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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.

Key words: Ibafloxacin, chickens, bioavilability, 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 (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). 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 aspects and bioavailability 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 hygienic 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 ibafloxacin standards. The plates were incubated at 37°C for 18-24 h. The inhibition zone diameters were measured and the ibafloxacin concentrations in the test samples were calculated from the standard curve.

The lower detectable limit of the ibafloxacin assay was 0.078 ug mL^{-1}. Semi-logarithmic plots of the inhibition zone diameter versus standard ibafloxacin 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 ibafloxacin concentrations 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ug 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:

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

Pharmacokinetic analysis: Serum concentrations of ibafloxacin 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:

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

Where:

C_p = The concentration of drug in the serum at time t
A = The intercept of the distribution phase with the concentration axis expressed as ug mL^{-1}
B = The intercept of the elimination phase with the concentration axis expressed as ug mL^{-1}
α = The distribution rate constant expressed in units of reciprocal time (h^{-1})
β = 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_{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 $\ln 2/K_{el}$ or $\ln 2/K_{ab}$, 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 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 = 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 = $AUC_{i.m}/AUC_{i.v} \times 100$.

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 ($AUC_{0\text{-}24\ h}$/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 MIC_{90} (0.032 ug mL^{-1}) of ibafloxacin 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 ibafloxacin 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.wt., the ibafloxacin 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 (Vd_{ss}) was 4.09 L kg^{-1} and mean residence time was 7.89 h. Ibafloxacin 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)

Parameters	Unit	IV	Parameters	Unit	IM
C_p^o	ug mL^{-1}	4.06±0.0300	C_{max}	ug mL^{-1}	1.060±0.010
A	ug mL^{-1}	2.56±0.0100	t_{max}	h	1.300±0.004
B	ug mL^{-1}	1.50±0.0400	K_{ab}	h^{-1}	2.450±0.020
α	h^{-1}	2.66±0.0500	K_{el}	h^{-1}	0.118±0.004
β	h^{-1}	0.118±0.002	$t_{0.5(ab)}$	h	0.283±0.002
K_{12}	h^{-1}	1.42±0.0200	$t_{0.5(el)}$	h	5.900±0.110
K_{21}	h^{-1}	1.06±0.0400	AUC	ug mL^{-1} h^{-1}	11.500±0.310
K_{el}	h	0.298±0.004	AUMC	ug mL^{-1} h^{-2}	94.100±3.700
$t_{0.5(\alpha)}$	h	0.261±0.004	MRT	h	8.730±0.210
$t_{0.5(\beta)}$	h	5.87±0.0800	MAT	h	1.070±0.220
V_c	L kg^{-1}	1.85±0.0200	F	%	79.600±0.090
Vd_{ss}	L kg^{-1}	4.09±0.0800	C_{max}/MIC	Ratio	33.200±0.220
Cl_B	L kg^{-1} h^{-1}	0.519±0.010	AUC/MIC	Ratio	358.900±9.500
MRT	h	7.89±0.1100	-	-	-
AUC	ug mL^{-1} h^{-1}	14.48±0.2500	-	-	-
AUMC	ug mL^{-1} h^{-2}	107.4 ±2.9000	-	-	-

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} 1st-order constant for transfer from central to peripheral compartment; K_{21} 1st-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; Vc apparent volume of the central compartment; Vd_{ss} volume of distribution at steady state; Cl_B total body clearance. K_{ab} 1st-order absorption 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

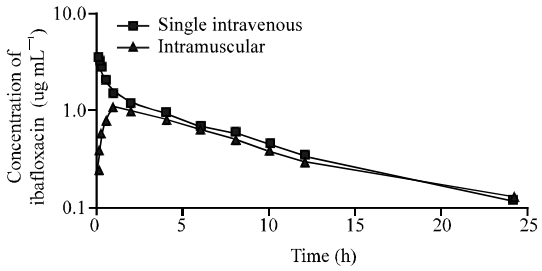

Fig. 1: Semi-logarithmic graph depicting the time-concentration of ibafloxacin in serum of normal chickens after a single intravenous and intramuscular injection of 7.5 mg kg^{-1} b.wt.

($t_{0.5(ab)}$) 0.283 h. Peak serum Concentration (C_{max}) was 1.06 μg mL^{-1} achieved after maximum time (t_{max}) 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 (Vd_{ss}) 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 Baggot (1978). Also, a low Volume of central compartment (V_c 1.85 L kg^{-1}) and high volume of distribution at steady state (Vd_{ss} 4.09 L kg^{-1}) indicated that the peripheral compartment is the major compartment for ibafloxacin distribution at steady state. This Vd_{ss} 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 (0.76 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 ($t_{0.5(ab)}$) 0.283 h. The pharmacokinetic properties of fluoroquinolones include rapid absorption (Vazquez *et al*., 1989; Hinz and Rottmann, 1990; Roland *et al*., 1995). The mean elimination half-life of ibafloxacin $t_{0.5(el)}$ 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 $t_{0.5(el)}$ of the drug. Absorption limits drug elimination (Gibaldi and Perrier, 1982). Also, this result supported by longer MRT (8.73 h). The mean $t_{0.5(el)}$ 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 (MIC_s) 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). Ibafloxacin 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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