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Single Oral-dose Pharmacokinetics of Ibafloxacin and Orbifloxacin in Chickens

M.A. Tohamy
Department of Pharmacology, Faculty of Veterinary Medicine,
Beni-suef University, Egypt

Abstract: Purpose: The single-dose pharmacokinetics of ibafloxacin and orbifloxacin were studied in clinically normal chickens after oral administration of 7.5 and 2.5 mg of ibafloxacin and orbifloxacin/kg of body weight, respectively. **Materials and methods:** Ibafloxacin and orbifloxacin concentrations were determined by microbiological assay method. **Results:** Following oral administration, ibafloxacin and orbifloxacin achieved maximum serum concentrations of 1.03 and 1.63 μg mL^{-1} achieved after maximum time of 1.3 and 1.32 h post-injection, respectively. The absorption half-lives ($t_{0.5(ab)}$) were 0.3 and 0.308 h, respectively. The elimination half-life ($t_{0.5(el)}$) and MRT values were 5.58 and 4.92, 8.50 and 7.54 h, respectively. The *in-vitro* serum protein-binding tendencies were 26.1 and 19.5% for ibafloxacin and orbifloxacin, respectively. Ibafloxacin and orbifloxacin could be useful in the treatment of systemic infections in chickens after specific assessment of susceptible microorganisms.

Key words: Ibafloxacin, orbifloxacin, oral, pharmacokinetics, chickens

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). 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). 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).

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. Also, the pharmacokinetics 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 chickens.

Consequently, this study describes some pharmacokinetic aspects of ibafloxacin and orbifloxacin in chickens following a single oral-dose of 7.5 and 2.5 mg kg^{-1} b.wt., respectively.

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. 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.

Chickens: Six clinically healthy Hubbard chickens weighing 1.6-2.0 kg b.wt. (45 day 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 oral dose of 7.5 mg kg^{-1} ibafloxacin (Coulet *et al*., 2002) and after washout period of two weeks, orbifloxacin 2.5 mg kg^{-1} (Marin *et al*., 2007) was given as a single oral dose. 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, 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: Ibafloxacin and orbifloxacin concentrations in serum samples were determined by the microbiological assay method described by Bennett *et al.* (1966) using *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 10031) as a test organisms, respectively. Standard curves were constructed using antibacterial-free sera collected from chickens. 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 and orbifloxacin standards. The plates were incubated at 37°C for 18-24 h. The inhibition zone diameters were measured and the ibafloxacin and orbifloxacin concentrations in the test samples were calculated from the standard curve. The lower detectable limit of the ibafloxacin and orbifloxacin assay was 0.078 μg mL^{-1}. Semi-logarithmic plots of the inhibition zone diameter versus standard ibafloxacin and orbifloxacin 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 and 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:

$$\text{Protein binding (\%)} = \frac{\text{Zone of inhibition in buffer-Zone of inhibition in serum}}{\text{Zone of inhibition in buffer}} \times 100 \quad (1)$$

Pharmacokinetic analysis: Serum concentrations of ibafloxacin and orbifloxacin for each individual chick after oral 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). 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). The 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 first Moment Curve (AUMC) were calculated by the method of trapezoids and extrapolation to infinity was performed. Mean Residence Time (MRT) was calculated as MRT = AUMC/AUC. Results were expressed as mean and Standard Error (S.E). Standard errors were calculated from the mean data according to Snedecor and Cochran (1976).

RESULTS

The mean serum concentrations time course of ibafloxacin and orbifloxacin after oral administration are depicted in Fig. 1. Pharmacokinetic parameters are showed in Table 1. Ibafloxacin and orbifloxacin were rapidly absorbed after oral administration with absorption half life ($t_{0.5(ab)}$) 0.3 and 0.308 h, respectively. Peak serum concentrations (C_{max}) were 1.03 and 1.63 μg mL^{-1} achieved after maximum times (t_{max}) of 1.34 and 1.32 h post administration, respectively. Both of the two drugs were eliminated from blood after oral administration with an elimination half-life 5.58 and 4.92 h, respectively. The *in-vitro* serum protein-binding tendencies were 26.1 and 19.5% for ibafloxacin and orbifloxacin, respectively.

Table 1: Pharmacokinetic parameters of ibafloxacin (7.5 mg kg^{-1}) and orbifloxacin (2.5 mg kg^{-1}) following a single oral administration in chickens (n = 6)

Parameter	Unit	Ibafloxacin	Orbifloxacin
C_{max}	μg mL^{-1}	1.03±0.03	1.63±0.005
t_{max}	h	1.34±0.002	1.32±0.004
K_{ab}	h^{-1}	2.31±0.04	2.25±0.02
K_{el}	h^{-1}	0.124±0.002	0.141±0.001
$t_{0.5(ab)}$	h	0.30±0.005	0.308±0.003
$t_{0.5(el)}$	h	5.58±0.10	4.92±0.03
AUC	μg mL^{-1} h^{-1}	10.63±0.29	15.20±0.10
AUMC	μg mL^{-1} h^{-2}	83.20±3.3	105.00±1.74
MRT	h	8.50±0.19	7.54±0.07

k_{ab}: First-order absorption rate constant; K_{el}: First-order 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; AUC Area under serum concentration-time curve; AUMC: Area under moment curve; MRT: Mean residence time

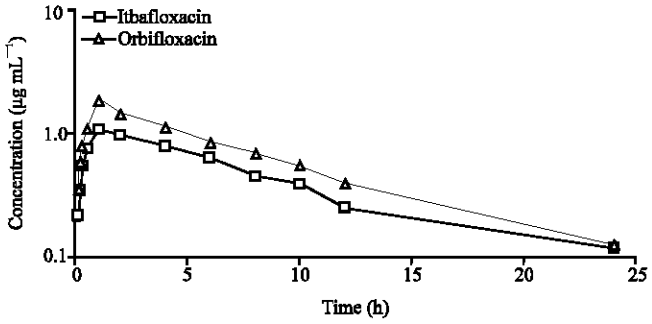

Fig. 1: Semi-logarithmic graph depicting the time-concentration of ibafloxacin (7.5 mg kg^{-1}) and orbifloxacin (2.5 mg kg^{-1}) in serum of normal chickens after a single oral administration

DISCUSSION

The pharmacokinetics of ibafloxacin and orbifloxacin in chickens are reported in the present study for the first time. Therefore, since pharmacokinetic studies of both drugs have 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.

Following oral administration of single dose of ibafloxacin and orbifloxacin, both of the two drugs were absorbed rapidly in chickens as indicated by short absorption half-lives ($t_{0.5(ab)}$) 0.3 and 0.308 h, respectively. The pharmacokinetic properties of fluoroquinolones include rapid absorption (Roland *et al*., 1995). The mean elimination half-lives of ibafloxacin and orbifloxacin $t_{0.5(el)}$ were longer (5.58 and 4.92 h, respectively) after single oral-dose. Also this result supported by longer MRT (8.50 and 7.54 h, respectively). The mean $t_{0.5(el)}$ of ibafloxacin (5.58 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*., 2006). The mean elimination half-life of orbifloxacin (4.92) 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 chickens than other species.

Following oral administration of single dose of orbifloxacin, The estimated C_{max} 1.63 µg mL^{-1} was 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 *in-vitro* protein binding tendencies of ibafloxacin and orbifloxacin to chicken's serum proteins were 27.1 and 19.5%, respectively. This indicated that both of the two drugs are 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 and orbifloxacin 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 spp. and Proteus mirabilis* (Coulet *et al*., 2002). Also, 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).

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.

These data allow concluding that ibafloxacin and orbifloxacin administered orally to chickens at a dose rate of 7.5 and 2.5 mg kg^{-1}, respectively could be useful in the treatment of systemic infections in chickens after specific assessment of susceptible microorganisms.

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