Pharmacokinetic study of flunixin and its interaction with enrofloxacin after intramuscular administration in calves

K. Abo-EL-Sooud[*1], L. AL-Anati [2]

1. Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Giza, Egypt. 2. The Institute of Environmental Medicine (IMM) Nobels väg 13, Solna, Karolinska Institutet, Box 210, 171 77 Stockholm, Sweden
[*]Corresponding author E-mail:kasooud@yahoo.com, Fax: 0020235725240 – 002035710305
Received: 13-04-2011, Accepted: 12-05-2011, Published Online: 05-09-2011
doi: 10.5455/vetworld.2011.449-454

Abstract

The Pharmacokinetic aspects of flunixin (FL) administered alone and in combination with enrofloxacin (EN), were studied in clinically healthy calves. The experiments were performed on two groups: FL alone {2.2 mg/kg, intramuscular (IM)}, and combination of FL (2.2 mg/kg, IM) and EN {2.5 mg/kg, IM}. Plasma concentrations of FL were determined using High Performance Liquid Chromatography (HPLC) method. Moreover, the effects of FL alone or in combination on liver and kidney functions were also assessed. Flunixin was rapidly absorbed intramuscularly with a half-life of absorption ($t_{1/2ab}$) of 0.094 h and the peak plasma concentration (C_{max}) was 1.27 g/mL was attained after 0.49 h (T_{max}). Enrofloxacin significantly altered the pharmacokinetics of FL by delaying its absorption and accelerate its elimination from body. Significant increases (32%) in the area under the curve (AUC) and (37%) in the elimination rate constant (K_{el}) from the central compartment and a significant decrease (27%) in the elimination half-life ($t_{1/2el}$) of FL were found following coadministration with EN, compared with administration of FL alone. The maximum plasma drug concentration (C_{max}) showed significant increase (28%) following the coadministration of EN with FL as compared to that following the administration of FL alone. It was concluded that the combination of FL and EN negatively altered the kinetics of FL and exaggerated the adverse effect on hepato-renal function in calves consequently; the concomitant use of FL and EN should be avoided in calves.
Keywords: Pharmacokinetics, Flunixin, Interaction, Enrofloxacin, Calves.

Introduction

Flunixin (FL) is nonsteroidal anti-inflammatory drug (NSAID) inhibiting cycloxygenase enzymes in the arachidonic acid cascade, thus block the formation of cycloxygenase derived eicosanoid inflammatory mediators (Landoni *et al*., 1995; Cheng *et al*., 1998). Due to its anti-inflammatory, analgesic, and antipyretic effects (Mckellar *et al*., 1989; Beretta *et al*., 2005), FL is widely used in veterinary medicine, particularly in ruminant to treat the musculoskeletal conditions, endotoxic shock, acute mastitis, endotoxemia, and calf pneumonia (Anderson *et al*., 1991; Welsh & Nolan, 1995; Odensvik & Magnusson, 1996; Rantala *et al*., 2002).

Enrofloxacin (EN) is a fluorquinolone antibacterial drug, extensively used in veterinary medicine (Schroder, 1989), due to its broad spectrum of activity against Gram-negative, some Gram-positive bacteria and mycoplasma species (Prescott & Yielding, 1990; Butaye *et al*., 1997; Pellerin *et al*., 1998). It has bactericidal activity at relatively low concentration, and high bioavailability following oral or parenteral administration (Dorfman, *et al*., 1995), with long serum half-life, thus it used once or twice daily (Abo-EL-Sooud, 2003). NSAIDs are frequently coadministered to enhance the rate of recovery in combination with antimicrobial drugs in calves suffering from endotoximia, pneumonia and other viral and bacterial respiratory diseases. However, it has been reported that the distribution and elimination of antimicrobials are altered when they are coadministered with NSAIDs (Whittem *et al*., 1996 & EL-Banna, 1999). Further, previous workers reported that the pharmacokinetics of FL are altered by the coadministration EN in dogs and mice (Ogino *et al*., 2005; Ogino & Arai, 2007), respectively. Therefore, the present investigation was undertaken with the following objectives:

(1) to establish the serum concentration time profile and pharmacokinetic parameters for FL after IM administration in calves;

(2) to determine pharmacokinetic parameters for FL

after co-administration with EN injected intramuscularly at the dose rate recommended for calves to establish any interaction between the drugs and (3) to evaluate the effects of FL alone and FL + EN on the liver and kidney functions.

Materials and Methods

Drugs and Chemicals: Flunixin meglumine (Finadyne®), is a product of Schering-Plough animal health Segre-France. Enrofloxacin (Avitryl®), is a product of Arab Veterinary industrial CO. Amman, Jordan. Methanol, Ethanol HPLC grade, and Distilled diethyl ether were purchased from Merck Darmstdt, Germany. Sodium dihydrogenate phosphate anhydrous, Potassium dihydrogenate phosphate anhydrous were purchased from Sigma-Aldrich Corp. St. Louis, MO, USA.

Animals: Ten clinically healthy, Friesian calves weighing 200-250 kg and 5-7 months old were used. Animals were fed on barseem, barely, and concentrated mixture in a pellet and water was *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. The study was reviewed and approved by the Animals Ethics Committee of University of Science and Technology, Irbid, Jordan.

Experimental design: The animals were randomly divided into two group's five calves each. All injections were made at zero time into the thigh muscles. The doses were selected on the bases that this is the recommended dose rate for use in calves. Animals of the first group received a single dose of FL at 2.2 mg/kg intramuscularly into the left gluteal muscles. Those of group two were injected FL (2.2 mg/kg IM) in the left gluteal muscle, one h before the injection of EN at a dose of 2.5 mg/kg in the right gluteal muscle.

Sampling: Ten ml venous whole blood samples were collected from the right jugular vein into 10 mL heparinized Vacutainers (Becton Dickinson vacutainers Systems) at 0 (blank sample), 0.166, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h after treatment. All the blood samples were centrifuged at 3000 *g* for 15 min to separate the plasma. The plasma samples were divided into two parts, the first part for estimation of hepato-renal functions directly and the second one was frozen at -70 ^{0}C until analysed by HPLC for FL assay.

Flunixin Assay: The concentration of FL in plasma samples were assayed by HPLC method previously reported by Odensvik & Johansson, (1995). Briefly, One ml of plasma was mixed with 2.0 ml of potassium phosphate buffer (potassium dihydrogenate phosphate anhydrous, 0.3 M, pH 3.5). Then FL was extracted by an addition of 5 ml distilled diethyl ether, after that the tubes were agitated for 30 minutes. Then the tubes were centrifuged for 10 minutes at 500-x g using cool centrifuge. The organic phase transferred to new tube and evaporated. The residue was dissolved in 200 μL mobile phase and injected into HPLC (Shimadzu HPLC equipment, Shimadzu Corp, Kyoto, Japan) provided with auto sampler. Flunixin separated through column (125X 4.0-mm ID) (LiChroCART, lichrosorb RP-18, 7 µm, Merck, Darmstadt, Germany) and the pre-column 4-mm (LiChroCART, RP-select B, 5 µ m, Merck, Darmstadt, Germany). Methanol and sodium 0.05 M phosphate buffer pH 5.8 (50/50 V/V) were used as mobile phase.

All chromatographic procedures were performed with a flow rate at 0.8 ml/min and a run time of 10 min. FL was detected by UV absorption at a wavelength of 254 nm. The retention time for FL was 7.4 min. The limit of quantification for the method was. Specificity of the method was assessed with no observed any interference with endogenous compounds or with the anticoagulant or with EN or ciprofloxacin, while the accuracy of our method was 98.61%. The mean absolute recovery of FL in plasma was found to be 93.75%.

Method validation: The precision and accuracy of the method were evaluated by repetitive analysis of the plasma samples (n=12) spiked with different known concentrations of FL. The percentage recoveries were determined by comparing the peak height of blank samples spiked with different amounts of drug and treated as any sample, with the peak height of the same standards prepared in phosphate buffer (n=6).

Intra-assay variations were determined by measuring six replicates (n=6) of three standard samples used for calibration curves. The intra-assay variation coefficient was < 2.0. Inter-assay precisions were determined by assaying the three standard samples on three separate days. The inter-assay variation coefficient was < 3.7. Recovery of FL from plasma was found to be 97%. The limit of quantification (LOQ) of FL in plasma was 39 ng/mL. The standard curve in calves' plasma was linear between 0.01 and 10 µg/mL with correlation coefficients (r^2) was 0.99. The peak height ratios of an unknown specimen (peak height of difloxacin/peak height of internal standard) were compared with that of the standard.

Evaluation of liver and kidney functions: The activity of plasma aspartate transaminase (AST) and alanine transaminase (ALT) and creatinine and urea concentrations were estimated in plasma of all animals for 5 consecutive days from the drug administrations. Determination of AST, ALT, urea and creatinine in plasma were measured colorimetrically using Bio Merieux Kits (Vitek, Inc., USA).

Pharmacokinetic analysis: A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration–time curves for each individual animal. Following IM administration, each individual curve of FL over time was analysed to determine the peak concentration C_{max} (extrapolated from the curve), and the time to peak concentration T_{max} was read from the data. The program calculated the non-compartmental parameters using the statistical moment theory (Yamaoka *et al*., 1978). The terminal elimination half-life ($t_{1/2el}$) and absorption half life ($t_{1/2ab}$) were calculated as $ln2/K_{el}$ or $ln2/K_{ab}$, respectively. The areas under the concentration-time curves (AUC) were calculated by the trapezoidal rule and further extrapolated to infinity. The mean residence time (MRT) was calculated as AUMC/AUC, where AUC is as defined previously and AUMC is the area under the first moment curve (Gibaldi & Perrier, 1982).

Statistical analysis: Data are presented as mean ± SE obtained from different five animals. An unpaired student's *t*-test was used for statistical analysis. *P* value (*<0.05, **< 0.01, ***< 0.001) compared with control values was considered statistically significant. Data were analyzed by Graphpad Prism software version 4.0 (San Diego, California, USA).

Results

Mean (±SE) concentrations of FL in plasma after the administration of FL and FL+EN intramuscularly are presented in Figure 1. After both FL and FL+EN administrations, plasma concentration–time curves were best fitted to an absorption and one-compartment elimination model in all animals. The pharmacokinetic parameters of FL in plasma after administration of FL and FL + EN combination were presented in Table 1.

Enrofloxacin significantly altered the pharmacokinetics of FL by delaying its absorption and accelerate its elimination from body. Significant increases (32%) in the area under the curve (AUC) and (37%) in the elimination rate constant (K_{el}) from the central compartment and a significant decrease (27%) in the elimination half-life ($t_{1/2el}$) of FL were found following coadministration with EN, compared with administration of FL alone. The maximum plasma drug concentration (C_{max}) showed significant increase (28%) following the coadministration of EN with FL as compared to that following the administration of FL alone. Transaminases (AST, ALT) activities, urea and creatinine were significantly increased till the 48 h and after that they are returning into the normal level 96 h after in both groups (Tables 2&3). Although, the concurrent administration of EN with FL was significantly exaggerated the hepato-renal functions in marked manner than FL alone.

Table-1: Mean ± SE of pharmacokinetic parameters of FL in plasma after administration of FL and FL + EN (n = 5)

Kinetic Parameters	Unit	Mean ± S.E. FL alone	FL + EN
K_{ab}	h-1	7.40 ± 0.46	1.80 ± 0.04***
$t_{1/2ab}$	h	0.094 ± 0.005	0.39 ± 0.006***
K_{el}	h-1	0.27 ± 0.01	0.37 ± 0.01***
$t_{1/2el}$	h	2.57 ± 0.12	1.87 ± 0.08**
MRT	h	3.54 ± 0.89	2.59 ± 0.78
C_{max}	mcg ml-1	1.27 ± 0.05	1.62 ± 0.06**
T_{max}	h	0.49 ± 0.01	1.03 ± 0.01***
AUC	mcg•h/mL	5.82 ± 0.74	7.68 ± 0.80***
AUMC	mcg•h2/mL	20.62 ± 1.83	19.87 ± 1.11

Values were significantly different at **P<0.01, ***P<0.001

k_{ab}: absorption rate constant; $t_{½ab}$: absorption half-life; k_{el}: elimination rate constant; $t_{½el}$: elimination half-life; MRT: mean residence time; C_{max}: maximum plasma concentration; T_{max}: time to peak concentration AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral.

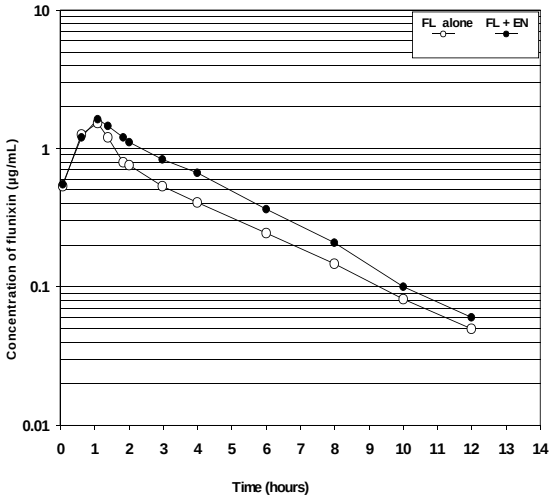

Figure-1: Semilogarithmic plot of plasma concentration time data for FL administered alone and in combination with EN. (n = 5).

Table-2. Mean ± SE of transaminases activity in plasma of calves after administration of FL and FL + EN (n=5)

Time (h)	AST(U/l)		ALT(U/l)	
	FL	FL+EN	FL	FL+EN
0	33.1 ± 2.12	33.20 ± 3.01	22.80 ± 1.5	23.10 ± 1.65
0.5	32.5 ± 2.10	36.20 ± 2.74	23.90 ± 3.05	23.20 ± 1.74
1	34.6 ± 1.40	40.50 ± 2.85	24.20 ± 2.01	24.10 ± 5.82
2	37.5 ± 1.10	45.40 ± 2.1*	24.20 ± 1.25	28.70 ± 1.2*
4	41.0 ± 10	47.60 ± 3.0*	25.90 ± 2.00	30.10 ± 1.4**
6	42.5 ± 0.90	50.20 ± 2.9**	27.00 ± 1.5	33.90 ± 1.7***
8	41.3 ± 1.20	68.80 ± 1.2***	28.20 ± 0.4	35.30 ± 1.0***
10	37.9 ± 1.20	69.80 ± 4.6***	28.50 ± 0.4	32.70 ± 1.6***
24	35.9 ± 0.98	47.40 ± 1.8**	29.90 ± 1.00	30.20 ± 1.00**
48	34.5 ± 1.00	45.20 ± 1.6*	27.20 ± 1.00	29.00 ± 1.1*
72	33.9 ± 2.10	37.40 ± 2.36	25.50 ± 0.97	28.30 ± 1.30
96	32.8 ± 1.50	35.20 ± 3.72	23.90 ± 2.60	26.50 ± 1.23

Values were significantly different at *P<0.05, **P<0.01, ***P<0.001

Discussion

Pharmacokinetic interactions between NSAIDs and antimicrobial drugs have received little attention in veterinary medicine, in spite of their frequent use in combination. However, pharmacokinetic interactions between phenylbutazone and the antibiotics benzylpenicillin and gentamicin have been studied in horses (Whittem *et al*., 1996). Phenylbutazone increased the serum concentrations of penicillin in one study but there was no effect of phenylbutazone on gentamicin pharmacokinetics.

The pharmacokinetics of FL after IM administration at a dosage of 2.2 mg/kg was described by a one-compartment model preceded by absorption, confirming the findings of Landoni *et al*. (1995). Absorption of FL from the IM injection site was rapid, as indicated by the short absorption half-life ($t_{1/2ab}$= 0.094 h) and time to attain maximum concentration (T_{max}= 0.49 h). Similar values were reported for FL in a previous study (Landoni *et al*., 1995).

Pharmacokinetic interactions of fluoroquinolones commonly occur with other drugs, usually by altering their hepatic biotransformation. Some quinolones preferentially inhibit cytochrome (CYP1A2), which is partially responsible for drug metabolism (Gillum *et al*., 1993). Although the mechanisms and scope of these interactions are becoming well characterized, the remaining challenge is how to best inform the clinician, so that the undesirable consequences of interactions may be prevented. NSAIDs are commonly used in combination with antimicrobials (Kopcha *et al*., 1992). The concurrent administration of NSAIDs with fluoroquinolones; such as fenbufen with enoxacin has been associated with seizures in human beings, but other NSAIDs with other member of fluoroquinolones have not developed seizures (Wolfsan & Hooper, 1991).

The pharmacokinetics behavior of FL has been previously studied in calves after intravenous

Table-3: mean ± SE urea and creatinine concentrations in plasma of calves after administration of FL and FL + EN (n=5)

Time (h)	Urea (g/l)		Creatinine (mg/100 ml)	
	FL	FL+EN	FL	FL+EN
0	0.41 ± 0.03	0.42 ± 0.04	1.32 ± 0.03	1.27 ± 0.01
0.5	0.40 ± 0.02	0.50 ± 0.03	1.39 ± 0.001	1.28 ± 0.03
1	0.43 ± 0.01	0.49 ± 0.01	1.41 ± 0.06	1.43 ± 0.01***
2	0.44 ± 0.09	0.63 ± 0.009***	1.41 ± 0.04	1.68 ± 0.05***
4	0.49 ± 0.006	0.78 ± 0.015***	1.51 ± 0.03	1.84 ± 0.01***
6	0.60 ± 0.04	0.80 ± 0.01***	1.60 ± 0.06	1.84 ± 0.02***
8	0.43 ± 0.04	0.77 ± 0.01***	1.53 ± 0.04	1.84 ± 0.03***
10	0.42 ± 0.06	0.67 ± 0.03**	1.49 ± 0.11	1.75 ± 0.05***
24	0.40 ± 0.01	0.56 ± 0.01*	1.40 ± 0.04	1.68 ± 0.04***
48	0.41 ± 0.04	0.48 ± 0.02	1.30 ± 0.06	1.55 ± 0.01***
72	0.41 ± 0.04	0.46 ± 0.04	1.20 ± 0.04	1.36 ± 0.05
96	0.42 ± 0.02	0.40 ± 0.02	1.20 ± 0.11	1.31 ± 0.07

Values were significantly different at *P<0.05, **P<0.01, ***P<0.001

administration (Landoni *et al*., 1995; Odensvik & Johansson, 1995). The disposition of FL after IM administration was steadied only in horse (Dyke *et al*., 1997). In our study FL was detected in the plasma 5 minutes post-injection in both groups. Inspection of plasma concentration time curve of FL alone or one hour before EN revealed that FL was rapidly absorbed. The $t_{1/2ab}$ of FL alone was 0.094 h whereas EN significantly delayed FL absorption. The $t_{1/2el}$ of FL in our study was shorter than that reported in horse (Dyke *et al*., 1997); this may be due to species variation. However, EN significantly accelerated the wash out of FL from the body, the elimination half live reduced by 27%. Our finding is contradicted with that found in mice by Ogino & Arai (2007); they found EN delay the elimination of FL. The peak plasma concentration (C_{max}) in our study was less than founded after oral administration in cats (Taylor *et al*., 1994). EN significantly elevated the peak of plasma concentration of FL (C_{max}) by 28%, which was attained 40 min later than that seen in FL alone. Our result in the same line with Ogino & Arai, 2007 finding, they found EN extended the time to reach the peak of plasma concentration of FL in ICR mice and dogs (Ogino *et al*., 2005; Ogino & Arai, 2007), but that peaks were lower than that of FL alone, this contradictory to our finding could be explained by difference in the experimental design, because they inject both drug at same time, while in our study EN injected one hour later than FL i.e. additional amount of FL could be absorbed from injection site, which caused elevation of FL peak. The MRT of FL in this study similar to that reported previously in horse (Dyke *et al*., 1997).

Concomitant administration of FL with EN was significantly increased plasma transaminases activity, this finding was in agreement with that of Tras *et al*., 2001, who found that treatment with EN induced temporary increases in AST and indirect bilirubin. Also, Arcieri *et al*., 1989 found ciprofloxacin induced changes in AST, ALT and alkaline phosphatase (Arcieri *et al*., 1989). FL did not alter most biochemical and hematological values in cat except ALT that was significantly elevated (Taylor *et al*., 1994).

The urea and creatinine concentrations were highly significant increased in plasma of calves received EN+FL combination. This finding agreed with that recorded for ciprofloxacin in rats (Basaran *et al*., 1993). They found that ciprofloxacin increased serum creatinine without significant histo-pathologic changes in the kidney. Also Mathews *et al*., 1996 found that serum creatinine was significantly elevated above base line at 24 hours post administration in dogs receiving FL. Our data indicated that the combination of FL with EN is harmful for the kidney for calves.

In summary our data clearly showed that EN negatively shifted the kinetic behavior of FL, due to the delay the peak of plasma concentration and increased the wash out of FL from body. That might cause reduction in the effectiveness of administered doses of both drugs. Addition to that, the combination of those drugs caused harmful effects on liver and kidney more than each alone. We concluded that the combination of FL and EN negatively altered the kinetics of both drugs and exaggerated the adverse effect on hepato-renal function in calves; therefore this combination should be avoided.

Acknowledgement

This work was funded by Deanship of Scientific Research, Jordan University of Science and Technology, grant no. (125/2001).

References

1. Abo El-Sooud, K. (2003) Influence of albendazole on the disposition kinetics and milk antimicrobial equivalent activity of enrofloxacin in lactating goats. *Pharmacological research*, 48, 389-395.
2. Anderson, K.L., Hunt, E. & Davis, B.J. (1991) The influence of anti-inflammatory therapy on bacterial clearance following intramammary Escherichia coli challenge in goats. *Veterinary Research Communications*, 15, 147-161.
3. Arcieri, G.M., Becker, N., Espositon, B., Griffith, E., Heyd, A., Neumann, C., Brien, B., & Schacht, P. (1989) Safety of intravenous ciprofloxacin, proceeding of symposium on ciprofloxacin: Major advances in Intravenous and oral Quinolones Therapy: Naples, F1 USA), 28-29.
4. Basaran, A., Erol, K., Basaran, N., Gunes, H.V., Acikalin, E. & Timuralp, G., (1993) Effects of ciprofloxacin on chromosomes, and hepatic and renal functions in rats. *Chemotherapy*, 39, 182-188.
5. Beretta, C., Garavaglia, G. & Cavalli M. (2005) COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. *Pharmacological research*, 52, 302-306.
6. Butaye, P., Ducatelle, R. & De Backer, P. (1997) In vitro activities of doxycycline and enrofloxacin against European Chlamydia psittaci strains from turkeys. *Antimicrobial Agents and Chemotherapy*, 41, 2800-2801.
7. Cheng, Z., Nolan, A.M. & McKellar, Q.A. (1998) Measurement of cyclooxygenase inhibition in vivo: a study of two non-steroidal anti-inflammatory drugs in sheep. *Inflammation*, 22, 353-366.
8. Dorfman, M., Barsanti, J. & Budsberg, S.C. (1995) Enrofloxacin concentrations in dogs with normal prostate and dogs with chronic bacterial prostatitis. *American Journal of Veterinary Research*, 56, 386-390.
9. Dyke, T.M., Sams, R.A. & Cosgrove, S.B. (1997) disposition of flunixin after intramuscular administration of flunixin meglumine to horses. *Journal of Veterinary Pharmacology and Therapeutics*, 20, 330-332.
10. EL-Banna, H.A. (1999) Pharmacokinetic interactions

between flunixin and sulphadimidine in horses. *Deutsche tierärztliche Wochenschrift*, 106, 400-403.
11. Gibaldi, M., Perrier, D., 1982. Non compartmental analysis based on statistical moment theory. Pharmacokinetics, 2nd edn. Marcel Dekker, New York. 409-424.
12. Gillum, J.G., Israel, D.S., & Polk, R.E. (1993) Pharmacokinetic drug interactions with antimicrobial agents. *Clinical Pharmacokinetics*, 25, 450-482.
13. Kopcha, M., Kaneene, J.B., Shea, M.E., Miller, R. & Ahl, A.S. (1992) Use of nonsteroidal anti-inflammatory drugs in food animal practices, *Journal of the American Veterinary Medical Association*, 201, 1868-1872.
14. Landoni, M.F., Cunningham, F.M. & Lees, P. (1995) Determination of pharmacokinetics and pharmacodynamics of flunixin in calves by use of pharmacokinetic/pharmaco-dynamic modelling. *American Journal of Veterinary Research*, 56, 786-794.
15. Mathews, K.A., Paley, D.M., Foster, R.A., Valliant, A.E. & Young, S.S. (1996) A comparison of ketorolac with flunixin, butorphanol, and oxymorphone in controlling postoperative pain in dogs. *The Canadian veterinary journal*, 37, 557-567.
16. Mckellar, Q.A., Galbrath, E.A., Bogan, J.A., Russell, C.S., Hooke, R.E. & Lees, P. (1989) Flunixin pharmacokinetics and serum thromboxane inhibition in the dog. *The Veterinary Record*, 124, 651-654.
17. Odensvik, K. & Johansson, M. (1995) High performance liquid chromatography method for determination of flunixin in bovine plasma and pharmacokinetics after single and repeated doses of the drug. *American Journal of Veterinary Research*, 56, 489-495.
18. Odensvik, K. & Magnusson, U. (1996) Effect of oral administration of flunixin meglumine on the inflammatory response to endotoxin in heifers. *American Journal of Veterinary Research*, 57, 201-204.
19. Ogino, T. & Arai, T. (2007) Pharmacokinetic interactions of flunixin meglumine and enrofloxacin in ICR mice. *Experimental Animal*, 56, 79-84.
20. Ogino, T., Mizuno, Y., Ogata, T. & Takahashi, Y. (2005) Pharmacokinetic interactions of flunixin meglumine and enrofloxacin in dogs. *American Journal of Veterinary Research*, 66, 1209-1213.
21. Pellerin, J.L., Bourdeau, P. & Sebbag, H. (1998). Epidemiosurveillance of antimicrobial compound resistance of *Staphylococcus intermedius* clinical isolates from canine pyodermas. *Comparative immunology, microbiology and infectious diseases*, 21, 115-133.
22. Prescott, J.F. & Yielding, K.M. (1990) In vitro susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. *The Canadian veterinary journal*, 54, 195-197.
23. Rantala, M., Kaartinen, L., Välimäki, E., Stryrman, M., Hiekkaranta, M., Niemi, A., Saari, L., & Pyörälä, S. (2002) Efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced Escherichia coli mastitis. *Journal of Veterinary Pharmacology and Therapeutics*, 25, 251-258.
24. Schroder, J. (1989) Enrofloxacin: a new antimicrobial agent. *Journal of the South African Veterinary Association*, 60, 122-124.
25. Taylor, P.M., Winnard, J.G., Jefferies, R. & Lees, P. (1994) Flunixin in the cat: a pharmacodynamic, pharmacokinetic and toxicological study. *The British veterinary journal*, 150, 253-262.
26. Tras, B., Maden, M., Bas, A.L., Elmas, M., Yazar, E. & Civelek, T. (2001) Investigation of biochemical and haematological side effects of enrofloxacin in dogs. *Journal of veterinary medicine. A, Physiology, pathology, clinical medicine*, 48, 59-63.
27. Welsh, E.M. & Nolan, A.M. (1995) Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. *Research in Veterinary Science*, 58, 61-66.
28. Whittem, T., Firth, E.C., Hodge, H. & Turner, K. (1996) Pharmacokinetic interaction between repeated dose phenylbutazone and gentamicin in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, 19, 454-459.
29. Wolfsan, J.S. & Hooper, D.C. (1991) Pharmacokinetics of quinolones: newer aspects. *European journals of clinical microbiology and infectious disease*, 10, 267-274.
30. Yamaoka, K., Nakagawa, T. & Uno, T. (1978) Statistical moments in pharmacokinetics. *Journal of pharmacokinetics and biopharmaceutics*, 6, 547-558.

* * * * * * * *