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Effect of Flunixin on the Disposition of Enrofloxacin in Calves

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Abstract: Background: Bacterial infections are frequently associated with inflammation symptoms, this often leads to the prescribed combination of anti-inflammatory with antibacterials, to relief inflammatory symptoms and treated bacterial agent. The aim of the study was to evaluate whether the concomitant administration of flunixin may alter enrofloxacin pharmacokinetic parameters after single Intravenous (IV) and Intramuscular (IM) injections. **Methods:** Enrofloxacin concentrations in serum were determined by a microbiological agar plate assay using *Bacillus subtilis* ATCC 6633 as the test organism. **Results**: Co-administration of flunixin with IV injection of enrofloxacin reduced the volume of distribution at steady state $V_{d(SS)}$ and total body clearance (Cl_B) by 33.9 and 30%, respectively. After IM injection of enrofloxacin, the elimination half-life ($t_{1/2el}$) and Mean Residence Time (MRT) were shorter in the flunixin-medicated calves. **Conclusion:** Concomitant administration of flunixin with enrofloxacin induced significant alterations in pharmacokinetic parameters in calves. Therefore, concurrent administration of flunixin with enrofloxacin should be avoided.

Key words: Enrofloxacin, Flunixin, pharmacokinetics, interaction

INTRODUCTION

Central nervous system reactions to new fluoroquinolones, such as convulsions due to interaction with Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), have attracted increased attention (Yamaguchi *et al*., 2007). Moreover, it has been reported that the distribution and elimination of antimicrobials are altered when they are coadministered with NSAIDs (El-Banna, 1999). Enrofloxacin is a synthetic antibacterial drug belongs to the fluoroquinolones. It has a broad spectrum of antibacterial activity that is excellent against Gram-negative and some Gram-positive bacteria as well as mycoplasma species in animals (Prescott and Yielding, 1990). It has highly bioavailable following either oral or parenteral administration in most species and achieves good penetration of body tissues and fluids (Dorfman *et al*., 1995). The pharmacokinetics of enrofloxacin has been determined in different animal species (Kaartinen *et al*., 1995; Mengozzi *et al*., 1996; El-Sooud, 2003; Gavrielli *et al*., 1995). The minimum inhibitory concentrations (MIC_{90}) of enrofloxacin, for *Pasteurella multocida* and *Staphylococcus aureus*, were reported to be 0.05 and 0.25 μg mL^{-1}, respectively (Yoshimura *et al*., 2001; Salmon *et al*., 1998). Flunixin is a NSAID, substituted derivative of nicotinic acid highly potent cyclo-oxygenase inhibitor (Beretta *et al*., 2005), it is widely used for treatment musculoskeletal conditions, colic and endotoxic shock as an adjunct to antimicrobial therapy for infections with bacteria elaborate endotoxin (Rantala *et al*., 2002). Consequently, the aim of the study was to evaluate whether the concomitant administration of flunixin may alter the pharmacokinetic parameters of enrofloxacin after single IV and IM injections.

MATERIALS AND METHODS

Animals: Ten clinically healthy, Freisian calves weighting 200-250 kg and 5-7 months old. The calves were fed on barseem, barely, darawa, and concentrated mixture in a pellet form and water *ad-libitum*. Calves were kept indoors under good hygienic conditions and under direct observation for a month before the start of experiment to insure complete clearance from any previous drug residues. All animals were clinically examined routinely and blood and faecal samples were collected to ensure that hey were parasite free.

Drug: Enrofloxacin (Avitryl®): product of Arab Veterinary industrial CO. Amman, Jordan.

Flunixin meglumine (finadyne®): product of Schering-Plough animal health Segre-France.

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Pharmacokinetic study: The calves were divided into two groups five animals each. First group was injected a single dose of enrofloxacin 2.5 mg kg^{-1} of body weight (b.wt.) intravenously. Second group was injected the same dose intramuscularly. After 1 month washout period, each of the 10 animals was given flunixin intramuscularly at a dose 2.2 mg kg^{-1} one-hour prior to with the injection of enrofloxacin in a dose of 2.5 mg kg^{-1} b.wt. in calves of the first group IV or the IM injections in the second group. Blood samples (5 mL) were collected from the right jugular vein just before and at and at 5, 10, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48 ,72 and 96 h after IV and IM injections. The clotted blood was centrifuged at 3000 rpm for 15 min to obtain clear serum that was kept at-20°C until, assayed within two days.

Enrofloxacin assay: Enrofloxacin concentrations in serum were determined by a microbiological agar plate assay (Tsai and Kondo, 2001) using *Bacillus subtilis* ATCC 6633 as the test organism. Standard curves of Enrofloxacin were prepared in pooled antibacterial-free serum. It was recognized that this assay fails to distinguish between enrofloxacin and its putative microbiologically active metabolite (ciprofloxacin) (Gavrielli *et al*., 1995) and therefore, results were expressed as serum enrofloxacin antimicrobial equivalent activity. Thus, the term enrofloxacin antimicrobial equivalent activity is used throughout the text rather than concentration.

All samples were directly added to the culture plate. The limit of quantitation by this method was 0.03 μg mL^{-1}. The response of Enrofloxacin was linear over the range of concentration between 0.01-10 μg mL^{-1} with a correlation coefficient (r^2) of 0.99. The intra-assay coefficient of Variation Coefficient (CV) was 4%.

The extent of protein binding was determined *in vitro* using the method of Craig and Suh (1980) which is based on the diffusion of the free antibiotic into the agar medium.

Pharmacokinetic analysis: The determination of the best-fit compartmental model and initial estimates of the model dependent pharmacokinetic parameters of $t_{½\alpha}$, $t_{½\beta}$, $t_{½ab}$, $t_{½el}$, and V_c was analyzed with the help of a computerized curve-stripping program (Rstrip, Micromath Scientific Software, version 5.0, Salt Lake City, UT and USA).

Statistical moments (Yamaoka *et al*., 1978) were also used to compute the non-compartmental models parameters of peak concentration (C_{max}), time to peak concentration (t_{max}), Mean Residence Time (MRT), elimination half-life ($t_{1/2el}$) and Area Under the Curve (AUC) from zero to infinity by the trapezoidal rule and in serum. The systemic bioavailability (F) is the fraction of the IM dose absorbed and was calculated as:

$$\frac{AUC\ i.m.}{AUC\ i.v.} \times 100$$

The relative bioavailability, was calculated as:

$$\frac{AUC_{IV\ (enrofloxacin\ with\ flunixin)}}{AUC_{IV\ (enrofloxacin\ alone)}} \times 100$$

And:

$$\frac{AUC_{\ IM\ (enrofloxacin\ with\ flunixin)}}{AUC_{\ IM\ (enrofloxacin\ alone)}} \times 100$$

Results are presented as Mean±SE.

RESULTS

Semilogarithmic plots of mean enrofloxacin antimicrobial equivalent activity in serum versus time following single IV injection in control and flunixin-treated calves are shown in Fig. 1. The pharmacokinetic parameters describing the disposition of enrofloxacin after a single IV injection of 2.5 mg kg^{-1} b.wt. in control and flunixin-treated calves are given in Table 1. All serum enrofloxacin data were described by two-compartmental open model. The drug was rapidly distributed with $T_{1/2\alpha}$ of 0.12 and 0.16 h in control and flunixin-treated calves, respectively. Co-administration of flunixin 1 h prior IV injection of enrofloxacin significantly reduced the Cl_B and $V_{d(SS)}$ ($p \geq 0.005$), respectively in flunixin-treated calves.

Following single IM injection of enrofloxacin at a dose of 2.5 mg kg^{-1} b.wt., the mean serum antimicrobial equivalent activity following IM injection of enrofloxacin in control and flunixin-treated calves are shown in Fig. 2.

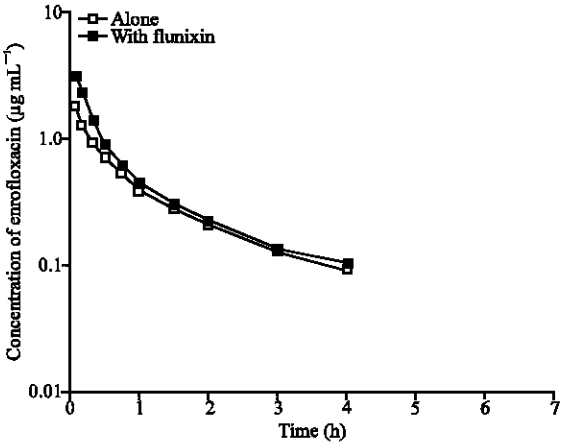

Fig. 1: Mean time-antimicrobial activity of enrofloxacin in serum of (μg mL^{-1}) following a single IV alone and with flunixin in calves

Table 1: Mean±S.E Pharmacokinetic parameters of enrofloxacin in calves after a single IV injection of 2.5 mg kg^{-1} b.wt. with or without IM injection of flunixin at 2.2 mg kg^{-1} b.wt. (n = 5)

		Mean±SE	
Kinetic parameters	Unit	Enrofloxacin alone	Enrofloxacin+flunixin
α	h^{-1}	6.77±1.20	4.50±0.17
$T_{1/2\alpha}$	h	0.12±0.01	0.16±0.05
β	h^{-1}	0.68±0.06	0.55±0.01
$T_{1/2\beta}$	h	1.07±0.09	1.30±0.03
MRT	h	1.27±0.08	1.25±0.02
V_C	L kg^{-1}	1.08±0.13	0.60±0.03*
K_{el}	h^{-1}	1.65±0.10	2.02±0.06*
K_{12}	h^{-1}	2.91±0.52	1.86±0.10
K_{21}	h^{-1}	2.90±0.74	1.26±0.08
K_{20}	h^{-1}	1.01±0.17	0.63±0.02
$V_{d(area)}$	L kg^{-1}	2.54±0.35	2.12±0.14
$V_{d(SS)}$	L kg^{-1}	2.24±0.17	1.48±0.04**
Cl_B	mL/min/kg	1.73±0.10	1.21±0.04**
AUC	µgmL/h	1.58±0.13	2.17±0.06**
AUMC	µgmL/h	1.85±0.01	2.61±0.13***
Relative bioavailability	%	137.34	

α: Distribution rate constant; $t_{1/2\alpha}$: Distribution half-life; β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; MRT: Mean residence time; k_{el}: Elimination rate constant; K_{12} and K_{21} first-order rate constants for drug distribution between the central and peripheral compartments; $V_{d(area)}$, volume of distribution calculated by area method; V_{dss}: Volume of distribution; Cl_B: Total body clearance; AUC: Area under the curve by the trapezoidal integral; AUMC: Area under moment curve by the trapezoidal integral *: p<0.01 **:p<0.005 ***: p<0.001

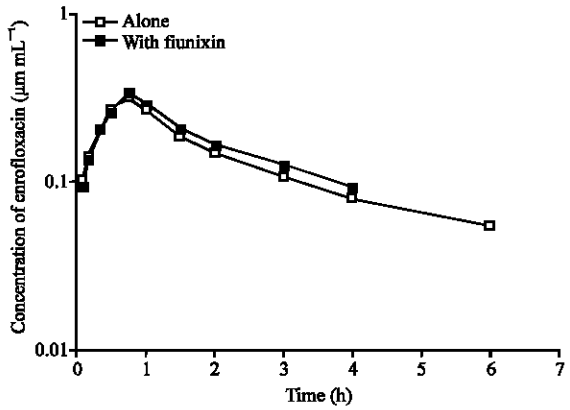

Fig. 2: Mean time-antimicrobial activity of enrofloxacin in serum of (µg mL^{-1}) following a single IM alone and with flunixin in calves

Also, antimicrobial equivalent activities were higher in control calves and persisted for longer time than in flunixin-treated ones. The C_{max} were 0.31 and 0.26 µg mL^{-1} attained at 0.94 and 0.69 h (T_{max}) for control and flunixin-treated calves, respectively. The pharmacokinetic parameters of enrofloxacin after a single IM injection following its independent administration or its coadministration with flunixin are presented in Table 2. The results showed that enrofloxacin was rapidly absorbed in control and flunixin-treated calves with absorption half-life ($T_{1/2ab}$) of 0.29 and 0.21 h, respectively.

Table 2: Mean±SE Pharmacokinetic parameters of enrofloxacin after IM injection of 2.5 mg kg^{-1} of b.wt. in calves with or without IM injection of flunixin at 2.2 mg kg^{-1} b.wt (n = 5 in each group)

		Mean±SE	
Kinetic parameters	Unit	Enrofloxacin alone	Enrofloxacin+flunixin
K_{ab}	h^{-1}	2.48±0.29	3.46±0.44
$T_{1/2ab}$	h	0.29±0.02	0.21±0.02**
K_{el}	h^{-1}	0.32±0.07	0.49±0.03
$T_{1/2el}$	h	2.24±0.14	1.45±0.11**
MRT	h	3.62±0.24	2.23±0.29**
C_{max}	µg mL^{-1}	0.31±0.01	0.26±0.01**
T_{max}	h	0.94±0.02	0.69±0.05**
AUC	µgmL/h	1.41±0.11	0.83±0.03***
AUMC	µgmL/h	4.93±0.80	1.87±0.22*
Relative bioavailability	%	58.87	

K_{ab}: Absorption rate constant, $t_{1/2ab}$: Absorption half-life, K_{el}: Elimination rate constant, $t_{1/2el}$: Elimination half-life, MRT: Mean residence time, C_{max}: Peak drug concentration, t_{max}: Time to peak concentration, AUC: Area under the curve by the trapezoidal integral nity and AUMC: Area under the moment curve by the trapezoidal integral *: p<0.01 **:p<0.005 ***: p<0.001

The elimination half-life ($T_{1/2el}$) and (MRT) ($p \geq 0.005$) were also shorter in the flunixin-treated calves. *In vitro* protein binding percent of enrofloxacin in serum of calves was ranged from 1.9 to 3.2% with an average of 2.3%.

DISCUSSION

This study used the bioassay technique to determine the enrofloxacin antimicrobial equivalent activity. The present investigation revealed that the serum concentration time curves of enrofloxacin were best fitted to follow a two compartment open model following single IV injections of 2.5 mg kg^{-1} b.wt. This finding was closely observed in animals (Kaartinen *et al*., 1995; Mengozzi *et al*., 1996; El-Sooud, 2003; Gavrielli *et al*., 1995). Enrofloxacin showed elevated values of the volume of distribution in both groups signifying further enrofloxacin fractions towards extravascular tissues and supporting the widespread penetration of enrofloxacin from blood into tissues. These findings may be related to low serum protein binding ability of enrofloxacin in calves. Greene and Budsberg (1993) suggested that the wide distribution of fluoroquinolones in body tissues at concentrations higher than the Minimal Inhibitory Concentration (MIC) for adequate periods of time for several important susceptible pathogenic bacteria of animal origin may be responsible for the high efficacy of this group of drugs.

Co-administration of flunixin with IV injection of enrofloxacin reduced the volume of distribution at steady state $V_{d(SS)}$ and total body clearance (Cl_B) by 33.9 and 30%, respectively. After IM injection of enrofloxacin, the elimination half-life ($t_{1/2el}$) and Mean Residence Time (MRT) were siginficantly shorter in the flunixin-medicated calves.

This was in agreement with El-Sooud (2003) who found that albendazole administration causes significant alterations in the disposition kinetic of enrofloxacin in lactating goats that may enhance the rate of enrofloxacin elimination from the body, and consequently diminish its efficacy. The relative bioavailability of enrofloxacin after IM injection was 58.87%, suggesting that flunixin is significantly decreased the extent of absorption and significantly enhanced the elimination of enrofloxacin. *In vitro* protein-binding percentage of enrofloxacin in serum of calves had an average of 2.3% indicating that the drug has a extremely low capacity to bind with serum proteins. This value is less than that reported in adult cattle (36-45%) (Kaartinen *et al*., 1995) but similar to that reported for camels (1.7%) (Gavrielli *et al*., 1995). This may be due to the binding affinity in young calf is significantly limited as the mean total protein concentration in blood is lower than adult animals and it is increasing with the age (Fraile *et al*., 1997).

Accordingly, Concomitant administration of flunixin with enrofloxacin induced significant alterations in pharmacokinetic parameters in calves. Therefore, concurrent administration of flunixin with enrofloxacin should be avoided.

REFERENCES

Beretta, C., G. Garavaglia and M. Cavalli, 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: An *in vitro* analysis. Pharm. Res., 52: 302-306.

Craig, A.W. and B. Suh, 1980. Protein Binding and the Antibacterial Effects. Methods for Determination of Protein Binding. In: Antibiotics in Laboratory Medicine, Lorian, V. (Ed.). Williams and Wilkins, Baltimore, MD, USA., pp: 265-297.

Dorfman, M., J. Barsanti and S.C. Budsberg, 1995. Enrofloxacin concentrations in dogs with normal prostate and dogs with chronic bacterial prostatitis. Am. J. Vet. Res., 56: 386-390.

EL-Banna, H.A., 1999. Pharmacokinetic interactions between flunixin and sulphadimidine in horses. Dtsch. Tierarztl. Wochenschr., 106: 400-403.

El-Sooud, K.A., 2003. Influence of albendazole on the disposition kinetics and milk antimicrobial equivalent activity of enrofloxacin in lactating goats. Pharm. Res., 48: 389-395.

Fraile, L.J., C. Martinez, J.J. Aramayona, A.R. Abadia, M.A. Bregante and M.A. Garcia, 1997. Limited capacity of neonatal rabbits to eliminate enrofloxacin and ciprofloxacin. Vet. Q., 19: 162-167.

Gavrielli, R., R. Yagil, G. Ziv, C.V. Creveld and A. Glickman, 1995. Effect of water deprivation on the disposition kinetics of enrofloxacin in camels. J. Vet. Pharmacol. Ther., 18: 333-339.

Greene, C.E. and S.C. Budsberg, 1993. Veterinary Use of Quinolones. In: Quinolone Antimicrobial Agents, Hooper, D.C. and J.S. Wolfson (Eds.). 2nd Edn., American Society for Microbiology, Washington, DC., pp: 473-484.

Kaartinen, L., M. Salonen, L. Alli, S. Pyorala, 1995. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injections in lactating cows. J. Vet. Pharmacol. Ther., 18: 357-362.

Mengozzi, G., L. Intorre, S. Bertini and G. Soldani, 1996. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and intramuscular administrations in sheep. Am. J. Vet. Res., 57: 1040-1043.

Prescott, J. and K.M. Yielding, 1990. *In vitro* susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. Can. J. Vet. Res., 54: 195-197.

Rantala, M., L. Kaartinen, E. Valimaki, M. Stryrman and M. Hiekkaranta *et al*., 2002. Efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced *Escherichia coli* mastitis. J. Vet. Pharmacol. Ther., 25: 251-258.

Salmon, S.A., J.L. Watts, F.M. Aarestrup, J.W. Pankey and R.J. Yancey Jr., 1998. Minimum inhibitory concentrations for selected antimicrobial agents against organisms isolated from the mammary glands of dairy heifers in New Zealand and Denmark. J. Dairy Sci., 81: 570-578.

Tsai, C. and F. Kondo, 2001. Improved agar diffusion method for detecting residual antimicrobial agents. J. Food Prot., 64: 361-366.

Yamaguchi, H., H. Kawai, T. Matsumoto, H. Yokoyama, T. Nakayasu, M. Komiya and J. Shimada, 2007. Post-marketing surveillance of the safety of levofloxacin in Japan. Chemotherapy, 53: 85-103.

Yamaoka, K., T. Nakagawa and T. Uno, 1978. Statistical moments in pharmacokinetics. J. Pharmacokinet. Biopharm., 6: 547-558.

Yoshimura, H., M. Ishimaru, Y.S. Endoh and A. Kojima, 2001. Antimicrobial susceptibility of *Pasteurella multocida* isolated from cattle and pigs. J. Vet. Med. B, 48: 555-560.