Bioavailability and Pharmacokinetics of Ibabloxacin in Ewes

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Abstract: Purpose: The single-dose pharmacokinetics of ibabloxacin was determined in clinically normal ewes after intravenous and intramuscular administration of 15 mg of ibabloxacin kg⁻¹ of body weight. Ibabloxacin concentrations were determined by a modified high-performance liquid chromatography (HPLC) method. Results: Steady-state volume of distribution and clearance of ibabloxacin after intravenous administration were 3.61 L kg⁻¹ and 0.660 L kg⁻¹ h⁻¹, respectively. Following intramuscular administration, ibabloxacin achieved maximum serum concentration of 1.89 µg mL⁻¹ achieved after maximum time of 1.43 h post-injection. The absolute bioavailability after intramuscular route was 86.6%. Conclusion: Ibabloxacin could be useful in the treatment of systemic infections in ewes after specific assessment of susceptible microorganisms.

Key words: Ibabloxacin, intravenous, microorganisms, 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).

Ibabloxacin is a new fluoroquinolone that was developed exclusively for veterinary use. It has the pharmacodynamic properties expected of a fluoroquinolone, that is, bactericidal activity and broad-spectrum antibacterial effects (Coulet et al., 2002). The pharmacokinetics (PK) of ibabloxacin have been evaluated in dogs (Coulet et al., 2002), in cats (Coulet et al., 2005) and in goats (Marin et al., 2007a), but not yet in ewes. Consequently, this study describes some pharmacokinetic aspects and bioavailability of ibabloxacin in ewes following Intravenous (IV) and Intramuscular (IM) administrations of a single dose of the drug at a dose rate of 15 mg kg⁻¹ b.wt. Moreover, to estimate an appropriate dosage regimen of ibabloxacin in ewes using the surrogate markers of pharmacokinetic-pharmacodynamic integration [maximum serum concentration/minimum inhibitory concentration (Cmax/MIC) and area under the curve/MIC (AUC/MIC)].

MATERIAL AND METHODS

Drug: Ibabloxacin was obtained as a pure substance from Intervet International Company, Cairo, Egypt and reconstituted in sterile aqueous solution to a final concentration of 5% prior to administration.

Animals: Six clinically normal ewes weighing 20-25 kg b.wt. (12-16 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: Animals were given a single intravenous dose of 15 mg kg⁻¹ ibabloxacin (Marin et al., 2007a) into the right jugular vein. 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 2000 revolution per minute for 45 min. Serum samples were stored at -20°C until assayed. After a washout period of two weeks, the animals injected intramuscular with the same dose into the deep gluteal muscle of hindquarter and blood was collected and processed as mentioned above.

Drug assay: Ibabloxacin concentrations in serum samples of ewes were determined by a modified high-performance liquid chromatography (HPLC) method for moxifloxacin that was previously described (Siefert et al., 1999).
the addition of 10 mL of the internal standard (1-pyrenebutanoic acid, 4-sulfo-2, 3, 5, 6-tetrafluorophenol ester and sodium salt, 1 mg mL⁻¹) to 200 mL of serum. 200 mL of acetonitrile was added. Proteins were precipitated by means of shaking in an ultrasonic bath followed by centrifugation for 45 min at 2000 revolution per minute. The supernatant was diluted 4-fold with 0.057 M disodium hydrogen phosphate buffer, pH 7.5 and injected directly into the HPLC/fluorescence apparatus (LC-10ASVP pump, RF-10AXL fluorescence detector and SIL-10 ADVP auto-injector; Shimadzu, Kyoto, Japan). The HPLC separation was performed with the use of a reverse-phase discovery C18 column (150 3 4.6 mm, particle diameter 5 mm; Supelco, Bellefonte, Pennsylvania, USA). The mobile phase was composed of acetonitrile (40%) and tetra-butyl-ammonium hydrogen sulfate solution, 5 g mL⁻¹ (60%), pH 3.5; an isocratic method with a flow rate of 1.0 mL min⁻¹ was used. Fluorescence detection was performed at an excitation wavelength of 330 nm and an emission wavelength of 368 nm.

Quality controls were prepared from a pool of blank ewe’s serum spiked with 8 concentrations (0.078 to 2.5 μg mL⁻¹) of ibafloxacin. The serum aliquots were stored at -20°C until assayed and then extracted as described for the test samples. After injection of 25 mL of each control into the chromatographic system, standard curves were obtained by un-weighted linear regression of the ibafloxacin peak areas versus known concentrations. Each point was established from an average of 5 determinations. The correlation coefficient (r) was greater than 0.96% for the calibration curves.

The percentage recovery of ibafloxacin was determined by comparing the peak areas of serum blank samples spiked with different amounts of drug and treated as any samples with the peak areas of the same standards prepared in phosphate buffer. Each point was established from an average of 5 determinations. The mean percentage recovery (and standard error) from serum was 94.45 (4.55). The assay precision (relative standard deviation RSD) was assessed by expressing the standard deviation of repeated measurements as a percentage of the mean value. Intraday precision was estimated from 6 replicates of 3 serum standard samples used for the calibration curves (RSD, 3.7%). Interday precision was estimated from the analysis of serum standard samples on 3 separate days (RSD, 4%). The limit of quantification was 0.078 μg mL⁻¹ for serum.

**In vitro serum protein binding:** The extent of serum protein binding was determined in vitro using ultrafiltration technique (Craig and Suh, 1991). Serum samples from ewes were fortified with known concentrations of ibafloxacin ranging between 0.078 and 2.5 μg mL⁻¹. One milliliter of each sample was placed on a conditioned semi-permeable membrane (Centriflow Cones CF-50; Amicon Corp., Lexington, MA, USA) resting on porous conical polyethylene support on the top of centrifuge tubes. The tubes were centrifuged at 2000 revolution per minute for 45 min. Serum samples and their corresponding ultra-filtrates were assayed using the same method (HPLC) as described above. The percentage of serum protein binding was calculated according to the following equation:

\[
\text{Protein binding} = \frac{\text{Total concentration} - \text{Ultrafiltrate concentration}}{\text{Total concentration}} \times 100
\]

**Pharmacokinetic analysis:** Serum concentrations of ibafloxacin for each individual ewe 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:

\[
C_t = Ae^{-\alpha t} + Be^{-\beta t}
\]

where, \(C_t\) 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; \(\alpha\) is the distribution rate constant expressed in units of reciprocal time (h⁻¹); \(\beta\) 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 (\(t_{1/2,\alpha}\)) and absorption half-life (\(t_{1/2,\beta}\)) were calculated as \(\ln 2/K_{\alpha}\) or \(\ln 2/K_{\beta}\) respectively, where \(K_{\alpha}\) and \(K_{\beta}\) 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_{tot}/MRT_{iv}. The total body clearance (CLb) was calculated as CLb = Dose/AUC and the absolute bioavailability (F) as F = AUC_{inf}/AUC_{iv} x 100.

Several pharmacodynamic parameters including the maximum serum concentration/minimum inhibitory concentration (C_{max}/MIC) ratio and the area under the 24 h serum concentration-time curve/MIC (AUC_{24h}/MIC) ratio have been proposed to predict the antimicrobial efficacy of fluoroquinolones in vivo (Turnidge, 1999; McKellar et al., 2004). The pharmacodynamic efficacy of ibafloxacin was determined by calculating the C_{max}/MIC and AUC/MIC ratios following IM administration using hypothetical MICs (0.032 μg mL^{-1}) of ibafloxacin against canine isolates of E. coli, Staphylococcus spp., and Proteus mirabilis. Results were expressed as mean and Standard Error (S.E). Standard errors were calculated from the mean data.

RESULTS AND DISCUSSION

The mean serum concentrations time courses of ibafloxacin after intravenous and intramuscular administration are depicted in Fig. 1. Pharmacokinetic parameters are shown in Table 1. After intravenous administration of 15 mg kg^{-1} b.wt., the ibafloxacin serum concentration time data obeys two-compartment open model. The distribution and elimination half-lives were 0.229 and 4.24 h, respectively. The steady state volume of distribution (Vss) was 3.61 L kg^{-1} and mean residence time was 5.48 h. Ibafloxacin was rapidly absorbed after intramuscular administration with absorption half life (t_{1/2(AB)}) of 0.309 h. Peak serum concentration (C_{max}) was 1.89 μg mL^{-1} achieved after maximum time (t_{max}) of 1.43 h post administration. The drug was eliminated from blood after intramuscular administration with an elimination half-life 5.89 h. The systemic bioavailability of ibafloxacin after intramuscular injection was 86.69%.

The pharmacokinetics of ibafloxacin in ewes is reported in the present study for the first time. Therefore, since pharmacokinetic studies of ibafloxacin has not been studied in this species, it is important to compare the results of this study mainly with those from studies of other fluoroquinolones in other species. The study revealed that serum ibafloxacin concentrations vs. time

![Fig. 1: Semi-logarithmic graph depicting the time-concentration of ibafloxacin in serum of normal ewes after a single intravenous and intramuscular injection of 15 mg kg^{-1} b.wt](image)

### Table 1: Pharmacokinetic parameters of ibafloxacin following a single intravenous (IV) and intramuscular (IM) administration of 15 mg kg^{-1} in ewes (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>IV</th>
<th>Parameter</th>
<th>Unit</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{0}</td>
<td>μg mL^{-1}</td>
<td>10.20±0.03</td>
<td>C_{max}</td>
<td>μg mL^{-1}</td>
<td>1.89±0.069</td>
</tr>
<tr>
<td>A</td>
<td>μg mL^{-1}</td>
<td>7.06±0.03</td>
<td>t_{max}</td>
<td>h</td>
<td>1.43±0.074</td>
</tr>
<tr>
<td>B</td>
<td>μg mL^{-1}</td>
<td>3.10±0.04</td>
<td>K_{D}</td>
<td>h</td>
<td>2.28±0.19</td>
</tr>
<tr>
<td>g</td>
<td>h</td>
<td>3.04±0.04</td>
<td>K_{D}</td>
<td>h</td>
<td>0.118±0.022</td>
</tr>
<tr>
<td>β</td>
<td>h</td>
<td>0.164±0.004</td>
<td>t_{0.5 h}</td>
<td>h</td>
<td>0.369±0.014</td>
</tr>
<tr>
<td>K_{12}</td>
<td>h</td>
<td>1.68±0.02</td>
<td>t_{200}</td>
<td>h</td>
<td>5.89±0.215</td>
</tr>
<tr>
<td>K_{21}</td>
<td>h</td>
<td>1.04±0.02</td>
<td>AUC</td>
<td>μg mL^{-1}</td>
<td>19.70±1.409</td>
</tr>
<tr>
<td>K_{0}</td>
<td>h</td>
<td>0.479±0.008</td>
<td>AUMC</td>
<td>μg mL^{-1}</td>
<td>169.00±13.22</td>
</tr>
<tr>
<td>T_{1/2D}</td>
<td>h</td>
<td>0.229±0.004</td>
<td>MRT</td>
<td>h</td>
<td>8.94±0.740</td>
</tr>
<tr>
<td>t_{0.5 h}</td>
<td>h</td>
<td>4.24±0.06</td>
<td>MAT</td>
<td>h</td>
<td>3.46±0.101</td>
</tr>
<tr>
<td>V_{e}</td>
<td>L kg^{-1}</td>
<td>1.48±0.004</td>
<td>F</td>
<td>%</td>
<td>86.60±6.54</td>
</tr>
<tr>
<td>V_{ss}</td>
<td>L kg^{-1}</td>
<td>3.61±0.02</td>
<td>C_{max}/MIC</td>
<td>Ratio</td>
<td>59.10±0.33</td>
</tr>
<tr>
<td>V_{ed}</td>
<td>L kg^{-1}</td>
<td>0.66±0.01</td>
<td>AUC/MIC</td>
<td>Ratio</td>
<td>61.50±14.2</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>5.48±0.098</td>
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<tr>
<td>AUC</td>
<td>μg mL^{-1}</td>
<td>2.80±0.46</td>
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<tr>
<td>AUMC</td>
<td>μg mL^{-1} h^{-1}</td>
<td>16.50±4.40</td>
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</table>

C_{0}: 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_{D}: Elimination rate constant; k_{200}: distribution half-life; k_{0.5 h}: elimination half-life; MRT: Mean residence time; AUC_{24h}: Area under serum concentration-time curve; AUMC: Area under moment curve; V_{e}: apparent volume of the central compartment; V_{ss}: Volume of distribution at steady state; CLb: Total body clearance; k_{12}: First-order absorption rate constant; C_{max}: Maximum serum concentration; t_{max}: Time to peak serum concentration; k_{0.5 h}: Absorption half-life; t_{200}: Elimination half-life; MAT: Mean absorption time; F: Fraction of drug absorbed systemically after IM injection.
decreased in a bi-exponential manner following intravenous injection, 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., 2007a). Serum concentration profiles showed a rapid initial distributive phase, followed by a slower elimination phase with an estimated mean elimination half-life of 4.24 h. This finding was similar to that recorded in dogs 4.23 h (Elias et al., 2009). In this respect, fluoroquinolones have a long serum half-life making them suitable for once or twice a day administration (Hooper and Wolfson, 1985; Vancutsem et al., 1990). The apparent volume of distribution at steady-state (Vd) is an accurate indication of the diffusion of the drug into the body tissues (Galinsky and Svensson, 1995). The distribution of ibafloxicin in the body of ewes recorded in this study was more than unity (>1 L kg⁻¹) following intravenous administration (3.61 L kg⁻¹), indicating that the drug was extensively distributed to extra-vascular tissues. This result supported by Baggot (1978). Also, a low volume of central compartment (V₁, 1.48 L kg⁻¹) and high volume of distribution at steady state (Vd, 3.61 L kg⁻¹) indicated that the peripheral compartment is the major compartment for ibafloxicin distribution at steady state. This Vd was in agreement with that of marbofloxac in ostriches 3.22 L kg⁻¹ (De Lucas et al., 2005).

Following intramuscular injection, the estimated Cₘₚₚ 1.89 µg mL⁻¹ was reasonably similar to that reported for orbifloxacin in goats 1.85 (Marin et al., 2007b) and camels 1.93 µg mL⁻¹ (Goudah and Abo-El-Soud 2008). The drug absorbed rapidly in ewes as indicated by short absorption half-life (tₙₐₙₐ) 0.309 h. The pharmacokinetic properties of fluoroquinolones include rapid absorption (Vazquez et al., 1989; Hinz and Rotthmann, 1990; Roland et al., 1995). The mean elimination half-life of ibafloxicin tₙₐₙₐ was significantly longer (5.89 h) than that calculated after intravenous injection. This may be due to the result of continued absorption of ibafloxicin from the site of intramuscular injection during the elimination phase, thereby, prolonging the tₙₐₙₐ of the drug. Absorption limits drug elimination (Gibaldi and Perrier, 1982). Also this result supported by longer MRT after intramuscular (8.94 h) than after intravenous injection (5.48 h). The mean tₙₐₙₐ of ibafloxicin (5.89 h) was similar to that of orbifloxacin in camels 5.95 h (Goudah and Abo-El-Soud, 2008). The in vitro protein binding tendency of ibafloxicin to serum proteins was 21.4%. This indicated that the drug is slightly bound to serum proteins. It was stated that fluoroquinolones binding to serum proteins is relatively low up to 30% (Wise et al., 1984).

Various empirical pharmacokinetic/pharmacodynamic ratios have been proposed to predict the success or failure of therapy. The effective use of the fluoroquinolones against clinically important animal pathogens is dependent on designing dosages that attain serum Cₘₚₐ/MIC ratios of 10.1 or AUC/MIC ratios of 125:1 (Walker, 2000; Toutain et al., 2002). Ibafloxicin pharmacokinetic/pharmacodynamic integration revealed significantly higher value for Cₘₚₐ/MIC and AUC/MIC ratios in ewes indicating the excellent pharmacokinetic characteristics of the drug in ewes.

These data allow concluding that ibafloxicin administered intravenously and intramuscularly to ewes at a dose rate of 15 mg kg⁻¹ was characterized by extensive absorption and high systemic bioavailability. Consequently, ibafloxicin could be useful in the treatment of systemic infections in ewes after specific assessment of susceptible microorganisms.

REFERENCES


