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Distribution of marbofloxacin within the bronchoalveolar region of healthy pigs

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ABSTRACT. The purpose of this study was to clarify the distribution of marbofloxacin (MBFX) within the bronchoalveolar region of pigs. Four clinically healthy pigs were intramuscularly injected with a single dose of MBFX (2 mg/kg). Samples of plasma and bronchoalveolar lavage fluid (BALF) were obtained for each pig at 0 (before administration), 3, 8 and 24 hr after administration of MBFX. As a result, the MBFX concentrations in pulmonary epithelial lining fluid (ELF) and in alveolar cells showed a similar pattern of concentrations during the experimental period. The MBFX concentrations both in ELF and alveolar cells were higher than in plasma. These results suggest that intramuscularly injected MBFX was well distributed in the bronchoalveolar region.

KEY WORDS: bronchoalveolar lavage fluid, distribution, marbofloxacin, pig

The respiratory diseases generally caused by infection with virus, bacteria, and mycoplasma, are common in modern swine production. Bronchopneumonia and subsequent pleuritis due to either bacterial or *Mycoplasma* spp. infections are frequent lesion of the lung in all age groups of pig [17]. Antimicrobials are generally used for treatment of respiratory diseases caused by bacteria and *Mycoplasma* spp. [10, 12, 13]. Therefore, information on the distribution of antimicrobials within the bronchoalveolar region of pigs is important and may aid in selection of proper antibacterial agents for treatment of pneumonia resulting in quicker recovery from respiratory diseases.

Marbofloxacin (MBFX) is a fluoroquinolone antibacterial agent. MBFX has been used for livestock animals in clinical practice [6, 12, 13, 20]. MBFX is a new type of fluoroquinolone and has been used for treatment of livestock animals since 2010 in Japan. There have been a few reports about the biodistribution of MBFX in animals. The distribution of MBFX within the bronchoalveolar region has been reported in dogs after oral administration [2], and in calves after intramuscularly injection [11]. Although some pharmacokinetic studies of MBFX have been performed to elucidate the biodistribution in pig [5, 21, 24], no reports were made concerning the concentrations of MBFX within the bronchoalveolar region. The purpose of this study was to elucidate the concentration of MBFX in bronchoalveolar lavage fluid (BALF) after administration of MBFX injections into healthy pigs.

Four clinically healthy female pigs were used in this study. Pigs were determined to be healthy if they exhibited good appetite and vitality, exhibited no coughing, fever or abnormalities of respiratory rate. The pigs use in the study were 12-weeks old and had body weights of 45.4 ± 3.7 kg (mean ± SD, range: 40.7–49.7 kg). The animals were cared for according to the Guide for the Care and Use of Laboratory Animals of the Joint Faculty of Veterinary Medicine, Kagoshima University. A commercial MBFX (Marbocyl 2% injectable solution, Meiji-Seika-Phama, Tokyo, Japan) was intramuscularly injected at a dose of 2 mg/kg to each pig. Measurements of body temperature, heart rates, and respiratory rates, as well as, sampling of blood from the peripheral vein were conducted at 0 (before administration), 3, 8 and 24 hr after the injection. Blood samples were collected into heparinized tubes (VP-H100K, Terumo, Tokyo, Japan) and Vacutainer tubes (VP-DK052K, Terumo) containing ethylenediaminetetraacetic acid dipotassium salt (EDTA-2K).

The blood collected into tubes containing EDTA-2K was used for determining white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb) and hematocrit (Ht). The measurements were taken within 30 min after the collection using an automated cell counter (Poch-100iV, Sysmex, Kobe, Japan). Plasma was separated from blood, which was collected into heparinized tubes, by centrifugation (4°C, 2,000 g for 10 min) and stored at −80°C until analysis. The urea concentrations within plasma were measured using the colorimetric method via assay kits (Quantichrom Urea Assay Kit, Bioassay Systems, Hayward, CA, USA). Using previously published reports as a guide [2, 15], bronchoalveolar lavage fluid (BALF) was collected using a flexible electronic endoscope (VQ TYPE 6112B, Olympus, Tokyo, Japan) at 0 (before administration), 3, 8 and 24 hr after

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administration of MBFX. Pigs were premedicated intramuscularly with 2 mg/kg of alfaxalone (Alfaxan, Meiji-Seika-Phama) and anesthetized with midazolam (0.2 mg/kg, IV) and isoflurane (2.5%, inhalation). Under the general anesthesia, a flexible electronic endoscope was inserted into a subsegment of lobe. Then 30 m*l* each of sterile 0.9% normal saline solution was infused into each lobe and immediate aspiration followed each infusion. This procedure was performed twice for each lobe. The second aspiration was pooled with the first one. BALF was randomly sampled from the left and right lobes, including two or three locations each. The BALF was immediately sent to a laboratory for cell counting, and then 1.5 m*l* of BALF from each of the 5 samples-was centrifuged at 400 g for 5 min. The supernatant and cell pellets were separated and frozen at −80°C until assays.

The concentration of MBFX was measured by the high-performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) method based on the previously established procedure reported by De Baere *et al*. [3]. Plasma samples (100 μl) were diluted 10 times with distilled water. Each BALF cell pellet sample was mixed with 0.5 m*l* of 1 mol sodium hydroxide in order to lyse cells and then each sample was mixed with 1.0 m*l* of 3% formic acid. Three hundred microliters of each sample (diluted plasma, supernatant of BALF and lysed BALF cell pellet) was mixed with 60 μl of internal standard (Lomefloxacin, Sigma-Aldrich, Tokyo, Japan; 300 *n*g/m*l* in 1% formic acid/methanol (4:1)) and 60 μl of methanol. The diluted sample (350 μl) was loaded into a solid-phase extraction column (Oasis HLB, Waters, Tokyo, Japan). The residue was dissolved in 250 μl of mobile phase. An aliquot (10 μl) of the extract was injected into the LC/MS/MS (Prominence, Shimadzu, Kyoto, Japan; 4000 QTRAP, AB, Sciex, Tokyo, Japan).

The MBFX concentration was determined for the pulmonary epithelial lining fluid (ELF) and the alveolar cells in BALF [8, 9]. The concentration of MBFX in ELF ($MBFX_{ELF}$) was calculated as follows:

$$MBFX_{ERF}=MBFX_{BALF} \text{ X } urea_{Plasma} / urea_{BALF}$$

where $MBFX_{BALF}$ was the concentration of MBFX in BALF, $urea_{Plasma}$ was the concentration of urea in plasma, and $urea_{BALF}$ was the concentration of urea in BALF.

The concentration of MBFX in alveolar cells ($MBFX_{AC}$) was determined as follows:

$$MBFX_{AC}=AC_{PELLET} / V_{AC}$$

where AC_{PELLET} was the concentration of MBFX in the alveolar cell pellet and V_{AC} was the mean volume of BALF cells. A volume of 1.28 $\mu l/10^6$ BALF cells was used based on previous studies [8, 9]. The area under MBFX concentration curve during the 0 to 24 hr timeframe ($AUC_{0–24}$) was calculated using the method based on the previously established procedure reported by Wang *et al*. [22].

Statistical analyses of data were conducted using analysis of variance (one-way ANOVA) followed by the Tukey-Kramer multiple comparison test to determine the differences in MBFX among three types of samples at the same sampling time. All statistical analyses were performed using the IBM SPSS Statistics 25 software (IBM, Tokyo, Japan), and $P<0.05$ was considered statistically significant.

The body temperature, heart rates, and respiratory rates of the pigs hardly fluctuated, and abnormal clinical findings were not recognized via visual inspection during the experiment. The WBCs, RBCs, Hb and Ht values of the pigs hardly fluctuated during the experiment. The mean MBFX concentrations in the plasma at 3, 8 and 24 hr after administration were 0.71 μg/m*l*, 0.50 μg/m*l* and 0.15 μg/m*l*, respectively (Fig. 1). The mean MBFX concentrations at 3, 8 and 24 hr after administration were 1.31 μg/m*l*, 1.72 μg/m*l* and 0.54 μg/m*l*, respectively in ELF, and were 0.92 μg/m*l*, 1.75 μg/m*l* and 0.77 μg/m*l*, respectively in alveolar cells. The mean MBFX concentration in ELF at 3 hr and 8hr and in alveolar cells at 8hr were significantly higher than those in plasma ($P<0.05$). The mean $AUC_{0–24}$ in the plasma, ELF, and alveolar cells were presented in Table 1. The mean $AUC_{0–24}$ in ELF and alveolar cells were significantly higher than that in plasma ($P<0.01$).

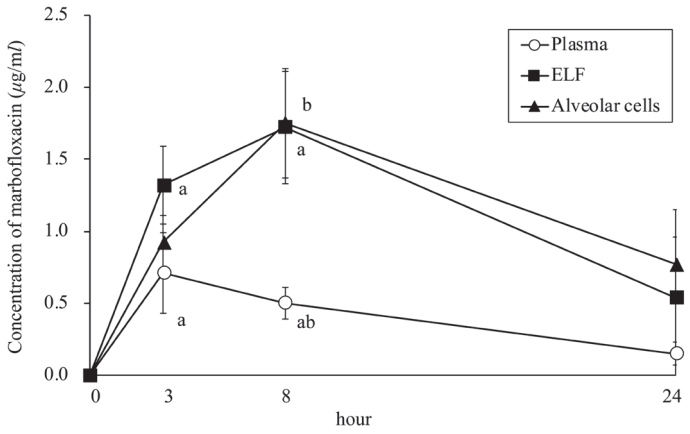

Fig. 1. Concentration of marbofloxacin after intramuscular injection as determined within the plasma, ELF, and alveolar cells in pigs. Data are shown as the mean ± SD. ELF; pulmonary epithelial lining fluid. Values with the same letters show the significant difference at the same sampling time ($P<0.05$). "a" and "b" represents statistical difference between plasma and ELF or alveolar cells, respectively.

Table 1. Area under the curve (AUC) of marbofloxacin from 0 to 24 hr after intramuscular injection in pigs

		$AUC_{0–24}$
Plasma	(μg·hr/m*l*)	$9.24 \pm 2.80^{a, b)}$
Pulmonary epithelial lining fluid	(μg·hr/m*l*)	$27.58 \pm 0.60^{a)}$
Alveolar cells	(μg·hr/m*l*)	$28.23 \pm 4.69^{b)}$

Data are shown as the mean ± SD. Values with the same letter indicate the significant difference (a, b: $P<0.01$).

In the present study, the dynamics of MBFX concentrations in plasma were similar to the previous report [5, 21, 24]. The MBFX concentrations in ELF and alveolar cells changed similarly during the experimental period. The MBFX concentrations and AUC_{0-24} in ELF and alveolar cells were higher than those found in plasma. These results suggest that intramuscularly administered MBFX into pigs was well distributed in the bronchoalveolar region.

In order for an antimicrobial agent to work effectively, it is important for it to reach the area where the bacterium is infected, and the concentration needs to exceed the minimum inhibitory concentration (MIC) for that specific bacteria [14]. The previous study reported that the MIC_{90} values of MBFX for *Pasteurella multocida, Actinobacillus pleuropneumoniae*, *Streptococcus suis* and *Mycoplasma hyopneumoniae* were 0.06 μg/m*l*, 0.06 μg/m*l*, 1.00 μg/m*l*, and 0.50 μg/m*l*, respectively [4, 13]. In the present study, the mean MBFX concentrations in ELF and alveolar cells were over 1.00 μg/m*l* at 8 hr after administration. Thus, the MBFX concentrations in the bronchoalveolar region achieved higher than the MIC_{90} for these bacteria.

Fluoroquinolones such as marbofloxacin exhibit concentration-dependent type of killing [16, 24]. Therefore AUC_{0-24} to MIC ratio (AUC/MIC) is used as an index of microbiocidal activity [16, 19, 20]. Although data are based on human clinical trials as well as laboratory animal infection models, for fluoroquinolones, AUC/MIC greater than 100–125 are generally associated with a treatment efficacy [1, 7, 16, 19, 23]. In previous field studies, good therapeutic effects of MBFX in treating respiratory diseases have been reported [10, 18]. Grandemange *et al*. evaluated clinical efficacy of a single 8 mg/kg dose of MBFX against naturally occurring respiratory disease associated principally with *A. pleuropneumoniae*. In the cited study, the MIC_{90} value of *A. pleuropneumoniae* isolated from the lung and BAL samples obtained before treatment was 0.06 μg/m*l* for MBFX, which was similar to those reported in other previous reports [4]. According to the calculations using the literature data and the value of AUC calculated in this study, AUC/MIC in plasma, ELF and alveolar cells for *A. pleuropneumoniae* were 154, 460 and 471, respectively. These data suggest that sufficient amounts of MBFX were likely to be distributed, especially in the intrapulmonary area, with the dosage of 2 mg/kg and was confirmed by the good therapeutic effect demonstrated in a previous study [18]. However, the results of the present study do not demonstrate the distribution of MBFX following multiple or high-dose administration. To evaluate the efficacy of MBFX treatment for respiratory diseases further, additional pharmacokinetic and pharmacodynamic studies, including microbiological research of other pathogens would be required.

In the present study, the distribution of MBFX in the bronchoalveolar region after intramuscular injection to healthy pigs was demonstrated. However, further studies of pigs with respiratory disease are needed to clarify the distribution of MBFX within the intrapulmonary area.

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