DISPOSITION OF LEVOFLOXACIN FOLLOWING ORAL ADMINISTRATION IN BROILER CHICKENS

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ABSTRACT

Pharmacokinetics of levofloxacin was determined following single dose intravenous (IV) and oral (PO) administration at the dose rate of 10 mg.kg^{-1} body weight in coloured broiler chickens. Drug concentration in plasma was determined using High Performance Liquid Chromatography. Following single dose intravenous administration, the drug was rapidly distributed ($t_{1/2\alpha}$: 0.29 ± 0.01 h) and eliminated ($t_{1/2\beta}$: 3.18 ± 0.07 h; Cl_B: 14.71 ± 0.12 mL.min^{-1}.kg^{-1}) from the body. Following oral administration, the drug was rapidly absorbed (C_{max}: 0.93 ± 0.02   µg.mL^{-1}; T_{max}: 2 h) and eliminated ($t_{1/2\beta}$: 3.64 ± 0.15 h) from the body. The mean residence time (MRT) and bioavailability following oral administration were 6.12 ± 0.13 h and 59.54 ± 1.97 %. The pharmacokinetic profile indicated that levofloxacin can be used to treat various bacterial infections in broiler chickens.

INTRODUCTION

Fluoroquinolones are an important group of antibacterial drugs used in veterinary medicine. They have broad-spectrum activity against bacteria, mycoplasma and rickettsia (1). Levofloxacin is a new third generation fluoroquinolone effective against species of Staphylococci, Streptococci, Enterobacteriaceae, *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Bacteroides*, *Clostridium*, *Haemophilus*, *Moraxella*, *Mycoplasma* and *Chlamydia* (2). Currently, it is widely used in human medicine but an increasing number of pharmacokinetic studies are now being undertaken in domestic animals with a view to also adopt this drug in veterinary medicine. The pharmacokinetics of levofloxacin has been worked out in cows, calves (3, 4, 5, 6) and cats (7). However, due to major physiological differences, the pharmacokinetic results of levofloxacin in these animals cannot be extrapolated to chickens. The drug has possible therapeutic applications through water medication in chickens. The present study was planned to determine pharmacokinetics of levofloxacin following intravenous (IV) and oral (PO) administration in broiler chickens.

MATERIALS AND METHODS

Experimental animals

The study was conducted on 10, 8-10 weeks old broiler chickens weighing 1.5- 2.0 kg.. The birds were maintained at Central Poultry Research Station (CPRS), College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat state, India. Birds were kept under observation for two weeks prior to commencement of experiment, and subjected to clinical examination in order to exclude the possibility of disease. The birds were kept in clean cages and were provided antibiotic-free standard broiler ration. Water was provided *ad libitum*. Standard managemental practices were followed to keep the birds free from stress. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Drugs and chemicals

Levofloxacin infusion (0.5 % Tavanic™, Aventis Pharmaceutical Ltd, Bangaluru, India) and levofloxacin oral tablet (Loxof™; Ranbaxy Laboratories Ltd., Himachal Pradesh, India) were used. Levofloxacin and enrofloxacin technical grade powder (Moxi Laboratory Pvt Ltd., Gujarat, India) were used for

standardization of the assay. Triethylamine, perchloric acid (70%), ortho-phosphoric acid (analytical grade), acetonitrile and water (HPLC grade) were procured from Merck Limited, Mumbai, India.

Experimental design

The experiment was conducted in a cross-over design with an interval of 15 days between 2 successive administrations of the drug. All birds were randomly allocated to receive either IV or oral dose of the drug. The drug was administered at a dose rate of 10 mg.kg^{-1} of body weight. For IV administration, levofloxacin infusion (0.5%) was injected through a wing vein using a needle (22G x 25mm). Birds were fasted for 24 hours before oral administration of the drug. For oral administration levofloxacin tablets (250 mg) were dissolved in sterile water.

Blood samples (1 mL) were collected using IV catheter (Venflon, 22G x 25mm) fixed into the contra-lateral wing vein and transferred to clean sterilized heparinized test tubes. Following IV administration, blood samples were collected at time 0 (before drug administration) and at 0.033 (2 minutes), 0.083 (5 minutes), 0.166 (10 minutes), 0.25 (15 minutes), 0.5 (30 minutes), 0.75 (45 minutes), 1, 2, 4, 8, 12, 18 and 24 hours. Following oral administration blood samples were collected at time 0 (before drug administration) and at 0.083 (5 minutes), 0.166 (10 minutes), 0.25 (15 minutes), 0.5 (30 minutes), 0.75 (45 minutes), 1, 2, 4, 8, 12, 18, 24 and 36 hours. Plasma was harvested soon after collection by centrifugation (3000 g at 4°C for 10 minutes), stored at -35°C and assayed within 24 hours.

Levofloxacin assay

The drug concentration in plasma was determined by reversed-phase high performance liquid chromatography (HPLC), using a published assay with some modifications (8). The HPLC system (Laballiance, USA) comprised of a quaternary gradient solvent delivery pump (model AIS 2000) and UV detector (model 500). Chromatographic separation was done on reverse phase C18 column (Thermo, 5 μ ODS; 250 X 4.6 mm ID) at room temperature. Data integration was performed using software Clarity (Version 2.4.0.190). The mobile phase consisted of a mixture of 1% triethylamine in water and acetonitrile (85:15 v/v) adjusted to pH 3.0 with ortho-phosphoric acid. Mobile phase was filtered through 0.45 μ filter and pumped into column at a flow rate of 1.5 mL.min^{-1} at ambient temperature. The effluent was monitored at 290 nm. Sample was prepared by taking 500 μl plasma samples in 2 mL clean micro-centrifuge tube. 20 μg enrofloxacin was added as an internal standard in each sample. Perchloric acid (50 μl) was added to precipitate plasma proteins. The mixture was shaken on a vortex mixer for 1 minute and centrifuged at 3000 g at 4 °C for 10 minute. The clean supernatant was collected and an aliquot of 20 μl of the supernatant was injected manually into the loop of HPLC system

Known amount of levofloxacin was added to unfortified plasma to prepare a range of concentrations from 0.01 to 50 μg.mL^{-1}. and treated as samples to prepare the calibration curve, and prepared by plotting the ratio (areas of peak of levofloxacin standards: areas of peak of internal standards) at the ordinate

and the drug concentration at abscissa. The calibration curve was prepared daily and it had a R^2 value > 0.99. The lower limit of quantification (LLOQ) was 0.01 μg.mL^{-1}. The assay was linear between 0.01 to 50 μg.mL^{-1}.

Precision and accuracy were determined with known concentrations of 0.05, 1.0, and 50 μg. μg.mL^{-1} in plasma (5 replicates each/day). The intra-day and inter-day coefficients of variation for 5 samples were satisfactory, with relative standard deviations (RSD) less than 8%. Intra-day and inter-day variations were within acceptable limits. The retention time of the drug was 6.4 min. The absolute recovery of levofloxacin was measured by comparison of the areas of levofloxacin after injection of supernatant fluid extracted from plasma (known concentrations) with those obtained after injection of the standard solution containing equivalent concentrations of the drug. Recovery of the drug from plasma was found to be more than 90%.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined for each bird by non-compartmental analysis with commercial software (PK solution 2.0, USA). Following oral administration of the drug, maximum concentration (C_{max}) and time to reach the maximum concentration (T_{max}) were determined from the concentrations-time curve. Bioavailability of the drug was calculated using the following formula (9): $F = AUC(PO)/AUC(IV)*100$

RESULTS

The semi-logarithmic plot of mean plasma concentration-time data for levofloxacin after a single dose IV and oral administration (10 mg.kg^{-1}) in broiler chickens is presented in Figure 1. Following single dose IV administration, the distribution half-life, elimination half-life, volume of distribution at steady state, area under curve and total body clearance were 0.29 ± 0.01 h, 3.18 ± 0.07 h, 3.25 ± 0.06 L.kg^{-1}, 11.33 ± 0.08 μg.h.mL^{-1} and 14.71 ± 0.12 mL.min^{-1}.kg^{-1}, respectively. Following oral administration, peak plasma drug concentration of 0.93 ± 0.02 μg.mL^{-1} (C_{max}) was observed at 2.0 h (T_{max}). The area under curve, elimination half-life and systemic bioavailability were 6.70 ± 0.08 μg.h.mL^{-1}, 3.64 ± 0.15 h and 59.54 ± 1.97 %, respectively following oral administration of the drug. Pharmacokinetic parameters following IV and oral administration of the drug in broiler chickens are presented in Table 1.

DISCUSSION

Following IV administration the elimination half-life of the drug in broiler chickens was similar to that of ofloxacin (4.44 h) and pefloxacin (3.25 ± 1.34 h) reported in chickens and ducks, respectively (10, 11), however it was lower than elimination half-life of ciprofloxacin (9.01 ± 0.79 h) and danofloxacin (6.73 h) reported in chickens (12,13). Thus, levofloxacin is more rapidly eliminated than other fluoroquinolones in broiler chickens. Total body clearance of the drug in the present study was similar to clearance of enrofloxacin, danofloxacin and pefloxacin (11, 13); however it was higher than ofloxacin (10) and ciprofloxacin (12) in chickens and ducks, respectively. The
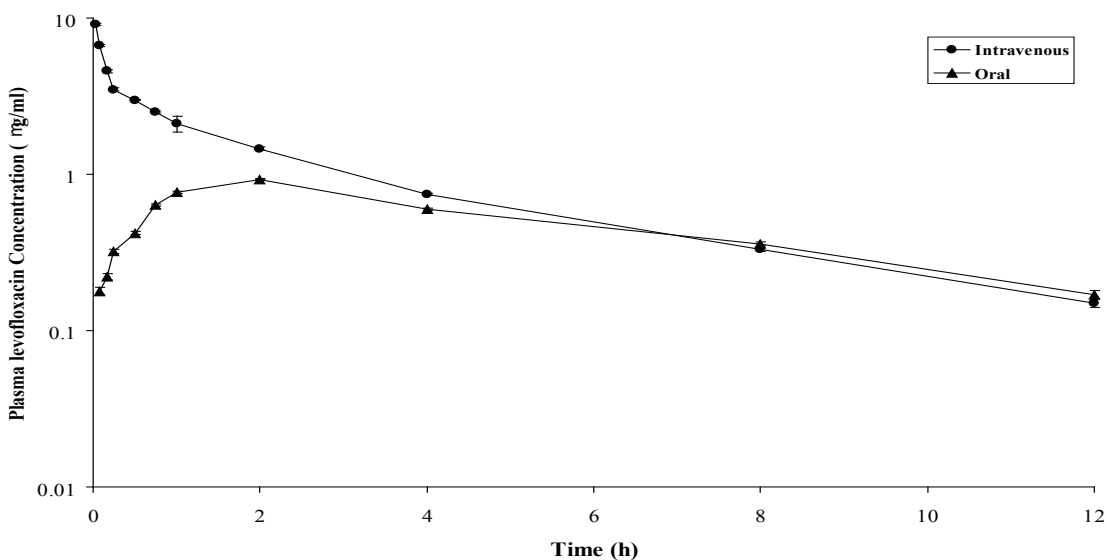
volume of distribution at steady state of the drug was found to be 3.25 ± 0.06 L.kg^{-1}, which was parallel to pefloxacin (3.73 ± 0.57 L.kg^{-1}) reported in male ducks (11), but lower steady state volume of distribution of ciprofloxacin was also found in chickens (12).

Following single dose oral administration, the observed peak plasma level (C_{max}) of the drug in the present study was lower than C_{max} of ofloxacin (3.65 µg.mL^{-1}) and enrofloxacin (1.88 µg.mL^{-1}) observed in chickens (10, 13). The elimination half-life and mean residence time of the drug were lower as compared to values reported for other fluoroquinolones in chickens and ducks (10, 11, 13). Following oral administration, the systemic bioavailability of levofloxacin (59.54 ± 1.97 %) in the present study was lower than bioavailability of ciprofloxacin (70.09 ± 9.8%), ofloxacin (110.01%), danofloxacin (99.2%) and enrofloxacin (89.2%) reported in chickens (10, 12, 13).

Success of antimicrobial therapy for concentration dependent fluoroquinolone antimicrobials such as levofloxacin can be largely determined by integrating the pharmacokinetics and pharmacodynamics through hybrid parameters like AUC/MIC and C_{max}/MIC. The effective use of the fluoroquinolones for clinically important pathogens depends on designing dosages that attain a C_{max}/MIC > 8-10 and an AUC/MIC > 100-125 (15,16,17). The values for AUC/MIC ratio and C_{max}/MIC ratio after oral administration were calculated using documented MIC values against Gram-positive and Gram-negative organisms. An average plasma concentration of 0.032-0.5 µg.mL^{-1} was reported as minimum therapeutic concentration (MIC$_{90}$) for levofloxacin against most bacteria (18). The MIC$_{90}$ of 0.03 and 0.06 µg.mL^{-1} of levofloxacin was taken into consideration for calculation of efficacy predictors. The C_{max}/MIC ratio of 31.0 and 15.50 and AUC/MIC ratio of 223.34 and 111.67 at MIC of 0.03 and 0.06 µg.mL^{-1}, respectively indicates potential clinical and bacteriological efficacy of levofloxacin in broiler chickens. Moderate bioavailability with good pharmacokinetic profile and pharmacokinetic-pharmacodynamic hybrid efficacy predictors for levofloxacin indicate that oral administration of levofloxacin at 10 mg.kg^{-1} may be highly efficacious against susceptible bacteria in broiler chickens.

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Fig. 1: Semilogarithmic plot of plasma levofloxacin concentrations versus time following single dose IV and oral administration (10 mg.kg^{-1}) in broiler chickens. Each point represents mean ± S.E. of 10 birds.

TABLES

Table 1: Pharmacokinetic parameters of levofloxacin after single dose IV and oral administration (10 mg.kg^{-1} of body weight) in broiler chickens (n = 10)

Pharmacokinetic parameters	Unit	Intravenous Mean ± S.E	Oral Mean ± S.E
$t_{\frac{1}{2}\alpha}$	h	0.29 ± 0.01	-
$t_{\frac{1}{2}\beta}$	h	3.18 ± 0.07	3.64 ± 0.15
$t_{\frac{1}{2}K(a)}$	h	-	0.95 ± 0.08
$AUC_{(0 - \infty)}$	µg.h.mL^{-1}	11.33 ± 0.08	6.70 ± 0.08
AUMC	µg.h^2.mL^{-1}	41.73 ± 1.15	41.87 ± 1.40
$V_{d(ss)}$	L.kg^{-1}	3.25 ± 0.06	-
$Cl_{(B)}$	mL.min^{-1}.kg^{-1}	14.71 ± 0.12	-
MRT	hour	3.69 ± 0.08	6.12 ± 0.13
C_{max}	µg.mL^{-1}	-	0.93 ± 0.02
T_{max}	hr	-	2.0
F	%	-	59.54 ± 1.97

$t_{1/2\alpha}$ = half-life of distribution phases; $t_{1/2\beta}$ = elimination half life; $t_{1/2k(a)}$ = absorption half-life; $AUC_{(0 - \infty)}$ = total area under plasma drug concentration-time curve; AUMC = area under first of moment curve; $V_{d(ss)}$ = volume of distribution at steady state; $Cl_{(B)}$ = total body clearance; MRT = mean residence time; C_{max} = observed maximum drug concentration; T_{max} = observed time of maximum concentration; F = bioavailability.