Pharmacokinetic interactions of flunixin meglumine and enrofloxacin in dogs

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Objective—To examine pharmacokinetic interactions of flunixin meglumine and enrofloxacin in dogs following simultaneously administered SC injections of these drugs.

Animals—10 Beagles (4 males and 6 females).

Procedure—All dogs underwent the following 3 drug administration protocols with a 4-week washout period between treatments: flunixin administration alone (1 mg/kg, SC); simultaneous administration of flunixin (1 mg/kg, SC) and enrofloxacin (5 mg/kg, SC); and enrofloxacin administration alone (5 mg/kg, SC). Blood samples were collected from the cephalic vein at 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12, and 24 hours following SC injections, and pharmacokinetic parameters of flunixin and enrofloxacin were calculated from plasma drug concentrations.

Results—Significant increases in the area under the curve (32%) and in the elimination half-life (29%) and a significant decrease (23%) in the elimination rate constant from the central compartment of flunixin were found following coadministration with enrofloxacin, compared with administration of flunixin alone. A significant increase (50%) in the elimination half-life and a significant decrease (21%) in the maximum plasma drug concentration of enrofloxacin were found following coadministration with flunixin, compared with administration of enrofloxacin alone.

Conclusions and Clinical Relevance—The observed decrease in drug clearances as a result of coadministration of flunixin and enrofloxacin indicates that these drugs interact during the elimination phase. Consequently, care should be taken during the concomitant use of flunixin and enrofloxacin in dogs to avoid adverse drug reactions. (*Am J Vet Res* 2005;66:1209–1213)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are inhibitors of cyclooxygenase that catalyze the incorporation of molecular oxygen into arachidonic acid to produce prostanoids (eg, thromboxanes, prostacyclin, and prostaglandin) and are effectively administered for inflammation and pain. However, despite their therapeutic benefits, it is well known that NSAIDs often induce gastrointestinal adverse effects. In 1997, flunixin meglumine was approved for the control of inflammation and fever in dogs in Japan.

Fluoroquinolones are unique antimicrobials that act through the inhibition of bacterial DNA gyrase and are effective for respiratory and urinary tract infections.[1] Most gram-negative aerobes as well as gram-positive bacteria and mycoplasmas are susceptible to fluoroquinolones. Enrofloxacin, a fluoroquinolone, is now frequently used to treat several species in veterinary clinics.[2-5]

Antimicrobial agents and NSAIDs are often concomitantly used in the treatment of endotoxemia. Flunixin has been reported to reduce fevers and improve clinical signs of endotoxemia.[6] It has been reported that the distribution and elimination of antimicrobial agents in horses were altered by coadministration with NSAIDs.[7,8] In contrast, no difference in pharmacokinetic parameters of enrofloxacin was reported after coadministration of enrofloxacin with flunixin in cows.[9] These discrepancies may be the result of species differences. Although sometimes used together to treat infections in dogs, the pharmacokinetic interaction of enrofloxacin and flunixin has not been studied in this species. The purpose of the study reported here was to evaluate the pharmacokinetic interaction between enrofloxacin and flunixin in dogs.

Materials and Methods

Animals—Ten Beagles (4 males and 6 females) between 3 to 7 years of age and 13 to 18 kg in body weight were used. Dogs were housed individually and fed a commercial dog chow and water ad libitum. All dogs were in good health throughout the experiments, and all experiments were conducted in accordance with the National Veterinary Assay Laboratory Guide for the Care and Use of Experimental Animals.

Experimental design—To minimize the number of dogs, the same 10 dogs received flunixin meglumine alone, flunixin and enrofloxacin, and enrofloxacin alone, with a 4-week washout period between treatments. This was considered to be a sufficient period to avoid possible drug-dependent interactions. The half-life of flunixin and enrofloxacin in dogs is 3.7 and 2.4 hours, respectively.[10,11]

This study included the following 3 experimental protocols: experiment 1, dogs received flunixin[a] alone (1 mg/kg, SC); experiment 2, dogs simultaneously received a dose of flunixin (1 mg/kg, SC) and a dose of enrofloxacin[b] (5 mg/kg, SC); and experiment 3, dogs received enrofloxacin alone (5 mg/kg, SC). Following SC injections in each experiment, blood samples (approx 2 mL) were collected from the cephalic vein at 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12, and 24 hours. Plasma was separated by centrifugation (2,000 × *g* for 10 minutes) and stored at –80°C until assayed. A blood sample before flunixin or enrofloxacin administration was not collected because any extremely low plasma concentrations of these drugs, including ciprofloxacin, a metabolite of enrofloxacin, would not be detectable by the analytic method used.

Analytic methods—Plasma flunixin concentrations were determined by use of **high-performance liquid chro-**

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matography (HPLC).[12] Briefly, 0.6 mL of potassium phosphate buffer (0.3 mmol/L; pH, 3.5) and 3 mL of distilled diethyl ether were added to 0.3 mL of plasma in a tube. Tubes were placed on a vertical agitator for 20 minutes. After centrifugation at 500 × *g* for 10 minutes, the organic phase was transferred to a new tube and evaporated. The residue was reconstituted with the HPLC mobile phase composed of equal volumes of methanol and a sodium phosphate buffer, (0.05 mmol/L; pH, 5.8). Samples were analyzed by use of HPLC with a 5-µm octadecylsilane column[c] at a flow rate of 1 mL/min. The UV light detector was set at 284 nm. Flunixin was resolved with baseline separation and without interfering peaks. Peak identities were confirmed by comparison with the standard. The plasma flunixin concentration was calculated by use of standard curves. The limit of quantification for flunixin was 10 ng/mL, and the recovery of flunixin was 95.6%. Standard curves had correlation coefficients of > 0.999 (between concentrations of 0.05 and 10 µg/mL). The intra- and interassay coefficients of variation were 1.2% and 1.5%, respectively.

Enrofloxacin and ciprofloxacin[d] were also assayed by HPLC; the HPLC column was similar to that used for the assay of flunixin. Plasma samples were extracted by use of a liquid-liquid extraction method.[13] Briefly, 0.3 mL of plasma was placed into a tube, followed by the addition of 3 mL of chloroform. Extraction was performed on a vertical agitator for 10 minutes. Samples were centrifuged at 2,000 × *g* for 10 minutes. The separated organic layer was transferred to another tube and evaporated. The residue was dissolved in the mobile phase of acetonitrile-methanol-aqueous solution (9:9:82 in vol/vol/vol) and separated by HPLC on a 5-µm octadecylsilane column[c] (4.6-mm inner diameter and 150 mm in length) at a flow rate of 1.0 mL/min. The aqueous solution consisted of phosphoric acid (0.4%) and was adjusted to pH 3.0 by the addition of triethylamine. The absorbance of the column effluent was monitored at 278 nm by use of a UV detector. Enrofloxacin and ciprofloxacin were resolved with baseline separation and without any interfering peaks. Peak identities were confirmed by comparison with standards. Plasma flunixin concentration was calculated by use of standard curves. Limits of quantification for enrofloxacin and ciprofloxacin were 10 ng/mL each. The recovery of enrofloxacin and ciprofloxacin was 91.5% and 89.3%, respectively. The standard curve for enrofloxacin was linear between 0.05 and 10 µg/mL (correlation coefficient, > 0.999). Standard curves for ciprofloxacin had correlation coefficients of > 0.999 (between concentrations of 0.01 and 1 µg/mL). The intra- and interassay coefficients of variation for enrofloxacin were 0.8% and 1.2%, respectively, and for ciprofloxacin, they were 1.4% and 2.0%, respectively.

Pharmacokinetics analysis—All pharmacokinetic analyses were performed by use of a weighted least squares and nonlinear regression analysis program.[e] Pharmacokinetics parameters were based on the 1-compartment model, which was determined by the information criterion of Akaike. Pharmacokinetic parameters, that is **area under the curve (AUC)**, **elimination half-life** ($t_{1/2(el)}$), **maximum plasma drug concentration** (C_{max}), and **time to reach** C_{max} (T_{max}), were calculated by use of the software program.[d] The **elimination rate constant** (k_{el}) from the central compartment was calculated from $k_{el} = 0.693/t_{1/2(el)}$.

Statistical analysis—Comparisons of pharmacokinetic parameters between single administration and coadministration of flunixin and enrofloxacin were performed by use of the Wilcoxon signed rank test for paired data. A value of $P < 0.05$ was considered significant.

Results

Safety—No adverse drug effects were observed in any dogs following administration of flunixin meglu-

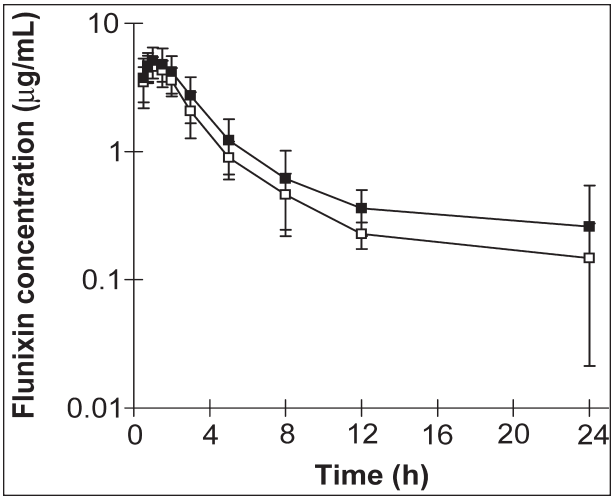

Figure 1—Mean ± SD plasma flunixin concentration in dogs after flunixin (1 mg/kg, SC) administration alone (open squares) or flunixin coadministration (closed squares) with enrofloxacin (5 mg/kg, SC).

Table 1—Pharmacokinetic parameters of flunixin following simultaneous SC injections of enrofloxacin and flunixin meglumine in dogs.

	Pharmacokinetic parameters				
Drug administration	**k_{el} (h^{-1})**	**$t_{1/2(el)}$ (h)**	**AUC (µg•h/mL)**	**T_{max} (h)**	**C_{max} (µg/mL)**
Flunixin alone					
Mean	0.598	1.159*	15.271	1.046	4.592
95% CI	0.408–0.788	0.832–1.486	12.286–18.256	0.876–1.216	3.938–5.246
Median	0.500	1.386	16.670	0.930	4.484
± SD	0.247	0.425	3.883	0.221	0.851
Flunixin and enrofloxacin					
Mean	0.462†	1.500*†	20.108†	1.142	5.219†
95% CI	0.334–0.59	0.909–2.091	15.35–24.866	0.826–1.458	4.334–6.104
Median	0.450	1.540	23.144	1.218	5.256
± SD	0.167	0.769	6.190	0.411	1.151

*Harmonic mean. †Significantly ($P < 0.05$) different from flunixin administration alone.

k_{el} = Elimination rate constant from central compartment. $t_{1/2(el)}$ = Elimination half-life. AUC = Area under the curve. T_{max} = Time to reach maximum plasma concentration. C_{max} = Maximum plasma drug concentration. CI = Confidence interval.

mine or enrofloxacin alone. Also, no adverse drug effects were observed in any dogs following coadministration of flunixin and enrofloxacin.

Pharmacokinetics of flunixin meglumine—Nine of the 10 dogs completed the experiments; 1 dog was excluded because of a failed drug injection. Mean plasma flunixin concentrations following flunixin administration alone or coadministration with enrofloxacin were determined (**Figure 1**). Flunixin was absorbed immediately after SC injection. Mean plasma flunixin concentrations after coadministration with enrofloxacin were always higher than plasma concentrations after administration of flunixin alone. A 1-compartment model described the plasma concentration-time data. Equation parameters derived from the pharmacokinetic constants of flunixin following administration of flunixin alone or coadministration of flunixin and enrofloxacin were determined (**Table 1**). Significant changes in the pharmacokinetics of flunixin following coadministration with enrofloxacin were detected. Coadministration of flunixin and enrofloxacin resulted in an increase of 32% in AUC and 29% in $t_{1/2(el)}$ for flunixin, and C_{max} for flunixin after coadministration with enrofloxacin was 14% higher than that after administration of flunixin alone. Accordingly, after coadministration, k_{el} for flunixin decreased by 23%. The T_{max} for flunixin was similar following flunixin administration alone and coadministration of flunixin with enrofloxacin.

Pharmacokinetics of enrofloxacin—Mean plasma enrofloxacin concentrations following enrofloxacin administration alone or coadministration with flunixin were determined (**Figure 2**). During the period from 0.75 to 2 hours, mean plasma enrofloxacin concentrations after administration of enrofloxacin alone were higher than plasma enrofloxacin concentrations after coadministration with flunixin. The plasma enrofloxacin concentrations during the period from 5 to 24 hours after administration of enrofloxacin alone were lower than plasma enrofloxacin concentrations after coadministration with flunixin. A 1-compartment model described the plasma concentration-time data. Pharmacokinetic parameters of enrofloxacin following enrofloxacin administration alone or coadministration with flunixin were determined (**Table 2**). Coadministration of enrofloxacin with flunixin resulted in a 50% increase in the $t_{1/2(el)}$ for enrofloxacin, whereas the C_{max} after coadministration with flunixin was 21% lower than that after the administration with enrofloxacin alone. Coadministration of enrofloxacin with flunixin also resulted in a 33% decrease in the k_{el} for enrofloxacin, whereas coadministration did not have any influence on the AUC and T_{max} for enrofloxacin.

Plasma ciprofloxacin concentration—Plasma ciprofloxacin concentration-time profiles after enrofloxacin administration alone or coadministration with flunixin were determined (**Figure 3**). The plasma ciprofloxacin concentration peaked at 8 hours following coadministration of enrofloxacin and flunixin, whereas it peaked at 3 hours following administration of enrofloxacin alone.

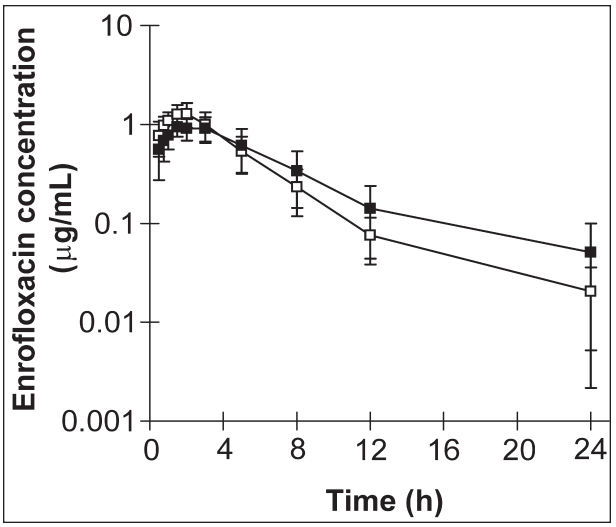

Figure 2—Mean ± SD plasma enrofloxacin concentration in dogs after enrofloxacin (5 mg/kg, SC) administration alone (open squares) or enrofloxacin coadministration (closed squares) with flunixin (1 mg/kg, SC).

Table 2—Pharmacokinetic parameters of enrofloxacin following simultaneous SC injections of enrofloxacin and flunixin meglumine in dogs.

	Pharmacokinetic parameters				
Drug administration	**k_{el} (h^{-1})**	**$t_{1/2(el)}$ (h)**	**AUC (μg•h/mL)**	**T_{max} (h)**	**C_{max} (μg/mL)**
Enrofloxacin alone					
Mean	0.427	1.623*	6.384	1.696	1.265
95% CI	0.319–0.535	1.243–2.003	5.103–7.665	1.385–2.007	1.064–1.466
Median	0.368	1.885	6.011	1.622	1.189
± SD	0.151	0.531	1.790	0.435	0.282
Enrofloxacin and flunixin					
Mean	0.285†	2.428*†	6.973	2.086	0.998†
95% CI	0.184–0.386	1.627–3.229	5.093–8.853	1.56–2.612	0.844–1.152
Median	0.249	2.898	7.061	2.091	0.995
± SD	0.141	1.120	2.628	0.735	0.215

†Significantly ($P < 0.05$) different from enrofloxacin administration alone.
See Table 1 for remainder of key.

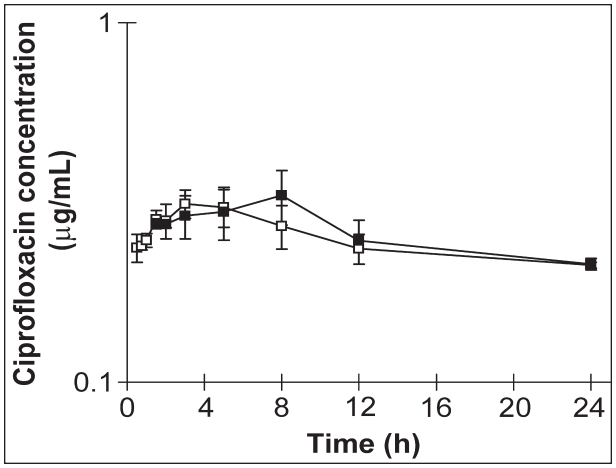

Figure 3—Mean ± SD plasma ciprofloxacin concentration in dogs after enrofloxacin (5 mg/kg, SC) administration alone (open squares) or enrofloxacin coadministration (closed squares) with flunixin (1 mg/kg, SC).

Discussion

Enrofloxacin, a fluoroquinolone antimicrobial agent, has been marketed for over 10 years in the United States, whereas flunixin meglumine, a potent NSAID,[14] has been administered for the control of inflammation and fever in veterinary medicine. Although pharmacokinetics of enrofloxacin and flunixin in other species have been reported,[15,16] little is known about the pharmacokinetic interaction between enrofloxacin and flunixin in dogs. In addition, it was also reported that the pharmacokinetics of enrofloxacin and flunixin differ greatly depending on age and endotoxin-induced fever.[17,18] Therefore, we have examined the pharmacokinetics of enrofloxacin and flunixin in dogs administered SC either alone or by simultaneous injection.

Results of our study revealed that the mean plasma flunixin concentrations in dogs after coadministration with enrofloxacin were always higher than plasma flunixin concentrations after administration of flunixin alone. This consistent increase in mean plasma flunixin concentrations after coadministration with enrofloxacin was reflected by increases in pharmacokinetic parameters, AUC and C_{max}. Increases in AUC and C_{max} following coadministration were also accompanied by a decrease in the k_{el} from the central compartment. Our results indicate that the SC, simultaneous injection of flunixin and enrofloxacin in dogs induces delayed elimination and clearance of flunixin. Delayed elimination and clearance of flunixin may have been a result of inhibition in the metabolism of ciprofloxacin, an accelerated transport of flunixin into the cells, or a retarded release of flunixin from plasma protein, all of which still remains to be examined.

The difference in the plasma enrofloxacin concentration between enrofloxacin administration alone and administration with flunixin was biphasic; in the earlier period for 0.75 to 2 hours after the injections, the plasma enrofloxacin concentration after coadministration with flunixin was lower than that after enrofloxacin administration alone. However, in the later period for 5 to 24 hours following enrofloxacin administration alone, it was lower than that after coadministration with flunixin. The plasma concentration after enrofloxacin administration alone and that after coadministration with flunixin reversed at 3 hours. Therefore, in contrast to the pharmacokinetic parameters for flunixin after coadministration with enrofloxacin, no difference in AUC between the administration of enrofloxacin alone and coadministration with flunixin was observed, probably because they were calculated from the overall plasma enrofloxacin concentrations from all sample collection times. Similarly, C_{max} for enrofloxacin decreased after coadministration with flunixin. However, k_{el} from the central compartment decreased, and $t_{1/2(el)}$ for enrofloxacin after coadministration with flunixin increased, similar to the increase observed for flunixin after coadministration with enrofloxacin. This indicates that coadministration of enrofloxacin with flunixin also results in the delayed elimination of enrofloxacin.

The plasma ciprofloxacin concentration in dogs after administration of enrofloxacin alone was not different from the plasma ciprofloxacin concentration after the coadministration of enrofloxacin and flunixin. However, the T_{max} for ciprofloxacin was different following enrofloxacin administration alone and administration with flunixin. Our results indicate that the total amount of metabolic conversion of enrofloxacin to ciprofloxacin is not inhibited in dogs by coadministration with flunixin; however, the metabolism rate of enrofloxacin is partially affected by the coadministration of enrofloxacin and flunixin.

Results of our study also revealed a decrease in the clearance of the drugs from the circulation following the coadministration of enrofloxacin with flunixin, suggesting that enrofloxacin and flunixin interact during the elimination phase of the drugs. It has been reported that the excretion of fluoroquinolones occurs via glomerular filtration and active tubular secretion by the anion transporter system.[19] We have also reported that the elimination of flunixin involves renal tubular secretion.[20] Therefore, it is probable that competitive inhibition between enrofloxacin and flunixin occurred during renal tubular secretion in our study following simultaneous administration of enrofloxacin and flunixin in dogs.

In conclusion, it is evident that the pharmacokinetics of flunixin and enrofloxacin are altered by coadministration. Thus, caution is necessary when enrofloxacin and flunixin are administered concurrently to ill dogs. An unexpected increase in plasma flunixin concentration may cause adverse drug effects because flunixin, even at therapeutic doses, induces gastric mucosal injury in dogs.[21] Therefore, the increase in plasma flunixin concentrations following coadministration with enrofloxacin may accelerate and exacerbate the occurrence of gastrointestinal injury. Consequently, care should be taken when dogs are treated concurrently with flunixin and enrofloxacin. Furthermore, it is recommended that the appropriate adjustments of dosages be instituted when flunixin and enrofloxacin are administered concomitantly in dogs.

a. Finadyne 1%, Dainippon Pharmaceutical Co Ltd, Osaka, Japan.
b. Baytril 2.5%, Bayer Corp, Tokyo, Japan.
c. CAPCELL PAK C18 UG120 (4.6-mm inner diameter and 150 mm in length), Shiseido Fine Chemicals Co Ltd, Tokyo, Japan.
d. Ciprofloxacin hydrochloride, Wako Pure Chemical Industries, Osaka, Japan.
e. WinNonlin 3.1, Pharsight Corp, Calif.

References

1. Vancutsem PM, Babish JG, Schwark WS. The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Vet* 1990;80:173–186.

2. Prescott JF, Yielding KM. In vitro susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. *Can J Vet Res* 1990;54:195–197.

3. Walker RD, Stein GE, Hauptman JG, et al. Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. *Am J Vet Res* 1992;53:2315–2319.

4. Hannan PCT, Windsor GD, de Jong A, et al. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. *Antimicrob Agents Chemother* 1997;41:2037–2040.

5. Tasker S, Helps CR, Day MJ, et al. Use of a Taqman PCR to determine the response of *Mycoplasma haemofelis* infection to antibiotic treatment. *J Microbiol Methods* 2004;56:63–71.

6. Anderson KL, Smith AR, Shanks RD, et al. Efficacy of flunixin megulumine for the treatment of endotoxin-induced bovine mastitis. *Am J Vet Res* 1986;47:1366–1372.

7. Whittem T, Firth EC, Hodge H, et al. Pharmacokinetic interaction between repeated dose phenylbutazone and gentamicin in the horse. *J Vet Pharmacol Ther* 1996;19:454–459.

8. EL-Banna HA. Pharmacokinetic interactions between flunixin and sulphadimidine in horses. *Dtsch Tierarztl Wochenschr* 1999;106:400–403.

9. Rantala M, Kaartinen L, Välimäki E, et al. Efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced *Escherichia coli* mastitis. *J Vet Pharmacol Ther* 2002;25:251–258.

10. Hardie EM, Hardee GE, Rawlings CA. Pharmacokinetics of flunixin meglumine in dogs. *Am J Vet Res* 1985;46:235–237.

11. Küng K, Riond JL, Wanner M. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and oral administration of enrofloxacin in dogs. *J Vet Phrmacol Ther* 1993;16:462–468.

12. Odensvik K, Johansson IM. High-performance liquid chromatography method for determination of flunixin in bovine plasma and pharmacokinetics after single and repeated doses of the drug. *Am J Vet Res* 1995;56:489–495.

13. Schneider M, Thomas V, Boisrame B, et al. Pharmacokinetics of marbofloxacin in dogs after oral and parental administration. *J Vet Pharmacol Ther* 1996;19:56–61.

14. Stegelmeier BL, Bottoms GD, Denicola DB, et al. Effects of flunixin meglumine in dogs following experimentally induced endotoxemia. *Cornell Vet* 1988;78:221–230.

15. 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.

16. Coakley M, Peck KE, Taylor TS, et al. Pharmacokinetics of flunixin megulumine in donkeys, mules, and horses. *Am J Vet Res* 1999;60:1441–1444.

17. Jensen RC, Fischer JH, Cwik MJ. Effect of age and status on pharmacokinetics of flunixin meglumine in Thoroughbreds. *Am J Vet Res* 1990;51:591–594.

18. Rao GS, Ramesh S, Ahmad AH, et al. Effects of endotoxin-induced fever and probenecid on disposition of enrofloxacin and its active metabolites ciprofloxacin after intravascular administration of enrofloxacin in goats. *J Vet Pharmacol Ther* 2000;23:365–372.

19. Neuman M. Comparative pharmacokinetics parameters of new systemic fluoroquinolones: a review. *Chemioterapia* 1987;6:105–112.

20. Miyazaki Y, Horii Y, Ikenaga N, et al. Possible active transport mechanism in pharmacokinetics of flunixin-meglumin in rabbits. *J Vet Med Sci* 2001;63:885–888.

21. Dow SW, Rosychuk RAW, McChesney AE, et al. Effects of flunixin and flunixin plus prednisone on the gastrointestinal tract of dogs. *Am J Vet Res* 1990;51:1131–1138.