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Disposition kinetic behaviour of marbofloxacin in broiler chickens

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Abstract
Marbofloxacin, a 'veterinary-use only' antimicrobial drug is having potential to treat bacterial infections in poultry species. The present study was aimed at to explore disposition kinetic behaviour of marbofloxacin following its single dose intravenous administration (5 mg/kg body weight) in broiler chickens. The concentrations of marbofloxacin in plasma samples were analyzed by Ultra High Performance Liquid Chromatography (UHPLC) and the data collected were subjected to non-compartmental kinetic analysis. Following single intravenous administration, marbofloxacin presented a relatively high volume of distribution ($V_{d(ss)}$ = 1.85 L/kg), suggesting its good tissue penetration, and a good total body clearance (CL_B) value of 0.31 L/h/kg, along with long elimination half-life ($t_{1/2\beta}$ = 5.54 h). The average value for area under curve (AUC), area under the first moment of the plasma drug concentration (AUMC) and mean residence time (MRT) were 17.03 µg·h/ml, 106.95 µg.h^2/ml and 6.09 h, respectively. The disposition parameters were favourable for the use of drug in poultry species.

Keywords: Broiler chickens, disposition kinetics, intravenous, marbofloxacin

Introduction
One of the agricultural sectors in India with the fastest growth rates is poultry, which serves as an excellent source of high-quality protein to meet dietary needs (Chatterjee and Rajkumar, 2015) [1]. The chicken industry is seriously threatened by bacterial infections which may hamper production targets; however, chicken producers have seen significant improvements in growth rate, feed efficiency, and mortality reduction in diseased flocks because to the therapeutic use of antimicrobial drugs (Hasan *et al.*, 2010) [2]. A wide variety of fluoroquinolones with outstanding broad-spectrum action against gram-negative as well as gram-positive bacteria and mycoplasma species are being used in the antimicrobial therapy for poultry (Hossain *et al.*, 2017) [3]. Marbofloxacin is a third-generation fluoroquinolone drug which is developed exclusively for veterinary use (Elzoghby and Aboubakr, 2015) [4]. It exhibits bactericidal action by targeting the bacterial DNA topoisomerases II (gyrase) and IV, which are responsible for supercoiling of DNA (Fernandez-Varon *et al.*, 2021) [5]. The objective of the current investigation was to study the disposition kinetic behaviour of marbofloxacin after single dose intravenous administration in broiler chickens.

Materials and Methods
Experimental birds
Eight healthy male broiler chickens weighing more than 1 kg were used in the experiment. The birds were monitored in quarantine for 10 days prior to the actual experimentation. IAEC (Institutional Animal Ethics Committee) of College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar approved the experimental protocol. Birds were kept at Poultry Unit, CVSc & AH, Kamdhenu University, Sardarkrushinagar, Gujarat. 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". A system of individual cages was used, and cage labels were used to identify the birds. Birds were fed the antibiotic-free grower and finisher ration. All of the experimental birds received unlimited access to clean, drinkable drinking water.

Drugs and Chemicals
The standard drug powder for marbofloxacin was procured from Nexia Enterprise, Mumbai, India. Water, methanol, tri-ethylamine (TEA), formic acid, acetonitrile and perchloric acid of HPLC grade were purchased from S.D. Fine Chemicals Ltd., Mumbai.

Experimental protocol

Total eight birds were used for the pharmacokinetic study of marbofloxacin. All birds (P1, P2, P3, P4, P5, P6, P7 & P8) were given drug by IV route. For PK study of marbofloxacin following single dose intravenous administration, about 0.5 - 0.6 ml blood was collected from each bird. Blood samples were collected from wing veins in heparinized sterilized test tube at 0 minute (pre-administration), 5 minutes (0.083 hours), 15 minutes (0.25 hours), 30 minutes (0.5 hours), 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 36 hours and 48 hours using 23 G needle. The plasma was separated after centrifugation of blood samples at 4000 RPM for 10 minutes. The plasma samples were transferred to 2 ml micro-centrifuge tubes and stored at -20ºC until assayed for marbofloxacin concentration using UHPLC assay.

Drug assay

In the current study, Dionex ultimate 3000® UHPLC system (Thermo Fisher, Germany) with an ultraviolet (UV) detector was used to assay the marbofloxacin concentration in plasma. Reverse phase C18 column (GL Science Inc., Japan, 4.6 mm ID) chromatographic separation was carried out at ambient temperature (30 - 32ºC). The sample injection loop had a 50 µl capacity. Software called "Chromeleon TM version 6.8" performed the data integration. After a minor change, in the method described by Carpenter *et al.* (2006) [6], chromatographic conditions were employed for the UHPLC detection of marbofloxacin. Buffer part of mobile phase was prepared by mixing formic acid with HPLC grade water to yield strength of 0.01 M formic acid buffer having final pH of 3.7 using tri-ethylamine (TEA). Before usage, it was degassed using an ultrasonic sonicator and filtered using filter paper (0.45 m pore size) using a vacuum pump. To prevent carry over effect, the HPLC sample syringe was intermittently washed during the sample run.

A method reported by Patel *et al.* (2018) [7] with a small modification was used to extract plasma samples for marbofloxacin quantification. For each sample, 150 µl of plasma were precisely measured out and transferred to 2 ml Eppendorf® micro-centrifuge tubes at room temperature. Then 150 µl of 20% perchloric acid was added, and the liquid was vortexed for 1 minute at 2400 RPM. The mixture was then centrifuged using a refrigerated centrifuge machine at 10,000 rpm for 10 minutes at 4 °C. Finally, 50 µl of the extracted sample as clear supernatant were manually feed into the UHPLC system for the drug assay. The marbofloxacin was detected from plasma at the average retention time of 5.6 min. Quantification of marbofloxacin in plasma samples was done in reference to the standard curve generated. The assay was sensitive and reproducible and linearity was observed from 0.0390 to 10 µg/ml with mean correlation coefficient (R^2) value of 0.9993. Precision and accuracy were estimated by analyzing three replicates at three different concentrations of plasma standards, i.e. 0.10, 1.00 and 10.00 µg/ml. The intra-day and inter-day coefficients of variation for three samples were satisfactory, with relative standard deviations (RSD) less than 4.10%.

Pharmacokinetic and statistical analysis

Pharmacokinetic (PK) parameters were computed with the software 'PK Solver 2.0'. It is a menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications (VBA). Non-compartmental analysis performed by this software is based on the basic theory of statistical moment concepts. The mean values along with standard error (Mean ± SE) were calculated for the plasma concentrations analyzed and data generated for important pharmacokinetic parameters.

Result and discussion

The assayed plasma concentrations of marbofloxacin at different time points after its single dose intravenous (IV) administration @ 5.0 mg/kg b.w. in broiler chickens are tabulated as the mean and standard error (S.E.) values with their range for all eight broiler chickens in Table 1. The semi-logarithmic graph of mean marbofloxacin concentration in plasma *versus* time following intravenous administration is appraised in Figure 1.

Table 1: Mean Plasma concentrations of marbofloxacin (5.0 mg/kg b.w.) with range following single dose intravenous administration in broiler chickens (n=8)

Time point (h)	Plasma marbofloxacin concentration (µg/ml)	
	Mean ± S.E.	Range
0.0833	6.13 ± 0.39	4.07 - 7.28
0.25	4.07 ± 0.21	3.32 - 5.03
0.50	3.24 ± 0.22	2.51 - 4.36
1	2.48 ± 0.23	1.97 - 3.67
2	1.65 ± 0.16	1.08 - 2.56
4	1.07 ± 0.12	0.75 - 1.76
8	0.58 ± 0.09	0.35 -1.02
12	0.29 ± 0.05	0.17 - 0.56
24	0.09 ± 0.02	0.04 - 0.17
36 & Beyond	Below Detection Limit	

Mean marbofloxacin concentration in plasma at first collection time point *i.e.* 0.0833 h (5 minutes) after single dose intravenous (IV) administration at dose rate of 5.0 mg/kg b.w. in broiler chickens was found to be 6.13 µg/ml which was declined by almost half to 3.24 µg/ml at 0.5 h. The mean values of plasma concentrations at 0.0833, 0.25, 0.50, 1, 2, 4, 8, 12 and 24 h were observed to be 6.13, 4.07, 3.24, 2.48, 1.65, 1.07, 0.58, 0.29 and 0.09 µg/ml respectively. The plasma concentrations were not detectable in the samples beyond 24 h collected at 36 and 48 h.

In the present study, mean plasma marbofloxacin concentration following single IV dose administration at 5.0 mg/kg b.w. of broiler chicken was above 0.25 µg/ml up to 12 h (0.29 µg/ml), which can cover MIC_{90} 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) [8, 9]. Similar trend of plasma drug concentration-time profile for marbofloxacin in broiler chickens after IV administration at the same dose (5.0 mg/kg b.w.) was also accounted by Patel *et al.* (2018) [7], and likewise, MIC_{90} cut-off point for *E.coli* (0.25 µg/ml) was maintained up to 12 h.

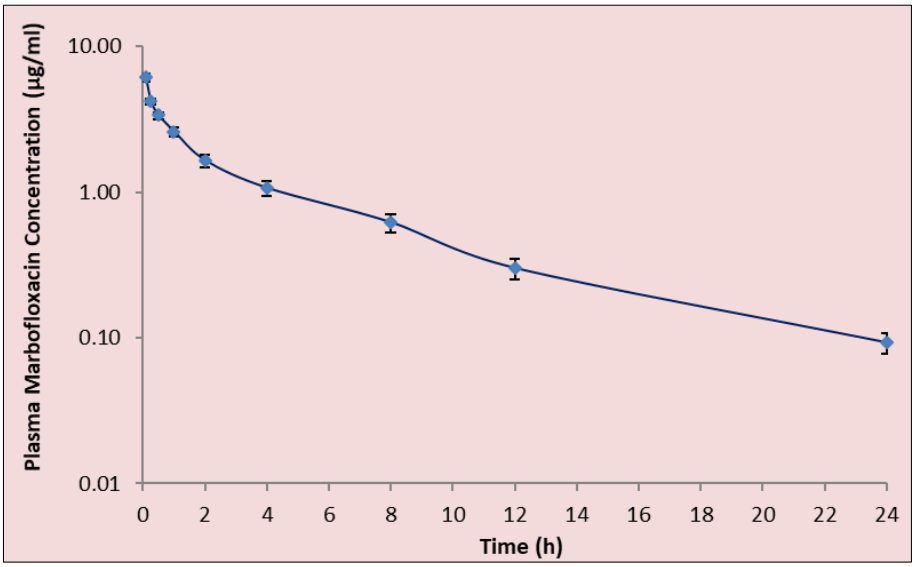

Fig 1: Semi-logarithmic plot of mean plasma marbofloxacin concentration *versus* time following single dose intravenous administration (5.0 mg/kg b.w.) in broiler chickens (n=8)

The pharmacokinetic (PK) parameters calculated from plasma concentrations of marbofloxacin after its single dose IV administration (5.0 mg/kg b.w.) in broiler chickens are shown in Table 2.

Table 2: Pharmacokinetic (PK) parameters of marbofloxacin following single dose intravenous administration (5.0 mg/kg b.w.) in broiler chickens (n=8)

PK Parameters	Unit	Values	
		Mean ± S.E.	Range
B	Per h	0.13 ± 0.004	0.12 - 0.15
$t_{1/2\beta}$	(h)	5.54 ± 0.15	4.72 - 6.00
$AUC_{0-\infty}$	µg.h/ml	17.03 ± 1.72	12.59 -26.70
AUMC	$\mu g.h^2/ml$	106.95 ± 15.69	60.76 - 191.66
MRT	h	6.09 ± 0.30	4.74 - 7.18
$V_{d(area)}$	L/kg	2.47 ± 0.18	1.62 - 3.03
$V_{d(ss)}$	L/kg	1.85 ± 0.09	1.34 - 2.18
Cl_B	L/h/kg	0.31 ± 0.03	0.19 - 0.40

(β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; $AUC_{0-\infty}$: 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)

The mean value of elimination rate constant (β) varied from 0.12/h to 0.15/h with mean value of 0.13/h. The mean value of half-life in the present study was calculated to be 5.54 h. Anadon *et al.* (2002) [10], El-Komy *et al.* (2016) [11] and Patel *et al.* (2018) [7] reported almost similar mean values of elimination half-life as 5.26, 5.43 and 5.55 h, respectively, for marbofloxacin after IV administration in broiler chickens, but in wild species of bird like vulture, higher value of elimination half-life ($t_{1/2\beta}$ = 12.51 h) was reported by Garcia-Montijino *et al.* (2011) [12] whereas low value of half-life as 1.47 h was reported in ostriches (De Lucas *et al.*, 2005) [13]. In mammalian species like dog, longer half-life (8.08 h) was reported (Yohannes *et al.*, 2015) [14]. The species variation for half-life is clearly evident and this may be due to variation in metabolism and excretion patterns of drug among different species of animals. Likewise half-life, a long mean value of mean residence time (MRT = 6.09 h) was observed in the present study which indicates good persistency of drug in body required for optimum therapeutic effects.

The average value for area under curve (AUC) of plasma marbofloxacin concentration *versus* time was found to be 17.03 µg·h/ml and mean value for area under the first moment of the plasma drug concentration (AUMC) was obtained to be 106.95 $\mu g \cdot h^2/ml$. Comparative lower value of AUC (14.7 µg·h/ml) in horse was reported by Tohamy and El-Gendy (2013) [15] whereas higher AUC value (26.03 µg·h/ml) in Japanese quails was reported by Aboubakr and Abdelazem (2015) [16] after IV administration at the same dose rate (5 mg/kg b.w.). The difference in AUC values might be related to species variation in physiology of fluid compartment and total physiological body space available to drug. The absorption patterns, lipid solubility of drug and specific tissues barriers influences the value of AUC. The mean values of apparent volume of distribution ($V_{d(area)}$) and volume of distribution at steady state ($V_{d(ss)}$) were 2.47 and 1.85 L/kg, respectively, indicating excellent tissue penetration property of marbofloxacin in broiler chickens. However, lower value of $V_{d(ss)}$ as 1.30 L/kg in broiler chickens was reported by Patel *et al.* (2018) [7] as compared to present study. However, in both the species the volume of distribution is very high, endorsing the wide distribution of marbofloxacin.

The total body clearance (Cl_B) is the sum of clearance of drug from each organ by the elimination processes including hepatic biotransformation and renal excretion. The mean value for total body clearance (Cl_B) in the present study was 0.31 L/h/kg (5.16 ml/min/kg). The reported clearance of marbofloxacin in horse (0.34 L/h/kg) (Tohamy and El-Gendy, 2013) [15] was comparable to the clearance value of present study. However, very high clearance rates (2.19 L/h/kg) were reported in domestic ostriches (De Lucas *et al.*, 2005) [13]. Faster drug clearance from the body in poultry than dog (0.23 L/h/kg) (Yohannes *et al.*, 2015) [14] seems to be main reason for shorter elimination half-life of marbofloxacin in dogs than the later species. It is clearly evident that, there is a marked interspecies difference, between dogs (mammal) and broiler chickens (avian), in the disposition kinetics of marbofloxacin given by the IV route. To sum up and conclude the findings, marbofloxacin exhibits optimum disposition kinetic behaviour in poultry to be used as therapeutic drug.

References

1. Chatterjee RN, Rajkumar U. An overview of poultry

production in India. Indian Journal of Animal Health. 2015;54(2):89-108.
2. Hasan AR, Ali MH, Siddique MP, Rahman MM, Islam MA. Clinical and laboratory diagnoses of common bacterial diseases of broiler and layer chickens. Bangladesh Journal of Veterinary Medicine. 2010;8(2):107-115.
3. Hossain M, Park HC, Jeong K, Kim DG, Kang J, Lee KJ. Pharmacokinetic and pharmacodynamic evaluation of marbofloxacin in pig against Korean local isolates of *Actinobacillus pleuropneumoniae*. BioMed Research International; c2017. p. 1-11.
4. Elzoghby RR, Aboubakr M. Pharmacokinetics, urinary excretion and milk penetration of marbofloxacin in lactating buffaloes. The Journal of American Science. 2015;11(4):23-28.
5. Fernandez-Varon E, Garcia-Romero E, Serrano-Rodriguez JM, Carceles CM, Garcia-Galan A, Carceles-Garcia C, *et al*. PK/PD analysis of marbofloxacin by monte carlo simulation against *Mycoplasma agalactiae* in plasma and milk of lactating goats after IV, SC and SC-Long acting formulations administration. Animals. 2021;11(4):1104.
6. Carpenter JW, Hunter RP, Olsen JH, Henry H, Isaza R, Koch DE. Pharmacokinetics of marbofloxacin in Blue and Gold Macaws (*Ara ararauna*). American Journal of Veterinary Research. 2006;67(6):947-950.
7. Patel HB, Patel UD, Modi CM, Bhadarka DH. Pharmacokinetics of marbofloxacin following single and repeated dose intravenous administration in broiler chickens. International Journal of Current Microbiology and Applied Sciences. 2018;7(6):2344-2351.
8. Spreng M, Deleforge J, Thomas V, Boisrame B, Drugeon H. Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use against canine and feline isolates. Journal of Veterinary Pharmacology and Therapeutics. 1995;18(4):284-289.
9. Kroemer S, Galland D, Guerin-Faublee V, Giboin H, Woehrle-Fontaine F. Survey of marbofloxacin susceptibility of bacteria isolated from cattle with respiratory disease and mastitis in Europe. Veterinary Record. 2012;170(2):53-53.
10. Anadon A, Martinez-Larranaga MR, Diaz MJ, Martinez MA, Frejo MT, Martinez M, Castellano VJ. Pharmacokinetic characteristics and tissue residues for marbofloxacin and its metabolite N-desmethyl-marbofloxacin in broiler chickens. American Journal of Veterinary Research. 2002;63(7):927-933.
11. El-Komy A, Attia T, El-Latif AA, Fathy H. Bioavailability pharmacokinetics and residues of marbofloxacin in normal and *E. coli* infected broiler chicken. International Journal of Pharmacology and Toxicology. 2016;4(2):144-149.
12. Garcia-Montijano M, Waxman S, de Lucas JJ, Luaces I, de San Andres MD, Rodriguez C. Disposition of marbofloxacin in vulture (*Gyps fulvus*) after intravenous administration of a single dose. Research in Veterinary Science. 2011;90(2):288-290.
13. De Lucas JJ, Rodriguez C, Waxman S, Gonzalez F, Uriarte I, San Andres MI. Pharmacokinetics of marbofloxacin after intravenous and intramuscular administration to ostriches. The Veterinary Journal. 2005;170(3):364-368.
14. Yohannes S, Awji EG, Lee SJ, Park SC. Pharmacokinetics and pharmacokinetic/pharmacodynamic integration of marbofloxacin after intravenous and intramuscular administration in Beagle dogs. Xenobiotica. 2015;45(3):264-269.
15. Tohamy MA, El-Gendy AAM. Some pharmacokinetic aspects and bioavailability of marbofloxacin in foals. Beni-Suef University Journal of Basic and Applied Sciences. 2013;2(1):46-50.
16. Aboubakr M, Abdelazem AM. Pharmacokinetics of marbofloxacin in Japanese quails (*Coturnix japonica*) after different routes of administration. Journal of American Science. 2015;11(4):136-142.