

Comparative study of the plasma pharmacokinetics and tissue concentrations of danofloxacin and enrofloxacin in broiler chickens

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The plasma pharmacokinetics of danofloxacin and enrofloxacin in broiler chickens was investigated following single intravenous (i.v.) or oral administration (p.o.), and the steady-state plasma and tissue concentrations of both drugs were investigated after continuous administration *via* the drinking water. The following dosages approved for the treatment of chickens were used: danofloxacin 5 mg/kg and enrofloxacin 10 mg/kg of body weight. Concentrations of danofloxacin and enrofloxacin including its metabolite ciprofloxacin were determined in plasma and eight tissues by specific and sensitive high performance liquid chromatography methods. Pharmacokinetic parameter values for both application routes calculated by noncompartmental methods were similar for danofloxacin compared to enrofloxacin with respect to elimination half-life ($t_{1/2}$; \approx 6–7 h), mean residence time (MRT; 6–9 h) and mean absorption time (MAT; 1.44 vs. 1.20 h). However, values were twofold higher for body clearance (Cl_B ; 24 vs. 10 mL/min. kg) and volume of distribution at steady state (Vd_{SS} ; 10 vs. 4 L/kg). Maximum plasma concentration (C_{max}) after oral administration was 0.5 and 1.9 μ g/mL for danofloxacin and enrofloxacin, respectively, occurring at 1.5 h for both drugs. Bioavailability (F) was high: 99% for danofloxacin and 89% for enrofloxacin. Steady-state plasma concentrations (mean \pm SD) following administration *via* the drinking water were fourfold higher for enrofloxacin (0.52 ± 0.16 μ g/mL) compared to danofloxacin (0.12 ± 0.01 μ g/mL). The steady-state AUC_{0-24h} values of 12.48 and 2.88 μ g.h/mL, respectively, derived from these plasma concentrations are comparable with corresponding area under the plasma concentration-time curve (AUC) values after single oral administration. For both drugs, tissue concentrations markedly exceeded plasma concentrations, e.g. in the target lung, tissue concentrations of 0.31 ± 0.07 μ g/g for danofloxacin and 0.88 ± 0.24 μ g/g for enrofloxacin were detected. Taking into account the similar *in vitro* activity of danofloxacin and enrofloxacin against important pathogens in chickens, a higher therapeutic efficacy of water medication for enrofloxacin compared to danofloxacin can be expected when given at the approved dosages.

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INTRODUCTION

Danofloxacin and enrofloxacin are fluorinated quinolone carboxylic acid derivatives of similar structure which were developed for use exclusively in animals. Like other fluoroquinolones, they exhibit a wide spectrum of antimicrobial activity, including some Gram-positive and Gram-negative bacteria and *Mycoplasma*. By inhibiting bacterial DNA gyrase, these synthetic drugs act as bactericidal antimicrobials at relatively low

concentrations, in particular against Gram-negative bacteria (Brown, 1996).

Danofloxacin and enrofloxacin are well absorbed in domestic animals both from parenteral injection sites and after oral administration (Scheer, 1987a; Cabanes *et al.*, 1992; Mann & Frame, 1992; Lynch *et al.*, 1994; Richez *et al.*, 1994). The drugs are widely distributed to various tissues in the body resulting in target tissue concentrations significantly higher than those achieved in blood (Giles *et al.*, 1991; Intorre *et al.*, 1997). There

are several reports describing their good therapeutic efficacy against various bacterial and mycoplasmal infectious diseases in poultry (Bauditz, 1987; Jordan *et al.*, 1993; Migaki *et al.*, 1993; Kempf *et al.*, 1995; Charleston *et al.*, 1998).

Good solubility in water (in the form of their salts) yielding stable solutions makes both drugs suitable for economic water medication. For chickens, danofloxacin and enrofloxacin are commercially available as concentrated formulations to be administered *via* the drinking water (soluble powder and oral solution, respectively). For this medication, the approved dosage recommendations for danofloxacin and enrofloxacin are different (danofloxacin 5 mg/kg and enrofloxacin 10 mg/kg of body weight per day), despite their similar *in vitro* antimicrobial activity (evaluated as minimum inhibitory concentration–MIC) against the target pathogens *Escherichia coli*, *Pasteurella multocida* and *Mycoplasma gallisepticum* (Hannan *et al.*, 1997; Watts *et al.*, 1997). This may be due to distinctive pharmacokinetics of the drugs.

The aim of this study was therefore to investigate the plasma pharmacokinetics and tissue concentrations in chickens following administration at the authorised dosages in a comparative experimental design. The plasma pharmacokinetics after single intravenous (i.v.) or single oral administration (p.o.) of danofloxacin and enrofloxacin, respectively, were determined. The steady-state concentrations of both drugs in plasma and eight different tissues (liver, kidney, lung, trachea, myocardium, skeletal muscle, skin, and caecal wall) of broiler chickens were also determined after continuous administration *via* the drinking water, the usual route of application in the field.

MATERIALS AND METHODS

Birds and husbandry

Two-hundred and eighty-two male broiler chickens of the Lohmann Meat B variety (LIR, Cuxhaven, Germany) were used in the study. The birds were purchased at one day of age and raised in pens. During a 3-week acclimatization period, the birds were monitored daily, and no clinical signs of disease were observed. The rooms were air-conditioned and the temperature was gradually lowered from 34 °C on the first day to 24 °C in the third week of life. Light regimen was established using bulbs with 30 lux for 20 h; a dark period was given between 0:00 h and 4:00 h. Water and commercial feed were available *ad libitum*. At the start of the experiments, the animals were 3-week-old. They were weighed (body weight ranged from 580 to 730 g) and randomly assigned to six groups: four groups of 24 birds each for the single i.v. or oral administration of danofloxacin and enrofloxacin, respectively, and two groups of 93 birds each for the continuous administration of danofloxacin or enrofloxacin *via* the drinking water.

Drugs

Danofloxacin was used as a powder (Advocin[®] Soluble Powder–16.7%; Pfizer Animal Health, Pfizer Laboratories Ltd, Sandton,

South Africa), dissolved in water for oral administrations, and as a 2.5% injectable solution (Advocin[®] 2.5%) for i.v. administration (after dilution with saline to 5 mg/mL). Likewise, enrofloxacin was used as a solution (Baytril[®] 10% Oral Solution; Bayer AG, Leverkusen, Germany) for oral administrations, and as a 2.5% injectable solution (Baytril[®], 2.5%) for i.v. injection (after dilution to 10 mg/mL).

Experimental design

Single intravenous and oral treatment

For the determination of i.v. pharmacokinetics, 10 mg/kg enrofloxacin and 5 mg/kg danofloxacin, respectively, were injected in the left brachial vein of 24 birds each. Identical dosages were administered by crop gavage to additional groups of 24 birds each for the determination of oral pharmacokinetics. The application volume was consistently 1.0 mL/kg body weight. Heparinized blood samples were obtained from the right brachial vein immediately prior to medication (time = 0), and then at 0.33, 0.67, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after treatment. To minimize the stress associated with repeated sampling, no more than three venipunctures per animal were performed. At each sampling time, blood was collected from six birds per treatment group. After centrifugation (1500 × g for 10 min), the plasma was stored at –70 °C until analysis.

Treatment via drinking water

Over a period of 5 consecutive days, danofloxacin and enrofloxacin were administered *via* the drinking water to 93 birds. To ensure daily dosages of 5 mg/kg bw of danofloxacin, and 10 mg/kg bw of enrofloxacin, respectively, drug concentrations added to the drinking water were based on daily water consumption rates and daily weight measures of the birds. Mean water consumption during the medication period decreased from approximately 400 mL/kg body weight to 280 mL/kg body weight in both groups. Therefore, drug concentrations of danofloxacin and enrofloxacin in the drinking water varied from 12 to 18 p.p.m and 25 to 36 p.p.m., respectively, as has been additionally confirmed by HPLC analysis of reserved water samples (after dilution with mobile phase containing the internal standard ofloxacin).

On the first day of treatment, prior to initiating the medication (at 0800 h, time = 0), blood and tissue samples were obtained from three birds in each treatment group. Blood samples were collected from 10 additional broiler chickens each at 1.5, 3, 4, 5, 6 and 7.5 h after time 0. In addition to sampling on the initial day of treatment, the aforementioned schedule was also repeated on days 3 and 5. After collection, blood samples were centrifuged (1500 × g for 10 min) and the separated plasma was stored at –70 °C until analysis.

On the third day of treatment, 10 birds from each group were killed at sampling times immediately prior to changing the medicated water supply (at 0800 h, time = 0), and at 1.5, 3 and 6 h thereafter. Samples of eight different tissues (liver, kidney, lung, myocardium, trachea, skeletal muscle, skin, caecal wall) were collected from each carcass. Tissue samples were stored at –70 °C until analysis.

Analytical procedures

Plasma and tissue concentrations of danofloxacin, enrofloxacin, and its microbiologically active metabolite ciprofloxacin were determined by high performance liquid chromatography (HPLC). The chromatographic system utilized during this study (LiChrograph, Merck, Darmstadt, Germany) consisted of a L-6200 A pump, an autosampler AS-2000 A, a fluorescence detector F-1050 (excitation – 278 nm, emission – 446 nm) and a PC with HPLC-manager software D-6000 for data processing.

Prior to analysis, the fluoroquinolones were extracted and further purified. Our procedure is based on methods published by Horie *et al.* (1994), and is briefly described in the following:

0.1 mL plasma sample, diluted with the internal standard (ofloxacin; Sigma, St. Louis, MO, USA) in 0.5 mL phosphate buffer solution (pH 2.2; 50 mM) was passed through a Bond Elut C18 cartridge (100 mg; Varian Sample preparation products, Harbor City, CA, USA) preconditioned with methanol and phosphate buffer solution. Thereafter, the cartridge was washed with phosphate buffer and then eluted with 1.0 mL of a methanol: 10 M formic acid mixture (9: 1; v/v). The eluate was evaporated at 30 °C to dryness with nitrogen. The residue was reconstituted with mobile phase and analysed by HPLC.

A 0.5 g sample of tissue, supplemented with internal standard solution (ofloxacin), was homogenized (Ultra turrax, Janke & Kunkel, Staufen, Germany) with 2.0 mL of an 0.2% metaphosphoric acid in water: acetonitrile mixture (7:3, v/v). After centrifugation (14000 × g for 10 min) the supernatant layer was transferred to a clean tube, the extract was concentrated to one third of volume (with nitrogen at 30 °C). This extract was passed through a Bond Elut C18 cartridge (200 mg) as previously described for plasma.

Chromatographic separation was achieved by reversed-phase HPLC with a LiChrospher-100 RP18/5 µm column (250 × 4 mm; Merck, Darmstadt, Germany) combined with a guard column (4 × 4 mm) of similar material. The mobile phase consisted of a mixture of 85% orthophosphoric acid: triethylamine: water: acetonitrile (in the ratio 0.3:0.3:81.0:18.4; v/v), adjusted to pH 2.2. The substances were isocratically eluted with a flow rate of 0.8–0.9 mL/min. An internal standard method, using peak area ratios of plasma or tissues from unmedicated birds fortified with known amounts of danofloxacin, enrofloxacin and ciprofloxacin, was used to achieve calibration. Calibration curves were linear up to the following concentrations:

danofloxacin, 1.5 µg/mL in plasma and 2.0 µg/g in tissues; enrofloxacin, 2.0 µg/mL in plasma and 4.0 µg/g in tissues; and ciprofloxacin, 1.25 µg/mL in plasma and 2.0 µg/g in tissues. Samples exceeding these concentrations were analysed after dilution. The lower limits of quantification were as follows: danofloxacin, 0.015 µg/mL in plasma and 0.02 µg/g in tissues; enrofloxacin, 0.060 µg/mL in plasma and 0.05 µg/g in tissues; and ciprofloxacin, 0.020 µg/mL in plasma and 0.02 µg/g in tissues. Recovery in plasma samples was determined as 90.9% for danofloxacin, 92.1% for enrofloxacin, 79.0% for ciprofloxacin, and 88.1% for the internal standard ofloxacin. The precision of the analytical method, determined at concentrations of 0.5 µg/mL for enrofloxacin and ciprofloxacin, and 0.1 µg/mL

for danofloxacin, was characterized by intra-assay coefficients of precision between 2.7 and 3.5% and interassay coefficients between 3.8 and 9.1%. In the different tissues, recovery of fluoroquinolones, determined at a concentration of 0.5 µg/g tissue, ranged between 64 and 96% with intra-assay coefficients of precision between 3.6 and 8.0% and interassay coefficients between 4.9 and 10.3%. As an example, the following recoveries were found in liver tissue: danofloxacin 84.0%, enrofloxacin 94.4%, ciprofloxacin 81.8%, and ofloxacin 87.6%.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis of plasma concentration–time data following single intravenous or oral application was performed with the TOPFIT version 2.0 computer program (Heinzel *et al.*, 1993). Due to our experimental design (three blood samples per chicken) the entire plasma concentration–time profile could not be assessed for one individual bird. Therefore, a concentration–time curve was calculated using the mean plasma drug concentration of six different birds at each sampling time. Based on this curve, mean pharmacokinetic parameters could be estimated.

The terminal elimination rate constant (λ_z) was calculated from the log-linear portion of the elimination curve using linear regression analysis. The terminal half-life was calculated according to the equation $t_{1/2} = \ln 2/\lambda_z$. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC$) were calculated using the linear trapezoidal rule for all measured data points and extrapolating the area to infinity by means of the elimination rate constant. The mean residence time (MRT) was calculated as $MRT = AUMC/AUC$; mean absorption time (MAT) as $MAT = MRT_{oral} - MRT_{i.v.}$; total body clearance (Cl_B) as $Cl_B = Dose_{i.v.}/AUC$; volume of distribution at steady state (Vd_{SS}) as $Vd_{SS} = Cl_B \times MRT$, and volume of distribution at pseudo distribution equilibrium (V_z) as $V_z = Cl_B/\lambda_z$. Comparing the corresponding oral and i.v. groups, the bioavailability (F) after oral administration was calculated as $F = (AUC_{oral}/AUC_{i.v.})$.

Statistical analysis

Plasma and tissue concentration values are given as mean ± standard deviation (SD). Data determined at steady state on the third day of water medication were analysed for differences in danofloxacin and enrofloxacin mean tissue and plasma concentrations. A computer-based version (C-Stat for Windows, Cherwell Scientific Publishing, Oxford, UK) of the nonparametric Mann–Whitney–U-test was used for the analysis. $P < 0.05$ was considered to be significant.

RESULTS

Single intravenous and oral treatment

The mean plasma concentrations (± standard deviation) of enrofloxacin and danofloxacin obtained after single intravenous and oral administration are shown in Figs 1 and 2, respectively.

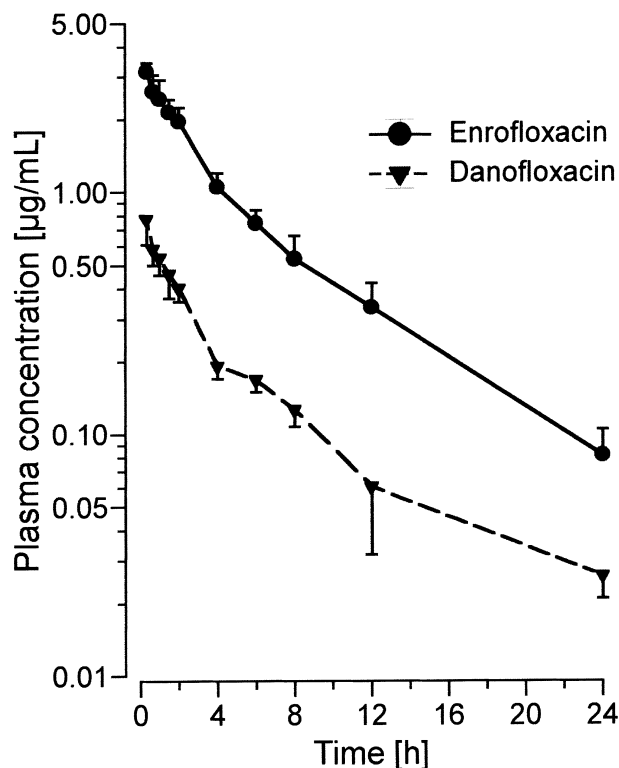


Fig. 1. Mean plasma concentrations of danofloxacin (5 mg/kg body weight) and of enrofloxacin (10 mg/kg body weight) in broiler chickens after single intravenous bolus administration. Each point represents the mean \pm SD of six birds.

Table 1. Mean pharmacokinetic parameters for danofloxacin (5 mg/kg bw) and enrofloxacin (10 mg/kg bw) after intravenous or oral administration to broiler chickens ($n = 6$)

Pharmacokinetic parameter	Danofloxacin		Enrofloxacin	
	i.v.	oral	i.v.	oral
C_{max}	$\mu\text{g/mL}$		0.47	1.88
t_{max}	h		1.5	1.5
λ_z	h^{-1}		0.103	0.105
$t_{1/2}$	h		6.73	6.62
Cl_B	$\text{mL}/\text{min}\cdot\text{kg}$		23.5	10.3
V_z	L/kg		13.7	5.0
$V_{d_{ss}}$	L/kg		10.2	3.9
$AUC_{0-\infty}$	$\mu\text{g}\cdot\text{h}/\text{mL}$		3.55	3.53
MRT	h		7.25	8.69
MAT	h		1.44	1.20
Bioavailability	%		99.2	89.2

Note: Standard deviations are not given because for each drug and route of administration only one concentration–time curve has been calculated based on the mean plasma drug concentration of six different birds per sampling time. C_{max} , maximum plasma concentration; t_{max} , time of C_{max} ; λ_z , terminal elimination rate constant; $t_{1/2}$, terminal elimination half-life; Cl_B , total body clearance; V_z , volume of distribution at pseudo distribution equilibrium; $V_{d_{ss}}$, volume of distribution at steady state; $AUC_{0-\infty}$, area under the plasma concentration–time curve; MRT , mean residence time; MAT , mean absorption time.

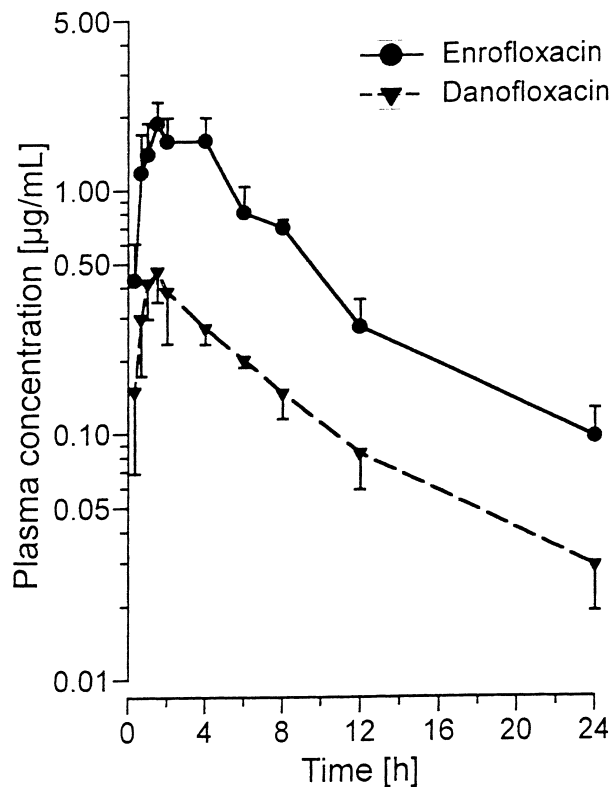


Fig. 2. Mean plasma concentrations of danofloxacin (5 mg/kg body weight) and of enrofloxacin (10 mg/kg body weight) in broiler chickens after single oral administration. Each point represents the mean \pm SD of six birds.

The pharmacokinetic parameters, describing the disposition of enrofloxacin and danofloxacin after i.v. and oral administration, are listed in Table 1.

Following i.v. administration of danofloxacin's approved dose, the 20 min postinjection plasma concentration ranged from 0.63 to 1.0 $\mu\text{g/mL}$ (mean 0.78 $\mu\text{g/mL}$) declining to 0.06 ± 0.03 $\mu\text{g/mL}$ after 12 h. At the corresponding sampling time, the enrofloxacin plasma concentration achieved 2.9–3.7 $\mu\text{g/mL}$ (mean 3.2 $\mu\text{g/mL}$) which declined during 24 h to 0.08 ± 0.02 $\mu\text{g/mL}$. For danofloxacin and enrofloxacin, plasma clearances were 23.5 and 10.3 $\text{mL}/\text{min}\cdot\text{kg}$, and steady-state distribution volumes were 10.2 and 3.9 L/kg, respectively.

Ninety minutes following oral administration of danofloxacin and enrofloxacin, plasma concentrations peaked at 0.47 $\mu\text{g/mL}$ (range 0.40–0.62 $\mu\text{g/mL}$) and 1.88 $\mu\text{g/mL}$ (range 1.54–2.58 $\mu\text{g/mL}$), respectively. The mean oral bioavailability was 99.2% for danofloxacin and 89.2% for enrofloxacin. For both danofloxacin and enrofloxacin, the route of administration (i.v. vs. oral) did not produce marked differences in plasma concentrations during the elimination phase which is reflected by nearly identical elimination half-lives for both routes.

Following both i.v. and oral administration of enrofloxacin, low concentrations of its metabolite ciprofloxacin were detected in plasma of most animals (between 0.02 and 0.08 $\mu\text{g/mL}$; data not shown). However, these concentrations of ciprofloxacin could not be quantified consistently as they were mostly around

or below the limit of quantification ($0.02 \mu\text{g/mL}$), in particular after oral administration and at sampling times longer than 8 h after administration.

Treatment via drinking water

Danofloxacin and enrofloxacin plasma concentration–time profiles, following administration of medicated drinking water, are shown in Fig. 3. Tissue concentrations of danofloxacin and enrofloxacin as well as ciprofloxacin are presented in Table 2 and Table 3, respectively.

Steady-state plasma concentrations were not attained within the sampling time period of the first day of administration. On the third day, steady-state was reached (Fig. 3), with mean concentrations (\pm SD) of danofloxacin and enrofloxacin of 0.12 ± 0.01 and $0.52 \pm 0.16 \mu\text{g/mL}$, respectively (Table 2 and 3). The ciprofloxacin plasma concentration was again very low (around the limit of quantification, with peak concentrations up to $0.07 \mu\text{g/mL}$; data not shown); therefore a mean value can not be given.

In most tissues the concentrations of both fluoroquinolones markedly exceeded the plasma concentrations (Table 2 and 3). The concentration of enrofloxacin significantly exceeded that of danofloxacin in six of eight tissues. For both drugs, including enrofloxacin's metabolite ciprofloxacin, the highest concentrations were measured in the excretory organs (liver and kidney) as well as in the trachea and in the caecal wall.

DISCUSSION

To our knowledge, this is the first study in which the pharmacokinetics of danofloxacin and enrofloxacin have been directly compared in chickens, following both single and

continuous administration. These drugs represent possible therapeutic alternatives for water medication in chickens. Although this comparison at different dosage levels may seem unusual, we suggest that comparing the results obtained at registered dosage regimens and correlating them with their *in vitro* activities is a useful tool to predict clinical efficacy.

Plasma concentrations of enrofloxacin after single oral administration to chickens at 10 mg/kg bw , as measured in this study, confirm results of other studies reported in the literature. We observed a peak plasma concentration of $1.88 \mu\text{g/mL}$, whereas a value of $2.44 \mu\text{g/mL}$ was reported by Anadón *et al.* (1995). The published peak plasma concentrations of danofloxacin following oral bolus administration (5 mg/kg bw) are: $0.66 \mu\text{g/mL}$ (Barthel, 1993) and $0.73 \mu\text{g/mL}$ (Lynch *et al.*, 1994), compared to $0.47 \mu\text{g/mL}$ in the present study.

Table 2. Steady state tissue concentrations of danofloxacin (dano) following administration *via* the drinking water. Danofloxacin dose equivalent 5 mg/kg bw per day; dosing over 5 days; sampling on the third day of administration

Tissue	<i>n</i>	Danofloxacin ($\mu\text{g/g}$) Mean \pm SD	dano _{tissue} /dano _{plasma}
Liver	16	1.31 ± 0.36	10.7
Kidney	16	0.81 ± 0.16	6.6
Lung	10	0.31 ± 0.07	2.6
Trachea	12	1.14 ± 0.18	9.4
Myocardium	10	0.15 ± 0.03	1.25
Muscle	12	0.30 ± 0.06	2.5
Skin	11	0.23 ± 0.05	1.8
Caecal wall	11	3.24 ± 1.67	26.6
Plasma	30	$0.12^{(x)} \pm 0.01$	

n, number of samples; ^(x), $\mu\text{g/mL}$.

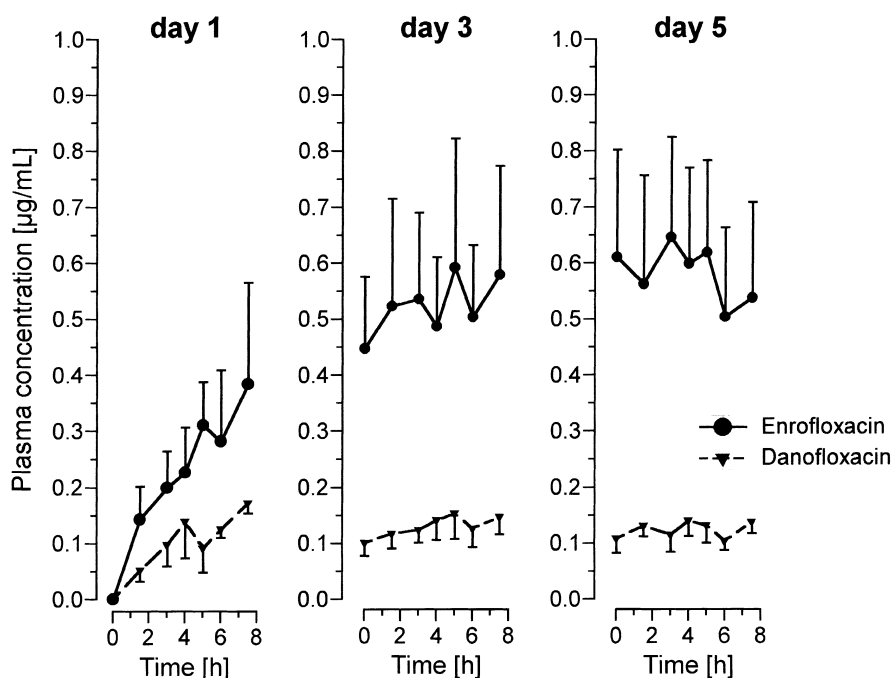


Fig. 3. Mean plasma concentrations of danofloxacin (5 mg/kg bw per day) and enrofloxacin (10 mg/kg bw per day) in broiler chickens following oral administration of medicated drinking water for 5 consecutive days. Each point represents the mean \pm SD of 10 birds. The sampling period between 0800 h and 1530 h (with 7 sampling time points per day) is indicated on the time axis.

Tissue	<i>n</i>	Enrofloxacin (µg/g) Mean ± SD	Ciprofloxacin (µg/g) Mean ± SD	Tissue ratio cipro/enro	enro _{tissue} / enro _{plasma}
Liver	30	2.82* ± 0.60	1.06 ± 0.44	0.38	5.4
Kidney	30	1.54* ± 0.36	0.18 ± 0.06	0.11	3.0
Lung	10	0.88* ± 0.24	0.02 ± 0.01	0.03	1.7
Trachea	10	1.57 ± 0.45	0.06 ± 0.02	0.04	3.0
Myocardium	15	1.05* ± 0.23	0.02 ± 0.02	0.02	2.0
Muscle	14	1.08* ± 0.31	0.03 ± 0.02	0.03	2.1
Skin	10	0.56* ± 0.15	0.02 ± 0.02	0.04	1.1
Caecal wall	10	3.65 ± 1.53	0.09 ± 0.01	0.03	7.0
Plasma	30	0.52 ^(x) * ± 0.16	< LOQ		

n, number of samples; < LOQ, < limit of quantification (0.02 µg/mL); ^(x), (µg/mL); *, significantly higher ($P < 0.05$) than the corresponding concentration of danofloxacin (see Table 2).

The terminal half-lives of enrofloxacin in chicken plasma following single i.v. or oral dosing, as reported in the literature, are: 18.7 h (i.v.) and 14.9 h (p.o.) (Conzelman *et al.*, 1987) and 10.3 h (i.v.) and 14.2 h (p.o.) (Anadón *et al.*, 1995). In our study, an enrofloxacin half-life of nearly 6 h was calculated for both routes of administration. These differences might be due to different assay methods (microbiological assay was used by Conzelman *et al.*, 1987) or different ages of the birds (6-week-old birds were used by Anadón *et al.*, 1995). However, half-lives of danofloxacin in chicken plasma in the current study (6.7 h–i.v., and 6.6 h–p.o.) were in accordance with values of 5.8 h (i.v.) and 7.2 h (p.o.) calculated by Lynch *et al.* (1994), and 6.98 h (p.o.) reported by Anadón *et al.* (1997).

Following single oral administration, both fluoroquinolones were rapidly absorbed, as is reflected by low *MAT* (mean absorption time) values: 1.4 h after danofloxacin and 1.2 h after enrofloxacin. The time of peak concentration was achieved by both drugs after 1.5 h. The bioavailability of both drugs (danofloxacin 99.2% and enrofloxacin 89.2%) indicates a high extent of absorption. The value obtained for danofloxacin in the current study is in good agreement with the value of 102% reported by Lynch *et al.* (1994). Likewise, the bioavailability value calculated for enrofloxacin is in close agreement with the value ($F = 84.5\%$) reported by Conzelman *et al.* (1987), but is markedly higher than the value of 64% published by Anadón *et al.* (1995).

After continuous medication *via* the drinking water, the steady-state plasma concentration of enrofloxacin achieved 0.52 µg/mL, exceeding the corresponding concentration for danofloxacin (0.12 µg/mL) fourfold. The derived steady-state AUC_{0-24h} values of 2.88 µg·h/mL (danofloxacin) and 12.48 µg·h/mL (enrofloxacin) are consistent with the *AUC* values obtained in our study after single oral administration (3.53 µg·h/mL for danofloxacin and 14.42 µg·h/mL for enrofloxacin). Our steady-state plasma concentrations for enrofloxacin are in accordance with other published data (Scheer *et al.*, 1997: 0.50 µg/mL; Ganière *et al.*, 1997: 0.84 µg/mL).

Comparative data for danofloxacin in chickens are sparse. Following pulse-dose administration of danofloxacin with drinking water at 5 mg/kg body weight/day during 3 days (6–8 h medication period daily), Barthel (1993) measured peak plasma

Table 3. Steady state tissue concentrations of enrofloxacin (enro) and its metabolite ciprofloxacin (cipro) following administration of enrofloxacin *via* the drinking water (enrofloxacin dose equivalent 10 mg/kg bw per day; dosing over 5 days; sampling on the third day of administration)

concentrations around 0.4 µg/mL which were declining rapidly and holding for the adjacent period only between 0.1 and 0.2 µg/mL. Assuming linear pharmacokinetics for danofloxacin, as was demonstrated for enrofloxacin (Walker *et al.*, 1992), it can be estimated from our results that, even by doubling the danofloxacin dosage to 10 mg/kg, a steady-state plasma concentration of only about 0.3 µg/mL would result.

The steady-state tissue concentrations for both danofloxacin and enrofloxacin generally exceeded corresponding plasma concentrations, in accordance with the literature. Ratios of tissue-to-plasma concentrations were higher for danofloxacin (range 1.3–26.6) than for enrofloxacin (range 1.1–7.0) which is in accordance with the estimated larger distribution volume value (V_z) of danofloxacin vs. enrofloxacin in our single dose experiments. Nevertheless, due to the fourfold higher steady-state plasma concentration of enrofloxacin compared to danofloxacin, in most tissues (with exception of trachea and caecal wall) the concentrations of enrofloxacin were significantly higher ($P < 0.05$) than those of danofloxacin.

For the successful treatment of respiratory tract infections high antimicrobial concentrations must be attained particularly in target tissues such as lung and trachea. In our study, the ratios of lung-to-plasma and trachea-to-plasma concentrations were 2.6 and 9.4, respectively, for danofloxacin, and 1.7 and 3.0, respectively, for enrofloxacin. Comparable lung-to-plasma concentration ratios between 2 and 3 in chickens were also previously reported (enrofloxacin: Scheer, 1987a; Anadón *et al.*, 1995; and danofloxacin: Barthel, 1993; Anadón *et al.*, 1997).

The antimicrobial activity of enrofloxacin and its metabolite ciprofloxacin against relevant veterinary bacterial pathogens is similar (Prescott & Yielding, 1990). In this study, plasma ciprofloxacin concentrations following enrofloxacin administration *via* the drinking water were found as being generally low, ranging below the limit of quantification up to 0.07 µg/mL. Ganière *et al.* (1997) obtained similar results: serum ciprofloxacin concentrations being ≤ 0.07 µg/mL while the mean enrofloxacin concentration amounted to 0.84 µg/mL. Investigating the pharmacokinetics of enrofloxacin in the Muscovy duck, Intorre *et al.* (1997) also measured very low ciprofloxacin concentrations between 0.02 and 0.07 µg/mL in plasma, both after oral and intramuscular administration.

Likewise, with the exception of the excretory organs, liver (38%) and kidneys (11%), tissue ciprofloxacin concentrations never exceeded 4% of the enrofloxacin values in the present study, which is in contrast to the results of Anadón *et al.* (1995). In the latter study, ciprofloxacin concentrations either equalled those of enrofloxacin or exceeded them up to three times. This discrepancy is unlikely to be caused by different sampling times (steady-state vs. one day after withdrawal) and, at this time, remains unexplained.

There are several publications (Scheer, 1987b; Kempf *et al.*, 1988; Raemdonck *et al.*, 1992; Cooper *et al.*, 1993; Bradbury *et al.*, 1993) reporting the *in vitro* activity of either danofloxacin or enrofloxacin against the following economically significant respiratory tract pathogens of poultry: *Escherichia coli*, *Pasteurella multocida* and *Mycoplasma gallisepticum*. MIC values derived from such studies are dependent on many factors and, therefore, comparisons between different studies are of limited value. In recent studies (Hannan *et al.*, 1997; Watts *et al.*, 1997), however, danofloxacin and enrofloxacin have been simultaneously tested against field isolates of these veterinary pathogens. It was reported that both fluoroquinolones exhibit nearly identical *in vitro* activity, based on the obtained MIC₉₀ values: against *E. coli* ≤ 0.015 µg/mL for danofloxacin, and 0.030 µg/mL for enrofloxacin; against *P. multocida* ≤ 0.015 µg/mL for both drugs; and against *M. gallisepticum* 0.1 µg/mL for both drugs.

Fluoroquinolones are active against bacterial pathogens in a concentration-dependent manner (Forrest *et al.*, 1993; Meinen *et al.*, 1995). The efficacy of fluoroquinolones can therefore be predicted by the ratio of the area under the drug concentration-time curve (AUC) to the MIC (AUC/MIC), or otherwise noted as AUIC (area under the inhibitory concentration curve). Assuming equal *in vitro* activity for danofloxacin and enrofloxacin (Hannan *et al.*, 1997; Watts *et al.*, 1997), AUIC values for enrofloxacin of about four times greater than for danofloxacin can be estimated on the basis of our results. Considering this, it is unlikely that danofloxacin at 5 mg/kg body weight per day might be capable of producing a treatment success in broilers equivalent to that observed with enrofloxacin at 10 mg/kg. This conclusion is in line with reported clinical data. Most recent results from comparative studies with experimentally infected chickens suggest that enrofloxacin's clinical success is significantly higher at dosages of 10 mg/kg when compared to danofloxacin given at 5 mg/kg (Carli *et al.*, 1997; Charleston *et al.*, 1998).

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