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SHORT COMMUNICATION

Pharmacokinetic behaviour of enrofloxacin and its metabolite ciprofloxacin after subcutaneous administration in cattle

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Abstract This study compared pharmacokinetic profiles in cattle dosed subcutaneously with two different formulations of enrofloxacin (5% and 10%) at a dose of 5 mg/kg. Plasma concentrations of enrofloxacin and its active metabolite, ciprofloxacin, were determined by a HPLC/u.v. method. The pharmacokinetic parameters of enrofloxacin and its metabolite were similar in both injectable formulations. Enrofloxacin peak plasma concentration (5%: 0.73±0.32; 10%: 0.60±0.14 μg/mL) was reached at 1.21±0.52 and 1.38±0.52 h to 5 and 10%, respectively. The terminal half-live and area under curve were 2.34±0.46 and 2.59± 0.46 h, and 3.09±0.81 and 2.93±0.58 μg·h/mL, to 5 and 10%, respectively. The AUC/ MIC_{90} and Cmax/MIC_{90} ratios for both formulations exceed the proposed threshold values for optimized efficacy and minimized resistance development whilst treating infections or septicaemia caused by *P. multocida* and *E. coli*.

Keywords Enrofloxacin · Ciprofloxacin · Cattle · Subcutaneous · Pharmacokinetic · Formulations

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Introduction

Fluoroquinolones are gaining widespread acceptance in veterinary medicine because of its wide spectrum of activity and favourable pharmacokinetic behaviour. They generally present very good activities against a broad spectrum of aerobic bacteria, including *Pasteurella* spp and against *Mycoplasma*. Generally, fluoroquinolones are characterized by excellent tissue penetration, high bioavailabilities and long terminal half-life. Enrofloxacin is used in beef breed cattle with respiratory and digestive diseases (McKellar 1996) and in dairy cattle suffering from acute *E. coli* mastitis (Hoeben et al. 2000).

The objective of this study was to determine the pharmacokinetic behaviour of enrofloxacin and its active metabolite, ciprofloxacin, following a single subcutaneous administration of two different formulations of enrofloxacin (5% and 10%) in cattle.

Matherial and methods

The study was performed in twelve healthy animals (limousin and holstein cross male beef cattle, on average 90 kg, 2–3 months old). The feedlot cattle were housed for an initial one-month acclimatisation period and were given mixed pelleted concentrate and hay and had access to water *ad libitum* Complete physical and analytical examinations and observations on general health were made each day throughout the study. The independent veterinary care of the animals was realized by Ms. Isabel Uriarte Inchausti. No drugs were administered during the period studied. Two commercial injectable solutions of enrofloxacin (Baytril® 5% and Baytril® 10%, Bayer AG) were injected subcutaneously at a single dose of 5 mg/kg in the neck region. Blood samples were collected from the right jugular vein at 0, 0.17, 0.33, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h after dosing. The plasma was separated and stored at −20°C until assay (analysis were performed within 4 weeks after sample collection).

Plasma concentrations of enrofloxacin and its active metabolite, ciprofloxacin, were simultaneously quantified in all samples using HPLC/u.v., according to a method described by de Lucas et al (2004). Linear calibration curves were obtained between 0.025–10 μg/mL concentration range (R^2>0.996) using ofloxacin as internal standard. The limit of quantification (LOQ) was 0.025 μg/mL for both compounds. Precision was calculated as the coefficient of variation of the average value found for each added concentration was <10% and accuracy ranged between 80 and 120%. The stability of the drug in spiked samples stored at ≤20°C for up 2 months was assessed.

Pharmacokinetic parameters were determined for each individual animal. Plasma concentrations of enrofloxacin and its metabolite were subjected to a non-compartmental

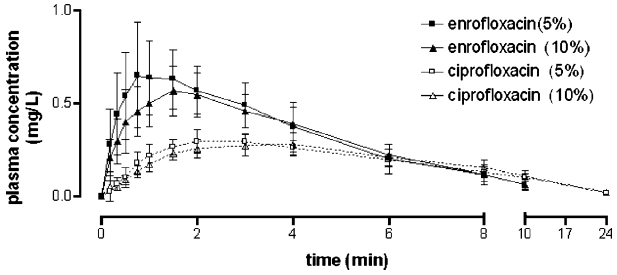

Fig. 1 Mean (±SD) plasma enrofloxacin and its active metabolite ciprofloxacin concentration *versus* time curves following single dose (5 mg/kg) subcutaneous administration of enrofloxacin (5% and 10% formulations) in cattle (n=8)

Table 1 Pharmacokinetic parameters of enrofloxacin and ciprofloxacin (as active metabolite) following single dose (5 mg/kg) subcutaneous administration of enrofloxacin (5% and 10% formulations) in cattle (n=8)

Pharmacokinetic parameter	Enrofloxacin 5%		Enrofloxacin 10%	
	Enrofloxacin	Ciprofloxacin	Enrofloxacin	Ciprofloxacin
Tmax (h)	1.21±0.52	2.63±0.71	1.38±0.52	3.50±0.67
Cmax (μg/mL)	0.73±0.25	0.32±0.06	0.60±0.14	0.29±0.06
λ (h^{-1})	0.31±0.06		0.28±0.05	
$t_{1/2\lambda}$ (h)	2.34±0.46		2.59±0.46	
AUC_t (μg·h/mL)	3.09±0.81	2.59±0.49	2.93±0.58	2.19±0.60
AUC_∞(μg·h/mL)	3.34±0.87		3.19±0.64	
MRT_t (h)	3.24±0.37	6.09±1.19	3.56±0.26	5.27±1.10
MRT_∞ (h)	3.97±0.73		4.38±0.56	
enrofloxacin+ciprofloxacin				
$Cmax_{enr+cip}$ (μg/ml)	0.97±0.28		0.83±0.16	
$AUC_{enr+cip}$ (μg·h/mL)	5.67±0.80		5.13±0.87	

Cmax: peak plasma concentration; Tmax: time to C_{max}; λ: terminal rate constant; $t_{1/2\lambda}$: terminal phase half-life; AUC: area under the plasma concentration-time curve; MRT: mean residence time.

analysis using PCnonlin V4.0 software package (Statistical Consultants Inc. Lexington, USA). Pharmacokinetic/Pharmacodynamic (PK/PD) parameters obtained were AUC/MIC_{90} and $Cmax/MIC_{90}$ (MIC_{90}=0.03 μg/mL for native populations of *P. multocida* and *E. coli*; Wallmann 2006).

Results

The plasma level concentrations versus time curves of enrofloxacin and ciprofloxacin, and the pharmacokinetic parameters of two formulations after subcutaneous administration in cattle are shown in Fig. 1 and Table 1, respectively. PK/PD values obtained, following subcutaneous administration of enrofloxacin for MIC_{90} value of 0.03 μg/mL, are present in Table 2.

Similar plasma concentrations and pharmacokinetic behaviour of enrofloxacin were obtained for both formulations. Statistical significant differences were observed between 5% and 10% formulation in Tmax of the active metabolite (2.63 h and 3.50 h, respectively).

Table 2 PK/PD values obtained following single dose subcutaneous administration of enrofloxacin, for MIC_{90} value of 0.03 ug/mL (MIC_{90} of *E. coli and P. multocida*)

Formulation	Enrofloxacin	Ciprofloxacin	Enrofloxacin + ciprofloxacin
5%			
Cmax/MIC	24.42±8.44	10.53±1.84	32.20±9.19
AUC/MIC	111.45±28.99	86.23±16.26	189.08±26.77
10%			
Cmax/MIC	19.83±4.73	9.65±1.95	27.52±5.30
AUC/MIC	106.37±21.47	73.04±20.13	170.86±28.97

Discussion

Mean AUC and Cmax values in our study (5 mg enrofloxacin/kg) are approximately, 2 and 2.5 times higher, respectively, than the values reported for a subcutaneous dose of 2.5 mg enrofloxacin/kg (McKellar et al. 1999), suggesting a linear dose~plasma pharmacokinetic response. Our results are similar than those described by McKellar et al. (1999), however, other authors observed a high values of AUC and Cmax in cows (Kaartinen et al. (1995): AUC=9.62 μg·h/mL; Cmax=3.20 μg/mL). The animals used in our study were similar than those used by McKellar et al. (1999) (male: 10 months, 250–270 kg); in contrast, Kaartinen et al. (1995) used lactating cows in their first or second lactation and microbiological assay as detection method. Methodological, physiological and age-related differences are likely to explain this discrepancy.

The absorption of enrofloxacin after subcutaneous administration obtained to us (Tmax= 1.21 h and 1.38 h, to 5 and 10% formulations, respectively) and to McKellar et al. (1999) (1.75 h) was faster than those described by Kaartinen et al. (1995) (3.20 h) in cows or by Ramesh et al. (2002) (2.9 h) in goats. In our study, enrofloxacin presents a similar half-life to that described in sheep (Rahal et al. 2006: $t_{1/2\beta}$=2.60 h; MRT=3.43 h) and goats (Ramesh et al. 2002: 2.84 h), however, long values of ciprofloxacin MRT were observed in our study, which could increase the efficacy. The values of elimination half-life obtained to Kaartinen et al. (1995) for cows were longer than those obtained in our study.

Also, the pharmacokinetic behaviour after subcutaneous administration in non-ruminant herbivorous, as alpaca, has been described by Gandolf et al. (2005). They observed higher values of Cmax (4.16 μg/mL) and AUC (41.90 μg·h/mL), a more delayed absorption (6 h) and a longer permanence ($t_{1/2}$=7.83 h and MRT=10.33 h) that those described in ruminant species.

The main antimicrobial active fraction, which is systemically available after SC enrofloxacin injection, consists of enrofloxacin, but the active metabolite also was detected. Ciprofloxacin is a more potent antimicrobial than the parent drug for many veterinary pathogens (Grobbel et al. 2007; Prescott and Yielding 1990). For this reason, it would be more appropriate to utilize the sum of AUCs and Cmaxs of enrofloxacin and ciprofloxacin in computing pharmacodynamic variables (Prescott and Yielding 1990).

In our study, ciprofloxacin comprised 32% and 30% of the total fluoroquinolone (enrofloxacin and ciprofloxacin) Cmax and 43% and 46% of the total fluoroquinolone AUC, for 10 and 5% formulations, respectively. It is important enough to be co-assessed for the PK-PD parameter AUC/MIC_{90} and $Cmax/MIC_{90}$ because the in vitro activity of enrofloxacin and ciprofloxacin may differ by 1 or 2 $\log_2$ dilutions for respiratory pathogens like *P. multocida* and *M. haemolytica*.

We used enrofloxacin MIC_{90} values of 0.03 μg/mL, for native populations of *P. multocida* and *E. coli* (Wallmann 2006). The ratio's AUC/MIC_{90} (189.08±26.77 and 170.86±28.97 h for 5 and 10% formulations, respectively) and $Cmax/MIC_{90}$ (32.20±9.19 and 27.52±5.30 for 5 and 10% formulations, respectively) do largely outweigh the proposed threshold values (125 h and 10, respectively, McKellar et al. 2004) for optimized efficacy and minimized resistance development whilst treating infections caused by *P. multocida* and *E. coli* (Table 2).

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