



NOTE

Internal Medicine

Distribution of marbofloxacin in the bronchoalveolar region in healthy calves

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ABSTRACT. The purpose of this study was to clarify the distribution of marbofloxacin (MBFX) within the bronchoalveolar region of calves. Four clinically healthy calves were intramuscularly injected with a single dose of MBFX (2 mg/kg). Samples of plasma and bronchoalveolar lavage fluid (BALF) were obtained for each calf at 0 (before administration), 1, 2, 6 and 24 hr after injection of MBFX. The injections and series of sample collections were conducted and repeated again after two weeks. The results show that the MBFX concentrations in the pulmonary epithelial lining fluid (ELF) were significantly higher than that in plasma and in alveolar cells at 2 hr after injection ($P < 0.05$). For concentrations of MBFX within the ELF, the mean area under the MBFX concentration curve calculated during the 0 to 24 hr timeframe (AUC_{0-24}) was significantly higher than the mean determined from samples collected from the plasma ($P < 0.05$). These results suggest that intramuscularly injected MBFX was well distributed in the bronchoalveolar region.

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

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The respiratory diseases, generally caused by viruses, bacteria, and mycoplasma infection, are some of the most common diseases among calves [14, 15]. Bacterial or mycoplasma infections in calves predominately result in alveolar pneumonia [14, 15]. Antimicrobials are generally used for the treatment of respiratory diseases caused by bacteria and mycoplasma [9, 18]. Therefore, information on the distribution of antimicrobials within the bronchoalveolar region of calves is important and may aid in selection of proper antibacterial agents for treatment of alveolar pneumonia resulting in quicker recovery from respiratory diseases.

Marbofloxacin (MBFX) is a fluoroquinolone antibacterial agent. Clinical use of MBFX for livestock animals has been reported [9, 12, 18]. 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 [4]. In cattle, only the concentration of MBFX in blood has been reported [2, 3, 11], and 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 calves.

Four clinically healthy Holstein bull calves were used. Calves were determined to be healthy if they exhibited good appetite and vitality, exhibited no coughing, fever or abnormalities of respiratory rate. The calves used in the study were 4-weeks old and had body weights of 53.9 ± 2.9 kg (mean \pm SD, range: 50.5–57.5). The animals were cared for according to the Guide for the Care and Use of Laboratory Animals of the Joint Faculty of Veterinary Medicine, provided by Kagoshima University. A commercial MBFX (Marbocyl 10% injectable solution, Meiji-Seika-Pharma, Tokyo, Japan) was intramuscularly injected at a dose of 2 mg/kg to each calf. Measurements of body temperature, heart rate, and respiratory rate, as well as, sampling of peripheral blood from the jugular vein were conducted at 0 (before administration), 1, 2, 6 and 24 hr after the injection. Blood samples were collected into heparinized tubes (VP-H100K, Terumo, Tokyo, Japan) and Vacutainer tubes (VP-NA052K, Terumo) containing dipotassium ethylenediaminetetraacetic acid (EDTA-2AK). The same procedure was conducted once and then again after two weeks.

The blood that was collected into tubes containing EDTA-2AK 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 collection using

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an automated cell counter (Poch-100iV, Sysmex, Kobe, Japan). Plasma was separated from the blood, which was collected into heparinized tubes, by centrifugation and it was stored at -80°C until analysis. The MBFX and urea concentrations within plasma were measured using the colorimetric method via assay kits (Quantichrom Urea Assay Kit, Bioassay Systems, Hayward, CA, U.S.A.). Using previously published reports as a guide [4, 13], bronchoalveolar lavage fluid (BALF) was collected using a flexible electronic endoscope (VQ TYPE 6112B, Olympus, Tokyo, Japan) at 0 (before administration), 1, 2, 6 and 24 hr after administration of MBFX. The upper airway of subjects, especially the epiglottis area, was sprayed with 2% lidocaine using a medicine spray tube passed through the endoscope. After the local anesthesia was administered, a flexible electronic endoscope was inserted into a subsegment of lobe. Then 30 ml 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. The volume of BALF was measured and recorded. BALF was collected from the left and right lobes, including three locations each. In total, six locations in the lung were sampled. The BALF was immediately sent to a laboratory for cell counting, and then 1.5 ml of BALF from each of the 6 samples (right middle lobe, third bronchiole of the right caudal lobe, fifth bronchiole of the right caudal lobe, second bronchiole of the left caudal lobe, third bronchiole of the left caudal lobe and fifth bronchiole of the left caudal lobe) was centrifuged at 400 g for 5 min. The supernatant and cell pellets were separated and frozen at -80°C until assays. All calves were used again after two weeks for the same procedure.

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.* [5]. Plasma samples (100 μl) were diluted 10 times with distilled water. Each BALF cell pellet sample was mixed with 0.5 ml of 1 mol sodium hydroxide in order to lyse cells and then each sample was mixed with 1.0 ml 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 ng/ml 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 [7, 8]. The concentration of MBFX in ELF (MBFX_{ELF}) was calculated as follows:

$$\text{MBFX}_{\text{ERF}} = \text{MBFX}_{\text{BALF}} \times \text{urea}_{\text{plasma}} / \text{urea}_{\text{BALF}}$$

where $\text{MBFX}_{\text{BALF}}$ was the concentration of MBFX in the BALF, $\text{urea}_{\text{plasma}}$ was the concentration of urea in the plasma, and $\text{urea}_{\text{BALF}}$ was the concentration of urea in the BALF.

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

$$\text{MBFX}_{\text{AC}} = \text{AC}_{\text{PELLET}} / V_{\text{AC}}$$

where $\text{AC}_{\text{PELLET}}$ was the concentration of MBFX in the alveolar cell pellet and V_{AC} was the mean volume of BALF cells. A volume of $1.28 \mu\text{l}/10^6$ BALF cells was used based on previous studies [7, 8]. 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.* [20].

Data are shown as the total values of the first and second procedures. 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 24 software (IBM, Tokyo, Japan), and $P < 0.05$ was considered statistically significant.

The body temperature, heart rates, and respiratory rates of the calves hardly fluctuated, and abnormal clinical findings were not recognized via visual inspection during the experiment. The WBCs, RBCs, Hb and Ht values of the calves hardly fluctuated during the experiment. The mean MBFX concentrations in the plasma at 1, 2, 6 and 24 hr after administration were 2.02, 2.04, 1.25 and 0.27 $\mu\text{g}/\text{ml}$, respectively (Fig. 1). The mean MBFX concentrations at 1, 2, 6 and 24 hr after administration were 2.37, 3.42, 1.76 and 0.39 $\mu\text{g}/\text{ml}$, respectively in the ELF, and were 1.71, 2.02, 1.23 and 0.37 $\mu\text{g}/\text{ml}$, respectively in the alveolar cells. The mean MBFX concentration in ELF at 2 hr was significantly higher than that in the plasma ($P < 0.05$). The mean AUC_{0-24} in the plasma, ELF, and alveolar cells were 21.3, 32.6, and 23.0 $\mu\text{g} \cdot \text{hr}/\text{ml}$ (Table 1), respectively. The mean AUC_{0-24} in the ELF was significantly higher than that in plasma ($P < 0.05$).

In the present study, the dynamics of MBFX concentrations in plasma were similar to the previous reports [2, 3, 12]. The MBFX concentrations in the plasma and in the alveolar cells changed similarly during the experimental period. The MBFX concentration and AUC_{0-24} in the ELF were higher than those found in the plasma. These results suggest that intramuscularly administered MBFX into calves 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) required for that specific bacteria [10]. The previous study reported that the MIC_{90} values of MBFX for *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* and *Mycoplasma bovis* were 0.12, 0.25, 0.06, and 2.00 $\mu\text{g}/\text{ml}$, respectively [10]. Fluoroquinolones such as marbofloxacin exhibit concentration-dependent type of killing [17, 21]. Therefore AUC_{0-24} to MIC ratios (AUC/MIC) is used as an index of microbicidal activity [16, 17, 19]. 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, 6, 17, 19, 21]. In the

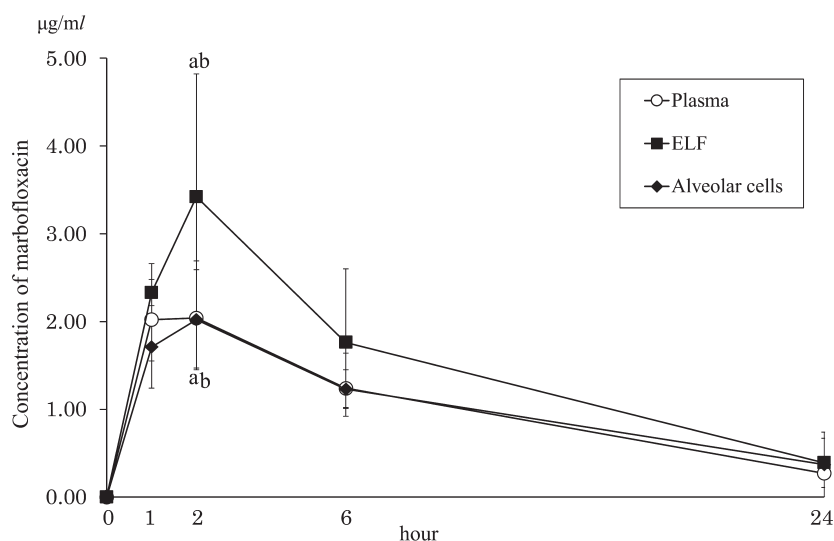


Fig. 1. Concentration of marbofloxacin after intramuscular injection as determined within the plasma, ELF, and alveolar cells. Data are shown as the mean \pm SD. ELF; pulmonary epithelial lining fluid. Values with the same letters show the significant difference at the same sampling time ($P < 0.05$).

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

		AUC ₀₋₂₄
Marbofloxacin in plasma	($\mu\text{g}\cdot\text{hr}/\text{ml}$)	21.29 \pm 7.28 ^a
Marbofloxacin in pulmonary epithelial lining fluid	($\mu\text{g}\cdot\text{hr}/\text{ml}$)	32.57 \pm 11.07 ^a
Marbofloxacin in alveolar cells	($\mu\text{g}\cdot\text{hr}/\text{ml}$)	23.02 \pm 4.51

Data are shown as the mean \pm SD. Values with the same letter indicate the significant difference (a: $P < 0.05$).

present study, AUC/MIC in ELF for *P.multocida*, *M. haemolytica* and *H.somni*, and AUC/MIC in alveolar cells for *P.multocida* and *H.somni* were above 125. On the other hand, the AUC/MIC in ELF for *M. bovis*, and AUC/MIC in alveolar cells for *M. bovis* and *M. haemolytica* were under 100. In calves with respiratory diseases, the distribution of MBFX in the bronchoalveolar region might be lower than that in this study due to the inflammatory condition of the calves. However, good therapeutic effects of MBFX in treating respiratory diseases in the field have been reported [9, 18].

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

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