Effect of Flunixin on the Disposition of Enrofloxacin in Calves

K. Abo El-Sooud and L. Al-Anati

1Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
2Department of Veterinary Basic Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Jordan

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 \( V_{ss} \) and total body clearance (CL) by 33.9 and 30\%, respectively. After IM injection of enrofloxacin, the elimination half-life \( t_{1/2} \) 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 (Dormian 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\(_{50}\)) of enrofloxacin, for Pasteurella multocida and Staphylococcus aureus, were reported to be 0.05 and 0.25 \( \mu \)g 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 residues. All animals were clinically examined routinely and blood and faecal samples were collected to ensure that they were parasite free.

Drug: Enrofloxacin (Avityl\(^\text{®}\)), product of Arab Veterinary industrial CO. Amman, Jordan.

Flunixin meglumine (finadyne\(^{\text{®}}\)): product of Schering-Plough animal health Segre-France.

Corresponding Author: K. Abo El-Sooud, Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
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.w.t.) 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.w.t. 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) (Garrielli 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\(_{\text{1/2a}}\), t\(_{\text{1/2b}}\), t\(_{\text{1/2c}}\), and V\(_{\text{p}}\) 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 (Yamacka et al., 1978) were also used to compute the non-compartmental models parameters of peak concentration (C\(_{\text{max}}\)), time to peak concentration (t\(_{\text{max}}\)), Mean Residence Time (MRT), elimination half-life (t\(_{\text{1/2e}}\)) 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:

\[
\frac{\text{AUC i.m.} \times 100}{\text{AUC i.v.}}
\]

The relative bioavailability, was calculated as:

\[
\frac{\text{AUC IV (enrofloxacin with flunixin)}}{\text{AUC IV (enrofloxacin alone)}} \times 100
\]

And:

\[
\frac{\text{AUC IM (enrofloxacin with flunixin)}}{\text{AUC IM (enrofloxacin alone)}} \times 100
\]

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.w.t. 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\(_{\text{1/2a}}\) 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\(_{\text{e}}\) and V\(_{\text{p}}\) (p<0.005), respectively in flunixin-treated calves.

Following single IM injection of enrofloxacin at a dose of 2.5 mg kg\(^{-1}\) b.w.t., 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 (μg mL\(^{-1}\)) following a single IV alone and with flunixin in calves](image-url)
Table 1: Mean±SE Pharmacokinetic parameters of enrofloxacin in calves after a single IV injection of 2.5 mg kg⁻¹ b.w.t. with or without IM injection of flunixin at 2.2 mg kg⁻¹ b.w.t. (n = 5)

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Unit</th>
<th>Enrofloxacin alone</th>
<th>Enrofloxacin+Flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>h⁻¹</td>
<td>6.77±1.20</td>
<td>4.50±0.17</td>
</tr>
<tr>
<td>T1/2α</td>
<td>h</td>
<td>0.12±0.01</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>β</td>
<td>h⁻¹</td>
<td>0.68±0.06</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td>T1/2β</td>
<td>h</td>
<td>1.07±0.69</td>
<td>1.30±0.03</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>1.27±0.08</td>
<td>1.25±0.02</td>
</tr>
<tr>
<td>V₁</td>
<td>L kg⁻¹</td>
<td>1.68±0.13</td>
<td>0.60±0.03*</td>
</tr>
<tr>
<td>K₄₃</td>
<td>h⁻¹</td>
<td>1.65±0.10</td>
<td>2.02±0.06*</td>
</tr>
<tr>
<td>K₁₂</td>
<td>h⁻¹</td>
<td>2.91±0.52</td>
<td>1.86±0.10</td>
</tr>
<tr>
<td>K₀₁</td>
<td>h⁻¹</td>
<td>2.90±0.74</td>
<td>1.26±0.08</td>
</tr>
<tr>
<td>K₀₂</td>
<td>h⁻¹</td>
<td>1.01±0.17</td>
<td>0.63±0.02</td>
</tr>
<tr>
<td>V₁(kao)</td>
<td>L kg⁻¹</td>
<td>2.56±0.35</td>
<td>2.12±0.14</td>
</tr>
<tr>
<td>V₁(kao)</td>
<td>L kg⁻¹</td>
<td>2.24±0.17</td>
<td>1.48±0.04**</td>
</tr>
<tr>
<td>Cl₄₃</td>
<td>mL/min/kg</td>
<td>1.73±0.10</td>
<td>1.21±0.04**</td>
</tr>
<tr>
<td>AUC</td>
<td>µg/mL h</td>
<td>1.58±0.13</td>
<td>2.17±0.06**</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg/mL h</td>
<td>1.85±0.01</td>
<td>2.61±0.13***</td>
</tr>
</tbody>
</table>

Relative bioavailability % 137.34

α: Distribution rate constant; T₁/₂α: Distribution half-life; β: Elimination rate constant; T₁/₂β: Elimination half-life; MRT: Mean residence time; K₄₃: Elimination rate constant; K₁₂ and K₀₂: first-order rate constants for drug distribution between the central and peripheral compartments; V₁(kao): Volume of distribution calculated by area method; V₁: Volume of distribution; Cl₄₃: Total body clearance; AUC: Area under the curve by the trapezoidal integral method; AUMC: Area under moment curve by the trapezoidal integral method * 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 Cₘ₉₅ were 0.31 and 0.26 µg mL⁻¹ attained at 0.94 and 0.69 h (Tₘ₉₅) 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 (T₁/₂α) 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 b.wt. in calves with or without IM injection of flunixin at 2.2 mg kg⁻¹ b.wt. (n = 5 in each group)

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Unit</th>
<th>Enrofloxacin alone</th>
<th>Enrofloxacin+Flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kₙ₄</td>
<td>h⁻¹</td>
<td>2.48±0.29</td>
<td>3.46±0.44</td>
</tr>
<tr>
<td>T₁/₂₄₄</td>
<td>h</td>
<td>0.29±0.02</td>
<td>0.21±0.02**</td>
</tr>
<tr>
<td>K₄₃</td>
<td>h⁻¹</td>
<td>0.32±0.07</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td>T₁/₂β</td>
<td>h</td>
<td>2.43±0.14</td>
<td>1.45±0.11**</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>3.62±0.24</td>
<td>2.24±0.29**</td>
</tr>
<tr>
<td>Cl₄₃</td>
<td>µg mL⁻¹</td>
<td>0.31±0.01</td>
<td>0.26±0.01**</td>
</tr>
<tr>
<td>Tₘ₉₅</td>
<td>h</td>
<td>0.94±0.02</td>
<td>0.69±0.05**</td>
</tr>
<tr>
<td>AUC</td>
<td>µg/mL h</td>
<td>1.14±0.11</td>
<td>0.83±0.03***</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg/mL h</td>
<td>4.93±0.80</td>
<td>1.87±0.22**</td>
</tr>
</tbody>
</table>

Relative bioavailability % 58.87

Kₙ₄: Absorption rate constant, T₁/₂₄₄: Absorption half-life, K₄₃: Elimination rate constant, T₁/₂β: Elimination half-life, MRT: Mean residence time, Cl₄₃: Peak drug concentration, Tₘ₉₅: Time to peak concentration, AUC: Area under the curve by the trapezoidal integral method and AUMC: Area under moment curve by the trapezoidal integral method * p<0.01 ** p<0.005 *** p<0.001

The elimination half-life (T₁/₂β) and (MRT) (p<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-Scoul, 2003; Gavielli 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 V₁(kao) and total body clearance (Cl₄₃) by 33.9 and 30%, respectively. After IM injection of enrofloxacin, the elimination half-life (T₁/₂α) and Mean Residence Time (MRT) were significantly shorter in the flunixin-mediated calves.
This was in agreement with El-Scooud (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%) (Gavielli 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