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Pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in cows following single dose intravenous administration

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Enrofloxacin is de-ethylated to the primary active metabolite ciprofloxacin. Both of these quinolone compounds work similarly and are active against many gram-negative organisms raising the possibility that the presence of both drugs is beneficial to the patient. This experiment was designed to evaluate the pharmacokinetic parameters of enrofloxacin and its active metabolite ciprofloxacin in cows following single dose intravenous (i.v.) administration.

Four healthy cross-bred Jersey cows (3–4 years, 370 ± 20 kg) bought from the livestock research center of G.B. Pant University of Agriculture and Technology, India, were kept for a preexperimental period for 2 weeks and subjected to physical and clinical examination. Enrofloxacin (Enrocin®, 100 mg/mL; Ranbaxy Animal Health India Ltd, New Delhi, India) was injected intravenously at a single dose of 5 mg/kg, in the jugular vein. Blood samples from the jugular vein on other side of neck were collected in heparinized tubes at 2, 10, 15, 30 min, 1, 2, 3, 6, 12, 24, and 30 h postinjection.

The plasma was separated and stored at −20 °C until assayed for enrofloxacin and its active metabolite, ciprofloxacin as described (Nielsen & Hansen, 1997) with slight modifications. To 1 ml plasma, 1.5 ml of acetonitrile was added, mixed thoroughly and centrifuged for 10 min at 2500 *g*. Then 0.5 ml of the supernatant was mixed with 1 ml of high-pressure liquid chromatography (HPLC) grade water. The mixture was filtered through a Millipore 0.2 μm filter and an aliquot of 20 μL of this mixture was injected into the HPLC system (Shimadzu Corporation, Kyoto, Japan; Model SPD 10 AT, LC10AT). The separation of enrofloxacin and ciprofloxacin was performed as per the method of Küng *et al.* (1993) with slight modifications using a C_{18} reverse phase column (4 × 150 mm) having particle size of 5 μm. An isocratic mobile phase consisting of acetonitrile:methanol:water (17:3:80, v/v) with 0.4% orthophosphoric acid (85%, v/v) and 0.4% triethylamine at a flow rate of 0.6 mL/min, was used to elute enrofloxacin and ciprofloxacin.

The lowest limit of quantification and detection for both compounds were 0.03 μg/mL. The plasma concentrations vs. time data of enrofloxacin and ciprofloxacin (active metabolite of enrofloxacin) of each animal were analyzed with the help of a nonlinear iterative curve-fitting computer programme (Statis, Ver. 3; M/s Clyde Soft, Glasgow, UK) as described by Gibaldi and Perrier (1982).

The plasma concentration of enrofloxacin (2.7 μg/mL) at 2 min after administration of drug intravenously was 27.4 times higher than the therapeutic concentration (0.1 μg/mL) in plasma. A quite comparable peak plasma concentration of enrofloxacin (2.7 μg/mL) has been reported in calves (1.6 μg/mL; Kaartinen *et al.*, 1997). The therapeutic concentration in the present study was considered to be 0.1 μg/mL as the mimimum inhibitory concentration (*MIC*) values of enrofloxacin and ciprofloxacin for many pathogens have been reported to be ≤0.1 μg/mL, (Prescott & Yielding, 1990). The therapeutic concentration (0.1 μg/mL) of enrofloxacin and ciprofloxacin (active metabolite) remained in the plasma for more than 12 and 8 h, respectively (Fig. 1).

The plasma concentration time profile following single dose (5 mg/kg) i.v. administration of enrofloxacin in all the four animals was adequately fitted to a two-compartment open model. The correlation coefficient of fit of curve (r^2) was 0.97 ± 0.003. The elimination rate constant in the present study was 0.282/h with an elimination half-life of 2.61 h indicating a slow decline in plasma concentration of enrofloxacin (Table 1). This finding agrees well with an elimination half-life of 2.7 h in calves (Davidson *et al.*, 1986). The $V_{d(area)}$ of enrofloxacin was observed to be 4.1 L/kg. The large V_d observed indicates that enrofloxacin is widely distributed in the extra vascular compartments. The clearance of enrofloxacin observed in the present study is comparable with that in dairy cows (21 mL/kg/min; Malbe *et al.*, 1996). The value of *AUC* of enrofloxacin in plasma was 4.4 μg·h/mL. In a model study of the pharmacodynamics of intravenous ciprofloxacin, the most important predictor for clinical and microbiological cure was

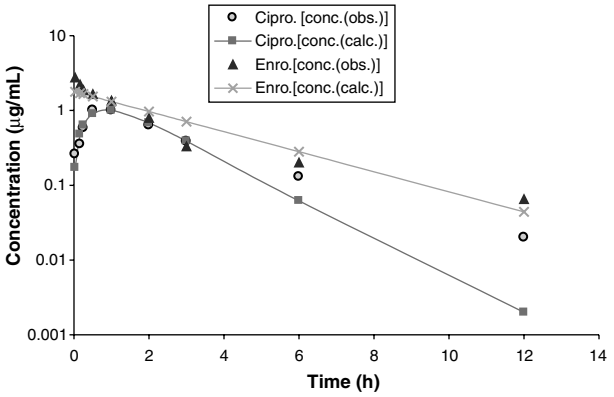

Fig. 1. Observed and calculated plasma concentration-time profile of enrofloxacin and ciprofloxacin (as metabolite) following single dose (5 mg/kg^{-1}) i.v. administration in cows ($n = 4$)

Table 1. Pharmacokinetic parameters of enrofloxacin following single dose (5 mg/kg) intravenous administration in cows ($n = 4$)

Pharmacokinetic parameters (units)	Enrofloxacin (mean ± SE)	Ciprofloxacin (mean ± SE)
A (µg/mL)	1.184 ± 0.240	–
B (µg/mL)	1.166 ± 0.112	0.839 ± 0.055
α/k_f (/h)	5.21 ± 1.351	0.997 ± 0.001
β (/h)	0.282 ± 0.022	0.227 ± 0.026
$t_{1/2\alpha}/t_{1/2}k_f$ (h)	0.133 ± 0.021	0.694 ± 0.123
$t_{1/2\beta}$ (h)	2.61 ± 0.281	3.061 ± 0.234
V_c (L/kg)	2.125 ± 0.319	–
$V_{d(area)}$ (L/kg)	4.090 ± 0.012	–
$V_{d(ss)}$ (L/kg)	0.447 ± 0.012	–
Cl_B (L/h/kg)	1.144 ± 0.214	–
AUC (µg·h/mL)	4.423 ± 0.812	3.692 ± 0.211
MRT (h)	3.480 ± 0.222	2.780 ± 0.157

A, zero time intercept of distribution slope in the two compartment model; B, zero time intercept of decline in plasma concentration of drug; α/k_f, distribution rate constant/metabolite rate forming constant; β, elimination rate constant; $t_{1/2\alpha}/t_{1/2}k_f$, distribution half-life/half-life for metabolite formation; $t_{1/2\beta}$, elimination half-life; Cl_B, clearance of drug from the body; V_c, volume of the central compartment; $V_{d(area)}$, apparent volume of distribution; $V_{d(ss)}$, volume of distribution at steady-state; *MRT*, mean residence time; *AUC*, total area under the concentration–time curve from zero to infinity.

an *AUC*/*MIC* ratio of >125 (Forrest *et al.*, 1993). A C_{max}/*MIC* ratio of enrofloxacin >3 in an *in vitro* pharmacokinetic model resulted in >99% reduction of bacterial count within 4 h, but in order to prevent bacterial regrowth within 24 h, a C_{max}/*MIC* ratio of 8:1 is required (Blaser *et al.*, 1987). The C_{max}/*MIC* and *AUC*/*MIC* ratio calculated in the present study would be far greater than 10 and 125, respectively, for a reported *MIC* value (≤0.1 µg/mL) of enrofloxacin against various species of bacteria.

Ciprofloxacin, an active metabolite of enrofloxacin, appeared within 2 min in the concentration of 0.26 µg/mL with a peak plasma concentration of 1 µg/mL at 30 min. The presence of ciprofloxacin in a concentration of 0.1 µg/mL up to 6 h could have therapeutic importance as it contributes to the *in vivo* activity of enrofloxacin (Flammer *et al.*, 1991), as the two compounds have similar antibacterial spectrum. The elimination half- life (3.1 h) of ciprofloxacin (as metabolite) was greater than the elimination half-life of enrofloxacin after i.v. administration of enrofloxacin. *AUC* of ciprofloxacin (3.7 µg·h/mL) was slightly less as compared with enrofloxacin (4.4 µg·h/mL) following i.v. administration.

Dosage regimens comprising larger doses given at less frequent intervals may be efficacious. Based on the pharmacokinetic data from the present study, an i.v. dosage regimen of enrofloxacin with a priming dose of 4 mg/kg followed by a maintenance dose of 3.5 mg/kg, every 8 h to maintain a therapeutic concentration of 0.1 µg/mL is calculated. However, several studies have reported that fluoroquinolones such as enrofloxacin and ciprofloxacin are concentration-dependent in their action (C_{max}/*MIC* > 8; *AUC*/*MIC* > 125) and hence have postantibiotic effect lasting for up to 4–8 h; therefore the dose interval can be kept at every 24 h. The recommended dosage regimen could be 3.5 mg/kg at 24 h interval. However, this drug may not be considered for use in food producing animals because of some regulatory jurisdictions.

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