Insight Pharmaceutical Sciences 1 (3): 34-38, 2011
ISSN 2040-705X

Bioavailability and Pharmacokinetics of Ibafloxacin in Ewes

M.A. Tohamy
Departmentof Pharmacology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

Abstract: Purpose: The single-dose pharmacokinetics of ibafloxacin was determined in clinically normal ewes after intravenous and intramuscular administration of 15 mg of ibafloxacin kg^{-1} of body weight. Ibafloxacin concentrations were determined by a modified high-performance liquid chromatography (HPLC) method. **Results:** Steady-state volume of distribution and clearance of ibafloxacin after intravenous administration were 3.61 L kg^{-1} and 0.660 L kg^{-1} h^{-1}, respectively. Following intramuscular administration, ibafloxacin 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:** Ibafloxacin could be useful in the treatment of systemic infections in ewes after specific assessment of susceptible microorganisms.

Key words: Ibafloxacin, 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).

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*., 2007a), but not yet in ewes. Consequently, this study describes some pharmacokinetic aspects and bioavailability of ibafloxacin 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 ibafloxacin in ewes 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)].

MATERIAL 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.

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} ibafloxacin (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: Ibafloxacin 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). After

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 0.067 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 ultra-filtration 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:

$$\text{Protein binding\%} = \frac{\text{Total concentration} - \text{Ultra filtrate concentration}}{\text{Total concentration}} \times 100$$

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:

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

where, C_p 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}; α is the distribution rate constant expressed in units of reciprocal time (h^{-1}); α 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 ($t_{0.5(el)}$) 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. 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 = $AUC_{i.m}/AUC_{i.v}\times100$.

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_{0-24h}/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 MIC_{90} (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 (Vd_{ss}) 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 ($t_{0.5(ab)}$) 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 86.6%.

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

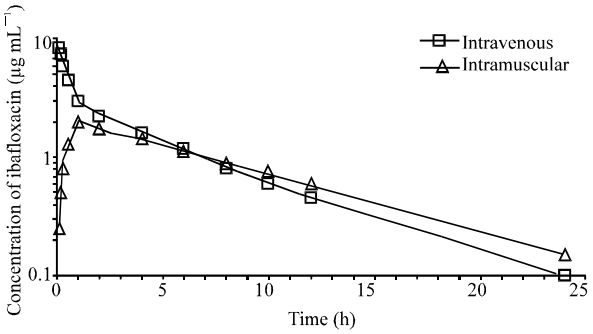

Fig. 1: Semi-logarithmic graph depicting the time-concentration of ibafloxacin in serum of normal ewes after a single intravenous and intramuscular injection of 15 mg kg^{-1} b.wt

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

Parameter	Unit	IV	Parameter	Unit	IM
C_p^o	μg mL^{-1}	10.20±0.03	C_{max}	μg mL^{-1}	1.89±0.099
A	μg mL^{-1}	7.06±0.03	t_{max}	h	1.43±0.074
B	μg mL^{-1}	3.10±0.04	K_{ab}	h	2.28±0.19
α	h	3.04±0.04	K_{el}	h	0.118±0.022
β	h	0.164±0.004	$t_{0.5(ab)}$	h	0.309±0.014
K_{12}	h	1.68±0.02	$t_{0.5(el)}$	h	5.89±0.215
K_{21}	h	1.04±0.02	AUC	μg mL^{-1} h	19.70±1.409
K_{el}	h	0.479±0.008	AUMC	μg mL^{-1} h	169.00±13.22
$T_{0.5(\beta)}$	h	0.229±0.004	MRT	h	8.94±0.740
$t_{0.5(\beta)}$	h	4.24±0.06	MAT	h	3.46±0.101
V_c	L kg^{-1}	1.48±0.004	F	%	86.60±6.54
Vd_{ss}	L kg^{-1}	3.61±0.02	C_{max}/MIC	Ratio	59.10±0.33
Cl_B	L kg^{-1} h^{-1}	0.66±0.01	AUC/MIC	Ratio	615.60±14.2
MRT	h	5.48±0.098			
AUC	μg mL^{-1} h^{-1}	2.80±0.46			
AUMC	μg mL^{-1} h^{-1}	16.50±4.40			

C_p: 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_{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}: First-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

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_{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 ewes recorded in this study was more than unity (>one L kg^{-1}) following intravenous administration (3.61 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 1.48 L kg^{-1}) and high volume of distribution at steady state (Vd_{ss} 3.61 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 marbofloxacin in ostriches 3.22 L kg^{-1} (De Lucas *et al*., 2005).

Following intramuscular injection, the estimated C_{max} 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-Sooud 2008). The drug absorbed rapidly in ewes as indicated by short absorption half-life ($t_{0.5(ab)}$) 0.309 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 significantly longer (5.89 h) than that calculated after intravenous 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 after intramuscular (8.94 h) than after intravenous injection (5.48 h). The mean $t_{0.5(el)}$ of ibafloxacin (5.89 h) was similar to that of orbifloxacin in camels 5.95 h (Goudah and Abo-El-Sooud, 2008). The *in vitro* protein binding tendency of ibafloxacin 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 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 ewes indicating the excellent pharmacokinetic characteristics of the drug in ewes.

These data allow concluding that ibafloxacin 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, ibafloxacin could be useful in the treatment of systemic infections in ewes after specific assessment of susceptible microorganisms.

REFERENCES

Baggot, T.D., 1978. Some aspects of clinical pharmacokinetics in veterinary medicine. J. Vet. Pharmacol. Therapeut., 1: 5-18.

Brown, S.A., 1996. Fluoroquinolones in animal health. J. Vet. Pharmacol. Ther., 19: 1-14.

Coulet, M., M. Van Borssum Waalkes, O.R. Leeuwenkamp, P. Cox and J. Lohuis, 2002. Pharmacokinetics of ibafloxacin after intravenous and oral administration to healthy beagle dogs. J. Vet. Pharmacol. Therapeutics, 25: 89-97.

Coulet, M., C. Morello, P. Cox and J. Lohuis, 2005. Pharmacokinetics of ibafloxacin in healthy cats. J. Vet. Pharmacol. Ther., 28: 37-44.

De Lucas, J.J., C. Rodriguez, S. Waxman, F. Gonzalez, I. Uriarte and M.I. San Andres, 2005. Pharmacokinetics of marbofloxacin after intravenous and intramuscular administration to ostriches. Vet. J., 170: 364-368.

Elias, G., L. Joong-Su, C. Zhi-Qiang, H. Mi-Hyun, C. Henrique and P. Seung-Chun, 2009. Integration of pharmacokinetic and pharmacodynamic indices of orbifloxacin in beagle dogs after a single intravenous and intramuscular administration. Antimicrobial Agents Chemoth., 53: 3024-3029.

Galinsky, R.E. and C.K. Svensson, 1995. Basic Pharmacokinetics. In: The Science and Practice of Pharmacy, Remington, J.P. (Ed.). 19th Edn. Mack Publishing Company, Easton, PA., pp: 724-740.

Gibaldi, M. and D. Perrier, 1982. Pharmacokinetics. 2nd Edn., Marcel Dekker Inc., New York, pp: 409-417.

Goudah, A. and K. Abo-El-Sooud, 2008. Pharmacokinetics and milk penetration of orbifloxacin after intravenous and intramuscular injections to dromedary lactating camels (*Camelus dromedaries*). J. Vet. Pharmacol. Ther., 31: 276-280.

Hannan, P.C.T., G.D. Windsor, A. Jong, N. Schmeer and M. Stegemann, 1997. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. Antimicrob. Agents Chemother., 41: 2037-2040.

Hinz, K.H. and S. Rottmann, 1990. Studies *in vivo* on the efficacy of enrofloxacin against *Mycoplasma gallisepticum*. Avian Pathol., 19: 511-522.

Hooper, D.C. and J.S. Wolfson, 1985. The fluoroquinolones: Pharmacology, clinical uses and toxicities in humans. Antimicrobial. Agents Chemoth., 28: 716-721.

Marin, P., C.M. Carceles, E. Escudero and E. Fernandez-Varon, 2007a. Pharmacokinetics and milk penetration of ibafloxacin after intravenous administration to lactating goats. Can. J. Vet. Res., 71: 74-76.

Marin, P., E. Escudero, E. Fernandez-Varon and C.M. Carceles, 2007b. Pharmacokinetics and milk penetration of orbifloxacin after intravenous, subcutaneous and intramuscular administration to lactating goats. J. Dairy Sci., 90: 4219-4225.

Mckellar, Q.A., S.F. Sanchezand D.G. Jones, 2004. Pharmacokinetic/ pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. J. Vet. Pharmacol. Ther., 27: 503-514.

Roland, N., T. Schmidt, K. Kaye, J.L. Froula and M.G. Tauber, 1995. Quinolone antibiotics in therapy of experimental pneumococcal meningitis in rabbits. Antimicrobial. Agents Chemoth., 39: 593-597.

Siefert, H.M., C. Kohlsdorfer, W. Steinke and A. Witt, 1999. Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: Tissue distribution in male rats. J. Antimicrobial. Chemoth., 43: 61-67.

Toutain, P.L., J.R.E. Del Castillo and A. Bousquet-Meclou, 2002. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. Res. Vet. Sci., 73: 105-114.

Turnidge, J., 1999. Pharmacokinetics and pharmacodynamics of fuoroquinolones. Drugs, 58: 29-36.

Vancutsem, P.M., J.G. Babish and W.S. Schwark, 1990. The fluoroquinolone antimicrobials, structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet., 80: 173-186.

Vazquez, F., R. Vazquez and M. Cervants, 1989. Effectiveness of enrofloxacin in the treatment of different bacterial infections in broiler stocks in Mexico. Proceedings of the 38th Western Poultry Disease Conference, (WPD'89), Tempe, Ariz, pp: 126-127.

Walker, R.D., 2000. Fluoroquinolones. In: Antimicrobial Therapy in Veterinary Medicine, Prescott, J.F. and J.D. Baggot (Eds.). 3rd Edn., Iowa State University Press, Ames, IA., pp: 315-338.

Wise, R., R. Lockley and J. Dent, 1984. Pharmacokinetics and tissue penetration of enoxacin. Antimicrobial. Agents Chemother., 26: 17-19.

Yamaoka, K., T. Nakagawa and T. Uno, 1978. Statistical moment in pharmacokinetics. J. Pharm. Biopharm., 6: 547-558.