

The disposition of marbofloxacin after single dose intravenous, intramuscular and oral administration to Muscovy ducks

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(Paper received 5 February 2010; accepted for publication 10 July 2010)

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Marbofloxacin is a fluoroquinolone antimicrobial agent developed exclusively for veterinary use. Marbofloxacin exhibits bactericidal activity against a broad spectrum of aerobic gram-negative and some gram-positive bacteria, and also against *Mycoplasma* spp. (Hannan *et al.*, 1997). Fluoroquinolones exhibit bactericidal action by targeting the bacterial DNA topoisomerases II (gyrase) and IV, which is responsible for supercoiling of DNA around RNA core to provide a suitable spatial arrangement of DNA within the bacterial cell (Drlica & Zhao, 1997). Like other fluoroquinolones, marbofloxacin is an organic acid with good tissue penetration, high volume of distribution, low binding to plasma proteins and activity at very low concentrations.

Marbofloxacin has similar or better antibacterial activity and shows a broad antibacterial spectrum compared with published data on the other fluoroquinolones (Spreng *et al.*, 1995). Marbofloxacin differs in particular from other fluoroquinolones on account of its oxadiazine ring, which is supposed to give the molecule some pharmacokinetic advantages such as a long elimination half-life ($t_{1/2\beta}$) and high bioavailability (Fitton, 1992).

The pharmacokinetic properties of marbofloxacin have been reported in several animal species like broiler chickens (Anadon *et al.*, 2002), ostriches (De Lucas *et al.*, 2005) and turkey (Haritova *et al.*, 2006). However, there have been no previous reports for marbofloxacin pharmacokinetics in Muscovy ducks. The Muscovy duck raised intensively in several parts of the world for meat production is not without attendant veterinary problems. Intorre *et al.*, 1997 reported that pasteurellosis and respiratory colibacillosis are very common infectious diseases that are responsible for high rates of mortality and morbidity in many avian species, including ducks, with important economic implications for breeding. In view of the marked species variations in the kinetic data for antimicrobial drugs, this study was undertaken to determine the disposition kinetics of marbofloxacin in Muscovy ducks after a single intravenous, intramuscular and oral administration of 2.0 mg/kg body weight. Meanwhile, plasma protein binding and bioavailability of marbofloxacin were estimated. Additionally, an appropriate dosage regimen of marbofloxacin in Muscovy ducks was suggested using the surrogate markers of pharmacokinetic-pharmacodynamic integration.

Marbofloxacin was used as 10% injectable aqueous solution purchased from Veterinary Pharmaceutical Laboratories, France (Marbocyl[®], Vetoquinol, Lure, France) (diluted to 0.5% in 5% dextrose solution for an accurate dosing) for i.v. and i.m. administration. The same sterile formulation was diluted with sterile distilled water to 1% and then used for oral administration. Marbofloxacin standard was provided by Vetoquinol (Lure, France), and ofloxacin (internal standard) was purchased from Sigma Chemical Company (St. Louis, MO, USA).

We used thirty clinically healthy male Muscovy ducks that were 16-week old, weighing 4.2 ± 0.36 kg and were obtained from a private farm, 2 weeks before the start of the study. During the acclimatization, they were fed antibacterial-free balanced commercial rations and drinking water was freely available. Also during the acclimatization period, the health status of Muscovy ducks was checked by daily observations without any clinical signs of disease being seen and did fecal examination to be free from intestinal parasites. The study was reviewed and approved by the Institutional Animal Care and Use Committee at Faculty of Veterinary Medicine.

Thirty Muscovy ducks were individually weighed before drug administration, and individual doses were calculated precisely. The Muscovy ducks were allocated to three equal groups of 10 each. Marbofloxacin was administered at a dosage of 2 mg/kg of body weight, according to the manufacturer's instructions of other animal species. Birds in group one was given a single i.v. dose of marbofloxacin at a dose of 2 mg/kg into the left brachial vein. Birds in the other two groups were given the same dose of marbofloxacin by i.m. and oral routes through the left semi-membranosus muscle, using a 23-gauge needle and into the crop by means of a feeding tube consisted of rubber catheter fit with a syringe, respectively. Half to one microliter venous whole blood samples were collected from the wing vein into 5-mL heparinized vacutainers (Becton Dickinson vacutainer Systems, Rutherford, NJ, USA). The sampling times were 0 (blank sample), 0.16, 0.25, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30 and 48 h after administration of marbofloxacin by i.v., i.m. and oral. All the blood samples were centrifuged at 3000 *g* for 15 min to separate the plasma. The plasma samples were frozen at -20 °C until analyzed by high-performance liquid chromatography (HPLC).

The plasma concentrations of marbofloxacin were determined using a reverse phase high-performance liquid chromatography with UV detection (295 nm) using the method described by Schneider *et al.*, 1996 and Abo-El-Sooud & Goudah, 2010. Separation was achieved by Discovery C₁₈ reverse-phase column (150 × 4.6 mm, 5 μm (Supelco, Bellefonte, PA, USA). The mobile phase was constituted of a mixture of acetonitrile/buffer citrate pH = 3.0 (15/85).

The calibration curve of plasma was prepared with seven different concentrations between 0.02 and 10 μg/mL, using blank Muscovy ducks plasma. The plasma standard curve of marbofloxacin was linear for concentrations ranging from 0.02 to 10 μg/mL.

The intra-assay and the inter-assay variation coefficients were <6 and <7.4, respectively, for plasma. Recovery of marbofloxacin from plasma was found to be 98%. The limit of quantification (LOQ) of marbofloxacin for plasma was 0.02 μg/mL.

The extent of plasma protein binding was determined *in vitro* using ultrafiltration (Craig & Suh, 1991); antimicrobial-free plasma from ducks fortified with known concentrations of marbofloxacin ranging between 0.02 and 5 μg/mL were used. Plasma samples and their corresponding ultrafiltrates were assayed by the same method (HPLC). The percentage of plasma protein binding was calculated according to the following equation:

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

A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration–time curves for each individual bird following the administration of marbofloxacin. For intravenous injection, the appropriate pharmacokinetic model was determined by the visual examination of individual concentration–time curves and by application of Akaike's information criterion (AIC) (Yamaoka *et al.*, 1978). The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively) and the volume of distribution at steady-state (V_{dss}) were calculated using standard equations (Gibaldi & Perrier, 1982).

Marbofloxacin plasma disposition curves after intramuscular and oral administration were analyzed, following the same procedure as used for intravenous analysis. Each individual curve of marbofloxacin over time was analyzed to determine the peak concentration (C_{max}) and the time to peak concentration (T_{max}). The program also calculated the noncompartmental parameters using the statistical moment theory (Yamaoka *et al.*, 1978). The terminal elimination half-life ($t_{1/2el}$) and absorption half-life ($t_{1/2ab}$) were calculated as $\ln 2/K_{el}$ or $\ln 2/K_{ab}$, respectively. The area under plasma 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 systemic clearance was calculated as $Cl = \text{Dose}/\text{AUC}$. The absolute bioavailability (F) was calculated as $\text{AUC}_{i.m.or} / \text{AUC}_{i.v} \times 100$.

The efficacy of marbofloxacin was determined by calculating the $C_{max} / \text{MIC}_{90}$ and $\text{AUC}_{0-24} / \text{MIC}_{90}$ ratios, following i.m. and oral administration of the drug using minimum inhibitory concentration (MIC) of enrofloxacin for *Pasteurella spp* in ducks ≤ 0.03 μg/mL (Watts *et al.*, 1993).

The statistical analysis was performed using the SPSS[®] 17.1 software package (SAS, Cary, NC, USA). Results are presented as mean ± SD. The nonparametric Wilcoxon test was used to compare the parameters obtained after i.v., i.m. and oral administration. Means were considered significantly different at $P < 0.05$.

Clinical examination of all birds before and after each trial did not reveal any abnormalities. None of the birds had treatment related adverse effects during the study. Akaike's information criterion test indicated that a two-compartment model best represented the plasma concentration versus time data after i.v., i.m. and oral administration of marbofloxacin in Muscovy ducks.

The Mean ± SD plasma concentration–time profiles of marbofloxacin following single i.v., i.m. and oral administrations of 2 mg/kg body wt are illustrated in Fig. 1. Mean ± SD values of pharmacokinetic parameters estimated from the curve fitting are shown in Table 1. There were significant differences between i.v. and the other two routes of administration for the distribution rate constant (α , k_{ab}), while there was a significant difference between the elimination half-life ($t_{1/2\beta}$) and mean residence time (MRT) between i.v. and i.m. administration, and there was a significant difference for AUC between i.v. and oral administration. Bioavailability of marbofloxacin after i.m. and oral administration was 103.42% and 72.35%, respectively. *In vitro*, plasma protein binding of marbofloxacin plasma ranged from 16.5% to 21.8% with an average of 18.4%.

Few reports are available on the pharmacokinetics of marbofloxacin in food-producing avian species. In Muscovy ducks, marbofloxacin could be useful to control common infectious diseases like bacterial septicemia and airsacculitis, which are major disease problems in ducks, resulting in substantial economic losses because of high mortality and condemnations.

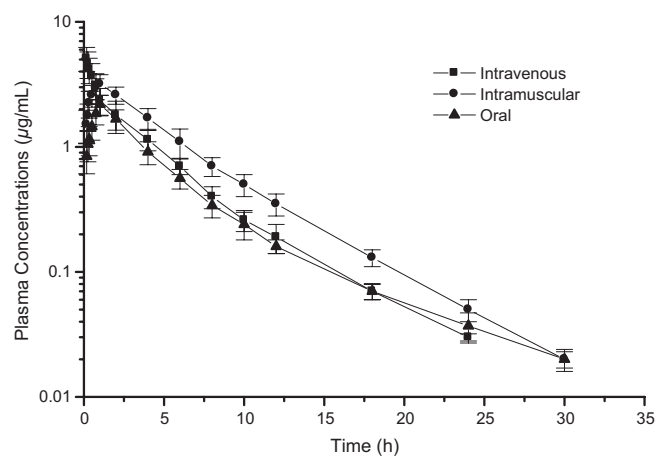


Fig. 1. Mean ± SD plasma Concentration vs. time of marbofloxacin in Muscovy ducks after i.v., i.m. and oral administration of 2 mg/kg body weight ($n = 10$).

Table 1. Mean \pm SD plasma pharmacokinetic parameters of marbofloxacin in Muscovy ducks ($n = 10$) following i.v., i.m. and oral administration at a dosage of 2 mg/kg body weight

Parameters	Unit	i.v.	i.m.	Oral
α (k_{ab})	Per h	2.05 \pm 0.17	2.57 \pm 0.15***	1.91 \pm 0.12*
$t_{1/2\alpha}$ ($t_{1/2ab}$)	h	0.34 \pm 0.12	0.27 \pm 0.13	0.36 \pm 0.13
β (k_{el})	Per h	0.23 \pm 0.12	0.22 \pm 0.11	0.29 \pm 0.11
$t_{1/2\beta}$ ($t_{1/2el}$)	h	2.83 \pm 0.28	3.15 \pm 0.26*	2.67 \pm 0.25
V_{dss}	L/kg	0.57 \pm 0.11	–	–
Cl_{tot}	L·h/kg	0.16 \pm 0.10	–	–
AUC	μ g·h/mL	15.17 \pm 2.19	16.14 \pm 3.40	11.51 \pm 1.94***
AUC _{0–24}	μ g·h/mL	15.03 \pm 2.13	16.10 \pm 2.15	11.26 \pm 1.63***
MRT	h	4.12 \pm 0.31	4.88 \pm 0.36***	3.89 \pm 0.41
C_{max}	μ g/mL	–	3.11 \pm 0.32	1.97 \pm 0.17
T_{max}	h	–	1.02 \pm 0.15	1.15 \pm 0.14
F	%	–	103.42 \pm 9.57	72.35 \pm 10.46

β (k_{el}), elimination rate constant; α (k_{ab}), distribution (absorption) rate constant; $t_{1/2\alpha}$, distribution half-life; $t_{1/2ab}$, absorption half-life; $t_{1/2\beta}$ ($t_{1/2el}$), elimination half-life; V_{dss} , volume of distribution; Cl_{tot} , total body clearance; AUC, area under the curve from zero to infinity by the trapezoidal integral; AUC_{0–24}, area under the curve from zero to 24 h; MRT, mean residence time; C_{max} , maximum plasma concentration; T_{max} , time to peak concentration; F(%), bioavailability. * $P < 0.05$, ** $P < 0.01$.

The causative agents of septicemia and airsacculitis include *Salmonella* spp., *Escherichia coli*, staphylococci and *Pasteurella* spp. Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with these diseases (Watts *et al.*, 1993).

Disposition of marbofloxacin after i.v., i.m. and oral administration in Muscovy ducks were best described by use of a 2-compartment model. In this work, marbofloxacin showed a moderate volume of distribution (V_{dss}), which suggest moderate tissue penetration. The obtained value was close to that recorded by Anadon *et al.*, 2002 in broiler (0.77 L/kg) and lower than those reported in ostriches and turkey (De Lucas *et al.*, 2005 and Haritova *et al.*, 2006), (3.22 and 1.41 L/kg, respectively), while higher values were reported by Dimitrova *et al.*, 2008; and Goudah & Mouneir, 2009 for pefloxacin and danofloxacin 3.74 and 5.41 L/kg, respectively, in ducks. The value of the total body clearance (0.16 L·h/kg) was similar to that reported by Anadon *et al.*, 2002 and Haritova *et al.*, 2006 in broiler and turkey (0.17 and 0.16 L·h/kg, respectively) and lower than those reported in ostriches (De Lucas *et al.*, 2005) 2.19 L·h/kg. Disappearance of marbofloxacin from the plasma of Muscovy ducks was characterized by an initial rapid distribution phase followed by a slower elimination phase. The $t_{1/2\beta}$ of marbofloxacin in Muscovy ducks (2.83 h after i.v. administration) was very much shorter than that obtained in broiler chickens and turkey (Anadon *et al.*, 2002 and Haritova *et al.*, 2006), (5.26 and 7.37 h, respectively), also it is longer than in ostriches (1.47 h) (De Lucas *et al.*, 2005). In comparison with other fluoroquinolones in ducks, this value was similar to that reported by Dimitrova *et al.*, 2008 for pefloxacin (2.84 h) and lower than that reported by Goudah & Mouneir, 2009 (3.91 h) for danofloxacin. These differences might be because of different assay methods, different age of animals, differences between species or different fluoroquinolones. In food animals, reduced persistence of the drug is preferred, as it has less potential to form residues and a lower

half-life could mean shorter withdrawal time. Hence, marbofloxacin could be preferable to other fluoroquinolones in ducks.

When given intramuscularly and orally, marbofloxacin was rapidly and efficiently absorbed in Muscovy ducks, and the time to peak plasma concentrations (T_{max}) were 1.02 and 1.15, respectively, this results were reasonably similar to that reported by De Lucas *et al.*, 2005 and Anadon *et al.*, 2002 for ostriches and broiler chickens 1.02 and 1.48 h, respectively, and lower than that reported by Haritova *et al.*, 2006 following oral administration of marbofloxacin in turkey, 6.00 h.

The observed C_{max} (3.11 and 1.97 μ g/mL following i.m. and oral administration, respectively) obtained in this study were higher than the values reported for the marbofloxacin in broiler chickens, ostriches and turkey by Anadon *et al.*, 2002; De Lucas *et al.*, 2005 and Haritova *et al.*, 2006, 1.05, 1.13 and 0.67 μ g/mL, respectively, and for other fluoroquinolones in ducks (Dimitrova *et al.*, 2008; Goudah & Mouneir, 2009). The obtained AUC value (16.14 μ g·h/mL) following i.m. administration was higher to that recorded for ostriches by De Lucas *et al.*, 2005 (2.25 μ g·h/mL), while the high value of AUC 11.51 μ g·h/mL, following oral administration, was consistent with that reported by Haritova *et al.*, 2006 in turkey (10.89 μ g·h/mL) and higher to that reported by Anadon *et al.*, 2002 (6.71 μ g·h/mL) for broiler. The observed higher C_{max} and AUC have important clinical implications when considering the values of surrogate markers to predict the clinical success of marbofloxacin in ducks (Toutain *et al.*, 2002).

Bioavailability expresses the rate and extent to which a drug administered by any nonvascular route gains access to the systemic circulation (Toutain & Bousquet-Melou, 2004). Intramuscular and oral bioavailability of marbofloxacin were 103.42% and 72.35%, respectively in Muscovy ducks of our study, while De Lucas *et al.*, 2005 and Anadon *et al.*, 2002 recorded lower values for ostriches and broiler (95.03% and

56.82%), respectively, and lower than that reported by Haritova *et al.*, 2006 in turkey 84.77% and higher than that reported for pefloxacin in ducks 68.71% (Dimitrova *et al.*, 2008). These high values indicate excellent absorption of marbofloxacin, following intramuscular and oral administration. The absorption process was rapid with an absorption rate constant (K_{ab}) of 2.57, 1.91/h and absorption half-life ($t_{1/2ab}$) of 0.27, 0.36 h, respectively.

A comparison of our pharmacokinetic results with those from Intorre *et al.*, 1997 and Dimitrova *et al.*, 2008 suggests that the antimicrobial activity of marbofloxacin is comparable to that of enrofloxacin and pefloxacin and slightly differ from danofloxacin (Goudah & Mouneir, 2009) in ducks.

Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in their distribution, excretion and therapeutic effectiveness (Turnidge, 1999). In our study, marbofloxacin displayed a low level of binding to plasma proteins in Muscovy ducks (18.4%). This finding is slightly lower than that obtained by Abo-El-Sooud & Goudah, 2010 in rabbits (26%). This difference may reflect species differences in the number of plasma protein binding sites or their affinity for these drugs (Lin, 1995). As low protein binding generally enables rapid and extensive distribution into the intra and extracellular space to exert its high antibacterial activity.

Although there are no published data concerning antibacterial activity of marbofloxacin against Muscovy ducks isolates, previous studies showed that the *in vitro* and *ex vivo* bactericidal activity of marbofloxacin against animal pathogens were similar (Garcia-Montijano *et al.*, 2006). Based on the available published reports on the value of surrogate markers to predict clinical success, a $C_{max}/MIC \geq 10$ or an $AUC_{0-24}/MIC \geq 125$ has been associated with optimum bactericidal effect. High C_{max}/MIC ratios also have been associated with a lower incidence of resistance development (Walker, 2000; Toutain *et al.*, 2002). Watts *et al.*, 1993 recorded that MIC of enrofloxacin for *Pasteurella spp* in ducks was about $\leq 0.03 \mu\text{g/mL}$. In this investigation, the dosage used of 2 mg/kg provided a plasma C_{max}/MIC ratio of 103.67- and 65.67-fold following i.m. and oral administration, respectively, which is well in excess of the ten value commonly recommended, while the AUC_{0-24}/MIC values for plasma of 536.67 and 375.33 h after i.m. and oral administration, respectively, are also likely to provide a good bacteriological outcome, so that the 2 mg/kg dosage may be regarded as appropriate for treating infections caused by bacteria with $MIC \leq 0.03 \mu\text{g/mL}$.

In conclusion, marbofloxacin i.m. and oral at 2 mg/kg dose showed favorable pharmacokinetic properties such as excellent bioavailability, a high C_{max} , AUC and rapid absorption in Muscovy ducks. Therefore, administration of marbofloxacin at a dosage of 2 mg/kg, intramuscularly and orally, every 24 h could be an effective treatment in ducks for a number of bacterial infections. However, the results need to be carried out in actual field conditions and the MIC concentrations of marbofloxacin for ducks isolates need to be studied to ascertain the aforementioned findings.

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