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Pharmacokinetic Interactions of Flunixin and Orbifloxacin in Buffalo Calves

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Abstract: Purpose and Context: Twelve apparently healthy buffalo calves were used to study the effect of flunixin as a non-steroidal anti-inflammatory drug on some pharmacokinetic aspects of orbifloxacin as a fluoroquinolone antimicrobial. **Results:** After intravenous injection of orbifloxacin alone and in combination with flunixin, there is no significant changes in the half-lives of distribution and elimination ($t_{0.5(\alpha)}$ and $t_{0.5(\beta)}$), volumes of distribution at steady state (Vd_{ss}), Mean Residence Time (MRT) and total body clearance (Cl_B) as evidenced from the values of 0.14 and 0.13 h, 4.98 and 4.95 h, 1.10 and 1.04 L kg^{-1}, 6.8 and 6.8 h, 0.16 and 0.15 L $kg^{-1}h^{-1}$, respectively. Following intramuscular administration, the maximum concentrations (C_{max}) 1.6 and 1.6 μg mL^{-1} were achieved at a maximum times (t_{max}) 1.30 and 1.31 h, respectively. No significant changes were detected in absorption ($t_{0.5(ab)}$) and elimination ($t_{0.5(el)}$) half-lives and Mean Residence Time (MRT) as a result of orbifloxacin co-administration with flunixin. The intramuscular bioavailability was 91.9 and 90.5% for orbifloxacin alone and in combination with flunixin, respectively. The result of *in-vitro* protein binding study indicated that 17.8% of orbifloxacin was bound to calve's serum proteins. **Conclusion:** These data allow concluding that orbifloxacin administered intravenously and intramuscularly to buffalo calves at a dose rate of 2.5 mg kg^{-1} was characterized by extensive absorption and high systemic bioavailability. Also, no significant alterations have been recorded in serum concentrations and pharmacokinetic parameters of orbifloxacin in buffalo calves by concurrent administration with flunixin and thus, dose regimens for orbifloxacin need not be altered when the two drugs are used in combination.

Key words: Pharmacokinetic, buffalo calves, intravenous, intramuscular

INTRODUCTION

Fluoroquinolones are antimicrobial drugs that generally have very good activities against a broad spectrum of aerobic bacteria, including *Pasteurella* spp., and against mycoplasma (Hannan *et al*., 1997). The main target site for their bactericidal action is the DNA-gyrase, an enzyme required for super-coiling of DNA to provide spatial arrangement of DNA in the bacterial cell. Fluoroquinolones have other good characteristics such as large volumes of distribution, low plasma protein binding, and relatively low MIC against susceptible target microorganisms (Brown, 1996). Orbifloxacin is a new synthetic third-generation fluoroquinolone that has been developed especially for use in veterinary medicine. In Japan, intramuscular administration of this drug has been shown to be effective and safe for the treatment of gastrointestinal and respiratory infections in cattle and swine (Nakamura, 1995).

Non-Steroidal Anti-Inflammatory Drugs ($NSAID_s$) are widely used strong analgesic and antipyretic agents (Igualada *et al*., 2007; Ogino and Arai, 2007). Flunixin meglumine which is used with meglumine as a solubilizer, belongs to the animal-special $NSAID_s$. Flunixin has been widely used for their anti-inflammatory and analgesic properties to treat musculo-skeletal conditions and colic in equine practice. It was also used routinely in ruminant veterinary practice, in the treatment of acute mastitis, endotoxaemia and calf pneumonia in cattle and goats and also, in endotoxin-induced reticulo-rumen stasis and tachycardia in cattle (Zu-Gong *et al*., 2007).

Antimicrobial agents and $NSAID_s$ are frequently co administered to treat endotoxaemia and to improve clinical signs (Ogino and Arai, 2007). The distribution and elimination of antimicrobial agents in horses (Whittem *et al*., 1996; El-Banna, 1999) and dogs (Ogino *et al*., 2005) were altered by being co-administered with NSAIDs. In contrast, no difference in the pharmacokinetic parameters of enrofloxacin (ENR) was reported after ENR was co-administered with flunixin meglumine in cows (Rantala *et al*., 2002) and in rabbits (Elmas *et al*., 2007).

The pharmacokinetics (PK) of orbifloxacin have been evaluated in goats (Marin *et al*., 2007), in horses (Davis *et al*., 2006; Haines *et al*., 2001), in pigs and cattle calves (Matsumoto *et al*., 1998a), in

rabbits (Marin *et al*., 2008), in dogs (Heinen, 2002; Iherke *et al*., 1999; Matsumoto *et al*., 1998b), in cats(Matsumoto *et al*., 1998b), in camels (Goudah and Abo-El-Sooud, 2008), in cattle (Elias *et al*., 2009) and recently, in sheep (Goudah *et al*., 2009) but not yet in buffalo calves. Consequently, this study describes some pharmacokinetic aspects and bioavailability of orbifloxacin in healthy buffalo calves following intravenous (IV) and intramuscular (IM) administrations of a single dose of the drug at a dose rate of 2.5 mg kg^{-1} b.wt. Also, to estimate the effect of co-administration of flunixin on some pharmacokinetic aspects of the drug.

MATERIALS AND METHODS

Drug: Orbifloxacin was obtained as a powder from Schering-Plough, Kenilworth, New Jersey, USA and reconstituted in sterile saline to a final concentration of 5% prior to administration. Flunixin 50 mg mL^{-1} (as meglumine) was supplied as an injectable solution (Flunix) from Parnell Laboratories (Aust) Pty. Ltd.

Animals: Twelve clinically healthy calves weighing 180-250 kg b.wt. (10-14 months age) were used. Animals were kept under good hygienic condition and none of them were treated with antibiotics for one month prior to the trial.

Experimental design: Calves were classified into two groups (each of 6 calves), the 1st group of calves were given orbifloxacin 2.5 mg kg^{-1} (Marin *et al*., 2007) as a single intravenous dose into the right jugular vein and single intramuscular dose into the deep gluteal muscle of hindquarter with a 2 weeks washout period between each route. The 2nd group of calves were given a single dose of flunixin 2.2 mg kg^{-1} (Elmas *et al*., 2007; Zu-Gong *et al*., 2007) followed immediately by orbifloxacin 2.5 mg kg^{-1} by intravenous and intramuscular routes with 2 weeks washout period between each route. Blood samples (10 mL each) were collected from the left jugular vein just before drug administration and at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after drug administration. The blood was allowed to clot at room temperature, then the serum was separated by centrifugation at 3000 revolution per minute for 15 min. Serum samples were stored at -20 °C until assayed.

Drug assay: Orbifloxacin concentrations in serum samples were determined by the microbiological assay method described by Arret *et al*. (1971) using *Klebsiella pneumoniae* (ATCC 10031) as a test organism (Heinen, 2002). Standard curves were constructed using antibacterial-free sera collected from calves. Six wells, 8 mm in diameter were cut at equal distances in standard petri dishes containing 25 mL seeded agar. The wells were filled with 100 µL of either the test samples or orbifloxacin standards. The plates were incubated at 37°C for 18-24 h. The inhibition zone diameters were measured and the orbifloxacin concentrations in the test samples were calculated from the standard curve.

The lower detectable limit of the orbifloxacin assay was 0.078 µg mL^{-1}. Semi-logarithmic plots of the inhibition zone diameter versus standard orbifloxacin concentrations in serum were linear with typical correlation coefficient of 0.970 (for the standard curve). The extent of protein binding was determined in vitro according to the method described previously by Craig and Suh (1991) with orbifloxacin concentrations 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 µg mL^{-1} in serum and phosphate buffer saline (pH 7.2). This method was based on the diffusion of free antibiotic into the agar medium. The differences in the diameters of the inhibition zones between the solutions of the drug in the buffer and serum samples were then calculated according to the following equation:

$$\text{Protein binding (\%)} = \frac{\text{Zone of inhibition in buffer} - \text{Zone inhibition in serum}}{\text{Zone of inhibition in buffer}} \times 100$$

Pharmacokinetic analysis: Serum concentrations of orbifloxacin for each individual calf after IV and IM administrations were subjected to a compartmental analysis using a nonlinear least-squares regression analysis with the help of a computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA). For IV and IM data, the appropriate pharmacokinetic model was determined by visual examination of individual concentration-time curves and by application of Akaike's Information Criterion (AIC) (Yamaoka *et al*., 1978). Following IV injection, the serum concentration-time relationship was best estimated as a two-compartment open model system (Baggot, 1978) according to the following bi-exponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where, C_p is the concentration of drug in the serum at time t; A is the intercept of the distribution phase with the concentration axis expressed as µg mL^{-1}; B is the intercept of the elimination phase with the concentration axis expressed as µg mL^{-1}; α is the distribution rate constant expressed in units of reciprocal time (h^{-1}); β is the elimination rate constant expressed in units of reciprocal time (h^{-1}) and e is the natural logarithm base.

After IM administration, data was analyzed by adopting a one-compartment open model. This program also calculated non-compartmental parameters using the statistical moment theory (Gibaldi and Perrier, 1982). The C_{max} (maximum serum concentration) and t_{max} (time of maximum serum concentration) were taken directly from the curve. The terminal elimination half-life ($t_{0.5(el)}$) and absorption half-life ($t_{0.5(ab)}$) were calculated as $ln2/K_{el}$ or $ln2/K_{ab}$, respectively, where K_{el} and K_{ab} are the elimination and absorption rate constants, respectively. The area under serum concentration-time curve (AUC) and area under the first moment curve (AUMC) were calculated by the method of trapezoids and extrapolation to infinity was performed. The Mean Residence Time (MRT) and Mean Absorption Time (MAT) were calculated as MRT = AUMC/AUC and MAT = $MRT_{i.m}$ - $MRT_{i.v}$. The total body clearance (Cl_B) was calculated as Cl_B = Dose/AUC and the absolute bioavailability (F) as F = $AUC_{i.m}/AUC_{i.v} \times 100$. Results were expressed as mean and Standard Error (S.E). The results obtained were statistically analyzed using student "t" test and analysis of variance (one way ANOVA). The results were expressed as mean and standard error. Standard errors were calculated from the mean data (Snedecor and Cochran, 1976).

RESULTS

Disposition of orbifloxacin in serum after intravenous administration either alone or in combination with flunixin was best fitted by the 2-compartment open pharmacokinetic model (Fig. 1, 2). The pharmacokinetic parameters o f orbifloxacin following a single intravenous and intramuscular administration of 2.5 mg kg^{-1} b.wt. alone and in combination with flunixin are recorded in Table 1 and 2, respectively. The results of the present study revealed that orbifloxacin was rapidly distributed following intravenous injection in calves alone or in combination with flunixin as indicated by short ($t_{0.5(\alpha)}$) 0.14 and 0.13 h, respectively. The elimination half-lives ($t_{0.5(\beta)}$) and total body clearances (Cl_B) were relatively

Table 1: Pharmacokinetic parameters of orbifloxacin (2.5 mg kg^{-1}) alone and in combination with flunixin (2.2 mg kg^{-1}) following a single intravenous administration in buffalo calves (n = 6).

Parameter	Orbifloxacin alone	Orbifloxacin with flunixin
C_p^o (μg mL^{-1})	5.80±0.05	5.82±0.05
A (μg mL^{-1})	3.80±0.03	3.78±0.05
B (μg mL^{-1})	2.00±0.02	2.03±0.02
α (h^{-1})	4.90±0.09	5.27±0.07
β (h^{-1})	0.14±0.002	0.14±0.01
K_{12} (h^{-1})	2.90±0.05	3.10±0.05
K_{21} (h^{-1})	1.80±0.03	1.93±0.02
K_{el} (h^{-1})	0.38±0.004	0.39±0.004
$t_{0.5(\alpha)}$ (h)	0.14±0.004	0.13±0.002
$t_{0.5(\beta)}$ (h)	4.98±0.05	4.95±0.04
V_c (L kg^{-1})	0.43±0.004	0.43±0.004
Vd_{ss} (L kg^{-1})	1.10±0.01	1.04±0.01
Cl_B (L kg^{-1} h^{-1})	0.16±0.002	0.15±0.01
MRT (h)	6.80±0.07	6.80±0.06
AUC (μg mL^{-1} h^{-1})	16.00±0.08	16.20±0.09
AUMC (μg mL^{-1} h^{-2})	103.30±1.43	102.50±1.30

C_p^o: Concentration at zero time (immediately after single IV injection); A, B: Zero-time intercepts of the biphasic disposition curve; α, β hybrid rate constants representing the slopes of distribution and elimination phases, respectively; k_{12}: First-order constant for transfer from central to peripheral compartment; k_{21}: First-order constant for transfer from peripheral to central compartment; K_{el}: Elimination rate constant; $t_{0.5(\alpha)}$ distribution half-life; $t_{0.5(\beta)}$: Elimination half-life; MRT mean residence time; AUC_{0-24}: Area under serum concentration-time curve; AUMC: Area under moment curve; V_c apparent volume of the central compartment; Vd_{ss}: Volume of distribution at steady state; Cl_B: Total body clearance

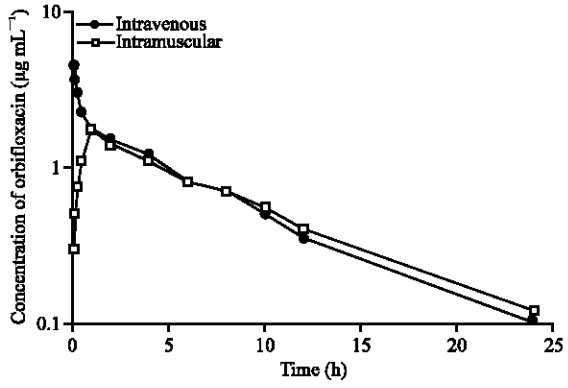

Fig. 1: Semi-logarithmic graph depicting the time-concentration of orbifloxacin in serum of normal buffalo calves after a single intravenous and intramuscular injection of 2.5 mg kg^{-1} b.wt.

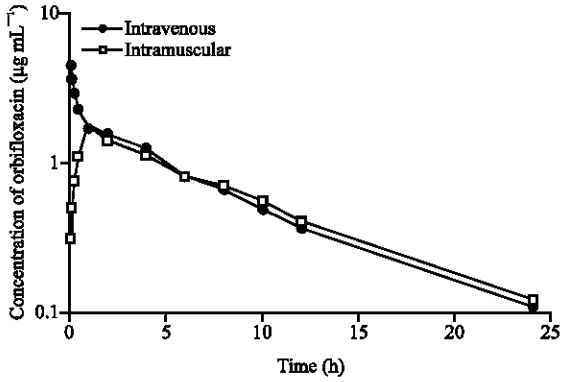

Fig. 2: Semi-logarithmic graph depicting the time-concentration of orbifloxacin (2.5 mg kg^{-1} b.wt) co-administered with flunixin (2.2 mg kg^{-1} b.wt) in serum of normal buffalo calves after a single intravenous and intramuscular injection

Table 2: Pharmacokinetic parameters of orbifloxacin (2.5 mg kg^{-1}) alone and in combination with flunixin (2.2 mg kg^{-1}) following a single intramuscular administration in buffalo calves (n = 6).

Parameter	Orbifloxacin alone	Orbifloxacin with flunixin
C_{max} (μg mL^{-1})	1.60±0.002	1.60±0.02
t_{max} (h)	1.30±0.004	1.31±0.01
K_{ab} (h^{-1})	2.39±0.03	2.31±0.03
K_{el} (h^{-1})	0.136±0.001	0.139±0.002
$t_{0.5(ab)}$ (h)	0.29±0.004	0.30±0.004
$t_{0.5(el)}$ (h)	5.10±0.05	5.00±0.07
AUC (μg mL^{-1} h^{-1})	14.70±0.11	14.70±0.16
AUMC (μg mL^{-1} h^{-2})	105.70±1.70	103.70±2.10
MRT (h)	7.80±0.08	7.60±0.09
MAT (h)	0.92±0.09	0.83±0.10
F (%)	91.90±0.69	90.50±1.30

k_{ab}: First-order absorption rate constant; K_{el}: Elimination rate constant; C_{max}: Maximum serum concentration; t_{max}: Time to peak serum concentration; $t_{0.5(ab)}$: Absorption half-life; $t_{0.5(el)}$: Elimination half-life; MAT: Mean absorption time; F: Fraction of drug absorbed systemically after IM injection

similar 4.98 and 4.95 h, 0.16 and 0.15 L kg^{-1} h^{-1}, respectively.

Following intramuscular administration, the drug was rapidly absorbed with $t_{0.5(ab)}$ of 0.29 and 0.30 h and maximum serum concentrations (C_{max}) of 1.6 and 1.6 μg mL^{-1} were achieved at (t_{max}) of 1.30 and 1.31 h, respectively. The elimination half-lives ($t_{0.5(el)}$) and systemic bioavailability were 5.1 and 5 h, 91.9 and 90.5% for orbifloxacin given alone and preceded by flunixin, respectively. The *in-vitro* serum protein-binding tendency was calculated to be 17.8%.

DISCUSSION

The pharmacokinetics of orbifloxacin in Egyptian buffalo calves is reported in the present study for the first time. The study revealed that serum orbifloxacin concentrations vs. time decreased in a bi-exponential manner following intravenous injection either alone or when used concomitantly with flunixin, demonstrating the presence of distribution and elimination phases and justifying the use of two-compartment open model. This finding is in agreement with other pharmacokinetic study with the drug in goats (Marin *et al*., 2007). Serum concentration profiles showed a similar rapid initial distributive phase, followed by a slower elimination phase with an estimated mean elimination half-life of 4.98 and 4.95 h, respectively. This finding was similar to that recorded in horse 5.08 h (Davis *et al*., 2006). The intravenous elimination half-life of orbifloxacin for this study was longer than those reported for goats 1.84 h (Marin *et al*., 2007), rabbits 2.5 h (Marin *et al*., 2008), sheep 3.16 h (Goudah *et al*., 2009) and cows 3.2 h (Elias *et al*., 2009) and this may be due to the difference in analytical method used. The apparent volume of distribution at steady-state (Vd_{ss}) is an accurate indication of the diffusion of the drug into the body tissues (Galinsky and Svensson, 1995). Orbifloxacin exhibits a relatively high volume of distribution at steady-state (1.10 and 1.04 L kg^{-1}), suggesting an extensive tissue distribution. This Vd_{ss} was in agreement with that of the drug in goats (Marin *et al*., 2007).

Following intramuscular injection, serum concentration-time curves of orbifloxacin alone and in combination with flunixin were remarkably similar, as recorded for C_{max}, t_{max}, $t_{0.5(ab)}$ and $t_{0.5(el)}$. The estimated C_{max} 1.6 and 1.6 μg mL^{-1} were reasonably similar to that reported for orbifloxacin in dog 1.37 (Heinen, 2002), goats 1.66 (Marin *et al*., 2007) and ewes 1.53 μg mL^{-1} (Goudah *et al*., 2009). The mean elimination half-life of orbifloxacin (5.1 and 5.0 h) was longer than those recorded in horse 3.42 h (Davis *et al*., 2006), goats 3.34 h (Marin *et al*., 2007) and ewes 3.84 h (Goudah *et al*., 2009) indicating a slower elimination in buffalo calves than other species. The systemic bioavailability of orbifloxacin in buffalo calves after intramuscular administration, alone and in combination with flunixin was nearly complete (91.9 and 90.5%). This value indicates the excellent absorption of the drug from that injection site and the absorption process was rapid with an absorption half-life 0.29 and 0.30 h, respectively.

The Minimum Inhibitory Concentrations (MIC_s) of orbifloxacin against calve's bacterial isolates have not yet been determined. Based on MIC data studied on bacterial isolates from mares, 0.12 μg mL^{-1} orbifloxacin showed high efficacy against some of the more common gram-negative equine isolates (*Escherichia coli*, *Pasteurella* spp. and *Salmonella* spp.) (Haines *et al*., 2001).

These data allow to conclude that orbifloxacin administered intravenously and intramuscularlyto buffalo calves at a dose rate of 2.5 mg kg^{-1} was characterized by extensive absorption and high systemic bioavailability. Consequently, orbifloxacin could be useful in the treatment of systemic infections in buffalo calves after specific assessment of susceptible microorganisms. Also, no significant changes have been recorded in kinetic parameters of orbifloxacin when given preceded by flunixin. Alterations of the dose or dose interval may not be necpessary when orbifloxacin is administered to calves concomitantly with a single dose of flunixin.

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