RESEARCH ARTICLE

Dosage Derivation of Marbofloxacin in Broiler Chickens based on Pharmacokinetic-Pharmacodynamic Integration

Ratn Deep Singh, Sanjay H. Vaghela, Sheen Tukra, Ankit R. Patel, Hitesh B. Patel, Vaidehi N. Sarvaiya, Shailesh K. Mody

Abstract

Marbofloxacin, a veterinary–exclusive antimicrobial drug of third–generation fluoroquinolone class, exhibits wide spectrum antimicrobial activity against Gram-negative as well as Gram-positive bacteria, and thus, is a promising agent to treat susceptible bacterial infections in poultry. Therefore, the present study was conducted to derive dosage regimen of marbofloxacin based on the results of intravenous and oral pharmacokinetic (PK) trial in sixteen healthy broiler chickens. Plasma concentrations of marbofloxacin were measured by optimized and in-house validated Ultra High Performance Liquid Chromatography (UHPLC) method. The PK parameters were calculated from plasma concentrations versus time data by non-compartmental analysis using 'PK Solver 2.0' software. Following oral administration of marbofloxacin at the dose rate of 5 mg/kg b.wt., the mean maximal plasma concentration (C_{max}: 2.193 µg/ml) was achieved at 1.68 h. Marbofloxacin showed long half-life (6.03 h), high volume of distribution (2.64 L/kg) and good oral bioavailability (88.10 %). The average plasma concentration of marbofloxacin ≥ 0.32 µg/ mL was maintained upto 12 h and 0.10 µg/ mL at 24 h. Based on the values of PK-PD integrated indices, it is concluded that oral dose of marbofloxacin @ 5.0 mg/kg b.wt., to be repeated at 24 h, would be efficient to treat common bacterial pathogens of broiler chickens having MIC ≤ 0.12 µg/mL.

Key words: Broiler chickens, Dosage, Marbofloxacin, Pharmacokinetic trial, PK-PD index.

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Introduction

Broiler chicken industry serves as a good source of high-quality protein to fulfill nutritional demands and is one of the fast-growing segments of the agricultural sector in India with the annual growth rates of 7-8 % or more (ICRA, 2020). Despite the fact that bacterial infections pose a serious threat to the chicken industry and may hamper production goals, chicken producers have seen notable improvements in growth rate, feed efficiency, and mortality reduction in infected flocks, as a result of the therapeutic use of antimicrobial drugs. One of such antibacterial class of drug used in poultry species is fluoroquinolones, dominated by the use of drugs like enrofloxacin and ciprofloxacin in India. Ciprofloxacin is a low-cost second-generation fluoroquinolone drug, having great efficacy against aerobic Gram-negative bacteria especially the Enterobacteriaceae. Compared to other widely used fluoroquinolones, it exhibited lower minimum inhibitory concentration (MIC) against pathogens like *Escherichia coli* (Singh *et al*., 2020). However, wide-spread use of enrofloxacin and ciprofloxacin has resulted in drug resistance problem in treating the colibacillosis infections in poultry (Roth *et al*., 2018). The emergent resistance among these drugs creates a vacuity in the antibacterial armory of poultry medicine. Thus, there is need for a new molecule of fluoroquinolone class of drug for judicious use in treating bacterial infection like colibacillosis of broiler chickens. However, looking at the tissue residue problem of drugs in broiler chickens, the drug must be safe and should have short withdrawal period and preferentially should be used in veterinary medicine only. Marbofloxacin, a third-generation, veterinary-exclusive, broad-spectrum antibacterial drug of fluoroquinolone fits these criteria. It is a concentration-dependent drug, exhibits a significant post-antibiotic effect (PAE) and can kill bacteria during both stationary and growth phase of bacterial multiplication. Marbofloxacin differs from other fluoroquinolones in chemical structure as the presence of an oxadiazine ring, which provides pharmacokinetic advantage such as a long elimination half-life. It exhibits bactericidal action by

Department of Veterinary Pharmacology and Toxicology College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardar krushinagar-385506, India

Corresponding Author: Ratn Deep Singh, Department of Veterinary Pharmacology and Toxicology College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardar krushinagar-385506, India, e-mail: rdsingh@kamdhenuuni.edu.in

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inhibiting bacterial DNA topoisomerases II (gyrase) and IV, and by preventing the supercoiling of DNA (Elzoghby and Aboubakr, 2015; Fernandez-Varon *et al.,* 2021). It has been found safe in poultry species. In broiler chickens, the marbofloxacin withdrawal time in edible tissues was estimated to be 4 days after its oral administration at 5 mg/kg b.wt. (Yang *et al*., 2014). The short withdrawal period of marbofloxacin makes this drug favourable to be used in treatment of susceptible bacterial infections including *Escherichia coli* in broiler chickens. However, pharmacokinetic trials are indispensable before recommending any drug to determine its proper dosage regimen. Hence the present study was undertaken to derive dosing protocol of marbofloxacin following its oral and IV administration in broiler chickens.

Materials and Methods

Ethical Statement: The experimental protocol of the present study was duly approved by Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar.

Birds and Experimental Design

Sixteen male broiler chickens of Cobb-400 strain ageing 4–5 weeks and weighing more than 1 kg were randomly selected for the experiment. The birds were provided 10-days quarantine and acclimatization period. In order to keep broiler chickens healthy and free from stress and disease, birds were kept under standard management circumstances (including housing, feeding, watering, etc.) in accordance with "CPCSEA Guidelines for Poultry/Birds Facility, 2020". Birds were fed the antibiotic-free grower and finisher ration. The experiment was conducted in 'cross-over' design involving two phases. One-week wash-out period was observed between two phases allowing the elimination of drug from the body.

Drug Administration and Sample Collection

For pharmacokinetic (PK) study, 1 % marbofloxacin (10 mg/mL) was given at a dose rate of 5 mg/kg b.wt. of broiler chickens through wing vein for IV route study and using oral gavage needle for oral drug administration. For drug administration, all 16 birds were randomly divided into two groups, *viz*. Group I and II; each group comprised of 8 birds. In first phase of study, drug was administered intravenously to group I birds and orally to group II birds. In second phase of experiment, drug was administered intravenously to group II birds and orally to group I birds. Blood samples were collected from the wing veins of the birds in sterilized pre-heparinized small tubes at 0 (before drug administration) and 5 (0.083 h), 15 (0.25 h), 30 (0.5 h) min., and 1, 2, 4, 8, 12, 24, 36 and 48 h after administration of marbofloxacin. Approximately 0.5 mL of blood was withdrawn at each collection point and plasma was harvested for the estimation of drug concentrations in it.

Plasma Drug Concentration Measurement

Plasma marbofloxacin levels in samples collected at different time points were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) equipment. Dionex ultimate 3000® UHPLC system (Thermo Fisher, Germany) using an ultraviolet (UV) detector and reverse phase C-18 column was used for the chromatographic separation and detection of the drug. The mobile phase consisted of mixture of 0.01 M formic acid and acetonitrile in the ratio of 82:18, which was pumped into column at a flow rate of 1.0 mL/min, on isocratic mode. Effluent was detected at wavelength of 297 nm with ultraviolet detector. Average retention time for detection of marbofloxacin was 5.6 min. For drug extraction from each sample, 150 µL of plasma were taken into 2 mL Eppendorf® micro-centrifuge tubes at room temperature. Then 150 µL of 20% perchloric acid was added for the deproteinization, and the liquid was vortexed for 1 min at 660 *g*. The mixture was then centrifuged using a refrigerated centrifuge machine at 11,430 *g* for 10 min at 4°C. Finally, 50 µL of the extracted sample as clear supernatant was manually feed into the UHPLC system for the drug assay. Linearity of the standard curve generated using known drug concentrations was reflected with mean correlation coefficient (R^2) value of 0.9993. The mean accuracy of the UHPLC method was 95.1 % whereas extraction recovery of the method ranged between 91.18 to 96.39 %. The values of intraday and inter-day precision (coefficient of variance) were not more than 0.52 and 1.62 %, respectively. Limit of detection (LOD) based on signal-noise ratio was 0.039 µg/mL.

Pharmacokinetic (PK) and Statistical Analysis

Chromatographic data generated from UHPLC were integrated with 'Chromeleon™ version 6.8' software. PK parameters were calculated using the software 'PK Solver 2.0' which is a menu-driven add-in program for Microsoft Excel (Zhang *et al*., 2010). Non-compartmental analysis was performed by the software based on the basic theory of statistical moment concepts. The mean values along with standard deviation (Mean ± SE) were calculated for the plasma concentrations analyzed. Dosage derivation was done using pharmacokinetic-pharmacodynamic (PK-PD) indices like C_{max}/MIC and AUC/MIC ratios, where C_{max} is maximum plasma concentration achieved after oral administration of drug, AUC is the area under curve and MIC is the targeted minimum inhibitory concentration of susceptible bacteria.

Results and Discussion

Plasma Concentrations

The plasma marbofloxacin concentrations at different time points, following single dose intravenous and oral administration @ 5 mg/kg b.wt. in broiler chickens (n=16), expressed as average values (mean ± SE) with their range are presented in Table 1. The comparison of

semi-logarithmic graph of mean marbofloxacin concentration in plasma versus time following intravenous and oral administration is depicted in Figure 1.

In broiler chickens, oral administration is a popular and convenient route for mass medication in diseased flocks; however, the intravenous route study was done in the present investigation along with oral route to calculate oral bioavailability, as it also requires value of AUC (area under curve) by intravenous route. Mean plasma marbofloxacin concentration at first collection time point *i.e.* 0.0833 h (5 min) following single dose oral administration @ 5.0 mg/kg b.wt. in broiler chickens was found to be 0.38 µg/mL which increased to maximum average concentration (C_{max}) of 2.04 µg/mL observed at 2 h. The mean plasma concentration was observed to be 0.1 µg/mL at 24 h for both the IV and oral routes. The plasma concentrations were not detectable in the samples beyond 24 h *i.e.* collected at 36 and 48 h. Mean plasma marbofloxacin concentration value remained above 0.25 µg/mL up to 12 h for both intravenous (0.30 µg/ mL) and oral (0.32 µg/ mL) routes, which can cover MIC value of 0.12 – 0.25 µg/mL against most of susceptible bacterial infections in broiler chickens (Spreng *et al.,* 1995; Kroemer *et al.,* 2012).

Pharmacokinetic (PK) Parameters

The PK parameters calculated from plasma concentrations of marbofloxacin after its single dose IV as well as oral administration (5.0 mg/kg b.wt.) in broiler chickens are shown in Table 2. In the present study, following oral administration, the mean elimination rate constant (β) was calculated as 0.12 per h with corresponding mean elimination half-life ($t_{½β}$) of 6.02 h. Similar mean $t_{½β}$ value was reported in turkey (6.23 h) by Haritova *et al.* (2006) and in Japanese quails (6.19 h) by

Table 1: Mean Plasma concentrations of marbofloxacin with range following single dose intravenous and oral administration in broiler chickens (n=16)

Time point (h)	Plasma marbofloxacin concentration (µg/mL)			
	Intravenous administration		Oral administration	
	Mean ± SE	Range	Mean ± SE	Range
0.0833	5.99 ± 0.23	4.96 –7.28	0.38 ± 0.05	0.12 – 0.73
0.25	4.39 ± 0.16	3.21 – 5.19	0.69 ± 0.09	0.19 – 1.76
0.50	3.34 ± 0.16	2.37 – 4.36	1.10 ± 0.10	0.51 – 2.03
1	2.52 ± 0.12	1.79 – 3.67	1.75 ± 0.13	0.86 – 2.79
2	1.70 ± 0.12	1.08 – 2.56	2.04 ± 0.14	1.19 – 3.10
4	1.10 ± 0.09	0.64 – 1.76	1.29 ± 0.11	0.69 – 2.22
8	0.64 ± 0.07	0.30 – 1.31	0.56 ± 0.04	0.34 – 0.88
12	0.30 ± 0.04	0.12 – 0.60	0.32 ± 0.03	0.18 – 0.57
24	0.10 ± 0.01	0.04 – 0.17	0.10 ± 0.01	0.05 – 0.16
36 & Beyond	Below Detection Limit			

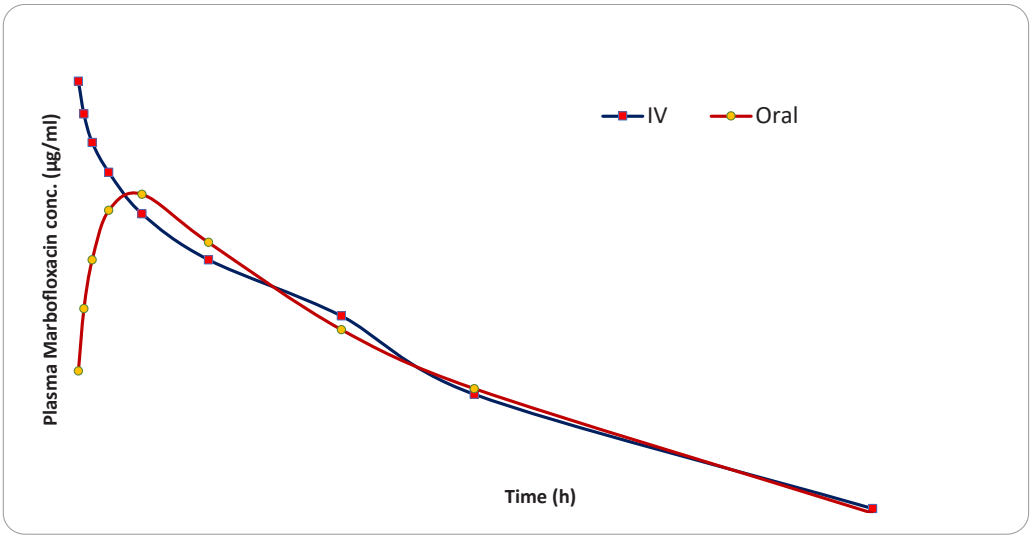

Fig. 1: Semi-logarithmic plot of mean plasma marbofloxacin concentration versus time following single dose intravenous and oral administration in broiler chickens (n=16)

Aboubakr and Abdelazem (2015). However, in mammalian species like dogs, longer elimination half life ranging between 12.5 to 14.7 h was reported (Schneider *et al*., 1996). Variation in half life observed between broiler birds and mammals may be due to differences in metabolic and elimination rate of drug. Glomerular filtration is not constant in birds, as it is in mammals, and likely has an impact on the pharmacokinetics in avian species.

The mean apparent volume of distribution ($V_{d(area)}$) following single dose oral administration was calculated to be 2.64 L/kg in broiler chickens. In contrast to this, lower $V_{d(area)}$ value of 0.96 ± 0.24 L/kg in cat and 1.81 ± 0.08 L/kg in goats were reported by Albarellos *et al.* (2005) and Bhardwaj *et al.* (2018), respectively. This comparison showed that marbofloxacin is having more tissue penetrability in avian species as compared to mammalian species like cat and goat, making it good choice in birds to treat deep-seated bacterial infection in broiler chickens. The mean values of AUC following single dose intravenous and oral administration were 17.47 and 15.13 µg•h/mL, respectively in the present study. The individual values of AUC_{IV} and AUC_{Oral} were used to calculate oral bioavailability which was found to be 88.06 ± 2.36 % as a mean ± SE value. Likewise, high oral bioavailability was also reported as 87.94 % in Japanese quails (Aboubakr and Abdelazem, 2015). The high bioavailability of marbofloxacin in broiler chickens indicates that the drug is useful for oral mass medication in this species.

Table 2: Pharmacokinetic (PK) parameters (mean ± SE) of marbofloxacin following single dose intravenous and oral administration in broiler chickens (n=16)

PK Parameters	*Unit*	*Intravenous route*	*Oral route*
β	Per h	0.12 ± 0.003	0.12 ± 0.01
$t_{1/2\beta}$	h	5.70 ± 0.15	6.03 ± 0.32
C_{max}	µg/mL	*not applicable*	2.19 ± 0.12
T_{max}	h	*not applicable*	1.68 ± 0.12
AUC	µg.h/ mL	17.47 ± 1.27	15.13 ± 0.94
AUMC	$\mu g.h^2$/ mL	114.53 ± 11.85	115.80 ± 8.63
MRT	h	6.36 ± 0.24	7.67 ± 0.34
$V_{d(area)}$	L/kg	2.50 ± 0.15	2.64 ± 0.19
$V_{d(ss)}$	L/kg	1.90 ± 0.09	*not applicable*
Cl_B	L/h/kg	0.31 ± 0.02	0.30 ± 0.02
$F_{(oral)}$	%	*not applicable*	88.06 ± 2.36

(β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; C_{max}: Maximum concentration achieved after oral administration; T_{max}: Time taken to achieve C_{max}; AUC: Area under curve; AUMC: Area under first moment of the plasma drug concentration; MRT: Mean Resident Time; $V_{d(area)}$: Apparent volume of distribution; $V_{d(ss)}$: Volume of distribution at steady state; Cl_B: Total body clearance; $F_{(oral)}$: Oral bioavailability)

The mean residence time (MRT) value (mean ± SE) following single dose oral administration of marbofloxacin was found to be 7.67 ± 0.34 h in the present study. A similar MRT value of 8.97 h was reported in horse (Bousquet-Melou *et al.* 2002). MRT is an important parameter to describe the time period of drug persistence in the body. Total body clearance of the drug following single dose oral administration was found to be 0.30 L/h/kg in the present study. In wild bird species like Blue-Gold macaw, higher clearance rate (Cl_B) of 1.08 L/h/kg was obtained (Carpenter *et al*., 2006). Fluoroquinolones are excreted by renal, biliary, or hepatic metabolic pathways and generally showed slower drug clearance rate in carnivorous species like dogs. In general, herbivores species are well-endowed with oxidative enzyme systems like cytochrome P450 group, thus, providing rapid drug clearance for drug elimination by hepatic metabolism (Toutain *et al*., 2010).

Dosage Derivation

The clinical effectiveness of any antibacterial drug depends on the correct dosage regimen depending on relationship between the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of that drug against a target pathogen. The integrated PK-PD indices like AUC/MIC and C_{max}/MIC ratios allow the prediction of the efficacy and potency of a concentration-dependent drug like marbofloxacin (Bhardwaj *et al*., 2018). The AUC/MIC ratio serves as predictor of efficacy, exhibiting rate of clinical cure > 80% when this ratio is higher than 100–125, which would cover important Gram negative pathogens. The other PK-PD index, C_{max}/MIC if having value ≥10, would lead to successful clinical results and are helpful in minimizing the risk of bacterial resistance. The calculated mean values of two important PK-PD indices for concentration-dependent antimicrobial drugs like marbofloxacin *i.e.* AUC/MIC and C_{max}/MIC for five different MICs are summarized in Table 3.

Table 3: Values of PK-PD indices *viz.* AUC/MIC and C_{max}/MIC calculated for marbofloxacin (5.0 mg/kg b.wt.) following oral administration in broiler chickens (n=16)

PK-PD Index	*Value*	*PK-PD Index*	*Value*
AUC/MIC^1	504.48	C_{max}/MIC^1	73.08
AUC/MIC^2	252.24	C_{max}/MIC^2	36.54
AUC/MIC^3	126.12	C_{max}/MIC^3	18.27
AUC/MIC^4	75.67	C_{max}/MIC^4	10.96
AUC/MIC^5	30.27	C_{max}/MIC^5	4.39

(MIC^1: 0.03 µg/mL; MIC^2: 0.06 µg/ mL; MIC^3: 0.12 µg/ mL

MIC^4: 0.20 µg/ mL and MIC^5: 0.50 µg/mL)

From the Table 3, it is clear that highest MIC value showing AUC/MIC ratio more than 125 and C_{max}/MIC ratio more than 10, is 0.12 µg/mL. Thus, it can be interpreted that marbofloxacin administered @ 5.0 mg/kg b.wt. in broiler chickens, once a day (to be repeated every 24 h) would be optimal dosage regimen for successful treatment of bacterial infections due to pathogens with MIC upto 0.12 µg/mL. This MIC range includes pathogens like *Escherichia coli* and *Pasteurella multocida* causing colibacillosis and fowl cholera in broiler chickens.

Conclusions

Marbofloxacin exhibited favourable oral pharmacokinetic behaviour in broiler chickens to be used as therapeutic drug against susceptible bacterial infections, as it showed long half-life (6.03 h), high volume of distribution (2.64 L/kg) and good oral bioavailability (88.10 %). The pharmacokinetic characteristics of marbofloxacin are different in avian species to that of mammalian species. The average plasma concentrations of marbofloxacin more than 0.32 and 0.10 µg/mL were maintained upto 12 h and 24 h, respectively following oral dose administration. Based on PK-PD indices, oral dose of marbofloxacin @ 5.0 mg/kg b.wt., to be repeated at 24 h, would be efficient to treat common bacterial pathogens of broiler chickens having MIC ≤ 0.12 µg/mL.

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