Bioavailability and Pharmacokinetics of Ibafloxacin Following Intravenous and Intramuscular Single-Dose Applications in Chickens

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Abstract: The single-dose pharmacokinetics of ibafloxacin was studied in clinically normal chickens after intravenous and intramuscular administration of 7.5 mg of ibafloxacin kg⁻¹ of body weight. Ibafloxacin concentrations were determined by Microbiological assay method. The distribution and elimination half-lives were 0.261 and 5.87 h, respectively. Steady-state volume of distribution and clearance of ibafloxacin after intravenous administration were 4.09 L kg⁻¹ and 0.519 L kg⁻¹ h⁻¹, respectively. Following intramuscular administration, ibafloxacin achieved maximum plasma concentration of 1.06 μg mL⁻¹ achieved after maximum time of 1.3 h post-injection. The systemic bioavailability after intramuscular injection was 79.6% and the extent of serum protein binding was 27.04%. Ibafloxacin could be useful in the treatment of systemic infections in chickens after specific assessment of susceptible microorganisms.

Keywords: Ibafloxacin, chickens, bioavailability, pharmacokinetics, infection, Egypt

INTRODUCTION

Fluoroquinolones are antimicrobial drugs that generally have very good activities against a broad spectrum of aerobic bacteria including Pasteurella sp. and against mycoplasma (Hamann 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). Ibafloxacin 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 ibafloxacin have been evaluated in dogs (Coulet et al., 2002), in cats (Coulet et al., 2005) and in goats (Marin et al., 2007) but not yet in chickens.

Consequently, this study describes some pharmacokinetic and bioavailability aspects of ibafloxacin in chickens following intravenous and intramuscular administrations of a single dose of the drug at a dose rate of 7.5 mg kg⁻¹ b.wt. Moreover to estimate an appropriate dosage, regimen of ibafloxacin in chickens using the surrogate markers of pharmacokinetic-pharmacodynamic integration [maximum serum Concentration/Minimum Inhibitory Concentration (C_max/MIC) and Area Under the Curve/MIC (AUC/MIC)].

MATERIALS AND METHODS

Drug: Ibafloxacin 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.

Chickens: The six clinically healthy Hubbard chickens weighing 1.75-2.10 kg b.wt. (45 days old) were used. Chickens were kept under good hygiene condition and fed antibacterial-free balanced commercial rations for one month prior to the trial. The drinking of water was freely available.

Experimental design: Chickens were given a single intravenous dose of 7.5 mg kg⁻¹ ibafloxacin (Coulet et al., 2002) into the wing vein. Blood samples (1 mL each) were collected from the wing 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 and then the serum was separated by centrifugation at 3000 revolution min⁻¹ for 15 min. Serum samples were stored at -20°C until assayed. After a washout period of 2 weeks, the chickens injected intramuscularly with the same dose and blood was collected and processed as mentioned before.

Drug assay: Ibafloxacin concentrations in serum samples were determined by the Microbiological assay method described by Bennett et al. (1966), using Escherichia coli (ATCC 25922) as a test organism. Standard curves
were constructed using antibacterial-free sera collected from chickens. The 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 ibafoxacin standards. The plates were incubated at 37°C for 18-24 h. The inhibition zone diameters were measured and the ibafoxacin concentrations in the test samples were calculated from the standard curve.

The lower detectable limit of the ibafoxacin assay was 0.078 μg mL⁻¹. Semi-logarithmic plots of the inhibition zone diameter versus standard ibafoxacin concentrations in serum were linear with typical correlation coefficient of 0.993 (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 ibafoxacin 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:

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

**Pharmacokinetic analysis:** Serum concentrations of ibafoxacin for each individual chick 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:
- \(C_t\) = The concentration of drug in the serum at time \(t\)
- \(A\) = The intercept of the distribution phase with the concentration axis expressed as μg mL⁻¹
- \(B\) = The intercept of the elimination phase with the concentration axis expressed as μg mL⁻¹
- \(\alpha\) = The distribution rate constant expressed in units of reciprocal time (h⁻¹)
- \(\beta\) = The elimination rate constant expressed in units of reciprocal time (h⁻¹)
- \(e\) = 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 \(C_{\text{mic}}\) (maximum serum concentration) and \(t_{\text{cat}}\) (time of maximum serum concentration) were taken directly from the curve. The terminal elimination half-life (\(t_{1/2}\)) and absorption half-life (\(t_{1/2}\)) were calculated as ln2/Kd or ln2/Ka, respectively where, Kd and Ka are the elimination and absorption rate constants, respectively. The Area under serum concentration-time curve (AUC) and Area under the 1st 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 = Σ[AUC/MIC] and MAT = Σ[AUMC/ AUC]. The total body clearance (Cl) was calculated as Cl = Dose/AUC and the absolute bioavailability (F) as F = AUCim/AUCiv×100.

Several pharmacodynamic parameters including the maximum serum Concentration/Minimum Inhibitory Concentration (Cmax/MIC) ratio and the area under the 24 h serum concentration-time curve/MIC (AUC24h/MIC) ratio have been proposed to predict the antimicrobial efficacy of fluoroquinolones in vivo (Turnidge, 1999; Mckellar et al., 2004). The pharmacodynamic efficacy of ibafoxacin was determined by calculating the Cmax/MIC and AUC/MIC ratios following IM administration using hypothetical MIC (0.032 μg mL⁻¹) of ibafoxacin against canine isolates of *E. coli*, *Staphylococcus sp.* and *Proteus mirabilis* (Coulet et al., 2002). Results were expressed as mean and Standard Error (SE). Standard errors were calculated from the mean data according to Snedecor and Cochran (1976).

**RESULTS**

The mean serum concentrations time course of ibafoxacin after intravenous and intramuscular administration is shown in Fig. 1. Pharmacokinetic parameters are shown in Table 1. After intravenous administration of 7.5 mg kg⁻¹ b.w.t., the ibafoxacin serum concentration time data obeys two-compartment open model. The distribution and elimination half-lives were 0.261 and 5.87 h, respectively. The steady state Volume of distribution (Vdss) was 4.09 L kg⁻¹ and mean residence time was 7.89 h. Ibafoxacin was rapidly absorbed after intramuscular administration with absorption half life.
Table 1: Pharmacokinetic parameters of ibafloxacin following a single Intravenous (IV) and Intramuscular (IM) administration of 7.5 mg kg⁻¹ in chickens (n = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>IV</th>
<th>Parameters</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀⁰</td>
<td>ug mL⁻¹</td>
<td>4.06±0.0300</td>
<td>C₀₀</td>
<td>ug mL⁻¹</td>
</tr>
<tr>
<td>A</td>
<td>ug mL⁻¹</td>
<td>2.56±0.0100</td>
<td>Lₚ₀</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>B</td>
<td>ug mL⁻¹</td>
<td>1.50±0.0400</td>
<td>Kₑ</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>t₀</td>
<td>h⁻¹</td>
<td>2.66±0.0500</td>
<td>Kₚ₀</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>β</td>
<td>h⁻¹</td>
<td>0.118±0.002</td>
<td>Lₚ₀₃ₐ</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>Kₑ</td>
<td>h⁻¹</td>
<td>1.42±0.0200</td>
<td>Lₚ₀₃ₐ</td>
<td>h</td>
</tr>
<tr>
<td>Kₑ₀</td>
<td>h⁻¹</td>
<td>1.06±0.0400</td>
<td>AUC</td>
<td>ug mL⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Kₑ₀</td>
<td>h</td>
<td>0.29±0.004</td>
<td>AUC/MIC</td>
<td>ug mL⁻¹ h⁻¹</td>
</tr>
<tr>
<td>t₀₅₀</td>
<td>h</td>
<td>0.25±0.004</td>
<td>MRT</td>
<td>h</td>
</tr>
<tr>
<td>t₀₅₀</td>
<td>h</td>
<td>5.87±0.0000</td>
<td>MAT</td>
<td>h</td>
</tr>
<tr>
<td>Vₚₕ</td>
<td>L kg⁻¹</td>
<td>1.85±0.2000</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>Vₚₕ</td>
<td>L kg⁻¹</td>
<td>4.09±0.0800</td>
<td>Cₘₑ/MIC</td>
<td>Ratio</td>
</tr>
<tr>
<td>Cₘₑ</td>
<td>L kg⁻¹ h⁻¹</td>
<td>0.51±0.010</td>
<td>AUC/MIC</td>
<td>Ratio</td>
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<tr>
<td>MRT</td>
<td>h</td>
<td>7.89±0.1100</td>
<td>-</td>
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<tr>
<td>AUC</td>
<td>ug mL⁻¹ h⁻¹</td>
<td>14.48±0.2500</td>
<td>-</td>
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<tr>
<td>AUMC</td>
<td>ug mL⁻¹ h⁻¹</td>
<td>167.4±2.9000</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

C₀₀ 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; Kₑ₀ 1st-order constant for transfer from central to peripheral compartment; Kₑ₁ 1st-order constant for transfer from peripheral to central compartment; Kₑ elimination rate constant; t₀₅₀ distribution half-life; t₁₇₅₀ elimination half-life; MRT mean residence time; AUCₚ₃₀₢ area under serum concentration-time curve; AUMCₚ₃₀₢ area under moment curve; Vₚₕ apparent volume of the central compartment; Vₚₕ volume of distribution at steady state; Cₘₑ total body clearance; Kₑ₀ 1st-order absorption rate constant; Cₘₑ maximum serum concentration; t₀₅₀ time to peak serum concentration; t₁₇₅₀ absorption half-life; t₁₇₅₀ elimination half-life; MAT mean absorption time; F fraction of drug absorbed systemically after IM injection.

Fig 1: Semi-logarithmic graph depicting the time-concentration of ibafloxacin in serum of normal chickens after a single intravenous and intramuscular injection of 7.5 mg kg⁻¹ b.wt.

(t₀₅₀) 0.283 h. Peak serum Concentration (Cₘₑ) was 1.06 μg mL⁻¹ achieved after maximum time (tₘₑ) of 1.30 h post administration. The drug was eliminated from blood after intramuscular administration with an elimination half-life 5.90 h. The systemic bioavailability after intramuscular injection was 79.6% and the extent of serum protein binding was 27.04%.

DISCUSSION

The pharmacokinetics of ibafloxacin in chickens 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 chickens and other species. The study revealed that serum ibafloxacin concentrations vs. time 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., 2007).

Serum concentration profiles showed a rapid initial distributive phase followed by a slower elimination phase with an estimated mean elimination half-life of 5.87 h. This finding was relatively similar to that recorded for marbofloxacin in broiler chickens 5.26 h (Anadon et al., 2002) and danofloxacin in rabbits 4.88 h (Fernandez-Varon et al., 2007). 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 (Vₚₕ) is an accurate indication of the diffusion of the drug into the body tissues (Galinsky and Svensson, 1995). The distribution of ibafloxacin in the body of chickens recorded in this study was >1 L kg⁻¹ following intravenous administration (4.09 L kg⁻¹) indicated that the drug was extensively distributed to extra-vascular tissues. This result supported by Baggett (1978). Also, a low Volume of central compartment (Vₚₕ, 1.85 L kg⁻¹) and high volume of distribution at steady state (Vₚₕ, 4.09 L kg⁻¹) indicated that the peripheral compartment is the major compartment for ibafloxacin distribution at steady state. This Vₚₕ was in agreement with that of enrofloxacin and sarafloxacin in broiler chickens 4 and 3.4 L kg⁻¹, respectively (Knoll et al., 1999, Ding et al., 2001). In the
present study, the total body Clearance ($Cl_b$) 0.519 L kg$^{-1}$ h$^{-1}$ agreed with that reported for danofloxacin in turkeys (0.587 L kg$^{-1}$ h$^{-1}$) (Haritova et al., 2006a) and rabbits (0.761 L kg$^{-1}$ h$^{-1}$) (Fernandez-Varon et al., 2007).

Following intramuscular injection, the drug absorbed rapidly in chickens as indicated by short absorption half-life ($t_{0.5A}$) 0.283 h. The pharmacokinetic properties of fluoroquinolones include rapid absorption (Vazquez et al., 1989; Hinze and Rottmann, 1990; Roland et al., 1995). The mean elimination half-life of ibafloxacin $t_{0.5E}$ was longer (5.90 h) after intramuscular injection. This may be due to the result of continued absorption of ibafloxacin from the site of intramuscular injection during the elimination phase, thereby prolonging the $t_{0.5E}$ of the drug. Absorption limits drug elimination (Ghaidi and Perrier, 1982). Also, this result supported by longer MRT (8.73 h). The mean $t_{0.5E}$ of ibafloxacin (5.90 h) was relatively similar to that previously recorded for sarafloxacin in broilers 6.81 h (Ding et al., 2001) and marbofloxacin in turkeys 6.23 h (Haritova et al., 2006b).

The systemic bioavailability of ibafloxacin in chickens after intramuscular administration was 79.6% and relatively similar to that recorded for ciprofloxacin in broiler chickens 75.5% (El-Gendi et al., 2001) and danofloxacin in turkeys 78.37% (Haritova et al., 2006a). This value indicates the good absorption of the drug from that injection site and the absorption process was rapid with an absorption half-life 0.283 h. The in vitro protein binding tendency of ibafloxacin to chicken’s serum proteins was 27.04%. 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).

The Minimum Inhibitory Concentrations (MICs) of ibafloxacin against chicken's bacterial isolates have not yet been determined. Based on MIC data studied on bacterial isolates from canine (0.032 mL$^{-1}$), ibafloxacin showed efficacy against canine isolates of E. coli, Staphylococcus sp. and Proteus mirabilis (Coulet et al., 2002). Fluoroquinolones are active against bacterial pathogens in a concentration-dependent manner. 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 $C_{max}$/MIC ratios of 10:1 or AUC/MIC ratios of 125:1 (Walker, 2000; Toutain et al., 2002). Ibaflaxcin pharmacokinetic/pharmacodynamic integration revealed significantly higher value for $C_{max}$/MIC and AUC/MIC ratios in chickens indicating the excellent pharmacokinetic characteristics of the drug in chickens.

CONCLUSION

The results found show that ibafloxacin administered intravenously and intramuscularly to chickens at a dose rate of 7.5 mg kg$^{-1}$ was characterized by extensive absorption and high systemic bioavailability. Consequently, ibafloxacin could be useful in the treatment of systemic infections in chickens after specific assessment of susceptible microorganisms.

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REFERENCES


