

Evaluation of the distribution of enrofloxacin by circulating leukocytes to sites of inflammation in dogs

D. M. Boothe, DVM, PhD; A. Boeckh, DVM, PhD; H. W. Boothe, DVM, MS

Objective—To determine the effect of WBC accumulation on the concentration of enrofloxacin in inflamed tissues in dogs.

Animals—6 adult Bloodhounds.

Procedures—Dogs were instrumented bilaterally with tissue chambers. Peripheral WBCs collected from each dog were exposed in vitro to radiolabeled enrofloxacin (^{14}C -ENR). Inflammation was induced with carrageenan in 1 chamber. Ten hours later, treated cells were administered IV to each dog such that ^{14}C -ENR was delivered at a mean \pm SD dosage of $212 \pm 43 \mu\text{g}$. Samples of extracellular fluid from inflammation and control chambers and circulating blood were then collected before (baseline) and for 24 hours after WBCs were administered. Samples were centrifuged to separate WBCs from plasma (blood) or chamber fluid. Radiolabeled enrofloxacin was scintigraphically detected and pharmacokinetically analyzed. Comparisons were made between extra- and intracellular chamber fluids by use of a Student paired *t* test.

Results— ^{14}C -ENR was not detectable in plasma, peripheral WBCs, control chambers, or baseline samples from inflammation chambers. However, ^{14}C -ENR was detected in extracellular fluid from inflammation chambers (mean \pm SD maximum concentration, $2.3 \pm 0.5 \text{ ng/mL}$) and WBCs (maximum concentration, $7.7 \pm 1.9 \text{ ng/mL}$). Mean disappearance half-life of ^{14}C -ENR from extracellular fluid and WBCs from inflammation chambers was 26 ± 10 hours and 17 ± 6 hours, respectively.

Conclusions and Clinical Relevance—WBCs were responsible for the transport and release of ^{14}C -ENR at sites of inflammation. Accumulation of drug by WBCs might increase the concentration of drug at the site of infection, thus facilitating therapeutic success. (*Am J Vet Res* 2009;70:16–22)

Researchers have used tissue chambers to evaluate movements of drugs into sites of inflammation.^{1,2} Such studies³ include those in which the effect of inflammation on antimicrobial delivery is evaluated. Identification of the mechanism of antimicrobial concentration at the site of inflammation is complicated by the multifactorial nature of the inflammatory response. Multiple components of inflammation, particularly in acute phases of inflammation, may influence drug concentration at sites of inflammation.

Increased vascular permeability and blood flow generally increase drug movement into sites of inflam-

Received January 4, 2008.

Accepted May 5, 2008.

From the Departments of Veterinary Physiology and Pharmacology (DM Boothe, Boeckh) and Small Animal Clinical Sciences (HW Boothe), College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843. Dr. D. M. Boothe's present address is Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849. Dr. Boeckh's present address is Fort Dodge Animal Health, PO Box 5366, Princeton, NJ 08543. Dr. H. W. Boothe's present address is Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL 36849.

Supported in part by Bayer Animal Health.

Presented in abstract form at the American College of Veterinary Internal Medicine Forum, Dallas, May 2002.

Address correspondence to Dr. D. M. Boothe.

ABBREVIATIONS

^{14}C -ENR	Radiolabeled enrofloxacin
$\text{AUC}_{0-\infty}$	Area under the concentration-time curve to infinity
C_{max}	Maximum concentration
k_d	Constant rate of disappearance
MEM	Minimum essential medium
MRT	Mean residence time
$t_{1/2d}$	Half-life of disappearance
T_{max}	Time to maximum concentration

mation. For example, the concentration of fluoroquinolone in cantharidin-induced human skin blisters is postulated to reflect increased blood flow and capillary permeability associated with the acute phase of inflammation.¹ Environmental pH, protein binding, and other factors facilitate influx or efflux of drug from the inflamed site.¹ For fluoroquinolones, an additional potential factor is accumulation of drug within phagocytic WBCs. Such a phenomenon has been reported for various fluoroquinolones.^{4–9} Studies^{7,9} of human WBCs exposed to fluoroquinolones in vitro have revealed intracellular accumulation of ofloxacin and ciprofloxacin. Enrofloxacin, its active metabolite ciprofloxacin, and marbofloxacin, which are all approved in animals, accumulate in WBCs.^{4,5,8,10,11} In dogs, increased concentrations reportedly occur in alveolar macrophages (en-

rofloxacillin, 10-fold; marbofloxacin, 20-fold) and WBCs (enrofloxacin, 60-fold), compared with concentrations of the same drugs in plasma.^{4,5,8} Although accumulation of other antimicrobials by WBCs has been reported,^{11,12} a potential advantage of fluoroquinolone accumulation in WBCs is its cytoplasmic location. As such, enhanced efficacy toward intracellular bacteria may result, as occurs with phagocytic WBCs containing enrofloxacin in swine infected with *Staphylococcus* spp.² Accumulation of fluoroquinolones within WBCs may also increase drug concentrations at sites of inflammation via passive diffusion, active transport, or death of WBCs.

It is difficult to isolate the impact of fluoroquinolone delivery by WBCs from other effects of inflammation that influence the delivery of drugs to inflamed tissue sites. The purpose of the study reported here was to evaluate the effect of concentrations of ¹⁴C-ENR in phagocytic WBCs on concentrations of enrofloxacin at inflamed sites in dogs by use of a tissue chamber model. It was hypothesized that the concentration of enrofloxacin at an inflamed site is associated with the concentration of ¹⁴C-ENR in WBCs.

Materials and Methods

Animals—Six research Bloodhounds (3 males and 3 females; age range, 2.0 to 2.5 years; body weight, 21.5 to 33 kg) were included in the study. All dogs were clinically normal on the basis of results of physical examination and clinical pathologic testing. Except for days on which the study took place, dogs were housed in separate runs. Dogs were fed a commercially available dry food^a once daily, had access to fresh water ad libitum, and received no medication other than a monthly oral heartworm preventative.^b The study protocol was reviewed and approved by the institutional laboratory animal care committee and the institutional office for radiologic safety, which regulates the use of radioactive materials.

Tissue chambers—Tissue chambers were modifications of those used in several species in other studies.^{1,2} Modifications included additional smaller perforations on the wall of the chamber, which were used to suture the chamber to the underlying tissue. Chambers were manufactured at Texas A&M University from a biocompatible rigid plastic,^c which was purchased as a solid cylinder (diameter, 41 mm) and modified to an appropriate shape. The cylinder was cut to 12 mm in height and machined to produce a concave form with smooth edges. The top of the chamber was open, and the walls and most of the bottom were fenestrated, allowing entry and exit of extracellular fluid and its constituents (Figure 1). Approximately a quarter of the bottom surface was unfenestrated, lessening the potential for puncture of underlying tissue during percutaneous sample collection. The top of the chamber was covered with 1 layer of a 0.5-mm biocompatible silicone sheet^d secured with a 28-gauge monofilament stainless steel suture that fit in a groove near the top of the chamber.

Two autoclaved tissue chambers were implanted SC, with 1 chamber over each scapula of each dog. Both scapular regions were clipped, and the skin was prepared aseptically. Skin over the mid portion of the

scapula was incised horizontally for 5 cm. Blunt dissection of the dorsal subcutaneous tissue formed a pocket for insertion of the chamber. Chambers were positioned in the pocket with the silicone sheeting located superficially and the unfenestrated portion of the chamber bottom located dependently. Chambers were secured to underlying muscle by means of 2 anchoring sutures of an absorbable monofilament synthetic material.^e Dogs were fitted with a stockinet to cover the surgical site overnight. Tissue chamber sites were allowed to heal for approximately 1 month prior to the experiment.

All dogs were evaluated within a 2-week period. Chambers on each dog (left or right) were randomly designated as either inflammation or control chambers. Within 24 hours before each phase of the experiment, a thorough physical examination, CBC, and serum biochemical analysis were performed. On the day of the study, each dog was housed in a stainless steel cage with a grid to collect voided urine and feces. Ten hours prior to each experiment (–10 hours), 1 mL of chamber fluid was removed aseptically by puncturing the skin and silicone sheet with a 22-gauge needle.^f The needle was directed toward the unfenestrated portion of the chamber to minimize trauma to underlying tissue. Inflammation was induced in the designated chamber via infusion of 1 mL of a freshly prepared solution of 1% carrageenan.^g Seven to 8 hours later, (–3 to –2 hours), 1 mL of fluid was collected from each chamber. Whole blood (200 mL) was collected aseptically via jugular venipuncture from each dog by use of a commercial blood collection system comprised of a vacuum bottle containing dextrose and sodium citrate.^h The volume of blood removed was replaced IV with an equivalent volume of isotonic saline (0.9% NaCl) solution via a cephalic catheter.ⁱ

Preparation of WBCs—Peripheral WBCs were incubated with ¹⁴C-ENR, which was supplied as a stock solution in acetonitrile at a concentration of 2.25 mg/mL (1.19 mCi; 53.2 mCi/mmol or 528 μ Ci/mL).^j The radiolabeled drug consisted of a carbon-14 on position 4; as such, the radiolabel was retained in the event of metabolism to ciprofloxacin. Therefore, for the purposes of the study reported here, ¹⁴C-ENR refers to radiolabeled enrofloxacin plus any potential radiolabeled metabolite.

For incubation purposes, ¹⁴C-ENR was diluted in sterile medium.^k The solution was freshly prepared on each day of the study by adding 25 μ L of stock solution of enrofloxacin to 5 mL of sterile MEM,^k for a final concentration of 11.25 μ g/mL.

Each sample of whole blood was decanted into a sterile 50-mL borosilicate glass tube^l and centrifuged at 1,150 \times g for 15 minutes. The WBC layer (buffy coat) was aspirated with a sterile Pasteur pipette and transferred into a sterile 5-mL borosilicate glass tube.^l Samples of WBCs were maintained at 5°C. Contaminant RBCs were lysed by adding 2 mL of sterile hypotonic saline (0.2% NaCl; 64.8 mOsm) solution for 30 seconds, and isotonicity was restored by adding 1 mL of sterile hypertonic saline (1.6% NaCl; 547.6 mOsm) solution. The resulting fluid was centrifuged aseptically at 700 \times g for 10 minutes to yield a pellet of WBCs. Supernatant was discarded, and the pellet was resuspended in

5 mL of sterile MEM, to which sufficient ^{14}C -ENR was added to render a final ^{14}C -ENR concentration of 11.25 $\mu\text{g}/\text{mL}$. The ^{14}C -ENR solution was freshly prepared on each study day and sterilized by filtration.^m

White blood cells were incubated in the ^{14}C -ENR solution at 37°C for 60 minutes in an environment of 6% CO_2 tension to allow uptake of ^{14}C -ENR by WBCs. After incubation, the suspension was centrifuged at 700 $\times g$ for 10 minutes at 5°C, and the ^{14}C -ENR-rich supernatant was removed. An aliquot of supernatant was set aside for analysis to confirm the concentration of ^{14}C -ENR. The remaining pellet of WBCs containing ^{14}C -ENR was washed briefly (inverted 3 times) with 5 mL of sterile, cold isotonic saline solution to remove any residual ^{14}C -ENR-rich supernatant and immediately centrifuged at 700 $\times g$ for 10 minutes. The supernatant was removed, and an aliquot was saved to determine the concentration of ^{14}C -ENR.

The resulting pellet of WBCs containing ^{14}C -ENR was resuspended in 5 mL of sterile, cold isotonic saline solution and, after removal of a 0.5-mL aliquot, was injected IV into the donor dog through a cephalic catheter. The saved aliquot was used to determine the concentration of ^{14}C -ENR, evaluate WBC viability with a vital stain (0.4% Trypan blue), and quantify numbers of WBCs by use of a modified Wright stain, hemocytometer, and optical microscopy. For quantitation of ^{14}C -ENR, the remaining sample of WBCs containing ^{14}C -ENR (460 μL) was centrifuged at 1,150 $\times g$ for 15 minutes, and the supernatant (which may have contained ^{14}C -ENR that had leaked from the WBCs) and WBC pellet (which provided information regarding the dose of ^{14}C -ENR given to each dog) were assayed, as described elsewhere.⁴

Evaluation of contents of tissue chambers—Fluid samples (1 mL) were collected from the inflammation and control chambers by means of a 22-gauge needle^l prior to (baseline; 0 hours) and 2, 4, 6, 12, and 24 hours after IV administration of WBCs containing ^{14}C -ENR. Simultaneously, a 22-mL sample of whole blood was collected from a jugular vein of each dog. To determine the concentration of ^{14}C -ENR in plasma, 2 mL of whole blood was added to heparin. To determine the concentration of ^{14}C -ENR in circulating WBCs, 20 mL of whole blood was added to 3.8% sodium citrate solution. Samples of chamber fluid were pipetted in preweighed microcentrifuge tubes,ⁿ and each sample was weighed with an analytic balance to estimate the volume. One milliliter was considered to weigh 1 g. As with the suspension of WBCs containing ^{14}C -ENR, an aliquot of each sample of chamber fluid was stained with 0.4% Trypan blue to quantify and assess cell viability, and a second aliquot was used for a differential count. The remainder of each sample was centrifuged at 1,150 $\times g$ for 15 minutes, and the supernatant, representative of extracellular fluid, was harvested.

Sample preparation—Pellets of WBCs from blood samples were resuspended to a volume of 1 mL by weighing the sample and adding a balanced salt solution^o to achieve a final net weight of 1 g. The volume of the WBC pellet was determined by multiplying the number of WBCs in the sample (determined by cell count)

by 135 μm^3 , which is the mean volume of circulating WBCs in dogs.¹³ Samples intended for analysis of WBCs containing ^{14}C -ENR were processed as described elsewhere.⁴ Blood samples used to determine plasma concentrations of enrofloxacin were centrifuged at 1,150 $\times g$ for 15 minutes and frozen until analysis.

Urine was collected for 48 hours (ie, 24 hours after collection of the last chamber sample) and treated as radioactive waste. Total volume of urine from each dog was recorded, and a 0.5-mL aliquot was saved for detection of ^{14}C -ENR. All samples intended for quantitation of ^{14}C -ENR were frozen at -20°C within 2 hours after collection. Samples were analyzed within 1 week after sample collection by means of liquid scintillation counting.

Assay validation—On the day of sample analysis, a calibration curve spanning the range of expected sample concentrations in serum was validated for each of the different fluids tested by adding known amounts of ^{14}C -ENR to ^{14}C -ENR-free aliquots of canine plasma, canine urine, cell lysate, chamber fluid, and MEM. The optimal ratio of sample and scintillation solution^p volume that would provide maximum signal enhancement (thus lowering the lower limit of quantitation) was de-

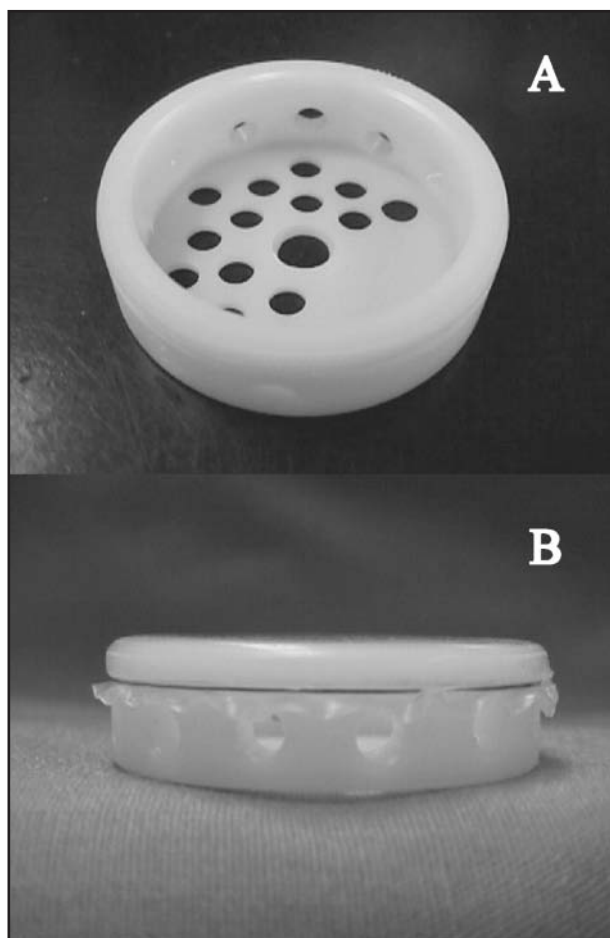


Figure 1—Photographs of the top (A) and side (B) of a tissue chamber used to evaluate the concentration of enrofloxacin in tissues at an experimentally induced inflamed site. The top of the chamber was covered with a 0.5-mm layer of silicone sheet that was secured in place with stainless steel suture.

terminated by preparing 2 standards in which concentrations of ^{14}C -ENR were within the range of those anticipated in samples of chamber fluid (1.125 and 4.5 ng/mL).

Calibration points for each fluid ranged from 0.002 to 8,260 ng/mL. Control concentrations were chosen to span the breadth of the ^{14}C -ENR concentrations detected in WBCs. Lower and upper limits of quantification for the ^{14}C -ENR assay were 0.22 and 6,630 ng/mL, respectively, reflecting the lowest and highest concentrations of the control samples predicted accurately by the standard curve. For samples that had ^{14}C -ENR concentrations that exceeded the upper limit of quantification, samples were reassayed after a 1:2 dilution in scintillation solution; appropriate control samples were similarly diluted. All control samples except those representing the lowest concentration in each tissue predicted values within 15% of actual concentrations; all lowest concentrations in each tissue predicted values within 18%.

The equation that described the linear regression line (standard curve) was as follows:

$$\text{Conc}_{\text{ENR}} = \frac{\text{CPM} - 10.04}{156.41}$$

in which Conc_{ENR} is the predicted concentration of enrofloxacin in the sample, and CPM is the number of counts per minute as measured by scintillation counting. The value of R^2 for the regression line was equal to 0.991.

Sample drug analysis—Cell pellet samples were defrosted in a hot water bath (56°C) and sonicated for 15 minutes to lyse cells and release ^{14}C -ENR. Sonication was repeated 3 times to ensure that no intact cells remained in the sample. Samples were allowed to return to room temperature (approx 24°C) to prevent condensation on the vials during scintillation counting. A portion (500 μL) of each liquid sample (plasma, urine, cell lysate, and chamber fluid) was transferred to scintillation counting vials,^q to which 4,500 μL of scintillation solution^p was added. Samples were capped and vortexed for 15 seconds to ensure proper mixing. For cell lysate samples, the entire sample was used for quantification of ^{14}C -ENR, regardless of the size of the sample. Cell lysate was transferred to vials by washing the microcentrifuge tube containing lysate with 0.5 mL of the scintillation solution to the volume of 5 mL. All samples were protected from exposure to light to minimize photoluminescence and prevent interference during scintillation counting.

Data regarding ^{14}C -ENR concentration versus time were subjected to computer pharmacokinetic analysis,^r in which $\text{AUC}_{0-\infty}$ was determined by means of a linear-log trapezoidal method. After completion of the study, chambers were excised, and surrounding tissue was histologically evaluated.

Statistical analysis—Descriptive statistics for ^{14}C -ENR in plasma and for extracellular fluid and WBCs from inflammation and control chambers are reported as mean \pm SD for all variables except half-life, which is reported as harmonic mean \pm pseudo-SD. Comparisons

were made between chambers and between tissues for C_{max} , T_{max} , MRT, k_d , and $t_{1/2d}$ by use of a paired Student t test.^s Values of $P < 0.05$ were deemed significant.

Results

Animals—All dogs were considered clinically normal. Values for CBCs and serum biochemical analyses were within reference ranges before the study began.

Tissue chambers—Twelve chambers were implanted in 6 dogs (2 chambers/dog). No adverse reaction such as bleeding or scratching was observed in any dog within 2 weeks after implantation. However, infections associated with 4 chambers developed in 3 dogs at 3 weeks after implantation. Those chambers were removed after unsuccessful attempts to salvage them. Because both chambers in 1 dog were associated with an infection, only 5 dogs were included in the study. In 2 of 5 dogs, only data from the inflammation chamber were evaluated; however, baseline data that were collected from all dogs prior to administration of WBCs containing ^{14}C -ENR served as negative control values.

In infected tissues, chambers were surrounded by purulent exudate. Microbiologic culture of material from those chambers revealed a *Klebsiella* sp in 1 chamber and *Staphylococcus intermedius* in another. *Escherichia coli* was isolated from both chambers of the dog removed from the study.

Characteristics of extracellular fluid and WBCs—Evaluation of WBC suspensions administered to each dog revealed 100% cell survival. Numbers of cells administered in the 4.5-mL aliquot ranged from 2.5×10^6 cells/mL to 10.1×10^6 cells/mL, yielding a mean \pm SD ^{14}C -ENR concentration of 47.08 ± 9.52 $\mu\text{g}/\text{mL}$ and a mean dose of 212 ± 43 $\mu\text{g}/\text{dog}$. Neutrophils were the predominant cells in all chamber fluid samples.

Radiolabeled enrofloxacin was not detected at any time in plasma, extracellular fluid or WBCs collected from control chambers, or baseline samples from inflammation chambers. In contrast, ^{14}C -ENR was detected in extracellular fluid and WBCs from inflammation chambers of all dogs at all times after WBCs containing ^{14}C -ENR were administered (Figure 2). Ra-

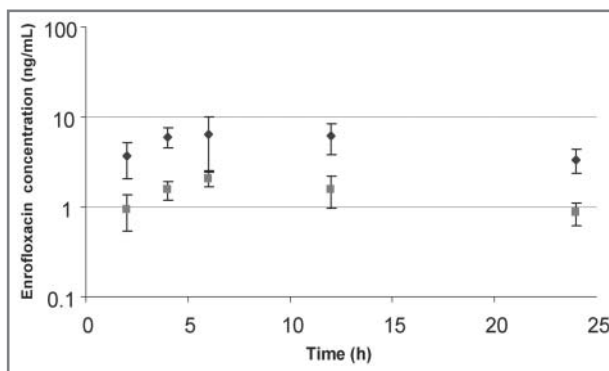


Figure 2—Graph of mean \pm SD concentrations of enrofloxacin in extracellular fluid (diamonds) and WBCs (squares) from inflammation chambers after WBCs containing ^{14}C -ENR were administered IV to 5 healthy dogs. Notice that the scale for the y-axis is logarithmic.

Table 1—Concentrations (ng/mL) of ¹⁴C-ENR in extracellular fluid and WBCs from inflammation chambers at various points before (–10 hours and 0 hours) and after IV administration of WBCs containing ¹⁴C-ENR in 5 healthy dogs.

Time (h)	Extracellular fluid		WBCs	
	Mean	SD	Mean	SD
–10	ND	ND	ND	ND
0	ND	ND	ND	ND
2	0.94	0.41	3.7	1.6
4	1.56	0.39	6.0	1.5
6	2.05	0.40	6.3	3.4
12	1.59	0.61	6.2	2.4
24	0.87	0.25	3.4	1.0

ND = Not detectable.

Table 2—Pharmacokinetic values for ¹⁴C-ENR in extracellular fluid and WBCs from inflammation chambers after IV administration of WBCs containing ¹⁴C-ENR in 5 healthy dogs.

Parameter	Extracellular fluid		WBCs	
	Mean	SD	Mean	SD
C _{max} (ng/mL)	2.29*	0.52	7.72	1.94
T _{max} (h)	10.2	8.6	6.4	3.2
AUC _{0–∞} (ng/mL/h)	98.4*	8.5	203.4	72.0
K _d (h ^{–1})	0.018	0.016	0.043	0.014
t _{1/2d} (h)	26	10	17	6
MRT (h)	42	15	27	12

*Mean values are significantly ($P < 0.01$) different between extracellular fluid and WBCs.

diolabeled enrofloxacin was detectable in extracellular fluid and WBCs obtained from inflammation chambers at 24 hours after administration of WBCs containing ¹⁴C-ENR (Table 1). Differences in pharmacokinetic values between extracellular fluid and WBCs in inflammation chambers were limited to C_{max} and AUC_{0–∞}, both of which were significantly ($P \leq 0.01$) higher in WBCs. Mean C_{max} of ¹⁴C-ENR in extracellular fluid from inflammation chambers was 2.29 ± 0.52 ng/mL at a T_{max} of 10.2 ± 8.6 hours. For WBCs, C_{max} and T_{max} were 7.72 ± 1.9 ng/mL and 6.4 ± 3.2 hours, respectively. Harmonic mean \pm pseudo-SD t_{1/2d} of ¹⁴C-ENR was 26 ± 10 hours in extracellular fluid and 17 ± 6 hours in WBCs (Table 2).

Radioactivity in urine decreased over time; $25.02 \pm 13.14\%$ of the administered ¹⁴C-ENR was excreted in the urine at 48 hours after injection. After the experiment, histologic evaluation of tissue surrounding excised chambers revealed dense fibrous connective tissue. At the time of chamber removal, evidence of acute inflammation was minimal.

Discussion

Bacterial infection is a common complication of tissue chamber implantation, reportedly occurring with 33% to 50% of implanted chambers.¹ In the study reported here, a third of the surgically implanted chambers (4/12) were associated with infection, necessitating their removal. Infection was detected 3 weeks after surgery.

Results of the present study suggested that the concentration of enrofloxacin in inflamed tissues was influenced by delivery of enrofloxacin by WBCs. Lack of detectable concentrations of enrofloxacin in serum during the study period indicates that the enrofloxacin detected within chambers originated from WBCs rather than diffusion from plasma.

Disappearance half-life of enrofloxacin in plasma of dogs evaluated in another study¹⁴ ranged from 4.6 to 5.2 hours, which is shorter than that measured in extracellular fluid obtained from tissue chambers in the present study (26 ± 10 hours). The likely reason for a greater half-life of enrofloxacin in chamber fluid is a potential delay in release of enrofloxacin by the WBCs. Indeed, time versus concentration curves of enrofloxacin in extracellular fluid were mirrored by those in WBCs. Appearance of enrofloxacin in chamber fluid is related to the rate of migration of WBCs to the site. Once at the site of inflammation, WBCs may not immediately release enrofloxacin, contributing to the enrofloxacin concentration in WBCs at the site but not to enrofloxacin concentration in extracellular fluid.

When comparing disappearance half-lives from different studies, analytic methodology needs to be considered. In the present study, enrofloxacin was not differentiated from its metabolite ciprofloxacin, which has a longer half-life in dogs.⁴ If WBCs metabolized enrofloxacin to ciprofloxacin, a longer half-life may have resulted. More likely causes of differences in t_{1/2d} of enrofloxacin in plasma and chamber fluid are factors that contribute to increased concentrations of enrofloxacin at sites of inflammation, including ionization and trapping of enrofloxacin, binding of enrofloxacin to cellular debris (eg, protein), and physical barriers impacting efflux of enrofloxacin. Fibrous tissue deposition around the chamber is 1 likely contributor to the longer t_{1/2d} of enrofloxacin in chamber fluid.

The t_{1/2d} of enrofloxacin was also longer in WBCs collected from the inflamed site (17 ± 6 hours) than that previously reported for circulating WBCs (4.92 ± 1.17 hours).⁴ This difference may be explained, in part, by continued migration of WBCs to the site of inflammation. Continued release of enrofloxacin by WBCs reaching the site of inflammation might mimic a pharmacokinetic “flip-flop” phenomenon, in which the rate of drug disappearance is dictated by its rate of appearance. Our study design did not allow confirmation of such a phenomenon, which would have required injection of WBCs containing ¹⁴C-ENR directly into tissue chambers.

The mechanism of efflux of fluoroquinolones from phagocytic WBCs is not clear, but bacteria are capable of active efflux of fluoroquinolones as a mechanism of antimicrobial resistance.¹⁵ A similar mechanism may exist in phagocytic WBCs. Another study¹² revealed that antimicrobial efflux in a macrophage cell line can be decreased via inhibition of an anionic transport system with probenecid. An active mechanism for the efflux of enrofloxacin from phagocytic cells is possible, although release of enrofloxacin upon death of WBCs is also possible.

In the study reported here, the disposition of enrofloxacin in WBCs from tissue chambers paralleled that

in extracellular fluid. Most circulating WBCs would not be expected to contain enrofloxacin because $\leq 10\%$ of the dogs' blood volume was collected and exposed to ^{14}C -ENR. The lack of detectable concentrations of enrofloxacin in samples of WBCs collected from peripheral blood is similarly explained. White blood cells containing ^{14}C -ENR were not differentiated from WBCs that did not contain enrofloxacin; therefore, the proportion of WBCs containing ^{14}C -ENR in the chamber was unknown.

With progression of inflammation from acute to chronic, changes in populations of WBCs occur. Neutrophils, which are the first cells to arrive at an inflamed site, would be expected to be replaced with monocytes, which are more reflective of chronic inflammation. A study³ in which WBCs labeled with ^3H -thymidine and ^{14}C were used revealed that neutrophils increase in number until about 15 hours after an inflammatory insult, then decline, and are surpassed by mononuclear cells between 25 and 30 hours after that insult. In the present study, WBCs containing ^{14}C -ENR were injected 10 hours after onset of inflammation. Neutrophils should have predominated until approximately 15 hours after WBCs were injected. Changes in WBC populations possibly occurred but were undetected because of the short duration of this study (ie, final samples were collected at 24 hours [34 hours after inflammatory stimulus]).

The amount of enrofloxacin excreted in the urine within 48 hours was approximately 25% of that administered. According to the $t_{1/2d}$ of enrofloxacin in the WBCs in this study (17 hours), approximately 80% of enrofloxacin should have been eliminated from WBCs (and thus plasma) at 48 hours. The difference may reflect other routes of elimination (eg, biliary). Lack of detectable enrofloxacin in the control chambers with noninflamed tissue supports the conclusion that transport and release of enrofloxacin at sites of inflammation were attributable to WBCs.

To evaluate whether enrofloxacin in WBCs can result in detection of enrofloxacin in inflamed versus noninflamed tissues, we eliminated other factors that could have accounted for the movement of enrofloxacin to those tissues. Such factors included those related to an increase in blood flow or capillary permeability, trapping of ions in an environment with an altered pH, and physical barriers to entry or exit of enrofloxacin at the inflamed site. Administration of enrofloxacin only via WBCs precluded any other source of enrofloxacin from having contributed to the drug concentration measured at the site of inflammation. Lack of a detectable plasma concentration of enrofloxacin ruled out contribution by plasma. Duration of enrofloxacin within the tissue chambers could have been influenced by factors other than WBC accumulation, including fibrous tissue deposition.

The concentration of enrofloxacin achieved in WBCs or extracellular fluid within tissue chambers in the present study should not be considered therapeutic; concentrations were 1,000-fold lower than concentrations measured during routine microbial culture and susceptibility testing. However, the intent of our study was not to evaluate potential efficacy of enrofloxacin when distributed to inflamed tissues via WBCs. Rather, the intent was to evalu-

ate whether efficacy of enrofloxacin administration might be enhanced or facilitated by distribution via phagocytic WBCs to sites of inflammation. Accordingly, the results of the present study suggested that clinical efficacy of enrofloxacin or other fluoroquinolones may reflect, in part, accumulation of the drug in WBCs and its subsequent transport in WBCs to the site of inflammation associated with infection. Additional studies designed to evaluate the impact of fluoroquinolone movement via WBCs on microbial response at the site of infection are warranted.

-
- a. Science Diet Canine Maintenance, Hills Pet Nutrition Inc, Topeka, Kan.
 - b. Heartgard Plus, Merial, Duluth, Ga.
 - c. Delrin thermoplastic, DuPont, Wilmington, Del.
 - d. Silastic, Dow Corning Corp, Alsip, Ill.
 - e. Monocryl, Ethicon Inc, Piscataway, NJ.
 - f. PosiGrip Huber needle, Access Technologies, Skokie, Ill.
 - g. Carrageenan, Sigma-Aldrich Inc, St Louis, Mo.
 - h. Metrix Co, Dubuque, Iowa.
 - i. Abbotath, Abbott Laboratories, North Chicago, Ill.
 - j. Bayer Corp, Shawnee Mission, Kan.
 - k. Eagle minimum essential medium, Sigma-Aldrich Corp, St Louis, Mo.
 - l. VWR International, Suwanee, Ga.
 - m. Syringe filter (0.2 μm), Whatman Inc, Florham Park, NJ.
 - n. Fisherbrand microcentrifuge tubes, Fisher Scientific, Fair Lawn, NJ.
 - o. Hank's balanced salt solution, Invitrogen Corp, Carlsbad, Calif.
 - p. Ultima Gold, PerkinElmer Inc, Waltham, Mass.
 - q. Tri-Carb pony vials, PerkinElmer Inc, Waltham, Mass.
 - r. Winnonlin, Pharsight Corp, Mountain View, Calif.
 - s. Microsoft Excel, Microsoft Corp, Redmond, Wash.
-

References

1. Clarke CR. Tissue-chamber modeling systems—applications in veterinary medicine. *J Vet Pharmacol Ther* 1989;12:349–368.
2. Wattrang E, Wallgren P, Fuxler L, et al. Tissue chambers—a useful model for in vivo studies of cytokine production in the pig. *Vet Immunol Immunopathol* 1997;56:133–150.
3. Movat HZ. The vascular phenomena of acute inflammation. In: Movat HZ, ed. *The inflammatory reaction*. New York: Elsevier Science, 1985;43–65.
4. Boeckh A, Boothe DM, Wilkie S, et al. Time course of enrofloxacin and its active metabolite in peripheral leukocytes of dogs. *Vet Ther* 2001;2:334–344.
5. Boothe HW, Jones SA, Wilkie WS, et al. Evaluation of the concentration of marbofloxacin in alveolar macrophages and pulmonary epithelial lining fluid after administration in dogs. *Am J Vet Res* 2005;66:1770–1774.
6. Carlier MB, Scorneaux B, Zenebergh A, et al. Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. *J Antimicrob Chemother* 1990;26(suppl B):27–39.
7. Garraffo R, Jambou D, Chichmanian RM, et al. In vitro and in vivo ciprofloxacin pharmacokinetics in human neutrophils. *Antimicrob Agents Chemother* 1991;35:2215–2218.
8. Hawkins EC, Boothe DM, Guinn A, et al. Concentration of enrofloxacin and its active metabolite in alveolar macrophages and pulmonary epithelial lining fluid of dogs. *J Vet Pharmacol Ther* 1998;21:18–23.
9. Pascual A, Garcia I, Perea EJ. Fluorometric measurement of ofloxacin uptake by human polymorphonuclear leukocytes. *Antimicrob Agents Chemother* 1989;33:653–656.
10. Schoevers EJ, van Leengoed LA, Verheijden JH, et al. Effects of enrofloxacin on porcine phagocytic function. *Antimicrob Agents Chemother* 1999;43:2138–2143.
11. Tulkens PM. Intracellular distribution and activity of antibiotics. *Eur J Clin Microbiol Infect Dis* 1991;10:100–106.

12. Cao CX, Silverstein SC, Neu HC, et al. J774 macrophages secrete antibiotics via organic anion transporters. *J Infect Dis* 1992;165:322–328.
13. Hogg JC, McLean T, Martin BA, et al. Erythrocyte transit and neutrophil concentration in the dog lung. *J Appl Physiol* 1988;65:1217–1225.
14. Boothe DM, Boeckh A, Boothe HW, et al. Plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin in dogs following single oral administration of enrofloxacin at 7.5, 10, or 20 mg/kg. *Vet Ther* 2002;3:409–419.
15. Hooper DC. Mechanisms of fluoroquinolone resistance. *Drug Resist Updat* 1999;2:38–55.