,500 revolutions/min for 30 seconds; 4 mL of dichloromethane and 0.5 mL of sodium phosphate buffer (pH, 7.5; 0.5M) were then added. After centrifugation^k at $1,200 \times g$ for 10 minutes, an organic phase was collected and mixed with sodium hydroxide (0.5M). After 2 centrifugations, aqueous phase was collected.

HPLC analysis—The concentrations of enrofloxacin in perfusate and glandular tissue samples were measured via reversed phase HPLC¹ with UV detection.^m Chromatographic analysis was performed with a separation columnⁿ (4 μm , 250 \times 4 mm) at room temperature (approx 21°C). The mobile phase was a mixture of a solution of sodium acetate (pH, 4.7; 0.1M) and acetonitrile (60:40 [vol/vol]), adjusted to a pH of 5 by addition of acetic glacial acid. The flow rate of the mobile phase was 1 mL/min, and the UV detector was set to 278 nm. For each sample, 2 injections of 50 μL were injected directly into the HPLC system.

Interday and intraday accuracy and precision were within 10%. The limits of quantification, deter-

mined according to described formulae,^{21,22} were 0.08 μg of enrofloxacin/mL of perfusate and 0.08 μg of enrofloxacin/g of tissue. The limits of detection were 0.04 μg of enrofloxacin/mL of perfusate and 0.08 μg of enrofloxacin/g of tissue. The mean extraction recovery of enrofloxacin from perfusate samples was $84.20 \pm 6.12\%$ and from tissue samples was $73.54 \pm 9.14\%$. The mean extraction recovery from perfusate samples was similar to that obtained for plasma by other authors ($89.6 \pm 5.5\%$).²¹

Statistical analysis—Data were analyzed with an asymmetry test to determine the normality of distribution and the Levene test to determine variance uniformity. When the data were normally distributed and there was uniformity in the variance, ANOVA was performed and the Duncan test was used to determine differences between data sets. When these data were not normally distributed or the variances were unequal, the Friedman test was used. To evaluate the viability of the isolated perfused udders, assessments of changes in glucose and lactate concentrations and pH in all isolated perfused sheep mammary glands during the period of perfusion and change in udder weight as a result of perfusion were performed. Mean enrofloxacin concentrations in glandular tissue at 180 minutes following intramammary and simulated systemic administration were compared. Values of $P \leq 0.05$ were considered significant for all analyses.

Results

Viability of the isolated perfused sheep udders—Glucose consumption, lactate production, and pH in the 26 isolated perfused sheep udders during the period of perfusion (180 minutes) were monitored (Figure 1). The viability of the udders was evident because there were no significant (Friedman test and ANOVA; $P \leq 0.05$) changes in the glucose concentration and pH of the perfusate within the perfusion period. Mean \pm SD glucose concentrations ranged from 58.54 ± 4.26 mg/dL at 30 minutes to 63.58 ± 11.60 mg/dL at 180 minutes, whereas mean pH ranged from 7.81 ± 0.60 at 30 minutes to 8.02 ± 0.45 at 90 minutes. The lactate production increased from an initial concentration of 0.55 ± 0.08 mmol/L at 30 minutes to 1.67 ± 0.45 mmol/L at 180 minutes. There were significant (ANOVA and Duncan test; $P \leq 0.05$) differences between the lactate concentration at the first sample collection time (30 minutes) and values at the other time points (60, 90, 120, 150, and 180 minutes). However, all lactate concentrations, both individual and mean values, were within the physiologic range for sheep,²³ which also indicated that the udders remained viable in the experimental model.

At the end of the perfusion phase, trypan blue solution was infused via the mammary artery to determine whether dermal perfusion was sufficient. In all 26 udders, stained dermal blood vessels were visible; thus, all udders were included in the present study. In addition, throughout the perfusion period, development of edema was not grossly evident.

Results of the histologic examination of the 52 mammary gland tissue samples (2 samples obtained

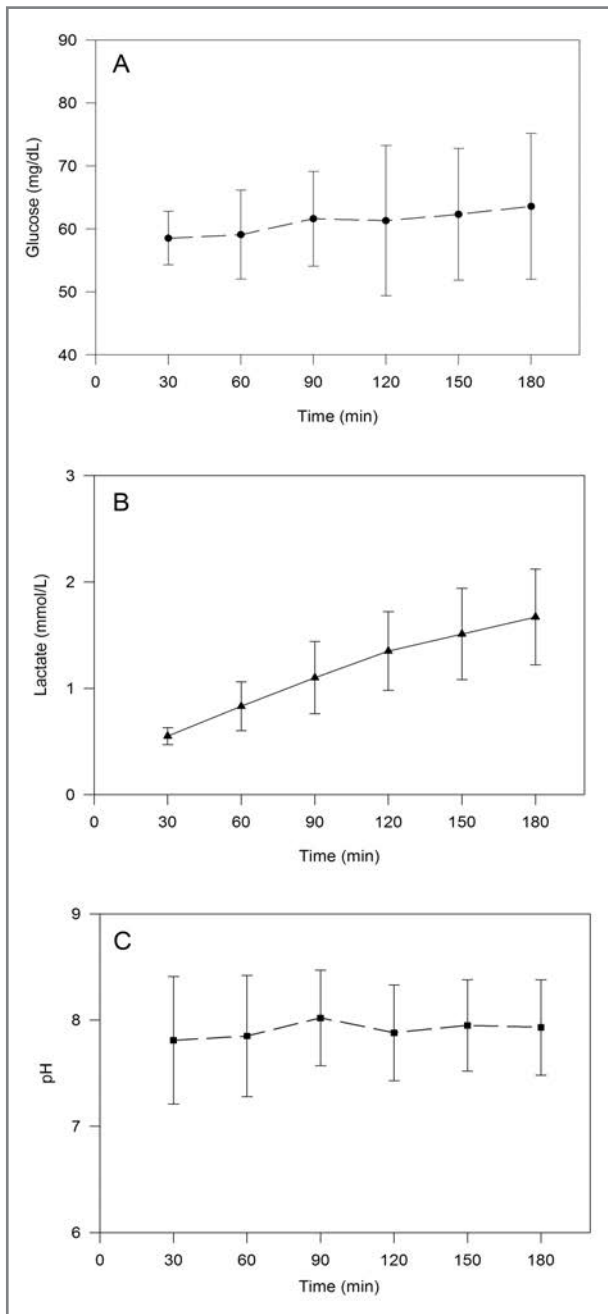


Figure 1—Mean \pm SD glucose (A) and lactate (B) concentrations and pH (C) in perfusate samples obtained from 1 mammary gland of each of 26 isolated perfused sheep udders during a 180-minute perfusion period following intramammary or simulated systemic administration of enrofloxacin (0 minutes). These variables were measured to assess udder viability during experiments in which 1 mammary gland/udder was perfused with Tyrode solution for 30 minutes (equilibration phase), after which 14 mammary glands received a preparation of enrofloxacin (1 g of enrofloxacin/5 g of ointment) applied by syringe through the teat canal (intramammary administration) and 12 mammary glands received administration of enrofloxacin via the perfusion fluid to simulate the in vivo concentration in the udder of a sheep after a single IV injection of enrofloxacin at a dose of 5 mg of enrofloxacin/kg of body weight (simulated systemic administration). Mammary gland perfusion continued for 180 minutes after the equilibration phase and drug administration. Glucose concentration and pH in the perfused mammary glands did not change over time; although lactate concentration was significantly ($P < 0.05$) increased from the 30-minute value at all other time points, the concentrations were within the reference range.

from each mammary gland perfused) collected to assess edema formation in the apparently viable udders revealed slight widening of interstitial spaces characteristic of negligible edema development. Overall, no major degenerative histologic changes were found in any perfused mammary gland sample (Figure 2) and findings did not differ from those reported for samples of mammary glands from healthy sheep.²⁰ These results are consistent with those reported for isolated perfused bovine udders by other investigators authors.^{14,15,24}

Udder weight was measured before and after the perfusion period. Among the 26 udders, perfusion resulted in a positive weight change of 6% to 11%. The mean \pm SD udder weight was 0.54 ± 0.03 kg before perfusion and 0.54 ± 0.04 kg after perfusion.

Intramammary treatment with enrofloxacin—At the end of the perfusion period (ie, 180 minutes after intramammary administration of enrofloxacin), mean concentrations of enrofloxacin in the glandular tissue samples obtained from isolated perfused sheep udders at 2, 4, 6, and 8 cm from the teat base (in a vertical direction) were determined (Figure 3). The concentration of enrofloxacin in the glandular tissue decreased exponentially with increasing vertical distance from the teat base. Mean drug concentration in perfused mammary gland tissue was 123.80, 54.48, 36.72, and 26.42 μ g of enrofloxacin/g of tissue at 2, 4, 6, and 8 cm from the teat base, respectively.

After intramammary administration, enrofloxacin was also detected in the Tyrode solution used to perfuse the mammary glands, indicating that there was transfer of enrofloxacin from the tissue to the perfusion fluid. The mean concentration of enrofloxacin in the perfusate samples collected during the perfusion period (180 minutes) steadily increased over time (Figure 4). Mean maximum concentration was detected at 180 minutes after the perfusion equilibration phase.

Simulated systemic treatment with enrofloxacin—At the end of the perfusion period (ie, 180 minutes after administration of enrofloxacin in the perfusion fluid [to simulate an IV injection]), mean concentrations of enrofloxacin in the glandular tissue samples obtained from isolated perfused sheep udders at 2, 4, 6, and 8 cm from the teat base (in a vertical direction) were determined (Figure 3). The mean drug concentrations in the glandular tissue at the end of the perfusion period at the 4 sample sites were very similar and ranged from 40.38 to 35.58 μ g of enrofloxacin/g of tissue. Although the enrofloxacin concentrations were slightly higher at the base of the mammary gland (at 6 and 8 cm from the teat base), the distribution of enrofloxacin in the perfused mammary gland after systemic administration was considered uniform.

After systemic administration, the mean concentration of enrofloxacin in the perfusate samples collected during the perfusion period (180 minutes) steadily decreased over time (Figure 4). Mean minimum concentration was detected at 180 minutes after the perfusion equilibration phase.

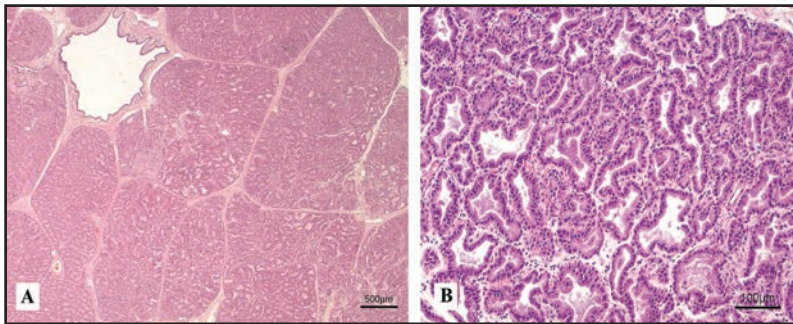


Figure 2—Photomicrographs of a histologic section of glandular tissue obtained from an isolated sheep udder at 180 minutes after intramammary administration of enrofloxacin and continuous perfusion. H&E stain; in panel A, bar = 500 μm and in panel B, bar = 100 μm .

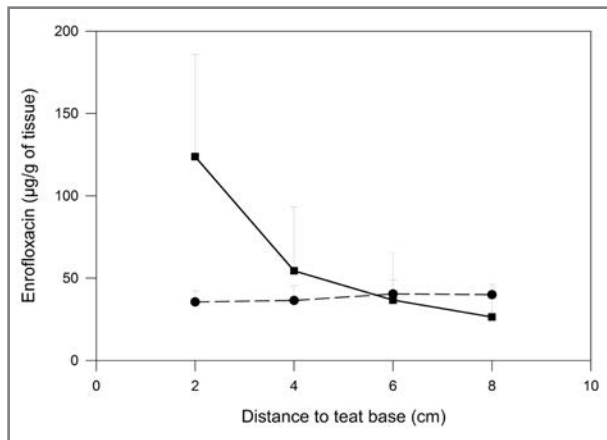


Figure 3—Mean \pm SD enrofloxacin concentration in glandular tissue samples ($\mu\text{g/g}$ of tissue) obtained from the perfused mammary gland in the isolated sheep udders in Figure 1 at 2, 4, 6, and 8 cm from the teat base after 180 minutes of perfusion following intramammary (squares; $n = 14$) or simulated systemic (circles; $n = 12$) administration of the drug. Data were obtained from 4 tissue samples at each site in each gland.

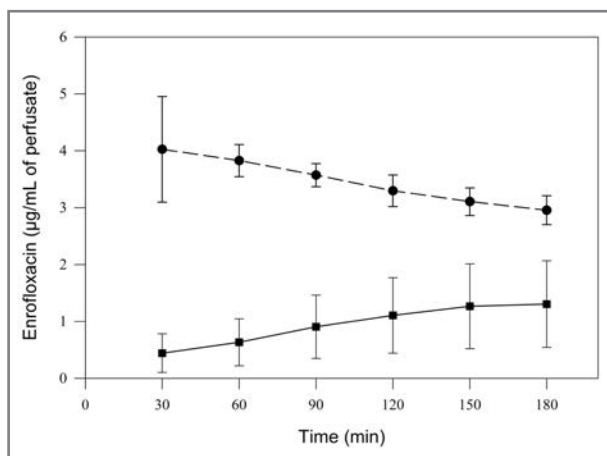


Figure 4—Mean \pm SD enrofloxacin concentration in perfusate samples ($\mu\text{g/mL}$ of perfusate) obtained from the perfused mammary gland in the isolated sheep udders in Figure 1 during the 180-minute period of perfusion following intramammary (squares; $n = 14$) or simulated systemic (circles; $n = 12$) administration of the drug. Data were obtained from 1 perfusate sample obtained from each gland at each time point.

Discussion

In the present study, we developed an *ex vivo* ex-

perimental model to evaluate the tissue distribution of enrofloxacin in sheep mammary glands after simulated systemic or intramammary administration of the drug. The viability of the udders during the experiments was confirmed by the evaluation of several biochemical variables, including glucose consumption, lactate production, and pH of the perfusate solution. Macroscopic and histologic evaluations revealed that the isolated sheep udders remained structurally intact during the perfusion period (180 minutes). Other authors have developed similar experimental models with bovine udders.^{14,15,18,19,24} In those investigations, evaluation of udder viability was performed by determination of the same variables as those used in the present study, and the results obtained were similar to findings of the present study.

When evaluating potential mammary gland treatments, collection of tissue samples from different regions of the udder is very important for assessment of the pharmacokinetics of a drug. Four sample sites/gland represents the minimum number of sample sites, because antimicrobials distribute more unevenly in glandular tissue than is assumed on the basis of milk analysis.^{25,26} As reported for bovine udders by other authors,¹⁸ isolated perfused sheep udders can be used to screen new candidate drug formulations for intramammary administration to select those suitable for testing *in vivo*.

After intramammary administration of enrofloxacin in the present study, there was a considerable variation in the tissue concentration, depending on location of the sample site (Figure 3). The mean concentration of enrofloxacin in the glandular tissue at 2 cm from the teat base was higher than values in tissues at 4, 6, or 8 cm from the teat base. This was probably a reflection of the proximity of the 2-cm site to the site of intramammary application of the ointment. This finding is similar to results reported for benzylpenicillin, oxacillin, ampicillin, and cefquinome administrations in isolated perfused bovine udders.^{15,18,24} The high variability of the tissue concentrations is an indicator of the high interindividual variability even when the udders are obtained from healthy sheep. These differences can be due, among other reasons, to differences in glandular size, which also frequently appears to be correlated with the volume of secretion. This variability reflects the normal situation in the field.²⁶ The udder tissue distribution of drugs is influenced also by the stage of lactation and health status of the udder as well as by the type of inflammation.²⁷ Additionally, it is known that the blood-udder barrier is more permeable in udders with acute mastitis than it is in healthy udders.²⁸

Following intramammary administration in the present study, the concentration of enrofloxacin decreased exponentially with increasing distance from the teat base. When enrofloxacin was added to the perfusion fluid to simulate systemic administration, the tissue concentrations at the 4 sample sites were very similar.

Overall, compared with findings following simulated systemic administration, the tissue concentrations of enrofloxacin achieved after intramammary administration were higher in samples obtained at 2 cm and slightly higher in samples obtained at 4 cm from the teat base. However, at the base of the mammary gland (at 6 and 8 cm from the teat base), the tissue concentrations achieved after intramammary administration were slightly lower than those obtained after systemic administration. Following intramammary administration, enrofloxacin was detected in the perfusate samples collected during the 180-minute perfusion period. Enrofloxacin concentrations in the perfusate samples collected after systemic administration were greater than those in the perfusate samples collected after intramammary administration at all sample collection times.

The isolated perfused sheep udder model developed in the present study appears to be a suitable *ex vivo* model for studying the pharmacokinetics of drugs administered via the intramammary route, as other authors have found for bovine udders.^{15,18,24} Results of the present study confirmed that the model also enables the measurement of tissue concentrations of active compounds after systemic treatment simulated by the addition of a drug in the perfusion fluid. Investigations involving isolated perfused sheep udders could be used to supplement conventional pharmacokinetic studies to provide data on the glandular tissue distribution of various drugs.

Moreover, after evaluating the results obtained by use of isolated perfused sheep udders, we concluded that this experimental model is appropriate for characterizing the passage of a drug from the perfusion fluid (Tyrode solution) to the glandular tissue and vice versa and is especially useful in conjunction with assessment of the distribution of the drug in glandular tissue by measuring its concentration at different distances from the teat base. The proposed experimental model would be very useful to investigate variations in glandular tissue concentrations that can occur depending on the dose and the characteristics of different formulations of a drug, both of which will determine changes in tissue distribution and in the blood-milk transfer of the drug. Because of the clinical use of intramammary treatments, the main application of this experimental model would be in the study of antimicrobial formulations for the treatment of mastitis.

In general, there are 3 indicators that predict the therapeutic efficacy of antibacterial agents. One is represented by the ratio of C_{\max} to MIC_{90} , another by the ratio of the area under the plasma concentration-time curve from 0 to 24 hours to MIC , and the third by the time that the circulating drug concentration exceeds the MIC .²⁹⁻³⁴ For concentration-dependent antibacterial agents, such as enrofloxacin, the best indicator for predicting therapeutic efficacy is the ratio of C_{\max} to MIC_{90} . According to various authors,^{35,36} the breakpoint for clinical efficacy of fluoroquinolones should be a $C_{\max}:MIC_{90}$ ratio > 8. The tissue drug concentrations achieved following both intramammary and simulated systemic administration in the present study were higher than the MIC_{90} of enrofloxacin against some pathogenic microorganisms that cause mastitis in sheep:

Staphylococcus aureus and *Mycoplasma agalactiae* (0.5 $\mu\text{g/mL}$), *Escherichia coli*, and *Streptococcus* spp (0.06 $\mu\text{g/mL}$) and *Pasteurella haemolytica* (0.03 $\mu\text{g/mL}$).³⁷

On the basis of the data obtained in isolated perfused sheep udders in the present study, we concluded that enrofloxacin administered at a dose of 5 mg/kg (simulated systemic administration) or 1 g (intramammary administration), alone or in combination, could be used for treatment of mastitis caused by these microorganisms. Clinical efficacy studies are needed to validate the usefulness of the *ex vivo* experimental model developed in this study for predicting therapeutic outcomes.

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- a. Syvaquinol, Syva Laboratories, León, Spain.
 - b. Propovet (10 mg/mL), Esteve, Spain.
 - c. T-61, Intervet Schering-Plough, Browmeer, The Netherlands.
 - d. Heparina Rovi 5%, Rovi, Spain.
 - e. Manitol 20%, Mein, Spain.
 - f. Accutrend Sensor, Roche Farma SA, Madrid, Spain.
 - g. Cobas Integra 400, Roche Farma SA, Madrid, Spain.
 - h. pH/mV metro micropH 2000, Crison Instruments SA, Barcelona, Spain.
 - i. Supelco C18 1-mL cartridges, Waters Corp, Milford, Mass.
 - j. Ultra Turrax T-25, IKA Works Inc, Wilmington, NC.
 - k. Centrifuged H-103N, Kokusan Denki Co, Shizuoka, Japan.
 - l. LC Module I Plus liquid chromatograph, Waters Corp, Milford, Mass.
 - m. UV detector, Waters Corp, Milford, Mass.
 - n. Nova Pak C18 column, Waters Corp, Milford, Mass.
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References

1. Abadia AR, Aramayona JJ, Muñoz MJ, et al. Ciprofloxacin pharmacokinetics in dogs following oral administration. *Zentralbl Veterinarmed A* 1995;42:505-511.
2. Garcia MA, Solans C, Aramayona JJ, et al. Determination of orbifloxacin in rabbit plasma samples by high-performance liquid chromatography with fluorescence detection. *J Chromatogr Sci* 1999;37:199-202.
3. McKellar QA, Gibson I, Monteiro A, et al. Pharmacokinetics of enrofloxacin and danofloxacin in plasma, inflammatory exudate, and bronchial secretions of calves following subcutaneous administration. *Antimicrob Agents Chemother* 1999;43:1988-1992.
4. Bregante MA, Saez P, Aramayona JJ, et al. Comparative pharmacokinetics of enrofloxacin in mice, rats, rabbits, sheep, and cows. *Am J Vet Res* 1999;60:1111-1116.
5. Perez S, Solans C, Bregante MA, et al. Pharmacokinetics and ocular penetration of grepafloxacin in albino and pigmented rabbits. *J Antimicrob Chemother* 2002;50:541-545.
6. Garcia MA, Solans C, Calvo A, et al. Determination of enrofloxacin and its primary metabolite, ciprofloxacin, in pig tissues. Application to residue studies. *Biomed Chromatogr* 2005;19:27-31.
7. Wolfson JS, Hooper DC. The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. *Antimicrob Agents Chemother* 1985;28:581-586.
8. Altreuther P. Data on chemistry and toxicology of Baytril. *Vet Med Rev* 1987;2:87-89.
9. Scheer M. Studies on the antibacterial activity of Baytril. *Vet Med Rev* 1987;2:90-99.
10. Scheer M. Concentrations of active ingredient in the serum and in tissue after oral and parenteral administration of Baytril. *Vet Med Rev* 1987;2:104-118.
11. Rollins LD, Mercer HD, Carter GG, et al. Absorption, distribution, and excretion of penicillin and dihydrostreptomycin in dairy cows following intramammary infusion. *J Dairy Sci* 1970;53:1407-1414.
12. Hardwick DC, Linzell JL. Some factors affecting milk secretion by the isolated perfused mammary gland. *J Physiol* 1960;154:547-571.

13. Linzell JL, Fleet IR, Mephram TB, et al. Perfusion of the isolated mammary gland of the goat. *Q J Exp Physiol Cogn Med Sci* 1972;57:139–161.
14. Kietzmann M, Löscher W, Arens D, et al. The isolated perfused bovine udder as an in vitro model of percutaneous drug absorption. Skin viability and percutaneous absorption of dexamethasone, benzoyl peroxide, and etofenamate. *J Pharmacol Toxicol Methods* 1993;2:75–84.
15. Ehinger AM, Kietzmann M. Tissue distribution of oxacillin and ampicillin in the isolated perfused bovine udder. *J Vet Med A Physiol Pathol Clin Med* 2000;47:157–168.
16. Pittermann W. In-vitro skin and mucous membrane tests in the focus of cosmetics research. Available at: www.gd-online.de/german/originals_d/Pittermann2000.html. Accessed Feb 22, 2010.
17. Zeitlin JJ, Eshraghi HR. The release and vascular action of bradykinin in the isolated perfused bovine udder. *J Physiol* 2002;543:221–231.
18. Ehinger AM, Schmidt H, Kietzmann M. Tissue distribution of cefquinome after intramammary and systemic administration in the isolated perfused bovine udder. *Vet J* 2006;172:147–153.
19. Kietzmann M, Braun M, Schneider M, et al. Tissue distribution of marbofloxacin after systemic administration into the isolated perfused bovine udder. *Vet J* 2008;178:115–118.
20. Banks WJ. Aparato tegumentario. In: *Histologia veterinaria aplicada*. 2nd ed. Mexico City: El Manual Moderno, 1986;430–434.
21. Manceau J, Gicquel M, Laurentie M, et al. Simultaneous determination of enrofloxacin and ciprofloxacin in animal biological fluids by high-performance liquid chromatography. Application in pharmacokinetics studies in pig and rabbit. *J Chrom* 1999;726:175–184.
22. Diaz D, Picco EJ, Encinas T, et al. Residuos tisulares de nico-tinato de norfloxacin administrado por vía oral en cerdos. *Arch Med Vet* 2001;33:37–42.
23. Tietz NW. *Clinical guide to laboratory tests*. 3rd ed. Philadelphia: WB Saunders, 1995;382–383.
24. Ehinger AM, Kietzmann M. Tissue distribution of benzylpenicillin after intramammary administration in the isolated perfused bovine udder. *J Vet Pharmacol Ther* 2000;23:303–310.
25. Funke H. The distribution of 35S-labelled benzylpenicillin in normal and mastitic mammary glands of cows and goats after local and systemic administration. *Acta Vet Scand* 1961;2(suppl 1):1–88.
26. Ehinger AM, Kietzmann M. Pharmakokinetische aspekte der mastitistherapie. *Berl Munch Tierarztl Wochenschr* 1998;9:337–343.
27. Ehinger AM, Bäumer W, Zerbe H, et al. Verteilung von intrazisternal appliziertem Benzylpenicillin im drüsengewebe des rindereuters: abhängigkeit vom Gesundheitszustand. *Tierartl Praxis* 2004;32:79–83.
28. Ziv G. Drug selection and use in mastitis: systemic vs local therapy. *J Am Vet Med Assoc* 1980;176:1109–1115.
29. Hyatt JM, McKinnon PS, Zimmer GS, et al. The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. *Clin Pharmacokinet* 1995;28:143–160.
30. Lode H, Borner K, Koeppel P. Pharmacodynamics of fluoroquinolones. *Clin Infect Dis* 1998;27:33–39.
31. Schentag JJ. Antimicrobial action and pharmacokinetics/pharmacodynamics: the use of AUC to improve efficacy and avoid resistance. *J Chemother* 1999;11:426–439.
32. Pickerill KE, Paladino JA, Schentag JJ. Comparison of the fluoroquinolones based on pharmacokinetic and pharmacodynamic parameters. *Pharmacotherapy* 2000;20:417–428.
33. Schentag JJ, Gilliland KK, Paladino JA. What have we learned from pharmacokinetic and pharmacodynamic theories? *Clin Infect Dis* 2001;32(suppl 1):39–46.
34. McKellar QA, Sanchez Bruni SF, Jones DG. Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *J Vet Pharmacol Ther* 2004;27:503–514.
35. Drusano GL, Johnson DE, Rosen M, et al. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas sepsis*. *Antimicrob Agents Chemother* 1993;37:448–490.
36. Forrest A, Nix DE, Ballow CH, et al. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* 1993;37:1073–1081.
37. Haritova A, Lashev L, Pashov D. Pharmacokinetics of enrofloxacin in lactating sheep. *Res Vet Sci* 2003;74:241–245.