Pharmacokinetics of Enrofloxacin Given by the Oral, Intravenous and Intramuscular Routes in Broiler Chickens

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ABSTRACT

Enrofloxacin was given to broiler chickens, 3 groups of 6 birds each, at a dose of 5 mg/kg. Routes of administration were intravenous (IM), intramuscular (IM) and oral (PO) and blood samples were collected from the jugular vein for determination of serum drug levels over a 54-hour period after administration. Drug levels were determined using *Bacillus subtilis* spore suspension on Meuller-Hinton antibiotic medium. Intravenous administration produced drug levels which followed a bi-exponential decay according to the model $C = 101e^{-1.84(t)} + 1.30e^{-0.06(t)}$. After IM administration, the mean C_{max} observed (2.01 μg/mL) occurred at 1 h and levels were detected for up to 48 h. The mean time to maximum concentration (T_{max}) for the birds occurred at 0.79 h. The model describing serum concentrations after IM administration was $C = 1.35e^{-0.48(t)} + 1.27e^{-0.07(t)} - 2.06e^{-2.1(t)}$. Serum concentrations after oral administration were lower and the mean ± standard error of mean, of the maximum concentrations (C_{max}) was 0.99 μg/mL at 2 h after administration. The mean residence times after the 3 routes of administration were not significantly different and ranged from 12.5–13.7 h. Bioavailability by the oral route was 80.1%. Dialysis of chicken plasma vs saline indicated that the protein binding was 22.7%.

RÉSUMÉ

Trois groupes de 6 poulets à griller ont reçu de l'enrofloxacin par voie intraveineuse (IV), intramusculaire (IM) ou orale (PO) à la dose de 5 mg/kg. Les concentrations sériques d'antibiotique furent déterminées à partir d'échantillons sanguins prélevés par ponction de la veine jugulaire durant une période de 54 h après l'administration de l'antibiotique. Une méthode microbiologique employant des spores de *Bacillus subtilis* fut utilisée pour ces déterminations. Les niveaux sériques atteints après l'adminstration IV d'enrofloxacin suivait une courbe de dégradation bi-exponentielle selon le modèle $C = 101e^{-1,84(t)} + 1,30e^{-0,06(t)}$. Lors de l'administration IM de l'antibiotique, la moyenne des C_{max} observée (2,01 μg/mL) fut notée après 1 h et les niveaux furent détectés jusqu'à 48 h après l'injection. Le temps moyen pour atteindre la concentration maximum (T_{max}) était de 0,79 h. Le modèle servant le mieux à décrire les concentrations sériques après administration IM était $C = 1,35e^{-0,48(t)} + 1,27e^{-0,07(t)} - 2,06e^{-2,1(t)}$. Les concentrations sériques obtenues après administration PO étaient plus faibles et la moyenne ± l'écart-type de C_{max} était de 0,99 μg/mL 2 h après l'administration de l'antibiotique. Le temps de détection moyen n'était pas significativement différent entre les 3 voies d'administration et variait entre 12,5 et 13,7 h. L'antibiotique avait une biodisponibilité de 80,1 % suite à une administration PO. Suite à une dialyse de plasma de poulet dans de la saline, il a été déterminé que l'attachement de l'antibiotique aux protéines plasmatiques était de 22,7 %.

(Traduit par le docteur Serge Messier)

INTRODUCTION

The first antimicrobials based on the 4-quinolone ring were introduced in the early 1960s. Nalidixic acid and its derivative, oxolinic acid (1) were mainly used in the treatment of urinary infections. These compounds were very active in vitro against most gram-negative bacteria, but showed no activity against *Pseudomonas aeruginosa* and their in vivo activity was limited by poor distribution within the body (2). The discovery of the fluoroquinolones changed this, as they had high tissue penetration (3) and were active against both gram-negative and -positive bacteria (4,5). The concentrations required to achieve therapeutic success were low, 0.1–1.0 μg/mL (6), however, more recent studies suggest a trend amongst certain organisms to markedly increased fluoroquinolone resistance, for example *Campylobacter* and *Salmonella* (7,8).

The fluoroquinolones are quite safe except in the young of certain mammalian species, where they have been shown to induce arthropathic changes at major joint surfaces (9).

The relative safety of the fluoroquinolones, their low minimum inhibitory concentrations (MIC), usually 0.1–2.0 μg/mL (10), their broad spectrum activity, and their property of leaving little or no residue in edible tissues have encouraged their use in veterinary medicine (11,12).

Despite the therapeutic potential of these drugs, their pharmacokinetics remain only partially understood in many species, including chickens. The purpose of this study was to evaluate the pharmacokinetic behavior of enrofloxacin when administered to chickens.

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MATERIALS AND METHODS

ANIMALS

Twenty-eight healthy broiler chickens were purchased from the Arkell Experimental Station, University of Guelph, and housed in individual cages in the isolation unit at the University. They were allowed to acclimatize for 1 week before the experiment started, and were offered, ad libitum, antibiotic-free broiler feed and water before and during treatment. Room temperature was 25°C and the daily light cycle rotated between 12 h of light and 12 h of darkness. Eighteen birds were treated with drug while 10 served as controls. Feed was withdrawn from birds earmarked for oral treatment 18 h before drug administration.

EXPERIMENTAL DESIGN I

Eighteen chickens randomly selected from the birds purchased were designated as the treatment group and housed in a separate pen. These were later divided into 3 subgroups of 6 birds each and given commercially available enrofloxacin (Baytril, 50 mg/mL, Haver Canada Inc.). The first subgroup was given a bolus of enrofloxacin into the left wing vein (IV), the second subgroup received an injection of enrofloxacin into the breast muscle (IM) and the third received drug by oral gavage (PO). The dose was 5 mg/kg. Blood was taken by venipuncture from the right wing vein at selected intervals after administration: 0, 5, 10, 15, 20, 30 and 45 minutes, and at 1, 2, 4, 6, 9, 12, 24, 30, 48 and 54 h. The blood was allowed to clot at room temperature, and was centrifuged for 10 min (Serofuge II centrifuge, Becton Dickinson & Co., Parsippany, New Jersey, USA). Serum was collected and stored at −20°C until analyzed. Samples were not taken at 5, 10, and 30 min after IM and PO administration.

FLUOROQUINOLONE ANALYSIS

The fluoroquinolone microbiological assay used for determining drug concentrations in chicken serum was a modification of the methods of Arret et al (13). The organism used was commercial *Bacillus subtilis* spore suspension. Twenty microlitres were diluted 1:1 in saline and placed in 5 mL of warmed, liquified, Meuller-Hinton antibiotic medium II. This was applied as the seed layer to a 5 mL base layer of the same media in petri dishes.

One hundred microlitre volumes of standard solutions or unknown samples were applied to the medium using 12.5-mm filter paper disks and incubated at 37°C for 18 h. Drug concentrations in the unknown samples were determined by measuring the zone of inhibition diameters and comparing them to the standard curve. Standard solutions used to prepare the standard curves ranged in concentration from 10 to 0.01 μg/mL.

PHARMACOKINETIC ANALYSIS

The log serum drug concentrations were plotted against time. The distribution (rapid decay) and elimination (slow decay) phases of the curves after IV administration were analyzed using least squares regression analysis (14), "curve stripping," and the method of residuals (15). The rates of absorption, distribution and elimination were determined following intramuscular administration using the above methods. Pharmacokinetic parameters were determined using the methods of Gibaldi and Perrier (15) and Baggot (16). Based on the number of linear decay components in the semi-log plot of the drug concentration/time data, a 2- or 3-compartment model was selected to describe the data. The Akaike Information Criterion (AIC) (17) was used to determine if a tri-exponential equation significantly reduced the weighted sum of squared deviations to justify its use over the bi-exponential equation. The parameters A and B represent the γ-intercepts of distribution and elimination in a bi-exponential decay curve, α and β, the first-order rate constants of distribution, and elimination, respectively. When a tri-exponential decay equation was used (eg, after an IM administration), AB represented the γ-intercept and k_{ab}, the rate of absorption. The maximum drug concentration (C_{max}) and the time to maximum drug concentration (T_{max}) were extrapolated for the IM and PO treatments from a spline plot of the drug concentration data (Slide Write 4.10Plus, Advanced Graphics Software, California, USA). Volume of the central compartment was determined by V_c = Dose/C_o, where C_o = A + B for a 2-compartment open model (18). The multicompartment volume term, $Vd_{(area)}$, was calculated using the area method [$Vd_{(area)}$ = dose/(AUC)β] where AUC is the area under the curve and β the rate of elimination. The rate constant of elimination (k_{el}) from the central compartment was determined as described by Gibaldi and Perrier (15).

Serum concentration time data after PO administration were analyzed using non-compartmental analysis. The following basic parameters were estimated, $AUC_{(t0\text{-}tn)}$ from time 0 until the last observation, plasma clearance (Cl), mean residence time ($MRT_{t0\text{-}tn}$) and volume of distribution at steady state (Vd_{ss}). To allow for comparisons with the IV and IM treatments, similar calculations were carried out on these routes as well. The AUC and area under the (first) moment curve (AUMC) were calculated for non-compartmental models as described by Rowland and Tozer (19). Bioavailability (F) estimations were determined using the relationship, F = $AUC_{(t0\text{-}tn)}$(PO or IM) × 100/$AUC_{(t0\text{-}tn)}$(IV). Plasma clearance was calculated using Cl = Dose/$AUC_{(t0\text{-}tn)}$, with the treatment doses after oral administration corrected for bioavailability. The Vd_{ss} and the MRT (reflects MRT the average length of time a drug molecule resides in the body) were determined by Vd_{ss} = Cl × MRT and MRT = $AUMC_{(t0\text{-}tn)}/AUC_{(t0\text{-}tn)}$, where AUMC = $\int C_p t dt$ and AUC = $\int C_p dt$, integrated from time 0 until the final sample was taken.

EXPERIMENTAL DESIGN II

The binding of fluoroquinolones to plasma protein was studied using a modified dialysis method (20). Lengths of dialysis tubing, internal diameter of 3 cm, were soaked in 0.85% salt solution for 2 to 3 h. Blood was obtained by decapitating Leghorn layers obtained from a flock maintained on drug-free feed and water. The blood was collected in heparinized containers, centrifuged and the supernatant plasma decanted and frozen at −20°C. To study binding, the frozen plasma was thawed and diluted to twice the volume with saline. Using the TS refractometer (American Optical, Scientific Instrument Division, Buffalo, New York, USA), the protein content of the

diluted plasma was determined [between 2.1 and 2.5 g/dL]. Ten millilitres of diluted plasma was pipetted into dialysis bags, the ends were tied securely, and a glass weight was added to one end. These were suspended in 150-mL beakers containing known concentrations of enrofloxacin (5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.07813 μg/mL), covered with parafilm and set in a shaker at 52 cycles/min at room temperature. The enrofloxacin solutions in the beakers and the contents in the dialysis bags were sampled after 24 h. Preliminary studies indicated that this was sufficient time to establish an equilibrium across the dialysis membrane.

The volumes of saline in the beakers and the dialysis bags were measured at the end of the experiment and the "volume-shift" was noted. This was taken into account when the standards used for drug analysis were prepared. Standards used for the quantitation of enrofloxacin in the beaker were made in saline; for the contents of the dialysis bags standards were made in plasma:saline (1:1) solutions. Drug levels were determined as described above using the microbiological assay.

Ratios of C_s/C_{pl} were calculated for the standard concentrations, where C_s was the concentration in saline and C_{pl} the concentration in saline:plasma. Using the formula $E = [1-(C_s/C_{pl})] \times 100$, the extent of protein binding (E) was calculated (21).

EXPERIMENTAL DESIGN III

Octanol:water partition coefficients for enrofloxacin at different pH's were obtained following the procedure outlined by Purcell et al (22). Britten-Robinson buffer (pH 2.3) was used in this study. By adding 0.2 M NaOH (Fisher Scientific, Canada Inc.) drop-wise to a series of Britten-Robinson buffer (pH 2.3) solutions, new buffers with pH's ranging from 3 to 10 were prepared. Aliquots of 9.9 mL at each pH were withdrawn, labeled and saved.

Five milligrams of enrofloxacin base powder (99% purity, Miles Canada Inc.) was dissolved in 5 mL of Britten-Robinson buffer (pH 10) and 0.1 mL of this solution was added to the 9.9 mL volumes of each of the buffers and mixed to make an enrofloxacin solution of 10 μg/mL at each pH. Five millilitres of the buffer solutions were pipetted into sixteen 100-mm test tubes and 5 mL of octanol (99% purity, Sigma Chemical Co., St. Louis, Missouri, USA) was added. The remaining 5 mL of the aqueous enrofloxacin solutions were used to make standard solutions for the antimicrobiological assay. The octanol and water phases were mixed thoroughly by inverting 200 to 250 times for approximately 10 min. Next, they were centrifuged at 2000 × *g* for 15 min to separate the phases and aliquots of the aqueous phases were collected and stored at 4°C until analyzed the following day. In addition, the drug concentrations were determined by the microbiological methods described above. The concentration of drug in the octanol was calculated as the difference between 10 μg/mL and the concentration of drug in the aqueous phase. This experiment was repeated 3 times.

The data were graphed using a spline plot and the pH's at which half of the drug was in the octanol and half in the aqueous phase were obtained.

STATISTICS

Means and standard errors were calculated for the pharmacokinetic parameters and tested for significant ($P \leq 0.05$) differences using analyses of variance (14). If significant differences were identified, Tukey's *w* procedure (14) was used to determine where these differences occurred.

TABLE I. Enrofloxacin pharmacokinetic parameters (mean ± SE) in chickens following administration of a 5 mg/kg dose

Parameter ($n = 6$)	IV	IM	PO
A (μg/mL)	1.01 ± 0.09	1.35 ± 0.44[4]	
α (/h)	1.84 ± 0.34	0.48 ± 0.03[a4]	
AB (μg/mL)		2.06 ± 0.65[4]	
k_{ab} (/h)		2.10 ± 0.71[4]	
B(μg/mL)	1.30 ± 0.08	1.27 ± 0.18[5]	
β (/h)	0.06 ± 0.01	0.07 ± 0.01[5]	
Body weight (kg)	2.98 ± 0.16	2.48 ± 0.28	1.58 ± 0.08
C_{max} (μg/mL)		2.10 ± 0.21	0.99 ± 0.08[a]
T_{max} (h)		0.79 ± 0.10	2.50 ± 0.86[b]
C_o (μg/mL)	2.30 ± 0.11		
V_c (L/kg)	2.20 ± 0.10		
$T_{1/2\alpha}$ (h)	0.36	2.13[4]	
$T_{1/2\beta}$ (h)	10.96	10.6[5]	
$T_{1/2ab}$ (h)		0.42[4]	
k_{el}(/h)	0.11 ± 0.01		
Vd_{area}(L/kg)	3.82 ± 0.24	3.79 ± 0.39[4]	
Vd_B (L/kg)	3.93 ± 0.24	4.26 ± 0.59[5]	
$Cl_{(t0\text{-}tn)}$(L/kg/h)	0.25 ± 0.31	0.25 ± 0.46[5]	0.18 ± 0.02
$AUC_{t0\text{-}tn}$ (μg·h/mL)	21.7 ± 1.59	21.4 ± 3.9[5]	17.4 ± 2.04
$MRT_{t0\text{-}tn}$ (h)	12.5 ± 0.77	12.5 ± 1.03[5]	13.7 ± 1.97
Vd_{ss}(L/kg)	2.92 ± 0.23	2.82 ± 0.36[5]	2.52 ± 0.28
Bioavailability		98.6%[5]	80.1%

Superscripts indicate significant differences ($P \leq 0.05$) using the one way ANOVA and Tukey's *w* test

[a] significantly different from IV

[b] significantly different from IM treatments

Superscript numbers indicate numbers of animals in the group when it is less than 6

RESULTS

PHARMACOKINETIC ANALYSIS OF ENROFLOXACIN IN CHICKENS

The serum concentrations of enrofloxacin after intravenous (IV) administration of a 5 mg/kg dose to chickens are shown in Figure 1. Enrofloxacin was detected up to 48 h after dosing. The highest mean concentration was 2.21 ± 0.18 μg/mL at 5 min and the lowest mean concentration was 0.09 ± 0.02 μg/mL at 48 h. The parameters describing enrofloxacin in the serum after IV administration are shown in Table I. These were determined for a 2-compartment open model after bolus administration. The equation of the line, $C = 1.01e^{-1.84(t)} + 1.30e^{-0.06(t)}$, which describes the means of the individual bird pharmacokinetic parameters (solid line), closely fits the mean data points (Fig. 1). The correlation coefficients ranging from 0.887 to 0.997 for α and 0.982 to 0.998 for β indicate a strong correlation between the dependent (drug concentration)

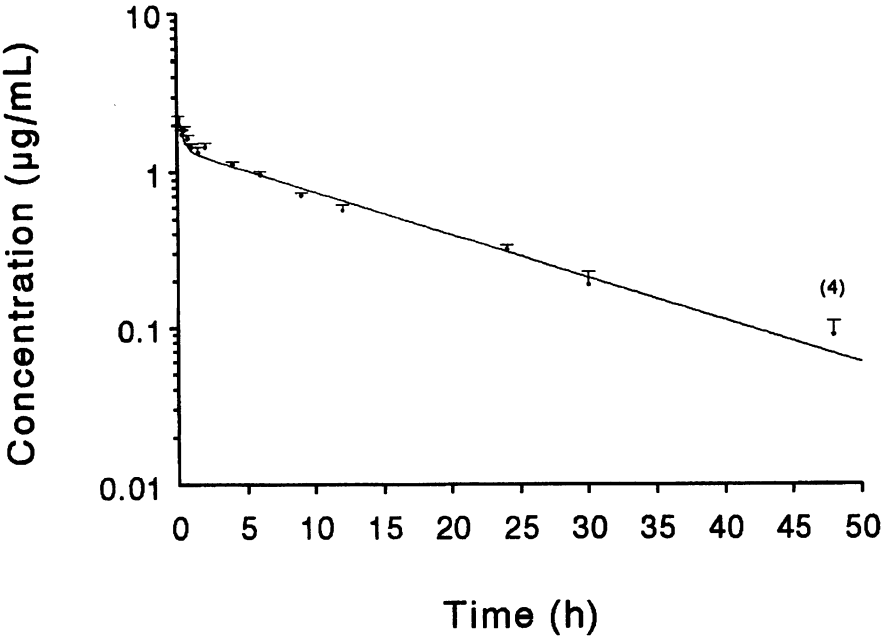

Figure 1. Plasma enrofloxacin concentrations (mean ± SE) following IV administration of a single 5 mg/kg dose. The curve represents the mean equation of the line describing the pharmacokinetic dispostion of enrofloxacin where $C = 1.01e^{-1.84(t)} + 1.30e^{-0.06(t)}$. The numbers indicate when the sample size for the parameter is less than 6.

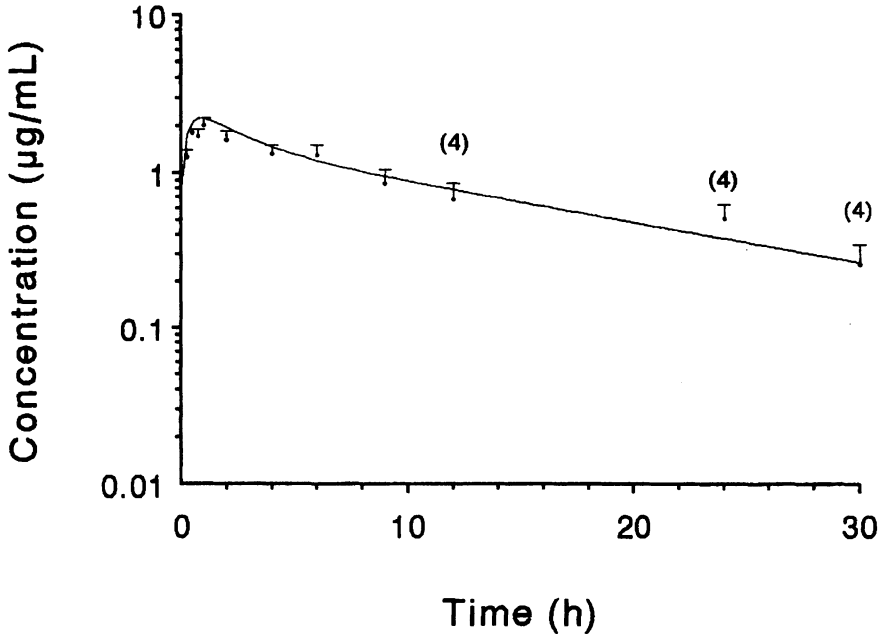

Figure 2. Plasma enrofloxacin concentrations (mean ± SE) following IM administration of a single 5 mg/kg dose. The curve represents the mean equation of the line describing the pharmacokinetic dispostion of enrofloxacin where $C = 1.35e^{-0.48(t)} + 1.27e^{-0.07(t)} - 2.06e^{-2.10(t)}$. The numbers indicate when the sample size for the parameter is less than 5.

and independent (time) variables in our model.

After intramuscular (IM) administration the maximum observed mean serum enrofloxacin concentration (C_{max}) was 2.01 ± 0.23 µg/mL. The mean C_{max} occurred at 1 h after drug administration and levels were detected for up to 48 h (0.08 ± 0.06 µg/ml). No drug was detected at 5 min and 10 min after IM administration. Time to reach the maximum serum concentration (T_{max}) was 0.79 ± 0.10 h. The serum concentrations of enrofloxacin following IM administration are presented in Figure 2.

TABLE II. Binding of enrofloxacin to chicken plasma protein following dialysis against saline for 24 h

Concentration of standard (µg/mL)	C_s/C_{pl}	% bound
0.078	NC	(1:2 dilution)
0.156	0.78	22%
0.313	0.78	22%
0.625	0.77	23%
1.25	0.78	22%
2.50	0.77	23%
5.0	0.76	24%
Mean ± SE	0.77 ± 0.003	22.7 ± 0.33

C_s – clearance from the serum; C_{pl} – clearance from the plasma; NC – not calculated

The serum concentrations were described by a 3 component exponential equation (one input and 2 decay components). Two birds, Nos. 9 and 12, did not fit the model due to lack of data in the time just after administration and the terminal points of drug decay were described by only a single exponent. The mean ± SE pharmacokinetic parameters calculated from the individual birds are presented in Table I. Bird No. 9 was not included in any of the calculations (other than body weight, C_{max} and T_{max}) because the AUC parameter, which is basic to the non-compartmental analysis, fell outside the 99% confidence limit for the group.

Significant differences were detected between IM and IV pharmacokinetic parameters only in the rate of distribution (α) which was significantly lower after IM administration. The equation of the line describing the serum/time concentration data was $C = 1.35e^{-0.48(t)} + 1.27e^{-0.07(t)} - 2.06e^{-2.10(t)}$ and is shown in Figure 2 (solid line) along with the mean ± SE serum concentration data. The correlation coefficients for the elimination slopes (β) of the individual birds ranged from 0.988 to 0.999, suggesting a strong correlation between the concentration and time. The correlation coefficients of absorption and distribution components were somewhat weaker at 0.924 to 1.0 for α and 0.844 to 1.0 for k_{ab}. Bird No. 9 was not included in Figure 2. The superscripts in Figure 2 indicate that at the 3 last sampling intervals, only 4 birds were used in the calculations of mean ± SE enrofloxacin concentrations. We were unable to collect one blood sample from the group of birds at each of these intervals, due to collapse of the wing veins.

Lower concentrations of enrofloxacin were detected in the serum of chickens after PO administration (Fig. 3). The mean pharmacokinetic parameters are shown in Table I. The maximum observed concentration in an individual bird was 1.3 μg/mL at 1.5 h and the mean C_{max} was 0.99 ± 0.08 μg/mL. The time to reach maximum serum concentration (T_{max}) averaged 2.50 ± 0.96 h after drug administration. The C_{max} in the PO study was significantly lower than the 2.1 ± 0.21 μg/mL C_{max} observed after IM treatment. The $AUC_{(t0\text{-}tn)}$ of 17.4 ± 2.04 μg·h/mL (PO), in this study was less than both the IV and IM area estimates using the trapezoidal methods but the differences were not significant at the $P \leq 0.05$ level. Clearance (0.18 ± 0.02 L/kg·h), MRT (13.7 ± 1.97 h) and Vd_{ss} (2.52 ± 0.28 L/kg) after PO administration were not significantly different than the other routes of administration.

The fraction of the drug absorbed after oral administration (bioavailability) was on average 80.1%.

BINDING OF ENROFLOXACIN TO CHICKEN PLASMA

The ratios of enrofloxacin in saline to enrofloxacin in saline:plasma (1:1) are shown after equilibration in Table II. For all standard concentrations tested, except the first which was not detectable, the C_s/C_{pl} ratios were less than 1. There were no differences in the equilibrium ratios over the range of concentrations tested (Table II). The percentage protein binding in the diluted plasma was estimated to range from 22–24% with a mean ± SE of 22.7 ± 0.33.

OCTANOL:BUFFER PARTITION STUDIES

Partitioning of fluoroquinolones into Britten-Robinson buffer and octanol was employed to study the octanol:water partitioning over a range of pH levels. Partitioning into the aqueous phase is shown in Figure 4. Almost all of the drug was found in the aqueous phase between pH 2.3 and 4.0. The amount of enrofloxacin in the aqueous phase decreased markedly between pH 5 and pH 7, the point of maximum lipid solubility. Just over 70% of the drug appeared in the octanol at pH 7.

Enrofloxacin concentrations in the aqueous phase increased again between pH 7 and 9. At pH 9, approximately 98% of the drug was in the aqueous phase. Perpendicular lines drawn to the points of intersection between the 50% solubility mark and the solubility curve cut the x (pH) axis at 5.9 and 8.3. These represent the pH levels at which the drug was distributed equally between the aqueous and octanol phases.

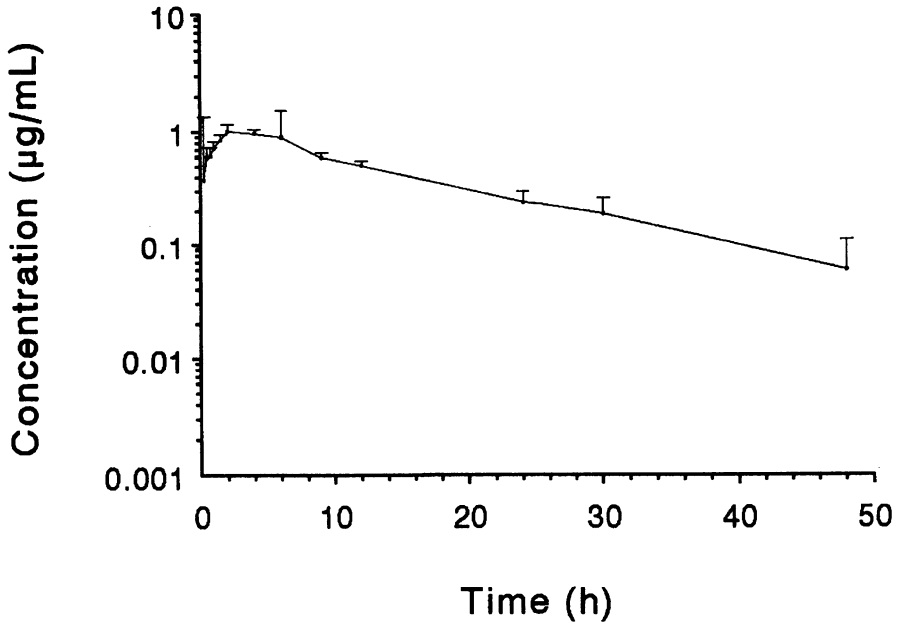

Figure 3. Plasma enrofloxacin concentrations (mean ± SE) following oral administration of a single 5 mg/kg dose. Six animals were used in the experiment.

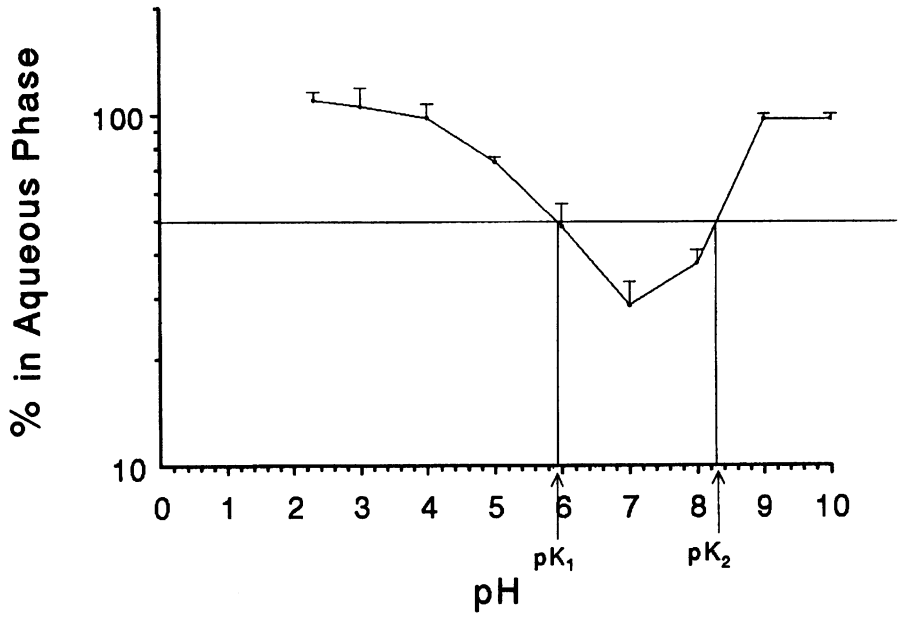

Figure 4. Percentage of enrofloxacin in the aqueous phase in an octanol/water partition study at pH's ranging from 3–10. The lines drawn perpendicular to the x-axis indicate the 2 estimates of the 50% dissociation pH's, pK_1 and pK_2. Each observation is the mean ± SE of 5 replicates.

DISCUSSION

The method used for measuring enrofloxacin in samples in this study was relatively simple and was able to detect drug at concentrations as low as 0.05 μg/mL for serum. The commercially-produced *Bacillus subtilis* spore suspension proved to be a convenient seed organism. Earlier

attempts to use *Sarcina lutea* were unsuccessful because the zones of inhibition were ill-defined, and the sensitivity poor. The assay described in this study was inexpensive and simple compared to HPLC techniques reported by other investigators (23,24). Dowling et al (25) reported success using *E. coli* as the test organism for detecting fluoroquinolones in samples. The *E. coli* gave detection limits of 0.01 μg/mL, apparently 5 times more sensitive than the method we used. Although regulatory limits for fluoroquinolones in chicken tissues and eggs have not been established in Canada and Ghana, tolerance limits for most antimicrobials are in the area of 0.1 μg/g for tissues and 0.3 μg/g for eggs (26). Our findings suggest that the method we describe can detect enrofloxacin at these concentrations.

Comparative pharmacokinetic studies in cattle, pigs and poultry suggest that biotransformation of enrofloxacin to microbiologically active metabolites does not impact serum concentration-time data generated using a microbiological plate assay (10). We encountered similar findings in a yet to be published study from our laboratory using HPLC identification of enrofloxacin and metabolites. However, in a study of norfloxacin in chickens (24) at a higher dose than used in the present study, antibacterial concentrations in the blood were believed to have been influenced by the presence of active metabolites in the samples.

The highest mean concentration of enrofloxacin (C_{max}) detected in chicken serum after intravenous administration was 2.30 ± 0.11 μg/mL and the lowest mean concentration detected was 0.09 ± 0.2 μg/mL. Since the minimum inhibitory concentration (MIC) of enrofloxacin has been shown to range from 0.008 to 0.75 μg/mL for more than 100 pathogens (10), the dose we gave IV should produce serum levels above the MIC for most bacterial infections. Levels above 0.75 μg/mL were seen in all birds up to 6 h after administration. Similar doses of ciprofloxacin administered IV to broiler chickens appear to give considerably higher blood levels (27).

There is little information regarding IM use of enrofloxacin in birds; however, the C_{max} reached in the present study, 2.10 ± 0.21 μg/mL, was close to what we observed following IV treatment, but less than the 3.87 ± 0.27 μg/mL obtained after 15 mg/kg IM enrofloxacin was administered to African grey parrots (28), or the 3.11 ± 0.22 μg/mL for homing pigeons after a 5 mg/kg dose (29). These data suggest good absorption of enrofloxacin when given IM to birds. The higher C_{max} reported by Flammer et al (28) probably reflects the larger dose, although the increase was not in proportion to the difference in dosages. Intramuscularly administered enrofloxacin gives blood levels above the MIC's reported for most pathogens. All chickens receiving the drug IM in our study had levels above 0.75 μg/mL for periods ranging from 4 h to 24 h.

Peak serum concentrations of enrofloxacin have been reported by others to occur at 1.64 h (24) and close to 2 h (29) after a single bolus administration. The peak serum concentration in the present study (C_{max} = 0.99 μg/mL) is less than half that reported by Anadon et al (23). This is likely due to the difference in dosages between the 2 studies (5 mg/kg vs 10 mg/kg). As with the IV and IM doses, all birds receiving the drug orally had serum levels above the 0.75 μg/mL level although for a shorter period of time. The times over which levels were above 0.75 μg/mL ranged from 2 h to 6 h after oral enrofloxacin administration.

The average T_{max} after IM enrofloxacin administration in the present study (0.79 h) was comparable with the reports in other species (10,28,29) and was significantly faster than after PO administration. The rapid absorption half-life ($T_{1/2ab}$) after IM treatment was also similar to other fluoroquinolone studies in chickens (30) and cattle (31).

The half-life and elimination parameters observed in the IV and IM treated birds were very similar (Table I). Conzelman et al (30) reported an elimination half-life of 18.7 h for chickens. This was much longer than the approximately 11 h elimination half-life we observed, the 10.27 h reported by Anadon et al (23) and the 7.3 h by Greene and Budsberg (12). These differences may be due to factors such as the age or strain of birds, because in none of the experiments did the birds precisely match up. The closest match in age, weight and production type were between our birds and those of Anadon et al (23). Interestingly, the half-lives were very nearly the same. Age, gender, diet, drug dose, etc., all can influence the elimination of drugs from animals (16). Enrofloxacin has been shown to inhibit some forms of the mixed function oxidase in broilers (32). Higher doses, therefore, have the potential to affect their own half-lives. Inhibition of mixed function oxidases should always be considered when enrofloxacin is used in animals, particularly when given in combination with other drugs (although the whole range of drugs affected by this interaction is not presently known). Using 4 or 5 half-lives as an indicator of the time until the drug is essentially gone from the body, it would require 44 to 55 h for the total amount of drug to be 93.75 or 96.86% eliminated from the birds, respectively (16).

The multicompartment volumes of distribution (3.82 L/kg after IV treatment and 3.79 L/kg after IM) calculated from our study were quite large but again comparable to the findings of others in chickens (23,30). It is also consistent with studies on different fluoroquinolones in other species (3,28,29). This suggests the presence of specific binding or affinity areas in the body where the drug accumulates outside the plasma. Specific tissue affinities have been suggested by Sheer (10). He reported that tissues retain the drug longer than serum (33), although recently, Duval and Budsberg (34) reported that canine cortical bone enrofloxacin levels tracked well below serum levels. In fish, bones and skin act only as a temporary reservoir for fluoroquinolones (35).

The observation, Figure 4, that enrofloxacin prefers a lipid environment at or near physiological pH, could influence the movement of drug from the blood and aqueous areas to tissues with a high lipid content. Fluoroquinolones are amphoteric by nature, and have at least 2 ionization sites (36). In fact, the data in Figure 4 virtually mirrors the findings of the microspeciation study of pefloxacin by Takacs et al (36), where the maximum partitioning into the lipid phase occurs at the neutral pH, when the

zwitterion [+/−] form reaches it maximum. Our study indicates that enrofloxacin exhibits its maximum octanol/water ratio at approximately pH 7 and lipid solubility decreases as the pH moves away from this point in either direction. The 50% partitioning [water:octanol], was estimated to occur at pH 6.0 (the acidic pKa_1) and at 8.2 (the basic pKa_2). The enrofloxacin Vd we observed in chickens could be influenced by the lipophilic form of the drug at physiological pH. However, given that the fat content of chickens is about 13–15%, and the partition ratio at physiological pH is 3.57/1 [octanol/buffer], partitioning into tissues with a high lipid content could account for only part of the Vd observed, even if we considered the serum to be totally fat free. Much of the distribution of enrofloxacin therefore must depend on other factors, most likely binding to sites of specific affinity (33,35).

Interspecies differences are important in enrofloxacin elimination and point to the risk of extrapolating doses and treatments from one species to another without suitable pharmacokinetic data. Greene and Budsberg (12) reported half-lives of 7.3, 1.4, 1.2, 2.1 and 3.3 h in chickens, turkeys, calves, dogs and horses, respectively, demonstrating a longer elimination half-life in chickens. In addition, the elimination half-life of enrofloxacin in chickens is longer than in the homing-pigeon, (3.82 h) (29), rabbit, (2.5 h) (37), and lactating cow, (1.7 h) (38).

The clearance calculated for enrofloxacin in the present study was similar to the 0.29 L/h/kg reported by Anadon et al (24). Fluoroquinolones, such as ofloxacin and lomefloxacin, are cleared mainly by the renal route, whereas norfloxacin, ciprofloxacin and enoxacin are cleared by both hepatic and renal routes (39). While we were not able to determine, from the literature, the exact clearance mechanisms for enrofloxacin in the chicken, it is likely, given that its first metabolite, ciprofloxacin, is cleared by both renal and hepatic routes, the same applies for enrofloxacin in chickens.

The area under the curve (AUC) estimation, using the method of trapezoids, is the critical step in the calculation of pharmacokinetic estimations using non-compartmental analysis (19). The AUC, after IM administration, was not significantly different than after the IV study ($P \leq 0.5$). The AUC after PO administration was lower than either the IV or IM routes although, once again, the difference was not significant. Since AUC in our calculation reflects the access of drug to the animal's circulation or "bioavailability," the IM data reflects almost complete absorption from the injection site in the 54 h after administration. It also suggests that both routes, IV and IM, are equally likely to guarantee therapeutically significant concentrations in blood at a given dose. While the uptake of enrofloxacin by gavage appears to be slower than after IM administration, the bioavailability, nevertheless, indicates that the drug is well absorbed by this route. The fact that bioavailability is less than 100% may reflect some "first pass" metabolism in the liver or binding of some drug to intestinal contents, such as divalent cations (16). Nevertheless, the levels of drug in the serum, and the bioavailability, both suggest that the PO route of administration is capable of producing therapeutically useful drug levels in many situations, even when given at the same dose as IV treatments. It should be emphasized that our bioavailability calculations are only estimates, since we used different groups of birds rather than a crossover design. We found that the fragile veins of the chicken collapse easily and this limits the number of times samples can be taken. Using different groups of chickens in pharmacokinetic studies has been reported by others (30).

The mean residence time (MRT), calculated for the IV and IM data in this experiment is virtually identical to that observed by Anadon et al (23). The MRT after PO administration was slightly longer but not significantly different from MRT's after IM or IV drug administration.

Although enrofloxacin has been licensed for veterinary use in many countries (12), the authors were unable to obtain data on plasma protein binding in animals. Total protein and albumin concentrations in chicken plasma are only about half those reported in mammals and the binding of drugs to plasma proteins is believed to be proportionately lower (21). Joos et al (41), using the dialysis method, reported plasma protein binding of ciprofloxacin to be between 16.4% and 28%. In the present study, the extent of enrofloxacin binding to diluted chicken plasma protein ranged from 22 to 24% and appeared not to change over the range of concentrations tested. The above authors (40) also failed to show an effect of ciprofloxacin concentration on protein binding. This leaves in question the significance of diluting the plasma with saline (1:1), as we did, on the calculation of the amount of drug-protein binding in whole chicken blood. Clearly the undiluted plasma would have twice the number of binding sites but over the range of concentrations we tested a relationship between drug concentration and protein binding (number of receptors) could not be observed. Nevertheless, the modest level of protein binding observed suggests that protein binding should not significantly affect the therapeutic activity of enrofloxacin or lead to toxic consequences when used in animals with lower than expected levels of plasma proteins.

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