



# Pharmacokinetics in plasma and alveolar regions of a healthy calf intramuscularly administered a single dose of orbifloxacin

Tetsuro KURAMAE<sup>1,2)</sup>, Shingo ISHIKAWA<sup>2,3)</sup>, Aki KONO<sup>3)</sup> and Seiji HOB0<sup>2,3)\*</sup><sup>1)</sup>Kuramae Animal Clinic, Kagoshima, Japan<sup>2)</sup>United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi, Japan<sup>3)</sup>Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan

**ABSTRACT.** This study analyzed the pharmacokinetics of orbifloxacin (OBFX) in plasma, and its migration and retention in epithelial lining fluid (ELF) and alveolar cells within the bronchoalveolar lavage fluid (BALF). Four healthy calves received a single dose of OBFX (5.0 mg/kg) intramuscularly. Post-administration OBFX dynamics were in accordance with a non-compartment model, including the absorption phase. The maximum concentration ( $C_{max}$ ) of plasma OBFX was  $2.2 \pm 0.1 \mu\text{g/ml}$  at  $2.3 \pm 0.5 \text{ hr}$  post administration and gradually decreased to  $0.3 \pm 0.2 \mu\text{g/ml}$  at 24 hr following administration. The  $C_{max}$  of ELF OBFX was  $9.3 \pm 0.4 \mu\text{g/ml}$  at  $3.0 \pm 2.0 \text{ hr}$  post administration and gradually decreased to  $1.2 \pm 0.1 \mu\text{g/ml}$  at 24 hr following administration. The  $C_{max}$  of alveolar cells OBFX was  $9.3 \pm 2.9 \mu\text{g/ml}$  at 4.0 hr post administration and gradually decreased to  $1.1 \pm 0.2 \mu\text{g/ml}$  at 24 hr following administration. The half-life of OBFX in plasma, ELF, and alveolar cells were  $6.9 \pm 2.2$ ,  $7.0 \pm 0.6$ , and  $7.8 \pm 1.6 \text{ hr}$ , respectively. The  $C_{max}$  and the area under the concentration-time curve for 0–24 hr with OBFX were significantly higher in ELF and alveolar cells than in plasma ( $P < 0.05$ ). These results suggest that OBFX is distributed and retained at high concentrations in ELF and alveolar cells at 24 hr following administration. Hence, a single intramuscular dose of OBFX (5.0 mg/kg) may be an effective therapeutic agent against pneumonia.

**KEY WORDS:** bronchoalveolar lavage fluid, calf, orbifloxacin, pharmacokinetic, pharmacodynamic

*J. Vet. Med. Sci.*

84(3): 400–405, 2022

doi: 10.1292/jvms.21-0517

Received: 23 September 2021

Accepted: 16 January 2022

Advanced Epub:

28 January 2022

*Mycoplasma* spp. and other bacterial pathogens can affect the respiratory system in calves. This can affect their development and life expectancy [1], presenting a major challenge in veterinary medicine [2]. Numerous cases of respiratory diseases among calves have been classified as alveolar pneumonia [2]. The treatment of alveolar pneumonia requires an antibacterial agent with adequate distribution to the intrapulmonary region, including alveolar or bronchoalveolar lavage fluid (BALF). Orbifloxacin (OBFX) is a quinolone-based agent with broad-spectrum antimicrobial activity against pathogens commonly associated with bovine respiratory diseases [16, 17, 24]. However, to determine if OBFX is an effective treatment for alveolar pneumonia, its pharmacokinetics and intrapulmonary distribution must first be characterized. Furthermore, intrapulmonary quinolone-based agent concentration that reaches the upper limit of the minimum inhibitory concentration (MIC) for the target pathogen should be achieved [18, 20, 22]. However, the concentration of OBFX in the alveolar region and its distribution remains unknown.

We previously reported the distribution of ERFX in plasma, pulmonary epithelial lining fluid (ELF), and alveolar cells in the BALF through analysis of BALF samples following subcutaneous administration to healthy calves [15]. This study aimed to elucidate the chronological dynamics of OBFX concentration in plasma, ELF, and alveolar cells by analyzing blood and BALF samples obtained from calves. We targeted OBFX in each matrix for pharmacokinetics (PK) analysis to investigate its distribution from plasma to ELF and alveolar cells.

## MATERIALS AND METHODS

Samples were collected from four healthy Holstein bull calves with body weights of  $48.6 \pm 3.6 \text{ kg}$  (mean  $\pm$  SD) at 4-week-old. This study was approved by the Ethics Committee of the Joint Faculty of Veterinary Medicine, Kagoshima University, and the animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals of the Joint Faculty of Veterinary

\*Correspondence to: Hobo, S.: k2088185@kadai.jp, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

©2022 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Medicine, Kagoshima University.

A single dose (5.0 mg/kg) of commercial OBFX (BICTAS Injection-5%, D.S. Pharma Animal Health, Osaka, Japan) was intramuscularly administered to the neck of each calf. Peripheral blood samples were collected from the jugular vein at 0 (before administration), 1, 2, 3, 6, 10, and 24 hr post administration, using heparinized Vacutainer tubes (VP-H050K, Terumo, Tokyo, Japan) and Vacutainer tubes (VP-NA052K, Terumo) treated with dipotassium ethylenediaminetetraacetic acid (EDTA-2AK). Body temperature, heart rate, and respiratory rate were measured, and visual examinations were carried out at each time point.

Blood collected in heparinized tubes was centrifuged to obtain plasma and stored at  $-80^{\circ}\text{C}$  until analysis. Blood samples collected in EDTA-2AK-treated tubes were analyzed for white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), and hematocrit (Ht) using an automated cell counter (Poch-100iv, Sysmex, Kobe, Japan) within 1 hr of collection. The blood collection time was determined using a previously described method [15].

BALF was collected at 0 (before administration), 1, 2, 6, and 24 hr post-administration using a flexible electronic endoscope (VQ TYPE 5112B, Olympus, Tokyo, Japan). The upper airway of the subjects, especially the epiglottis area, was sprayed with 2% lidocaine using a medicine spray tube passed through the endoscope. After administering local anesthesia, a flexible electronic endoscope was inserted into a subsegment of the lobe. Two 30 ml-aliquots of sterile 0.9% normal saline solution were injected into the lobe, immediately aspirated and the two samples pooled.

BALF was collected from three sites in both the left and right lobes, totaling six sites per calf (right middle lobe, third and fifth bronchioles of the right caudal lobe, and second, third and fifth bronchioles of the left caudal lobe) and promptly transported to the laboratory for analysis. After the cell count was determined, a 1.5 ml aliquot of each BALF sample was centrifuged at  $400 \times g$  for 5 min. The supernatant and cell pellets were separated and frozen at  $-80^{\circ}\text{C}$  until use. The urea concentrations within plasma were measured using the colorimetric method via assay kits (Quantichrom Urea Assay Kit, Bioassay Systems, Hayward, CA, USA).

OBFX concentrations were measured via high-performance liquid chromatography (HPLC) with reference to the previously reported method [4]. Plasma samples (100  $\mu\text{l}$ ) were diluted 10-fold with distilled water. Each BALF cell pellet was mixed with 0.5 ml of 1 M sodium hydroxide for cell lysis and then mixed with 1.0 ml of 3% formic acid. Three-hundred microliters of each sample (diluted plasma, BALF supernatant, and lysed BALF cell pellet) was mixed with 60  $\mu\text{l}$  of the internal standard [Lomefloxacin, Sigma-Aldrich, Tokyo, Japan; 300 ng/ml in 1% formic acid/methanol (4:1)]. Each sample (350  $\mu\text{l}$ ) was loaded into a solid-phase extraction column (Oasis HLB, Waters, Tokyo, Japan). The residue was dissolved in 250  $\mu\text{l}$  of the mobile phase, and an aliquot (10  $\mu\text{l}$ ) of the extract was injected into the HPLC system (Prominence, Shimadzu, Kyoto, Japan). The excitation and emission wavelengths used with the fluorescence detector were 350 nm and 460 nm, respectively.

Treatment of samples and validation of the chromatographic method through the determination of specificity, linearity, accuracy, precision, detection, and quantitation limits were similar to those of the previously reported method [4]. Calibration curves were prepared by fortifying the blank matrix with reference drug standards of orbifloxacin (Merck KGaA, Darmstadt, Germany) to validate the HPLC analysis and perform quality control assessments during the assay. The retention time of orbifloxacin was 17.2 min. No interfering peaks in all blank samples were noted in the elution position of orbifloxacin. A linear relationship existed in the calibration curve at both lower (1–256 ng/ml) and higher (256–1,000 ng/ml) concentrations. Linearity of the standard curve was confirmed by the test for lack of fit and the value of  $r^2$  was 0.999. Orbifloxacin yielded recovery from samples ranging from  $99.8 \pm 2.9\%$  to  $100.8 \pm 3.7\%$ . The within-run precision (percent coefficient of variation, % CV) was less than 1.2% for concentrations greater than or equal to 100 ng/ml and less than 2.6% at lower concentrations. The limit of detection was determined to be 1.0 ng/ml.

The OBFX concentration was determined in the ELF and alveolar cells in BALF [9, 10]. The concentration of OBFX in ELF ( $\text{OBFX}_{\text{ELF}}$ ) was determined as follows:

$$\text{OBFX}_{\text{ELF}} = \text{OBFX}_{\text{BALF}} \times \text{urea}_{\text{PLASMA}} / \text{urea}_{\text{BALF}}$$

where  $\text{OBFX}_{\text{BALF}}$  is the concentration of OBFX in the BALF and  $\text{urea}_{\text{plasma}}$  is the concentration of urea in BALF.

The concentration of OBFX in alveolar cells ( $\text{OBFX}_{\text{AC}}$ ) was determined as follows:

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

where  $\text{AC}_{\text{PELLET}}$  is the concentration of OBFX in the alveolar cell pellet and  $V_{\text{AC}}$  is the mean volume of cattle BALF cells.

A volume of  $1.28 \mu\text{l}/10^6$  alveolar cells, as established in another bovine study, was used for the volume of BALF cells [8, 9]. A cytospin smear (Cytospin4, Thermo Fisher Scientific, Waltham, MA, USA) was prepared following the standard method to confirm the BALF cell fractions, and 200 WBCs were counted.

The bronchoalveolar lavage cell pellet was resuspended in PBS and the total cell count per ml in BALF was determined using trypan blue (Sigma Aldrich Co., St. Louis, MO, USA) and haemocytometer (Neuber's), under a light microscope [19, 23].

PK parameters were determined for each animal after intramuscular administration of OBFX (5.0 mg/kg) based on the OBFX concentrations in plasma, ELF, and alveolar cells. OBFX levels were determined using a non-compartmental model, including the absorption phase. The area under the concentration-time curve between 0 and 24 hr or infinity time ( $\text{AUC}_{0-24}$  or  $\text{AUC}_{\text{inf}}$ ) and the area under the first moment curve between 0 and 24 hr or infinity time ( $\text{AUMC}_{0-24}$  or  $\text{AUMC}_{\text{inf}}$ ) were determined using a linear trapezoidal method. The mean residence time between 0 and 24 hr or infinity time ( $\text{MRT}_{0-24}$  or  $\text{MRT}_{\text{inf}}$ ) was determined as  $\text{AUMC}_{0-24} / \text{AUC}_{0-24}$  ( $\text{AUMC}_{\text{inf}} / \text{AUC}_{\text{inf}}$ ). The terminal phase half-life ( $t_{1/2}$ ) was determined to be  $0.693/\text{terminal slop} (\lambda_z)$ . As a general rule, the  $\lambda_z$  constant was calculated using three or more measurement points below  $C_{\text{max}}$ .

The MICs of OBFX for the two pneumonia-inducing bacteria (*Pasteurella multocida* (*P. Multocida*) and *Mannheimia haemolytica* (*M. Haemolytica*)) [11] were obtained from health research on cattle in Japan as PD parameters. The MICs of *P. multocida* and *M. haemolytica* were 0.25 and 1.0  $\mu\text{g}/\text{ml}$ , respectively.

Statistical analyses of data were conducted using analysis of variance (one-way ANOVA) followed by the Tukey-Kramer

multiple comparison tests to determine the differences in OBFX among the three types of samples at the same sampling time point. ANOVA was used to determine the differences in OBFX among the three types of PK parameters and AUC/MIC ratios. All statistical analyses were performed using IBM SPSS Statistics 24 software (IBM Corp., Armonk, NY, USA), and statistical significance was set at  $P < 0.05$ . Data are shown as mean  $\pm$  SD.

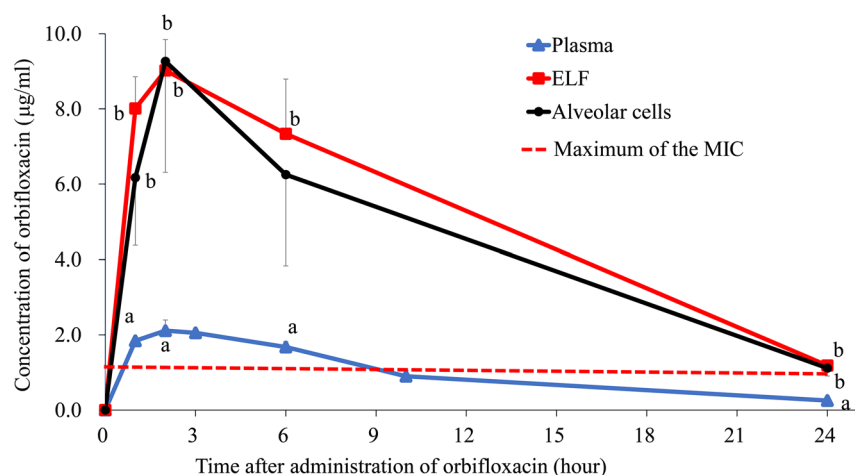
## RESULTS

The body temperature, heart rate, respiratory rate, WBC, RBC, Hb, and Ht levels of each calf did not fluctuate significantly, and abnormal clinical findings were not found upon visual inspection throughout the study.

The BALF recovery rates, before administration, and at 1, 2, 6, and 24 hr post administration were  $52.5 \pm 16.5$ ,  $40.6 \pm 9.9$ ,  $44.2 \pm 7.2$ ,  $51.9 \pm 3.7$ , and  $52.5 \pm 6.4\%$ , respectively. Hence, the BALF recovery rate retained essentially unchanged. Similarly, the recovery rate of alveolar cells did not significantly change before administration, and at 1, 2, 6, and 24 hr post-administration, with values of  $4.5 \pm 2.7$ ,  $5.5 \pm 1.3$ ,  $4.8 \pm 2.0$ ,  $5.7 \pm 1.4$ , and  $6.8 \pm 2.0$  ( $\times 10^5/\text{ml}$ ), respectively.

In all specimens of the BALF cell smear, as for the compartmentation, 95% or more were macrophages.

OBFX concentrations in plasma, ELF, and alveolar cells are shown in Fig. 1. The corresponding PK parameters are listed in Table 1. The  $C_{\max}$  of plasma OBFX was  $2.2 \pm 0.1$   $\mu\text{g}/\text{ml}$  at  $2.3 \pm 0.5$  hr post administration and gradually decreased to  $0.3 \pm 0.2$   $\mu\text{g}/\text{ml}$  at 24 hr following administration. The  $C_{\max}$  of ELF OBFX was  $9.3 \pm 0.4$   $\mu\text{g}/\text{ml}$  at  $3.0 \pm 2.0$  hr post administration and gradually decreased to  $1.2 \pm 0.1$   $\mu\text{g}/\text{ml}$  at 24 hr following administration. The  $C_{\max}$  of Alveolar cell OBFX was  $9.3 \pm 2.9$   $\mu\text{g}/\text{ml}$  at  $4.0 \pm 0.0$  hr



**Fig. 1.** Concentrations of orbifloxacin (OBFX) in plasma, epithelial lining fluid (ELF), and alveolar cells 0 to 24 hr after a single intramuscular dose of OBFX (5.0 mg/kg) in calves (n=4). Data are shown as mean  $\pm$  standard deviation. Values with the different letters indicate significant differences among the plasma, ELF, and Alveolar cells at the same sampling time (a–b:  $P < 0.05$ ). —Maximum of the minimum inhibitory concentration (MIC), MIC of *Mannheimia haemolytica* (Harada, K. *et al.* [11]).

**Table 1.** Pharmacokinetic (PK) parameters of orbifloxacin (OBFX) in plasma, epithelial lining fluid (ELF), and alveolar cells following single intramuscularly dose of OBFX (5.0 mg/kg) in calves (n=4)

PK Parameters	Plasma	ELF	Alveolar cells
$C_{\max}$ ( $\mu\text{g}/\text{ml}$ )	$2.2 \pm 0.1^{\text{a}}$	$9.3 \pm 0.4^{\text{b}}$	$9.3 \pm 2.9^{\text{b}}$
$T_{\max}$ (hr)	$2.3 \pm 0.5$	$3.0 \pm 2.0$	$4.0 \pm 0.0$
$\text{AUC}_{0-24}$ ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	$23.8 \pm 1.8^{\text{a}}$	$122.0 \pm 15.2^{\text{b}}$	$108.2 \pm 35.1^{\text{b}}$
$\text{AUC}_{\text{inf}}$ ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	$26.5 \pm 2.8^{\text{a}}$	$134.0 \pm 14.7^{\text{b}}$	$121.2 \pm 34.8^{\text{b}}$
$\text{AUMC}_{0-24}$ ( $\mu\text{g}\cdot\text{hr}^2/\text{ml}$ )	$177.1 \pm 23.8^{\text{a}}$	$793.5 \pm 102.0^{\text{b}}$	$706.3 \pm 185.0^{\text{b}}$
$\text{AUMC}_{\text{inf}}$ ( $\mu\text{g}\cdot\text{hr}^2/\text{ml}$ )	$274.7 \pm 99.6^{\text{a}}$	$1,204.9 \pm 100.1^{\text{b}}$	$1,172.0 \pm 293.3^{\text{b}}$
$t_{1/2}$ (hr)	$6.9 \pm 2.2$	$7.0 \pm 0.6$	$7.8 \pm 1.6$
$\text{MRT}_{0-24}$ (hr)	$7.4 \pm 0.8$	$6.5 \pm 0.2$	$6.6 \pm 0.6$
$\text{MRT}_{\text{inf}}$ (hr)	$10.2 \pm 2.9$	$9.1 \pm 0.6$	$9.9 \pm 2.4$

Data are shown as the mean  $\pm$  SD. AUC: area under the concentration-time curve; AUMC: area under the first moment curve;  $C_{\max}$ : peak concentration;  $T_{\max}$ : time to  $C_{\max}$ ;  $t_{1/2}$ : half-life; MRT: mean residence time. Values with the different letters indicate significant differences among the plasma, ELF, and Alveolar cells in PK parameters (a–b:  $P < 0.05$ ).

**Table 2.** Area under the concentration-time curve between 0 and 24 /minimum inhibitory concentration (MIC)<sub>90</sub> ratio for orbifloxacin (OBFX) following a single intramuscularly dose of OBFX in cattle (n=4), using MIC<sub>90</sub> values of 0.25 and 1.0 µg/ml for *Pasteurella multocida* and *Manheimia haemolytica* (Harada, K *et al.* [11]), respectively

Bacteria Name	Plasma	ELF	Alveolar cells
<i>P. multocida</i>	95.2 ± 7.3 <sup>a)</sup>	488.0 ± 60.8 <sup>b)</sup>	432.8 ± 140.4
<i>M. haemolytica</i>	23.8 ± 1.8 <sup>a)</sup>	122.0 ± 15.2 <sup>b)</sup>	108.2 ± 35.1 <sup>b)</sup>

Data are shown as the mean ± standard deviation. ELF; epithelial lining fluid. Values with the different letters indicate significant differences among the plasma, ELF, and Alveolar cells in PK parameters (a–b:  $P < 0.05$ ).

post administration and gradually decreased to  $1.1 \pm 0.2$  µg/ml at 24 hr following administration (Fig. 1).

Additionally, the  $C_{max}$  in ELF and alveolar cells was significantly higher than that in plasma ( $P < 0.05$ ). The  $T_{max}$  among in plasma, ELF, and alveolar cells were no statistical differences. The OBFX concentration in ELF and alveolar cells at 1, 2, and 24 hr was significantly higher than that in plasma ( $P < 0.05$ ). Similarly, at 6 hr in ELF, the OBFX concentration was also higher than that in plasma ( $P < 0.05$ ) (Fig. 1). The  $AUC_{0-24}$  ( $AUC_{inf}$ ) in plasma, ELF, and alveolar cells was  $23.8 \pm 1.8$  ( $26.5 \pm 2.8$ ),  $122.0 \pm 15.2$  ( $134.0 \pm 14.7$ ), and  $108.2 \pm 35.1$  ( $121.2 \pm 34.8$ ) µg·hr/ml (Table 1), respectively, with that in ELF and alveolar cells, was significantly higher than that in plasma ( $P < 0.05$ ). The  $AUMC_{0-24}$  ( $AUMC_{inf}$ ) in plasma, ELF, and alveolar cells was  $177.1 \pm 23.8$  ( $274.7 \pm 99.6$ ),  $793.5 \pm 102.0$  ( $1,204.9 \pm 100.1$ ), and  $706.3 \pm 185.0$  ( $1,172.0 \pm 293.3$ ) µg·hr<sup>2</sup>/ml (Table 1), respectively, with that in ELF and alveolar cells also were significantly higher than in plasma ( $P < 0.05$ ). The  $t_{1/2}$  in plasma, ELF, and alveolar cells was  $6.9 \pm 2.2$ ,  $7.0 \pm 0.6$ , and  $7.8 \pm 1.6$  hr, respectively; however, no statistical differences were observed. The  $MRT_{0-24}$  ( $MRT_{inf}$ ) in plasma, ELF, and alveolar cells was  $7.4 \pm 0.8$  ( $10.2 \pm 2.9$ ),  $6.5 \pm 0.2$  ( $9.1 \pm 0.6$ ), and  $6.6 \pm 0.6$  ( $9.9 \pm 2.4$ ) hr, respectively; however, no statistical differences were observed.

The ratios of MIC to  $AUC_{0-24}$  ( $AUC_{0-24}/MIC$ ) in plasma, ELF, and alveolar cells of bacterial species are shown in Table 2. The  $AUC_{0-24}/MIC$  in plasma, ELF, and alveolar cells of *P. multocida* was  $95.2 \pm 7.3$ ,  $488.0 \pm 60.8$ , and  $432.8 \pm 140.4$ , respectively (Table 2), with the ratio being significantly higher in ELF than in plasma ( $P < 0.05$ ). The  $AUC_{0-24}/MIC$  in plasma, ELF, and alveolar cells of *M. haemolytica* was  $23.8 \pm 1.8$ ,  $122.0 \pm 15.2$ , and  $108.2 \pm 35.1$  (Table 2), respectively, with the ratio being significantly higher in ELF and alveolar cells than in plasma ( $P < 0.05$ ).

## DISCUSSION

PK analysis in this study revealed a higher  $C_{max}$  and a later  $T_{max}$  for OBFX in plasma than those previously reported [16]. The  $t_{1/2}$  was shorter and the  $AUC_{0-24}$  for OBFX was greater than that reported in a previous report [16]. However, they used 3–5-month-old calves, while we used 4-week-old calves in our study. Shoaf *et al.* [25] reported changes in the activity of metabolic enzyme such as cytochrome P-450 during the early developmental period. Therefore, differences in the PK parameters of OBFX could be due to age differences associated with the hepatic drug-metabolizing activity.

The OBFX distribution in the alveolar region was evaluated by comparing the parameters of the ELF, alveolar cells, and plasma. The  $AUC_{0-24}$  and  $C_{max}$  in the ELF and alveolar cells were significantly higher than those in plasma. Accordingly, we considered that OBFX administration to calves would potentially result in a very high drug distribution to the alveolar region. The  $C_{max}$  of OBFX in the plasma was 3.7–4.4- and 2.9–5.8-fold lower than that in the ELF and BALF cells, respectively. The volume of distribution of OBFX (2.5 mg/ml) in cows is 1.61 l/kg [4], and that of OBFX (3.0 mg/ml) is 0.92 l/kg [7]. The OBFX concentration in the alveolar region was much higher than that in the blood, which can be attributed to the volume of distribution. Distribution volume is an indicator of organizational migration; therefore, OBFX can be considered to have excellent tissue migration ability.

In addition, new quinolone-based antimicrobial agents are rapidly taken up by macrophages; the relevant drug concentrations were reportedly higher in the cellular fractions than in the tissue [5, 13]. Furthermore, the neutrophil extracellular trap phenomenon (NETs) is enhanced by antimicrobial agents. Such drugs are incorporated into neutrophils, and their bactericidal activity is strengthened [12, 13]. Our results demonstrated that OBFX concentrations were higher in alveolar cells than in plasma. Therefore, in the case of pneumonia, the administration of OBFX may increase the OBFX concentration in alveolar cells from the inflamed site and prevent bacterial invasion.

The treatment of infectious diseases requires identifying the affected organs and tissues, relevant pathogen, and appropriate therapeutic strategies. In cases of pneumonia, predicting the antimicrobial effects of an agent is crucial. This prediction is based on both effective PK and pharmacodynamic (PD) analyses. Therefore, combined PK/PD approaches are widely used in clinical practice [3]. In human medicine, a higher concentration of antimicrobials, known as the mutant prevention concentration, is administered to eliminate all bacteria, including resistant bacteria generated by mutations in response to the administration of doses approaching the MIC [6]. The same approach has been adopted in veterinary medicine [9, 18, 20, 22].

The basic PK/PD parameters for predicting the effects of clinical treatment are the  $C_{max}/MIC$ ,  $AUC_{0-24}$  to  $MIC_{90}$  ( $AUC/MIC$ ) ratio, and duration beyond the MIC (%T > MIC) [12, 20, 22]. The antimicrobial effects of quinolone-based drugs (concentration-dependent systems) correlate with their  $AUC/MIC$  ratios. Therefore,  $AUC/MIC$  ratios are useful indicators in PK/PD analysis. In human medicine, the  $AUC/MIC$  ratio for Gram-positive bacteria ranges from 30 to 50 or more [14], and the  $AUC/MIC$  ratio

of Gram-negative bacteria is 125 or more [14]. An AUC/MIC ratio of at least 125 is recommended for concentration-dependent antimicrobial agents in veterinary medicine [18]. Therefore, if AUC / MIC is 125 or more, it is defined as exceeding MPC.

The AUC<sub>0-24</sub>/MIC was evaluated, and the therapeutic effect against each bacterial species was assessed. The AUC<sub>0-24</sub>/MIC ratios of *P. multocida* in ELF and alveolar cells were 488.0 ± 60.8 and 432.8 ± 140.4, respectively, with OBFX exceeding the MPC in both cases. For *M. haemolytica*, the AUC<sub>0-24</sub>/MIC ratio of in ELF and alveolar cells was 122.0 ± 15.2 and 108.2 ± 35.1, respectively, which was lower than the recommended ratio. In plasma, the ratios were also below 125 and significantly lower than those in ELF and alveolar cells for both bacteria ( $P < 0.05$ ). Therefore, intramuscular administration of OBFX (5.0 mg/kg) was considered to have excellent antibacterial activity against *P. multocida*.

OBFX did not exceed the MPC against *M. haemolytica* in ELF and alveolar cells. However, OBFX concentrations against *M. haemolytica* for ELF and alveolar cells at 24 hr post-administration were 1.2 ± 0.1 and 1.1 ± 0.2 µg/ml, respectively, suggesting that they exceeded the MIC approximately 24 hr after administration. Specifically, antibacterial activity against *M. haemolytica* was achieved at concentrations higher than the MIC and lower than the MPC, which is the mutant selection window (MSW) [10, 18, 20, 21], in which resistant bacteria are readily selected for and induced. The OBFX concentration in ELF and BALF cells decreased to 1.0 µg/ml or less at 24.5 ± 0.9 hr after administration from the half-life. Therefore, OBFX in the alveolar region at 24 hr following administration was considered to have an antibacterial effect equal to or higher than MIC (1.0 µg/ml [11]).

In the PK/PD analysis, the AUC<sub>0-24</sub>/MIC ratios in the alveolar region indicated that this regimen exhibited therapeutic effects against *P. multocida* and *M. haemolytica*. Future field measurements of MIC are needed for further investigation of treatment methods. In clinical practice, an antibiogram is generated for each farm, and a clinically therapeutic effect against these bacteria may be expected when its MIC is less than 1.2 µg/ml.

In this study, we measured the concentration of OBFX in plasma, ELF, and alveolar cells after the single dose of intramuscular administration of OBFX (5.0 mg/kg). PK parameter analysis suggested that this treatment was effective against bacterial species with a MIC of 1.0 µg/ml or less. However, additional studies are needed to clarify aspects, such as the migration of OBFX in calves with pneumonia, prior to the practical application of this treatment.

**CONFLICT OF INTEREST.** The authors have financial conflicts of interest to disclose concerning the study. This study was supported by the DS Pharma Animal Health Ltd.

## REFERENCES

1. Brown, S. A., Chester, S. T. and Robb, E. J. 1996. Effects of age on the pharmacokinetics of single dose ceftiofur sodium administered intramuscularly or intravenously to cattle. *J. Vet. Pharmacol. Ther.* **19**: 32–38. [Medline] [CrossRef]
2. Bryson, D. G. 1985. Calf pneumonia. *Vet. Clin. North Am. Food Anim. Pract.* **1**: 237–257. [Medline] [CrossRef]
3. Craig, W. A. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin. Infect. Dis.* **26**: 1–10, quiz 11–12. [Medline] [CrossRef]
4. Elias, G., Lee, J. S., Hwang, M. H., Park, Y. S., Cho, K. H., Kim, Y. H. and Park, S. C. 2009. Pharmacokinetics and pharmacokinetic/pharmacodynamic integration of orbifloxacin in Korean Hanwoo cattle. *J. Vet. Pharmacol. Ther.* **32**: 219–228. [Medline] [CrossRef]
5. Deguchi, Y., Sun, J., Tauchi, Y., Sakai, S. and Morimoto, K. 2003. Distribution characteristics of grepafloxacin, a fluoroquinolone antibiotic, in lung epithelial lining fluid and alveolar macrophage. *Drug Metab. Pharmacokinet.* **18**: 319–326. [Medline] [CrossRef]
6. Dong, Y., Zhao, X., Domagala, J. and Drlica, K. 1999. Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **43**: 1756–1758. [Medline] [CrossRef]
7. Gebru, E., Lee, S. J., Kim, J. C. and Park, S. C. 2011. Allometric scaling of orbifloxacin disposition in nine mammal species: a retrospective analysis. *J. Vet. Med. Sci.* **73**: 817–820. [Medline] [CrossRef]
8. Giguère, S., Huang, R., Malinski, T. J., Dorr, P. M., Tessman, R. K. and Somerville, B. A. 2011. Disposition of gamithromycin in plasma, pulmonary epithelial lining fluid, bronchoalveolar cells, and lung tissue in cattle. *Am. J. Vet. Res.* **72**: 326–330. [Medline] [CrossRef]
9. Gotfried, M. H., Danziger, L. H. and Rodvold, K. A. 2001. Steady-state plasma and intrapulmonary concentrations of levofloxacin and ciprofloxacin in healthy adult subjects. *Chest* **119**: 1114–1122. [Medline] [CrossRef]
10. Griffin, D., Chengappa, M. M., Kuszak, J. and McVey, D. S. 2010. Bacterial pathogens of the bovine respiratory disease complex. *Vet. Clin. North Am. Food Anim. Pract.* **26**: 381–394. [Medline] [CrossRef]
11. Harada, K., Usui, M. and Asai, T. 2014. Application of enrofloxacin and orbifloxacin disks approved in Japan for susceptibility testing of representative veterinary respiratory pathogens. *J. Vet. Med. Sci.* **76**: 1427–1430. [Medline] [CrossRef]
12. Hoeben, D., Burvenich, C. and Heyneman, R. 1997. Influence of antimicrobial agents on bactericidal activity of bovine milk polymorphonuclear leukocytes. *Vet. Immunol. Immunopathol.* **56**: 271–282. [Medline] [CrossRef]
13. Jerjomiceva, N., Seri, H., Völlger, L., Wang, Y., Zeitouni, N., Naim, H. Y. and von Köckritz-Blickwede, M. 2014. Enrofloxacin enhances the formation of neutrophil extracellular traps in bovine granulocytes. *J. Innate Immun.* **6**: 706–712. [Medline] [CrossRef]
14. Kuti, J. L. 2016. Optimizing antimicrobial pharmacodynamics: a guide for your stewardship program. *Rev. Med. Clin. Las Condes* **27**: 615–624.
15. Kuramae, T., Otomaru, K., Hirata, M., Ishikawa, S., Noguchi, M., Ikedo, T., Horinouchi, C., Hayashi, J., Tsumagari, K. and Hobo, S. 2020. Pharmacokinetics in plasma and alveolar regions of healthy calves subcutaneously administered a single dose of enrofloxacin. *J. Vet. Med. Sci.* **82**: 1197–1203. [Medline] [CrossRef]
16. Matsumoto, S., Nakai, M., Yoshida, M. and Katae, H. 1998. Absorption, distribution and excretion of orbifloxacin in swines and calves. *Nippon Juishikai Zasshi* **51**: 13–18 (in Japanese with English abstract).
17. Matsumoto, S., Nakai, M., Yoshida, M. and Katae, H. 1999. A study of metabolites isolated from urine samples of pigs and calves administered orbifloxacin. *J. Vet. Pharmacol. Ther.* **22**: 286–289. [Medline] [CrossRef]
18. McKellar, Q. A., Sanchez Bruni, S. F. and Jones, D. G. 2004. Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. *J. Vet. Pharmacol. Ther.* **27**: 503–514. [Medline] [CrossRef]

19. Mehta, A. K., Gaur, S. N., Arora, N. and Singh, B. P. 2007. Effect of choline chloride in allergen-induced mouse model of airway inflammation. *Eur. Respir. J.* **30**: 662–671. [[Medline](#)] [[CrossRef](#)]
20. Page, S. W. and Gautier, P. 2012. Use of antimicrobial agents in livestock. *Rev. Sci. Tech.* **31**: 145–188. [[Medline](#)] [[CrossRef](#)]
21. Pascual, A., García, I., Ballesta, S. and Perea, E. J. 1997. Uptake and intracellular activity of trovafloxacin in human phagocytes and tissue-cultured epithelial cells. *Antimicrob. Agents Chemother.* **41**: 274–277. [[Medline](#)] [[CrossRef](#)]
22. Papich, M. G. 2014. Pharmacokinetic-pharmacodynamic (PK-PD) modeling and the rational selection of dosage regimes for the prudent use of antimicrobial drugs. *Vet. Microbiol.* **171**: 480–486. [[Medline](#)] [[CrossRef](#)]
23. Saw, S., Kale, S. L. and Arora, N. 2012. Serine protease inhibitor attenuates ovalbumin induced inflammation in mouse model of allergic airway disease. *PLoS One* **7**: e41107. [[Medline](#)] [[CrossRef](#)]
24. Sato, T., Okubo, T., Usui, M., Higuchi, H. and Tamura, Y. 2013. Amino acid substitutions in GyrA and ParC are associated with fluoroquinolone resistance in *Mycoplasma bovis* isolates from Japanese dairy calves. *J. Vet. Med. Sci.* **75**: 1063–1065. [[Medline](#)] [[CrossRef](#)]
25. Shoaf, S. E., Schwark, W. S., Guard, C. L. and Babish, J. G. 1987. The development of hepatic drug-metabolizing enzyme activity in the neonatal calf and its effect on drug disposition. *Drug Metab. Dispos.* **15**: 676–681. [[Medline](#)]