Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.046

SHORT COMMUNICATION

Pharmacokinetic / Pharmacodynamic Evaluation of Marbofloxacin for Common Pathogens in Swine Following Single Intramuscular Administration of Different Doses

Julie Rambaldi[1], Andrea Barbarossa[1*], Eva Morbidelli[1,2] and Anna Zaghini[1]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum – University of Bologna, Via Tolara di Sopra 50, Ozzano Emilia (BO) 40064, Italy; [2]Fatro S.p.A. Via Emilia, 285, Ozzano Emilia (BO) 40064, Italy
*Corresponding author: andrea.barbrarossa@unibo.it

ARTICLE HISTORY (17-324)

Received: September 12, 2017
Revised: November 25, 2017
Accepted: January 10, 2018
Published online: April 17, 2018

Key words:
Marbofloxacin
One shot
Pig
PK/PD
Tolerability

ABSTRACT

The aims of the present work were to study the pharmacokinetics of marbofloxacin 10% solution after single i.m. administration at doses of 6, 8, 10 mg/kg b.w. in swine of 20 weeks of age, to estimate the PK/PD indices using bibliographic MIC_{90} values and to assess the tolerability of the drug. Marbofloxacin C_{max} increased in a dose dependent manner, however animals receiving the 10 mg/kg b.w. dose revealed slightly lower AUC_i compared to those receiving 8 mg/kg b.w. This suggests a potential alteration of the elimination mechanisms, evidenced by Cl_{tot} values after administration of the three dosages. According to the results of the study, the three tested dosages are well tolerated and active for 2-3 days against the most common pathogens in swine. In particular, 8 mg/kg appeared to be the most suitable dose, since 6 mg/kg might be insufficient against the less susceptible pathogens and 10 mg/kg seemed not to provide any advantage.

To Cite This Article: Rambaldi J, Barbarossa A, Morbidelli E and Zaghini A, 2018. Pharmacokinetic/ pharmacodynamic evaluation of marbofloxacin for common pathogens in swine following single intramuscular administration of different doses. Pak Vet J, 38(2): 222-224. http://dx.doi.org/10.29261/pakvetj/2018.046

INTRODUCTION

Marbofloxacin is a synthetic third-generation fluoroquinolone with a broad spectrum of activity, licensed only for animals. It is particularly indicated to treat respiratory infections, metritis-mastitis-agalactia syndrome, and to treat skin and soft tissue infections in animals (Yang *et al*., 2017). Fluoroquinolones are concentration dependent antimicrobial agents. Their therapeutic success is correlated with both the ratio of the area under the curve and minimum inhibitory concentration (AUC/MIC), and the ratio of maximum concentration and MIC (C_{max}/MIC) (Vilalta *et al*., 2014). Due to this characteristic, in addition to the most traditional i.m. regimen of 2 mg/kg b.w. once daily for 3-5 days, a single dose regimen of 8 mg/kg b.w. was developed. Such regimen should assure the therapeutic success at a population level, minimizing the appearance and development of antimicrobial resistance (Schneider *et al*., 2014). Pharmacokinetics of marbofloxacin has already been described in many species and for different doses, administration routes and formulations. However, information regarding pharmacokinetics/pharmaco-dynamics and tolerability of high doses of i.m. marbofloxacin in pigs are lacking. Thus, the aims of the present work were to determine the pharmacokinetic parameters of marbofloxacin 10% solution after single intramuscular (i.m.) administration at doses of 6, 8, 10 mg/kg b.w. in swine, estimate the PK/PD indices from bibliographic MIC_{90} values and assess marbofloxacin general and local tolerability.

MATERIALS AND METHODS

The trial was approved by the Institutional Animal Care and Use Committee (IACUC) and the Committee for Animal Protection of the Ministry of Health of the Czech Republic (32/2016) and conducted in compliance with Good Laboratory Practice requirements. Eighteen clinically healthy pigs (commercial breed) of 20 weeks old and 52.5-70.0 kg bodyweight were allocated in three experimental groups (A, B and C). Each group consisted of 6 animals: 3 males and 3 females. Pigs were housed in self-contained animal units, fed twice daily with a commercial non-medicated feed; fresh water was available *ad libitum*. Groups A, B and C were treated in parallel with a single i.m. dose of 6, 8, 10 mg/kg b.w., respectively, of marbofloxacin 10% solution (Masterflox 100 mg/mL, solution for injection, FATRO S.p.A., Ozzano dell'Emilia, Bologna, Italy), injected in the neck

area. Approximately 8mL of blood were collected by venipuncture from the jugular veins into heparinized tubes, centrifuged at 3180×*g* for 10 minutes at room temperature and the obtained plasma was stored at -20°C until analysis.

General tolerance was evaluated by clinical observation of health status, appetite and behavior of each subject. Local tolerability was assessed by visual examination and palpation of the injection site area following administration: appearance (e.g. erythema, hair loss, scaling, pigmentation and edema), pain, heat, induration and swelling were evaluated using specific rating scales. Both general and local tolerability were assessed at 1, 2, 3, 12, 24 and 48 hours following marbofloxacin administration.

Marbofloxacin concentrations in plasma were determined using a validated liquid chromatography-tandem mass spectrometry method based on deproteinization with trichloroacetic acid. Chromatographic separation was achieved using an Agilent 1290 HPLC binary pump, with a Phenomenex Synergi 4µm Hydro RP 80A (150 × 3 mm) reversed phase column, fitted with a Phenomenex Luna C18 guard column (4 × 2 mm). The mobile phase consisted of water/acetonitrile 80/20 (*v/v*) with 0.1% formic acid, at a flow rate of 0.4 mL/min. The instrument was coupled with an AB SCIEX API 3200 triple quadrupole mass spectrometer, operating in positive electrospray ionization. Ionspray voltage and source temperature were 5.5kV and 400°C, respectively. The transition observed for marbofloxacin was 363.3>320.0 *m/z*, obtained with a cone voltage of 35V and a collision energy of 35eV. The upper limit of quantification, lower limit of quantification and limit of detection for marbofloxacin were 12,000 ng/mL, 50 ng/mL and 10 ng/mL, respectively. The intra and inter-assay coefficients of variation were always respectively <4.4% and <7.2%. Mean accuracy values were within the range -9.1% and 2.4%. The coefficients of correlation of the calibration curves (R2) were always ≥0.98. Pharmacokinetic parameters for marbofloxacin were calculated using Win Non Lin Version 5.3 software (Pharsight Corporation, CA, USA). The calculated PK/PD indices were AUC/MIC_{90} for selected time intervals (0-24 h, 24-48 h, 48-72 h) divided by 24 h, as suggested by Toutain *et al.* (2007) for long acting formulations, and C_{max}/MIC_{90}. These indices were calculated using MIC_{90} values for the most common pathogens in swine (Grandemange *et al.*, 2017).

RESULTS AND DISCUSSION

No mortality, signs of toxicity, illnesses or behavioral abnormalities were observed throughout the study. Moreover, the clinical examination of the animals and the local observation and palpation of the injection sites confirmed the good general and local tolerability for all the tested doses.

A non-compartmental model with first order absorption best described the marbofloxacin concentration versus time relations after single i.m. dosing (Fig. 1 shows the overlay plot). The main pharmacokinetic parameters of marbofloxacin are listed in Table 1. The drug was rapidly absorbed in all groups and the achieved maximum concentrations were comparable with data available in literature (Ding *et al.*, 2010; Schneider *et al.*, 2014; Yang *et al.*, 2017). As shown in Table 1, C_{max} increased in a dose dependent manner. However, this was not the case for AUC_{24}, differently from what previously reported (Schneider *et al.*, 2014). In such study, pigs of 27 weeks of age administered with 4, 8 and 16 mg/kg b.w. of marbofloxacin i.m. revealed an AUC_i of 56.9, 115 and

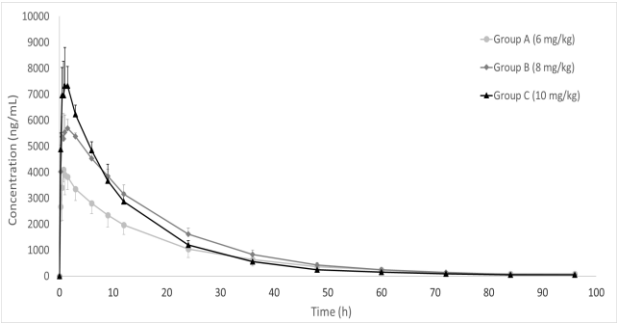

Fig. 1: Plasma concentration vs. time curves of marbofloxacin in pigs after i.m. administration of a single dose of 6, 8 and 10 mg/kg b.w. in groups A (*n* = 6), B (*n* = 6) and C (*n* = 6), respectively. Samples were taken before the treatment, and at 15, 30 and 45 minutes, and 1, 1.5, 3, 6, 9, 12, 24, 36, 48, 60, 72, 84 and 96 hours after the administration of the drug. Error bars indicate SD.

Table 1: Selected pharmacokinetic parameters (mean±SD) obtained from plasma concentrations of marbofloxacin in pigs after i.m. administration of a single dose of 6, 8, 10 mg/kg b.w. in groups A (*n* = 6), B (*n* = 6), and C (*n* = 6), respectively

Parameter	Group A (6 mg/kg)	Group B (8 mg/kg)	Group C (10 mg/kg)
C_{max} (µg/mL)	4.75±0.748	6.15±0.684	8.10±1.568
T_{max} (h)	0.92±0.34	1.00±0.45	1.04±0.40
K_{el} (1/h)	0.04±0.02	0.05±0.01	0.07±0.02
$t_{1/2}$ (h)	20.3±9.9	14.2±2.8	10.9±2.2
Cl_{tot} (L/h·kg)	0.083±0.028	0.071±0.007	0.098±0.013
V_d (mL/kg)	2199±848	1443±269	1546±389
AUC_{0-24} (h·µg/mL)	51.8±9.7	82.0±3.7	83.0±10.0
AUC_{24-48} (h·µg/mL)	28.5±11.7	41.7±9.9	29.8±8.5
AUC_{48-72} (h·µg/mL)	13.6±8.9	16.4±6.2	9.6±4.7
AUC_i (h·µg/mL)	79.5±25.6	113.6±11.2	103.4±14.5

C_{max}: peak concentration; T_{max}: time of peak concentration; K_{el}: rate constant of the elimination phase; $t_{1/2}$: last elimination half-life; Cl_{tot}: total body clearance; V_d: apparent distribution volume; AUC_t: area under the curve to the last quantifiable concentration; AUC_i: area under the curve to infinity.

Table 2: Efficacy predictors (AUC/MIC_{90}·24h and C_{max}/MIC_{90}) estimated for marbofloxacin i.m. at 6, 8, 10 mg/kg against two common pathogens in swine. AUC/MIC ratio is expressed using the AUC extrapolated for the selected time interval and is scaled by the 24 h considered

	AUC/MIC_{90}·24h			C_{max}/MIC
	0-24 h	24-48 h	48-72 h	
Group A (6 mg/kg)				
A. pleuropneumoniae	36	20	9	79
B. bronchispetica	4	2	1	10
H. parasuis	36	20	9	79
P. multocida	72	40	19	159
Group B (8 mg/kg)				
A. pleuropneumoniae	57	29	11	103
B. bronchispetica	7	3	1	12
H. parasuis	57	29	11	103
P. multocida	114	58	23	205
Group C (10 mg/kg)				
A. pleuropneumoniae	58	21	7	135
B. bronchispetica	7	2	1	16
H. parasuis	58	21	7	135
P. multocida	115	41	13	270

MIC_{90}, lowest concentration (µg/mL) of marbofloxacin for which 90% of the isolates were inhibited: *A. pleuropneumoniae* = 0.06; *B. bronchispetica* = 0.5; *H. parasuis* = 0.06; *P. multocida* = 0.03. (Grandemange *et al.*, 2017).

228 µg·h/mL, respectively, showing dose proportionality. In our case, group C (10 mg/kg b.w.) revealed slightly lower AUC_i than group B (8 mg/kg b.w.), suggesting a potential alteration of the mechanisms implied in the elimination phase. The marbofloxacin Cl_{tot} after i.m. administration of 6, 8, 10 mg/kg b.w. were, respectively, 0.083±0.028, 0.071±0.007, and 0.098±0.013 L/h·kg. These values are lower than those obtained in other studies conducted on pregnant and lactating sows, and piglets of 3 month of age (Ding *et al*., 2010). Marbofloxacin is excreted predominantly in urine with minimal biotransformation, and this might explain why in young and pregnant animals the total body clearance is higher, as suggested by Dvorchik (1982). The overall drug extraction ratio (E) was calculated as the ratio between total body clearance and cardiac output, which can be obtained using the relationship: Cardiac output (mL/kg/min) = 180. Body Weight $(kg)^{-0.19}$ (Toutain and Bousquet-Melou, 2004). The E values were below 0.05 for all the tested doses, indicating a low drug extraction ratio. After i.m. administration of 6 mg/kg b.w., the elimination half-life of marbofloxacin was longer (20.3±9.9 h) compared to the injection of 8 and 10 mg/kg b.w. (14.2±2.8 h and 10.9±2.2 h, respectively). This again suggests that the elimination processes may prevail over the distribution processes at higher doses, possibly affected by the decreased reabsorption deriving from the unfavourable concentration gradient at the tubular level. However, it must be remarked that a certain variability was observed among subjects in group A (although the statistical analysis did not reveal any outlier), which may have unexpectedly increased discrepancies with the other two groups for some PK parameters.

To the best of our knowledge, there is a lack of information concerning the PK/PD of high doses of marbofloxacin against swine isolates. It is known that AUC/MIC_{90} and C_{max}/MIC_{90} well correlate with the efficacy of the fluoroquinolones (Craig, 1998; Toutain *et al*., 2007). In the present study, the AUC/MIC_{90} ratios extrapolated for each group at selected time intervals (0-24 h, 24-48 h and 48-72 h) satisfied the requirements to predict a clinical cure (index ≥5) with all the three tested doses (Toutain *et al.*, 2007), although only for infections caused by highly susceptible bacteria (see Table 2). On the contrary, the ratio calculated using the MIC_{90} for the less susceptible *B. bronchiseptica* was above 5 only for the 0-24 h interval, and only at 8 and 10 mg/kg doses. However, in consideration of the prolonged *in vivo* PAE of fluoroquinolones (Craig, 1998) and since AUC/MIC_{90} resulted always >1 no matter what dose, a residual efficacy of the treatments during the 24-72 h interval can be expected. The calculated C_{max}/MIC_{90} values against all the considered pathogens were greater than the recommended threshold of 8-10, which has been shown to prevent the emergence of resistant mutants during therapy with fluoroquinolones (Craig, 1998). In addition, for all the considered pathogens except *B. bronchiseptica* the ratio between plasma concentration and MIC_{90} remained above 10 for at least 36 h for the 8 and 10 mg/kg treatments.

According to literature, the indices of clinical outcome are not sufficiently sensitive for the determination of the optimal dosage regimen for bacteriological treatments. A useful tool to overcome this limitation is represented by PK/PD approaches and surrogate indices in healthy animals (Toutain & Lees, 2007). The results of this study not only provide further data on the pharmacokinetic profile of marbofloxacin in pigs, but also suggest that high doses (6, 8 and 10 mg/kg) of marbofloxacin administered i.m. are well tolerated and active for 2-3 days against the considered pathogens. In particular, 8 mg/kg seems the most adequate dose to treat common infections in swine, since 6 mg/kg might be insufficient against the less susceptible pathogens and 10 mg/kg does not seem to provide any advantage.

Authors contribution: EM, JR and AZ conceived and designed the study. AB and JR analyzed the data and drafted the manuscript. All authors interpreted the data, critically revised the manuscript and approved the final version.

REFERENCES

Craig WA, 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 26:1-10.

Ding H, Li Y, Chen Z, *et al.*, 2010. Plasma and tissue cage fluid pharmacokinetics of marbofloxacin after intravenous, intramuscular and oral single-dose application in pigs. J Vet Pharmacol Ther 33:507-10.

Dvorchik BH, 1982. Drug disposition during pregnancy. Int J Biol Res Pregnancy 3:10-3.

Grandemange E, Perrin PA, Cvejic D, *et al.*, 2017. Randomised controlled field study to evaluate the efficacy and clinical safety of a single 8 mg/kg injectable dose of marbofloxacin compared with one or two doses of 7.5 mg/kg injectable enrofloxacin for the treatment of *Actinobacillus pleuropneumoniae* infections in growing fattening pigs in Europe. Porcine Health Manag 3:10.

Schneider M, Paulin A, Dron F, *et al.*, 2014. Pharmacokinetics of marbofloxacin in pigs after intravenous and intramuscular administration of a single dose of 8 mg/kg: dose proportionality, influence of the age of the animals and urinary elimination. J Vet Pharmacol Ther 37:523-30.

Toutain PL and Bousquet-Melou A, 2004. Plasma clearance. J Vet Pharmacol Ther 27:415-25.

Toutain PL and Lees P, 2007. Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. J Vet Pharmacol Ther 27:467-77.

Toutain PL, Bousquet-Melou A and Martinez M, 2007. AUC/MIC: a PK/PD index for antibiotics with a time dimension or simply a dimensionless scoring factor? J Antimicrob Chemother 60:1185-8.

Vilalta C, Giboin H, Schneider M, *et al.*, 2014. Pharmacokinetic/ Pharmacodynamic evaluation of marbofloxacin in the treatment of *Haemophilus parasuis* and *Actnobacillus pleuropneumoniae* infections in surgery and fattener pigs using Monte Carlo simulations. J Vet Pharmacol Ther 37:542-9.

Yang F, Liu Y, Li Z, *et al.*, 2017. Tissue distribution of marbofloxacin in pigs after a single intramuscular injection. J Vet Sci 18:169-73.