Original Article
J Vet Sci 2017, 18(2), 169-173 · https://doi.org/10.4142/jvs.2017.18.2.169

Tissue distribution of marbofloxacin in pigs after a single intramuscular injection

Fan Yang[1,*,†], Yiming Liu[2,†], Zhili Li[3], Yuqin Wang[1], Baobao Liu[1], Zhensheng Zhao[1], Bianhua Zhou[1], Guoyong Wang[1]

Colleges of [1]Animal Science and Technology, and [3]Food and Bioengineering, Henan University of Science and Technology, Luoyang 471023, China
[2]Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 10081, China

Tissue distribution of marbofloxacin was studied in pigs after a single intramuscular injection at 2.5 mg/kg body weight. Samples of plasma, muscle, liver, kidney, heart, lung, and muscle at the injection site were randomly collected from five pigs at 2, 6, 10, 24, 48, 72, and 96 h after administration. Marbofloxacin concentrations were determined by using high-performance liquid chromatography with ultraviolet detection and were subjected to non-compartmental analysis to obtain kinetic parameters. The elimination half-life ($t_{1/2\lambda z}$) of marbofloxacin at the injection site was 22.12 h, while those in kidney, plasma, liver, lung, heart, and muscle were 16.75, 21.48, 21.84, 24.00, 24.45, and 28.91 h, respectively. Areas under the concentration-time curve from 0 h to ∞ ($AUC_{0-\infty}$s) were calculated to be 31.17 h · μg · mL^{-1} for plasma and 32.97, 33.92, 34.78, 37.58, 42.02, and 98.80 h · μg · g^{-1} for heart, muscle, lung, liver, kidney, and injection site, respectively. The peak concentration (C_{max}) of marbofloxacin was 1.62 μg/mL in plasma and 1.71, 1.74, 1.86, 1.93, 2.45, and 7.64 μg/g in heart, lung, muscle, kidney, liver, and injection site, respectively. The results show that marbofloxacin was fast absorbed, extensively distributed, and slowly eliminated from pigs after a single intramuscular administration.

Keywords: kinetic parameters, marbofloxacin, pigs, tissue distribution

Introduction

Marbofloxacin is a fluoroquinolone antibiotic developed only for application in veterinary medicine [14] that acts by inhibiting bacterial DNA-gyrase. It has been demonstrated that marbofloxacin is potent *in vitro* against Mycoplasma, most Gram-negative and some Gram-positive bacteria, and some intracellular pathogens, but has limited or little activity against anaerobes [3,16]. Most porcine respiratory pathogens, such as *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*, are very sensitive to marbofloxacin. Currently, in China, marbofloxacin has been not licensed for use in pigs. However, because of its spectrum of activity against swine pathogens, it is being used in an extra-label manner to treat pigs' respiratory diseases and sows' metritis–mastitis–agalactia syndrome.

Similar to other fluoroquinolones, marbofloxacin has a low plasma protein binding rate [7], a large volume of distribution with potent activity [6,9], and high drug concentrations in tissues and body fluids [1]. The pharmacokinetics of marbofloxacin in plasma and urine has been studied in pigs under different administration routes [14], and it was shown that marbofloxacin is well absorbed with a bioavailability of 91.5% after intramuscular administration, and its body clearance rate decreases significantly with pig age after intravenous administration. Another study showed easy penetration of marbofloxacin into pig tonsils [19]. In addition, a pharmacokinetics study in pigs' tissue cage fluid demonstrated efficient distribution of marbofloxacin into tissues [5]. Some studies have been published regarding the tissue residues of marbofloxacin in other species [10,12,21]. However, there is a paucity of systematic information in the literature regarding the distribution of marbofloxacin in the respiratory system. Moreover, there is also a paucity of literature regarding the distribution of marbofloxacin in the edible tissues of pigs.

Information about tissue distribution of an antibiotic would be very valuable for improving treatment efficacy and avoiding problems associated with residues. Therefore, the purpose of this study was to investigate marbofloxacin's tissue distribution in pigs after a single intramuscular administration.

Received 23 Feb. 2016, Revised 28 May 2016, Accepted 21 Jul. 2016
*Corresponding author: Tel: +86-379-69884468; Fax: +86-379-64563979; E-mail: yfscau@126.com
†The first two authors contributed equally to this work.
pISSN 1229-845X
eISSN 1976-555X

Materials and Methods

Animals

A total of 40 eight-week-old castrated cross-bred (Duroc × Landrace × Yorkshire) pigs weighing 20 to 23 kg were used in the study. The pigs were allowed to acclimate for seven days with free access to water and a drug-free pelleted diet. Five days before the start of the study, a complete wellness examination including a physical examination and blood samples was performed. Blood was collected from each pig, and a complete blood count (CBC) and chemistry panel (CP) were performed. No clinically significant abnormalities were noted on examination and all blood parameters were within normal ranges. The study (animal study protocol 201506007) was approved by the Institutional Animal Care and Use Committee (IACUC) of Henan University of Science and Technology. All animals were humanely handled.

Chemicals and reagents

Commercial marbofloxacin suitable for intramuscular injection (5 mL: 5 g, Lot No. 091008) was kindly provided by Hebei Yuanzheng Pharmaceutical (China). Marbofloxacin standard (99.82%, Lot No. H050408) was provided by the National Institutes for Food and Drug Control (China). Formic acid and acetonitrile of high-performance liquid chromatography (HPLC) grades were both purchased from Merck (Germany). The other reagents used in this study were all of analytical grades. Deionized water was purified by using a Milli-Q system (Millipore, USA).

Drug administration and sampling

Pigs were randomly divided into eight groups with five pigs in each group. Group 1 was used as a control. Pigs in the other groups were weighed and intramuscularly injected on the right side of the neck with marbofloxacin at a dosage of 2.5 mg/kg body weight. To obtain tissue and plasma samples, five pigs which received the intramuscular injection were randomly killed at 2, 6, 10, 24, 48, 72, and 96 h, respectively, whereas those in control group were sacrificed at 96 h. Samples of plasma and tissues, including muscle, lung, heart, kidney, liver, and muscle at the injection site, were collected from each animal. Tissue samples were thoroughly minced and stored at −20°C prior to use.

Marbofloxacin determination

The marbofloxacin concentrations in plasma and tissues were measured by using a previously described method [20]. Briefly, 6 mL of trichloromethane was used to extract the marbofloxacin from 0.6 mL of plasma. Three grams of homogeneous tissue samples were extracted separately with 3 parts of trichloromethane (5 mL each) and 0.1 M sodium phosphate buffer (pH 7.4; 3 mL each). After shaking for 5 min and centrifugation at 3,180 × g for 10 min, the organic phase was pooled for tissues and collected for plasma, then evaporated using a stream of nitrogen at 30°C and reconstituted in 1 mL mobile phase for tissues and 0.6 mL mobile phase for plasma.

The processed samples (50 μL) was injected into a Hypersil BDS-C18 column (4.6 mm × 250 mm, 5 μm; Elite Analytical Instruments, China) which was kept at 30°C. The mobile phases for HPLC analysis comprised 12.5% water, 12.5% acetonitrile, and 75% buffer which consisted of 1% formic acid and 0.5% triethylamine. Elution flow rate was set as 1 mL/min. An ultraviolet detector set to a wavelength of 295 nm was used to determine marbofloxacin presence.

Tissue kinetic analysis

Plasma and tissues concentrations *versus* time profiles were calculated based on mean marbofloxacin concentrations and non-compartmental modeling by using Phoenix WinNonlin (ver. 6.1; Pharsight, USA). The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated by using a trapezoidal method. The peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the concentration *versus* time profiles. The elimination rate constant (λ_z) was estimated by linear regression of mean drug concentrations *versus* time. The C_{max} values in different tissues were compared by using SPSS (ver. 20.0; IBM, USA), and a multiple-range test was used to determine the significance of differences between mean concentrations. A p value lower than 0.05 was considered significant.

Results

The HPLC method was selective for marbofloxacin, and no endogenous interferences were found on chromatograms. The limit of quantitation (LOQ) for marbofloxacin was determined based on a signal-to-noise ratio greater than 10, and the LOQ values were 0.05 μg/g in tissues and 0.02 μg/mL in plasma. Marbofloxacin concentration was linear in tissues within the range of 0.05−5.00 μg/g ($r > 0.998$) and in plasma within the range of 0.02−5.00 μg/mL ($r > 0.997$). Mean recovery of marbofloxacin ranged from 90.82%−96.43% in plasma and 84.36%−90.27% in tissues. Repeatability was measured as within-run and between-run coefficients of variation, values of which were less than 7.52% in both plasma and tissues.

The concentrations of marbofloxacin in plasma and tissues are presented in Table 1 and Figs. 1−3. Marbofloxacin was detected at injection site, liver, muscle, and plasma up to 96 h after intramuscular administration and in kidney, lung, and heart up to 72 h after intramuscular administration. The highest concentration (7.64 ± 1.06 μg/g) of marbofloxacin was measured in injection site, followed by liver (2.45 ± 0.09 μg/g), kidney (1.93 ± 0.06 μg/g), muscle (1.86 ± 0.08 μg/g), lung (1.74 ± 0.02 μg/g), heart (1.71 ± 0.03 μg/g), and plasma (1.62 ± 0.13

Table 1. Plasma and tissue concentrations (μg/mL and μg/g, respectively) of marbofloxacin in pigs (n = 5) after a single intramuscular injection at 2.5 mg/kg body weight

Time (h)	Injection site	Liver	Kidney	Muscle	Lung	Heart	Plasma
2	7.64 ± 1.06	2.45 ± 0.09	1.93 ± 0.06	1.86 ± 0.08	1.74 ± 0.02	1.71 ± 0.03	1.62 ± 0.13
6	3.95 ± 0.40	1.06 ± 0.06	1.54 ± 0.03	1.14 ± 0.04	1.21 ± 0.02	1.12 ± 0.01	1.09 ± 0.07
10	1.96 ± 0.25	0.75 ± 0.03	1.25 ± 0.06	0.80 ± 0.05	0.75 ± 0.02	0.71 ± 0.02	0.65 ± 0.03
24	0.82 ± 0.03	0.46 ± 0.05	0.54 ± 0.01	0.35 ± 0.03	0.41 ± 0.03	0.39 ± 0.02	0.38 ± 0.01
48	0.36 ± 0.05	0.25 ± 0.01	0.21 ± 0.04	0.18 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.18 ± 0.02
72	0.17 ± 0.03	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
96	0.08 ± 0.03	0.05 ± 0.01	ND	0.07 ± 0.01	ND	ND	0.03 ± 0.01

ND, not detectable.

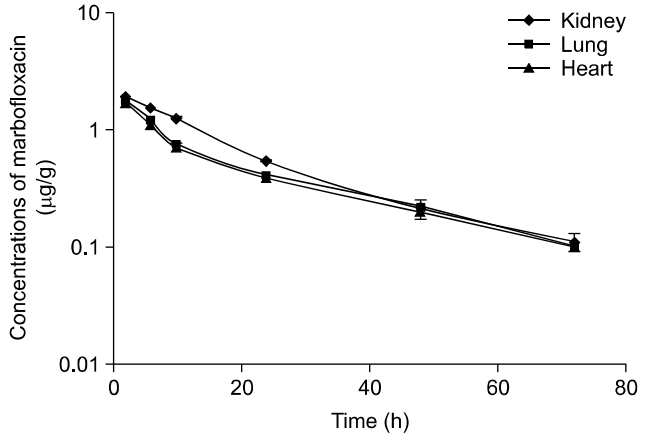

Fig. 1. Concentrations of marbofloxacin in kidney, lung, and heart after a single intramuscular injection (2.5 mg/kg body weight) in pigs.

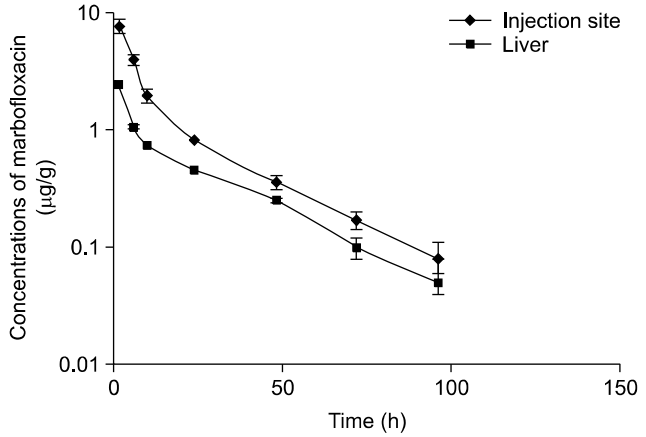

Fig. 2. Concentrations of marbofloxacin in liver and at injection site after a single intramuscular injection (2.5 mg/kg body weight) in pigs.

μg/mL). The kinetic parameters after a single intramuscular injection are listed in Table 2 for injection site, plasma, and each tissue type.

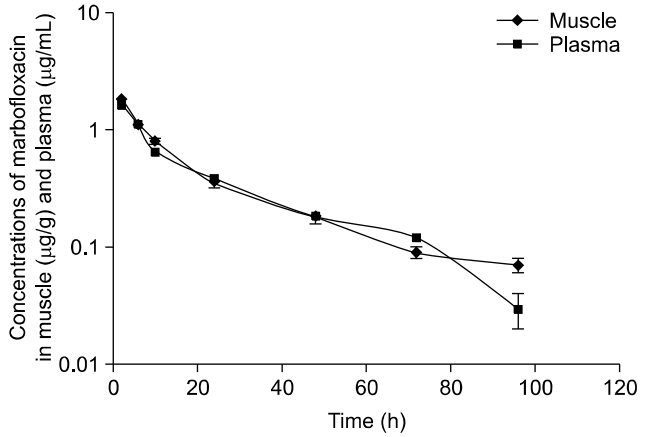

Fig. 3. Concentrations of marbofloxacin in muscle and plasma after a single intramuscular injection (2.5 mg/kg body weight) in pigs.

Discussion

Based on the plasma concentrations *versus* time data, a $t_{1/2\lambda z}$ of 21.48 h was calculated for marbofloxacin when it was intramuscularly injected to pigs at 2.5 mg/kg body weight, a $t_{1/2\lambda z}$ that is longer than those reported by Ding *et al*. [5] (17.3 ± 5.38 h) and Schneider *et al*. [14] (15.1−15.4 h). The total body clearance of marbofloxacin decreases in older pigs [14], which may be the reason for the longer $t_{1/2\lambda z}$ reported here. Pigs used here were eight weeks old and younger than those used by Schneider *et al*. [14]. In addition to age, differences between preparations of marbofloxacin used in the studies may be another reason for the inconsistent $t_{1/2\lambda z}$ results. In addition to that in pigs, marbofloxacin pharmacokinetics have been investigated in other species. After intramuscular injection, the $t_{1/2\lambda z}$ was 1.96, 3.15, 3.65, 7.16, 7.72, and 17.50 h in ostriches [4], ducks [8], sheep [15], camels [11], rabbits [13], and calves

Table 2. Tissue and plasma kinetic parameters of marbofloxacin in pigs (n = 5) after a single intramuscular injection at 2.5 mg/kg body weight

Parameters	Units	Heart	Kidney	Liver	Lung	Muscle	Injection site	Plasma
λ_z	h^{-1}	0.028	0.041	0.032	0.029	0.024	0.031	0.032
$t_{1/2\lambda z}$	h	24.45	16.75	21.84	24.00	28.91	22.12	21.48
T_{max}	h	2	2	2	2	2	2	2
C_{max}	μg/g or μg/mL	1.71 ± 0.03[a]	1.93 ± 0.06[b]	2.45 ± 0.09[b]	1.74 ± 0.02[a]	1.86 ± 0.08[a,b]	7.64 ± 1.06[c]	1.62 ± 0.13[c]
$AUC_{0-\infty}$	h · μg · g^{-1} or h · μg · mL^{-1}	32.97	42.02	37.58	34.78	33.92	98.80	31.17
$AUMC_{0-\infty}$	h^2 · μg · g^{-1} or h^2 · μg · mL^{-1}	975.88	959.94	1027.21	1027.30	1059.90	1818.36	879.75
MRT	h	29.60	22.84	27.34	29.53	31.24	18.40	28.23
AUC_{tissue}/ AUC_{plasma}	Unitless	1.06	1.35	1.21	1.12	1.09	4.71	1
$C_{max\text{-}tissue}$/ $C_{max\text{-}plasma}$	Unitless	1.05	1.19	1.51	1.07	1.14	3.17	1

λ_z, first order rate constant associated with the terminal phase; $t_{1/2\lambda z}$, terminal half-life; T_{max}, time to reach peak concentration; C_{max}, maximum observed concentration; $AUC_{0-\infty}$, area under the concentration-time curve from the time of dosing to infinity; $AUMC_{0-\infty}$, area under the moment curve from the time of dosing to infinity; MRT, mean residence time extrapolated to infinity; $AUC_{tissue}/AUC_{plasma}$, the ratio of $AUC_{0-\infty}$ for tissue to that for plasma; $C_{max\text{-}tissue}/C_{max\text{-}plasma}$, the ratio of C_{max} in tissue to that in plasma. [a,b,c]Values not sharing a common superscript letter are significantly different ($p < 0.05$).

[17], respectively. These results indicate that the elimination of marbofloxacin from pigs is slower than that in other species. Such differences are relatively common and often associated with the following factors: breed, gender, age, body weight, disease, and heritable traits. Pharmacokinetic differences may also result from different eating habits, water intake, and exercise, which may lead to individual differences in blood flow. In a previous report [6], marbofloxacin was given orally to experimentally infected chickens, and its concentrations in tissues (except brain) exceeded those in plasma, which is consistent with the present results. A tissue residue study of marbofloxacin conducted in healthy chickens also indicated that marbofloxacin easily penetrated into all tissues [2].

The ratios of C_{max} in tissues to that in plasma were between 1.05 and 3.17, and the $AUC_{0-\infty}$ values in tissues were also higher than that in plasma (1.06 to 4.71 times higher), indicating that marbofloxacin was easily distributed to tissues. The ratios of $AUC_{0-\infty}$ and C_{max} values in tissues and plasma of infected chickens [6] were both higher than those observed in the present study of pigs. In addition to species difference, the presence of an infection perhaps affected concentrations by increasing the permeability of tissues, leading to more extensive distribution. After one single intramuscular injection, the highest marbofloxacin concentration (7.64 ± 1.06 μg/g) was observed at the injection site, and, thereafter, the concentration presented a sustained downward trend. According to the report by Vilalta *et al*. [18], the intramuscular absorption rate constant of marbofloxacin ranged from 5.06 h^{-1} to 5.85 h^{-1} in pigs. This is consistent with the present results, which also indicate fast absorption of marbofloxacin. Based on the high residual concentrations in the injection site, the withdrawal time of marbofloxacin after intramuscular injection may be longer than that after oral administration. Moreover, the depletion of marbofloxacin residue after multiple intramuscular dosages should be studied to estimate further its withdrawal time in pigs.

When using fluoroquinolones to treat an infection, the ratio between AUC and minimum inhibitory concentration (AUC/MIC) and that between C_{max} and MIC (C_{max}/MIC) are both associated with successful therapeutic resolution. The MICs of marbofloxacin were reported to be 0.03−0.06 μg/mL and 0.015−0.03 μg/mL against *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*, respectively [18]. According to the kinetic parameters determined in the present study, and the MIC data reported by Vilalta *et al*. [18], an application of 2.5 mg/kg marbofloxacin *via* intramuscular administration every 8 h in pigs could provide sufficient plasma concentrations to inhibit *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*. Such a multiple dosing regimen is suggested and should be followed in clinical veterinary medicine.

In conclusion, this study demonstrates that marbofloxacin is rapidDly absorbed, widely distributed, and slowly eliminated from pigs after a single intramuscular dose. Based on the derived kinetic parameters and MICs, intramuscular injection of marbofloxacin at 2.5 mg/kg per 8 h interval might be highly efficacious against susceptible bacteria in pigs.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (grant No. 31402253) and Doctoral Scientific Research Foundation of Henan University of Science and Technology (grant No. 09001677).

Conflict of Interest

The authors declare no conflicts of interest.

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