Indian Journal of Animal Sciences 76 (1): 6–9, January 2006

Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin and their interaction with diclofenac sodium in cows

R VARMA[1], A H AHMAD[2], V AHUJA[3], V GAUTAM[4], PARDEEP K[5] and K P SINGH[6]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal 263 145 India

Received: 25 August 2004; Accepted: 21 September 2005

ABSTRACT

The pharmacokinetics of enrofloxacin was studied in 5 cows following single dose of intramuscular administration (5 mg.kg^{-1}) alone and along with diclofenac sodium (250 mg, total dose). Therapeutic concentration (0.1 μg.mL^{-1}) was maintained in plasma up to 12 and 24 h for enrofloxacin alone and enrofloxacin along with diclofenac sodium respectively. Following concurrent administration of enrofloxacin along with diclofenac sodium, the $t_{1/2}$ K_e (9.2 h), T_{max} (2 h), MRT (13.2 h) and Cl (1.4 L.kg^{-1}) were significantly higher compared to those following enrofloxacin given alone ($t_{1/2}$ K_e–5.9 h, T_{max}–0.4 h, MRT–6.8 h, Cl–0.82 L.kg^{-1} h^{-1}). However, when enrofloxacin was administered along with diclofenac sodium, the AUC (3.8 μg.h.mL^{-1}) and C_{max} (0.2 μg.mL^{-1}) were significantly lower, compared to when enrofloxacin was given along (AUC–6 μg.hmL^{-1} and C_{max}–1.5 μg.mL^{-1}). The plasma concentration of ciprofloxacin (metabolite of enrofloxacin) was significantly lower following administration of enrofloxacin along with diclofenac sodium compared to enrofloxacin administered alone. Our results indicate that co-administration of diclofenac sodium with enrofloxacin could lead to drug interaction i.e. altering the pharmacodynamic and pharmacokinetics of enrofloxacin.

Key words: Ciprofloxacin, Cows, Diclofenac sodium, Enrofloxacin, Interaction, Pharmacokinetics

Fluoroquinolones are currently the most actively studied groups of antimicrobials. Enrofloxacin is an antimicrobial drug with an extended spectrum that has been successfully used to combat infections in various farm animals (Bauditz 1987). The drug is partially metabolized in the liver to ciprofloxacin, a primary metabolite, which itself is a potent antimicrobial agent (Bergan *et al.* 1988, Tyczkowska *et al.* 1989).

The drug interaction becomes an important consideration in the selection of the most suitable antimicrobial agent for concomitant use in animals receiving other chemotherapeutic agents. Jayakumar (2000) indicated that fluoroquinolone antimicrobial agents interact with other drugs. Hence, the present study was designed to determine the effect of diclofenac sodium on the pharmacokinetics of enrofloxacin in cattle.

MATERIALS AND METHODS

Healthy, non lactating cows (5) of 3–4 years in age and weighing 30±15 kg were used in this experiment. They were kept on green fodder supplemented with concentrate ration and provided with water *ad lib*. A pre-experimental period of 2 weeks was given before the commencement of experiment to acclimatize them to the new environment.

Enrofloxacin was injected as a single dose (5 mg.kg^{-1}) intramuscularly for pharmacokinetic studies. Blood samples were collected in heparinized tubes at 0, 2, 10, 15, 30 min and 1, 2, 3, 6, 12, 24 and 30 h after injection. After a washout period of 15 days, the pharmacokinetics of enrofloxacin along with diclofenac sodium was studied in the same animals. Enrofloxacin (5 mg.kg^{-1}) and diclofenac sodium (250 mg, total dose) were administered intramuscularly simultaneously at different sites. Blood samples were collected in heparinized tubes before and after administration of the drug at predetermined time intervals. The blood samples were centrifuged for 10 min at 2500× g for separation of plasma. Plasma samples were stored at –20°C till analyzed. The analysis was done within a week of collection of the samples.

Extraction of enrofloxacin from plasma was done as per Nielsen and Gyrd-Hansen (1997) with slight modifications. Deproteinization of the plasma was carried out by mixing 0.5 ml plasma and 0.75 ml acetonitrile, followed by 1 min vortexing and subsequent centrifugation at 2 500 × g for 10 min. The clear supernatant thus obtained was transferred to a tube and double the volume of HPLC water added. The mixture was filtered through a 0.2 μm membrane filter.

Present address: [1]Assistant Professor, Department of Pharmacology, Veterinary College, Faizabad, Uttar Pradesh.
[2]Professor and Head, [3,6]Ph.D Scholar and SRF, Department of Pharmacology, College of Veterinary and Animal Sciences.
[4]Ph.D Scholar, Nebraska Medical School, Ohama, USA.
[5]Technical Officer, SBI, Panchkula, Chandigarh.

The concentration of the drugs in the various samples was done by high performance liquid chromatography. The method developed by Kung *et al.* (1993) was used with slight modifications. The HPLC system comprised LC–10AT double plunger pump with a manual loop injector, 20 μl of filtrate being injected for HPLC analysis.

Enrofloxacin and its metabolite ciprofloxacin were separated using a C_{18} reverse phase column, particle size 5μm (4×150 mm) as the stationary phase. A mixture of acetonitrile—methanol-water (17:3:80, v/v), containing 0.4% orthophosphoric acid (85%, v/v) and 0.4% triethylamine was used as mobile phase. The flow rate of the mobile phase was kept at 0.6 mL.min^{-1}. Chromatography was performed at 20±2°C with UV detection at 278 nm. The retention times for enrofloxacin and ciprofloxacin were 7–9 and 5–6 min respectively.

Drug standards were prepared by dissolving 5 mg of pure enrofloxacin in 5 ml of 0.1 N NaOH solution to give a stock solution of 1 mg.mL^{-1}. Stock solution of ciprofloxacin was also prepared by the same method. Further dilutions of enrofloxacin and ciprofloxacin were made in water from the stock solution. Acetonitrile was added to the dilutions of enrofloxacin and ciprofloxacin in the ratio of 1:1.5. After vortexing and centrifugation, 0.5 ml of each supernatant was mixed with 1 ml of water and diluted out so that the final concentrations obtained were 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06 and 0.03 μg.mL^{-1}. A standard calibration curve was obtained by plotting the concentration against the peak area. Plasma standards of enrofloxacin (0.03–8.0 μg.mL^{-1}) and ciprofloxacin (0.03–0.8 μg.mL^{-1}) were also prepared from the stock solution. Pooled cattle plasma being spiked with the appropriate dilutions of enrofloxacin and ciprofloxacin was treated in the same way as the plasma samples. The calibration curves were linear with regression coefficient (r^2) of 0.95. Enrofloxacin standards including diclofenac sodium were also prepared to check for interference, but no interference was observed.

The recoveries of enrofloxacin and ciprofloxacin were ≥90% with intra- and inter-assay coefficients of variation of ≤6% respectively. The limit of quantification and detection was 0.03 μg.mL^{-1}.

The plasma concentration-time profile of enrofloxacin and its metabolite ciprofloxacin obtained during the study were subjected to analysis by fitting them to various compartmental models. The pharmacokinetic analysis was done by nonlinear iterative curve-fitting computer software 'Statis' 3 and 'Pharmkit'. These softwares are based on the methods described by Gibaldi and Perrier (1982). The statistical comparison of plasma concentrations at various times between enrofloxacin alone and along with diclofenac sodium was carried out by students 't' test (Snedecor and Cochran 1980). The pharmacokinetic data were compared by non parametric Wilcoxon's rank sum test (Steel and Torrie 1960). The dosage regimen of enrofloxacin have been calculated by the following formula (Baggot 1980),

Priming dose $= C_{therapeutic} \cdot Vd \cdot e^{\beta}$

Maintenance dose $= C_{therapeutic} \cdot V_d \cdot (e^{\beta.t}-1)$

Minimum steady state concentration $\{C_{ss\,(min)}\} = D/V_d \cdot (e^{\beta.t}-1)$

Maximum steady state concentration $\{C_{ss\,(max)}\} = D/V_d \cdot (1-e^{-\beta.t})$

where, $C_{therapeutic}$ is the desired therapeutic concentration of the drug in plasma, D is the priming dose, V_d is the apparent volume of distribution, e represents the base of natural logarithm, ß is the elimination rate constant, and t is the dosage interval.

RESULTS AND DISCUSSION

The plasma drug concentration-time profile of enrofloxacin and ciprofloxacin (as metabolite of enrofloxacin) alone and along with diclofenac sodium following single dose (5 mg.kg^{-1}) i/m administration of enrofloxacin in cows is depicted in Table 1. The peak plasma concentration (1.4±0.006 μg.mL^{-1}) of enrofloxacin following single dose i/m administration, was observed at 0.5 h, which declined to 0.012±0.001 μg.mL^{-1} at 24 h. This route of

Table 1. Mean (±SE) plasma concentration (μg.mL^{-1}) of enrofloxacin and ciprofloxacin (as active metabolite) along with diclofenac sodium following single dose (5 mg.kg^{-1}) intramuscular administration in cows (n=5)

Time after administration of drug (h)	Enrofloxacin	Ciprofloxacin	Enrofloxacin along with diclofenac sodium	Ciprofloxacin along with diclofenac sodium
0.03	0.480±0.009	0.014±0.01	–	–
0.16	0.790±0.03	0.032±0.009	–	–
0.25	1.242±0.006	0.072±0.08	0.0.37±0.008	–
0.50	1.433±0.009	0.152±0.006	0.080±0.003	–
1	0.880±0.006	0.290±0.005	0.127±0.006	0.037±0.002
2	0.400±0.003	0.432±0.002	0.207±0.001	0.072±0.003
3	0.207±0.002	0.380±0.002	0.191±0.002	0.084±0.004
6	0.176±0.003	0.220±0.006	0.180±0.004	0.120±0.002
12	0.072±0.002	0.076±0.009	0.125±0.006	0.082±0.002
24	0.017±0.001	–	0.082±0.007	0.035±0.004
30	–	–	0.032±0.005	0.014±0.001

Table 2. Pharmacokinetic parameters of enrofloxacin and ciprofloxacin (as an active metabolite) alone and along with diclofenac sodium following single dose (5 mg.kg^{-1}) intramuscular administration in cows (n=5)

Pharmacokinetic parameters	Unit	Enrofloxacin (mean±SE)	Ciprofloxacin (Mean±SE)	Enrofloxacin along with diclofenac sodium (mean±SE)	Ciprofloxacin along with diclofenac sodium (mean±SE)
A	μg.mL^{-1}	0.709±0.008	0.642±0.048	0.290±0.012**	0.240±0.0226**
K_a	h^{-1}	11.454±0.022	0.594±0.086	1.727±0.012**	0.333±0.055*
K_e	h^{-1}	0.117±0.002	0.100±0.012	0.077±0.002*	0.096±0.007
$t_{½}$ K_a	h	0.062±0.001	1.173±0.089	0.402±0.011**	1.995±0.373
$t_{½}$ K_e	h	5.91±0.271	6.99±0.234	9.196±0.256**	7.345±0.592
Cl/F	L.h^{-1}. kg^{-1}	0.825±0.037	–	1.317±0.049*	–
Vd/F	L.kg^{-1}	7.072±0.251	–	17.33±0.841**	–
AUC	μg.h.mL^{-1}	6.007±0.099	6.45±0.096	3.806±0.171**	1.767±0.207**
MRT	h	6.791±0.055	5.906±0.403	13.17±0.589**	14.101±1.300**
C_{max}	μg.mL^{-1}	1.468±0.025	0.443±0.020	0.207±0.010**	0.120±0.008**
T_{max}	h	0.437±0.009	1.75±0.250	2.00±0.000**	6.000±0.000**

*P<0.05, **P<0.01. Differences between data for enrofloxacin and metabolite ciprofloxacin in diclofenac and non-diclofenac treated animals at 5% (*P<0.05) and 1% (**P<0.01) level of significance. A=Zero time intercept of distribution slope in the two compartment model, K_a = absorption rate constant in one compartment, K_e=elimination rate constant, $t_{½}$ Ka = absorption half-life, $t_{½}$ Ke = elimination half-life, Cl/F = clearance of drug when the fraction of drug absorbed is not known (im route), Vd/F, volume of distribution, AUC = total area under the concentration time-curve, MRT = mean residence time, C_{max} = maximum plasma concentration, T_{max} = time to reach maximum plasma concentration.

administration of enrofloxacin produced a therapeutically effective concentration of 0.1 μg.mL^{-1} (Bergan *et al.* 1988) within 0.03 h and was maintained for 8–12 h. This finding is in close approximation to the values in lactation cows where the therapeutic concentration (0.1 μg.mL^{-1}) in serum was detected up to 8–12 h after i/m administration (Kaartinen *et al.* 1995).

The plasma concentration time profile, following i/m administration of enrofloxacin in cattle was adequately described by a one-compartment open model, (regression coefficient of fit of curve was 0.96±0.008) based on the assumption that the distribution phase of the drug is overlapped by its absorption phase (Toutain and Alvineri 1980). The pharmacokinetic values of enrofloxacin and its active metabolite ciprofloxacin alone and along with diclofenac sodium are presented in Table 2. The value of K_e (elimination rate constant) and $t_{1/2}$ K_e (elimination half-life) of enrofloxacin were similar to those estimated in lactating cows (Kaartinen *et al.* 1997). The volume of distribution (Vd/F) following i/m administration in the present study was 7 L.kg^{-1}, which can be compared with the values in chickens (5.04–6.52 L.kg^{-1}, Anadon *et al.* 1995) and bustards (3.3–7.18 L.kg^{-1}, Bailey *et al.* 1998). The high volume of distribution suggests that enrofloxacin easily penetrates tissues.

Ciprofloxacin (metabolite of enrofloxacin) appeared in the plasma after 0.03 h. Based on the C_{max} values, 30% of enrofloxacin was metabolized to ciprofloxacin. Ciprofloxacin attained peak concentration of 0.4 μg.mL^{-1} at 2 h following i/m administration of enrofloxacin. The plasma concentration-time profile of ciprofloxacin was adequately fitted to a one-compartment model with regression coefficient of fit of curve as 0.95±0.006. Comparing the pharmacokinetic parameters of enrofloxacin and ciprofloxacin in the present study, it was observed that elimination half-life ($t_{1/2\ Ke}$) of ciprofloxacin was higher (7 h) than that of the parent drug (5.9 h) following i/m administration of enrofloxacin. This agrees reasonably well with the elimination-half life for ciprofloxacin (10 h) and enrofloxacin (3.6 h) in sheep (Mengozzi *et al.* 1996).

The ultimate aim of disposition kinetics studies is to define a suitable dosage regimen. Based on the pharmacokinetic data of enrofloxacin, an intramuscular dosage regimen with a priming dose of 2.88 mg.kg^{-1} (C^{ss}_{max} of 0.54 μg.mL^{-1} and C^{ss}_{min} of 0.13 μg.mL^{-1}) and maintenance dose of 2.17 mg. kg^{-1} (C^{ss}_{max} of 0.41 μg.mL^{-1} and C^{ss}_{min} of 0.10 μg.mL^{-1}), with a dosing interval of 12 h and therapeutic concentration of 0.1 μg.mL^{-1} was calculated, and is recommended.

Co-administration of diclofenac sodium significantly (P<0.01) altered the plasma concentrations and pharmacokinetics of enrofloxacin. Metaclopromide also alters the pharmacokinetic parameters of ciprofloxacin (Wingender *et al.* 1985). Ranitidine and antimuscarinic piprenzipine also alters the absorption of quinolones (Hoffkin *et al.* 1985). Hard water also altered the pharmacokinetics of enrofloxacin (Sumano *et al.* 2004).

Following concurrent administration of enrofloxacin along with diclofenac sodium, significant (P<0.01) reductions in the zero time intercept of elimination phase (A), elimination rate constant (K_e), absorption rate constant (K_a), area under curve (AUC) of enrofloxacin were observed, together with significant ((P<0.01) increases in the values

of the absorption half-life ($t_{1/2\ Ka}$), elimination half-life ($_{1/2}$ K_e), volume of distribution ($V_{d/F}$) and body clearance ($^{Cl/F}$). However, enrofloxacin and ciprofloxacin concentration could be detected up to 30 h when enrofloxacin was administered along with diclofenac sodium, as compared to 24 h, when enrofloxacin was administered alone. The metabolic conversion ratio based on the AUC values of ciprofloxacin was reduced significantly ($P<0.05$), when enrofloxacin was administered along with diclofenac sodium. Nitesh *et al.* (2003) did not observe any interaction between enrofloxacin, ciprofloxacin (as metabolite) and diclofenac sodium, following single dose (4 mg.kg^{-1}, i/v) administration of enrofloxacin and diclofenac (1 mg.kg^{-1}, i/v) in female buffalo calves of 12–18 months of age. The variation in the findings between this reported study and the present study might be attributed to difference in the species, age and sex of the experimental animals. NSAIDs are known to influence the pharmacokinetics of other drugs by displacing the plasma protein bound fraction of the drugs (Zarfin *et al.* 1985) and by causing liver microsomal enzyme induction, resulting in increased drug metabolism (Baggot 1982). Based on the pharmacokinetic data of enrofloxacin along with diclofenac sodium, an intramuscular dosage regimen with a priming dose of 4.36 mg.kg^{-1} (C^{ss}_{max} of 0.42 μg.mL^{-1} and C^{ss}_{min} of 0.17 μg.mL^{-1}) and maintenance dose of 2.63 mg.kg^{-1} (C^{ss}_{max} of 0.23 μg.mL^{-1} and C^{ss}_{tren} of 0.10 μg.mL^{-1}), with a dosing interval of 12 h and therapeutic concentration of 0.1 μg.mL^{-1} was calculated, and is recommended.

It can be deduced from the present study that co-administration of diclofenac with enrofloxacin could lead to drug interaction i.e. altering the pharmacodynamics and pharmacokinetics of enrofloxacin in cows.

ACKNOWLEDGEMENT

The authors would like to thank M/s Ranbaxy Animal Health, New Delhi, for providing us with technical grade enrofloxacin, its formulation chemicals, columns etc. for HPLC analysis during the conduct of this study.

REFERENCES

Anadon A, Martinez Larrange M R, Diaz M J, Bringas P, Martinez M A, Fernandez-Cruz M L, Fernandez M C and Fernandez R. 1995. Pharmacokinetic and tissue residues of enrofloxacin in chickens. *American Journal of Veterinary Research* **56**: 501–06.

Baggot J D. 1982. Disposition and fate of drugs in the body. *Jones Veterinary Pharmacology and Therapeutics*. 5th edn. Iowa State University Press, Ames.

Baggot J D. 1980. Distribution of antimicrobial agents in normal and diseased animals. *Journal of American Veterinary Medical Association* **176**: 1085–90.

Bailey T A, Sheen R S, Silvanose C, Samour J H, Garner A and Harron D W G. 1998. Pharmacokinetics of enrofloxacin after intravenous, intramuscular and oral administration in houbara bustard. *Journal of Veterinary Pharmacology and Therapeutics* **21**: 288–97.

Bauditz R. 1987. Results of clinical studies with Baytril in calves and pigs. *Veterinary Medicine Review* **2**: 122–29.

Bergan T, Dalholf A and Rohwedder R. 1988. Pharmacokinetics of ciprofloxacin. *Infection* **16**: 3–13.

Gibaldi M and Parrier D. 1982. *Pharmacokinetics*, Marcel Dekker, New York.

Hoffkin G, Lode H, Prinzing C, Broner K and Koeppe P. 1985. *Antimicrobial Agents and Chemotherapy* **27**: 375–79.

Jayakumar K, Honnegowda and Narayana K. 2000. Dosage regimen for ciprofloxacin when administered after phenylbutazone in lactating cow. *Indian Veterinary Journal* 77: 954–56.

Kaartinen L, Salonen M, Alli L and Pyorala S. 1995. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cattle. *Journal of Veterinary Pharmacology and Therapeutics* **18**: 357–62.

Kaartinen L, Pyonala S, Moilanen M and Raisanen S. 1997. Pharmacokinetics of enrofloxacin in new born and one week old calves. *Journal of Veterinary Pharmacology and Therapeutics* **20**: 479–82.

Kung K, Riond J L and Wanner M. 1993. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and oral administration of enrofloxacin in dogs. *Journal of Veterinary Pharmacology and Therapeutics* **16**: 462–68.

Mengozzi G, Intorre L, Bertini S and Soldani. 1996. Pharmacokinetics of enrofloxacin and its metabolite, ciprofloxacin after intravenous and intramuscular administration in sheep. *American Journal of Veterinary Research* **57**: 1040–43.

Nielsen P and Gyrd-Hansen N. 1997. Bioavailability of enrofloxacin after oral administration to fed and fasted pigs. *Pharmacology and Toxicology* **80**: 246–50.

Nitesh Kumar, Singh S D and Jayachandran C. 2003. Pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin and its interaction with diclofenac after intravenous administration in buffalo calves. *Veterinary Journal* **165**(3): 302–06.

Snedecor G W and Cochran W G. 1980. *Statistical Methods*. 61. Oxford IBH Company, Bombay.

Steel R G D and Torrie J H. 1960. Non parametric statistics. *Principles and Procedures of Statistics*. Mc Graw-Hill Book Co., New York.

Sumano L H, Gutierrez O L, Aguilera R, Rosiles M R, Bernard B M J and Gracia M J (2004). Influence of Hard Water on the Bioavailability of Enrofloxacin in Broilers. *Poultry Science* **83**: 726–31.

Toutain P N and Alvineri M. 1980. Residual profile of phenylbutazone in rabbits. *Journal of Veterinary Pharmacology and Therapeutics* **3**: 4–8.

Tyczkowska K, Hedeen K M, Aucoin D P and Aronson A L. 1989. High Performance liquid chromatographic method for the simultaneous determination of Enrofloxacin and its primary metabolite ciprofloxacin in canine serum and prostatic tissue. *Journal of Chromatography* **493**: 337–346.

Wingender W, Forester D, Breemann D, Rohwedder, R, Graeffe K M, Schacht P and Scharbrodt V. 1985. *Proceedings of the 14th International Congress of Chemotherapy*. Kyoto, pp. 1585–86.

Zarfin Y, Koren G, Maresky D, Perlman M and MacLeod M D. 1985. *Journal of Pediatrics* **106**: 511.