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Formulation of a rational dosage regimen of ceftiofur hydrochloride oily suspension by pharmacokinetic-pharmacodynamic (PK-PD) model for treatment of swine *Streptococcus suis* infection

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ABSTRACT

Background: Our previously prepared ceftiofur (CEF) hydrochloride oily suspension shows potential wide applications for controlling swine *Streptococcus suis* infections, while the irrational dose has not been formulated.

Objectives: The rational dose regimens of CEF oily suspension against *S. suis* were systematically studied using a pharmacokinetic-pharmacodynamic model method.

Methods: The healthy and infected pigs were intramuscularly administered CEF hydrochloride oily suspension at a single dose of 5 mg/kg, and then the plasma and pulmonary epithelial lining fluid (PELF) were collected at different times. The minimum inhibitory concentration (MIC), minimal bactericidal concentration, mutant prevention concentration (MPC), post-antibiotic effect (PAE), and time-killing curves were determined. Subsequently, the area under the curve by the MIC (AUC_{0-24h}/MIC) values of desfuroylceftiofur (DFC) in the PELF was obtained by integrating *in vivo* pharmacokinetic data of the infected pigs and *ex vivo* pharmacodynamic data using the sigmoid E_{max} (Hill) equation. The dose was calculated based on the AUC_{0-24h}/MIC values for bacteriostatic action, bactericidal action, and bacterial elimination.

Results: The peak concentration, the area under the concentration-time curve, and the time to peak for PELF's DFC were 24.76 ± 0.92 μg/mL, 811.99 ± 54.70 μg·h/mL, and 8.00 h in healthy pigs, and 33.04 ± 0.99 μg/mL, 735.85 ± 26.20 μg·h/mL, and 8.00 h in infected pigs, respectively. The MIC of PELF's DFC against *S. suis* strain was 0.25 μg/mL. There was strong concentration-dependent activity as determined by MPC, PAE, and the time-killing curves. The AUC_{0-24h}/MIC values of PELF's DFC for bacteriostatic activity, bactericidal activity, and virtual eradication of bacteria were 6.54 h, 9.69 h, and 11.49 h, respectively. Thus, a dosage

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Conflict of Interest
The authors declare no conflicts of interest.

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regimen of 1.94 mg/kg every 72 h could be sufficient to reach bactericidal activity.

Conclusions: A rational dosage regimen was recommended, and it could assist in increasing the treatment effectiveness of CEF hydrochloride oily suspension against *S. Suis* infections.

Keywords: Ceftiofur hydrochloride; pigs; pharmacokinetic (PK); pharmacodynamic (PD) model; *Streptococcus suis*

INTRODUCTION

Streptococcus suis is mainly responsible for respiratory disease, which is characterized by acute hemorrhagic septicemia, endocarditis, meningitis, arthritis, lactation piglet diarrhea, and abortion [1]. In recent years, *S. suis* has spread worldwide. At least 35 capsular serotypes of *S. suis* have been identified globally. The 1, 2, 3, 4, 7, 9, and 14 types are the main serotypes [2-4]. The serotypes of *S. suis* prevalent in various countries are different [5-7]. The *S. suis* has caused large economic losses in the worldwide pig industry because of its high morbidity and mortality [8,9]. Therefore, exploration of an effective antimicrobial drug and its related formulation is necessary to treat swine *S. suis* infections. Cephalosporins have been used in animal production as a potent antimicrobial agent against *S. suis*. For example, ceftiofur (CEF) hydrochloride, a broad-spectrum third-generation cephalosporin, has excellent antibacterial activity against both Gram-positive and Gram-negative bacteria, especially for *S. suis* [10]. It is widely used in the treatment of swine respiratory diseases, such as *S. suis*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Salmonella choleraesuis* due to the advantage of rapid absorption, maintaining effective drug concentration in lungs for a comparatively long time compared to first- or second-generation cephalosporins, slow elimination, and high bioavailability [11]. Therefore, CEF is expected to have potential wide applications for controlling swine respiratory disease caused by *S. suis*. In our previous study, a CEF hydrochloride oily suspension was explored to improve its pharmacokinetics. It significantly improved drug absorption, prolonged the drug's sustained-release performance, and reduced irritation [12], while its rational dose regimen is still not studied. Pharmacokinetic-pharmacodynamic (PK-PD) modeling is an important investigative tool that can help optimize the dosage regimens of drugs by linking the dosage regimens of the drugs to their clinical effects [13]. At present, PK-PD has been used to establish dosage regimens for eliminating bacteria, reducing carrier status, and increasing resistance in the veterinary field [13,14]. Furthermore, both the US Food and Drug Administration and European Medicine Agency have recommended the use of PK-PD models to formulate a scientific dosing scheme of a new drug [15-17].

In this study, the antibacterial activity of CEF oily suspension against swine *S. suis* and its pharmacokinetics in pigs were systematically investigated. The surrogate's index (area under the curve by the minimum inhibitory concentration [$AUC_{0–24h}$/MIC]) of antibiotic efficacy, taking into account minimum inhibitory concentration (MIC; PD) and exposure antibiotic metrics (PK), were calculated by the *ex vivo* PK-PD model. Finally, the recommended daily dose of the new formulation was calculated based on PK-PD models.

MATERIALS AND METHODS

Chemicals and reagents

CEF (99.7%) and desfuroylceftiofur (DFC; 98%) reference standard were purchased from China Institute of Veterinary Drug Control (China) and Dr. Ehrenstorfer, respectively. CEF hydrochloride was obtained from Shandong Jiulong Fine Chemical Co., Ltd (China). Methyl alcohol and acetonitrile of liquid chromatography grade as well as trifluoroacetic acid (TFA) were bought from TEDIA (USA). Phosphoric acid was provided by Sinopharm Chemical Reagent Co., Ltd (China). The water for high-performance liquid chromatography (HPLC) was prepared with a Milli-Q system. Nicotinamide adenine dinucleotide and fetal calf serum were obtained from Guangzhou Ruite Biological Technology Co., Ltd (China). Various media, broth, and agar were provided by Qingdao Hope Bio-Technology Co., Ltd (China). Other chemicals and reagents not specified in the text were of analytical grade or equivalent.

Bacteria

Twenty-nine strains of *S. suis* isolated from pig nostrils in pig farms were obtained by the National Reference Laboratory of Veterinary Drug Residues (HZAU) (China) and identified by conventional methods. Isolates were subcultured thrice on a tryptose soya agar base supplemented with 5% sheep blood and incubated at 37°C for 18–24 h [18].

Animals

The study was carried out using twelve healthy male (castrated) pigs, weighing 22–25 kg and 12–13 weeks old. All the experimental protocols concerning the handling of pigs were in accord with the requirements of the Institutional Animal Care and Use Committee of Huazhong Agricultural University, and the approval number for the experiment was HZAUSW-2016-007. Animal housing was kept at 16°C–28°C and 50%–80% relative humidity. The pigs were placed in separate metabolism cages, had free access to water, and were fed antibiotic-free feed twice a day.

Pharmacokinetics

The twelve pigs were stochastically separated into two groups (n = 6/group): the *S. suis* infection group and the healthy group. The healthy group without oral gavage of *S. suis* was established based on clinical symptom observations and a negative *S. suis* status. The *S. suis* infection group was established by oral gavage with 100 mL of *S. suis cvcc* 607 culture suspension containing 10^9 CFU/mL. After inoculation, the pigs were observed for clinical symptoms. Clinical manifestations of fever, decreased appetite, shortness of breath, cough, asthma, presence of serous or purulent secretions, and other clinical symptoms were observed. At the same time, nasal swab samples were obtained to determine the infection of *S. suis*.

After the *S. suis* infection model was established, the *S. suis* infection pigs and healthy pigs were intramuscularly administered CEF hydrochloride oily suspension at a dose of 5 mg/kg. Atropine (0.05 mg/kg), ketamine (5 mg/kg), and propofol (3 mg/kg) were given intramuscularly and intravenously 30 min before drug administration of the oily suspension. Pulmonary epithelial lining fluid (PELF) was collected as previously described at different fixed times [19] with an electronic fiberoptic bronchoscope inserting in the right middle lung lobe. Then, 50 mL of normal saline was instilled into that lobe and aspirated into a 50 mL centrifugal tube after 20 sec. The PELF samples were collected in heparinized tubes at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, and 120 h post intramuscular dosing. Simultaneously, blood was collected from the front cavity vein of each pig into heparinized tubes at the same time

points. The collected plasma and PELF samples were divided into two aliquots and stored at -20°C for subsequent PK-PD studies.

HPLC
Once administered, CEF was generally undetectable in plasma and rapidly metabolized into DFC in the body [20]. Thus, DFC concentrations (small quantity of CEF remained in samples was transformed into DFC by sample preparation) in plasma and PELF samples were determined by using a Waters 2695 series reverse-phase HPLC. A ZORBAX SB C_{18} column (250 × 4.6 mm, i.d. 5 µm; Agilent Technology, USA) was used for separation. The mobile phases were 0.1% TFA (w/v) mixed with acetonitrile (86/14; V/V). A 20-µL aliquot of the reconstituted sample was injected into the HPLC system. The wavelength and flow rate were 266 nm and 1 mL/min, respectively.

Sample extraction
The solution used to extract the drug from plasma and PELF samples was borate buffer (0.05 M, pH = 9.0). Seven milliliters of the extracting solution were added to 0.5 mL of the sample, and the mixture was placed in a water bath at 50°C for 15 min. Then, the mixture was taken out from the water bath every 3 min and vortexed for 10 sec. Subsequently, the mixture was filtrated by using Oasis HLB solid-phase extraction and evaporated to dryness at 50°C under nitrogen. Then, the residue was reconstituted in a 0.5 mL mobile phase. After the reconstituted solution was filtered by a 0.22 µm syringe filter membrane, a 20-µL aliquot of the filtrate was injected into the HPLC system for analysis.

Pharmacokinetic analysis
The PK parameters of CEF oily suspension in plasma and PELF were determined by WinNonlin software (version 5.2.1; Pharsight Corporation, USA). Drug concentration vs. time curves were plotted on semi-logarithmic graphs to choose the appropriate PK models. The most suitable compartmental model was evaluated by applying the minimum Akaike's information criterion. The non-compartmental model was the most appropriate model for all tested pigs and was used to compute the main PK parameters, including the time to peak concentration (T_{max}), the peak concentration (C_{max}), the area under the concentration-time curve (AUC), etc.

Determination of MIC, minimal bactericidal concentration (MBC), mutant prevention concentration (MPC), and post-antibiotic effect (PAE)
The MIC was determined by using the micro-dilution method of the Clinical and Laboratory Standard Institute (CLSI-M07A8-2010). *Escherichia coli* ATCC 25922 was recommended by CLSI as the quality control strain, and the MIC_{50} and MIC_{90} of 29 clinical strain of *S. suis* were calculated.

The pathogenicity of *S. suis* was determined by lethal tests in mice. The pathogenicity of different strains of *S. suis* (*cvcc* 607, SC-19, and SC-109) were determined by the death of mice intranasally inoculated with the same amount of bacteria (5×10^9 CFU). It was found that *S. suis cvcc* 607 caused the largest number of deaths (8/10) compared with the other two strains (5/10 and 3/10). Thus, the MIC and MBC for the *S. suis cvcc* 607 isolate of the highest pathogenicity were determined *in vitro* and *ex vivo* using the micro-dilution technique. Determination of MBC was performed by inoculating a supplemented agar plate with 100 µL of suspension with no obvious bacteria from the initial MIC testing. Inoculated plates were inverted and incubated at 37°C. The MBC was determined as the concentration that reduced the viable organism count by ≥ 3$\log_{10}$ over 24 h. The drug carry-over effect was reduced by ≥ 250-fold sample dilution in the agar plate.

The agar dilution method was used to determine the MPC. For *S. suis cvcc* 607, 10^{10} CFU/mL was inoculated onto the supplemented agar plates containing serial dilutions of CEF and DCF ($1 \times$ MIC, $2 \times$ MIC, $4 \times$ MIC, $8 \times$ MIC, $16 \times$ MIC, and $32 \times$ MIC). The plates were then incubated at 37°C, and the MPC defined as the lowest concentration that yielded no visible bacterial growth after 72 h.

For PAE determination, logarithmically growing cultures of *S. suis cvcc* 607 at an initial inoculum of 1×10^6 CFU/mL were exposed to a CEF and DCF concentration equivalent to $1 \times$ MIC, $2 \times$ MIC, $4 \times$ MIC for 1 or 2 h. The media containing CEF and DCF was removed by 1,000-fold dilution with broth medium, and the continued suppression of bacterial growth was monitored over time. The PAE was defined as the time required for the antimicrobial-treated bacterial to increase in number by 1 $\log_{10}$ CFU/mL minus the value determined for the non-treated cultures of the same bacteria.

In vitro and *ex vivo* time-killing study

The *in vitro* killing curves of CEF against *S. suis cvcc* 607 were established by plotting time versus $\log_{10}$ CFU/mL. The strain *S. suis cvcc* 607 at the stationary phase was added to 10 mL of TSB, giving a starting inoculum of 10^6 CFU/mL. CEF was added to obtain a serial concentration corresponding to $1/4 \times$ MIC, $1/2 \times$ MIC, $1 \times$ MIC, $2 \times$ MIC, $4 \times$ MIC, $8 \times$ MIC, $16 \times$ MIC, and $32 \times$ MIC. The tubes were placed at 37°C and the bacterial count (CFU/mL) was determined by agar dilution method for each tube after incubation of 1, 2, 4, 6, 8, 12, and 24 h. Briefly, each culture sample was subjected to 10-fold serial dilution, and then 100 μL of each dilution spread onto agar plates. The plates were incubated at 37°C, and the viable colonies were counted after 24 h. Each concentration was performed in triplicate. The limit of detection (LOD) was 10 CFU/mL.

Similarly, the *ex vivo* killing curves were determined as described above using PELF samples obtained from pigs at different time points after intramuscular administration. The tubes containing bacterial culture and PELF samples were incubated at 37°C, and the viable organism levels were determined at 1, 2, 4, 8, 12, and 24 h. Results are expressed as CFU/mL with a LOD of 10 CFU/mL.

PK-PD integration

The AUC_{0-24h}/MIC was used as the combined PK-PD parameter according to the above pharmacokinetic and pharmacodynamic study. Using the following inhibitory sigmoid E_{max} model to integrate the *ex vivo* AUC_{0-24h}/MIC ratio and bacteria count change (CFU/mL) in PELF during 24 h incubation. This model is described as follows:

$$E = E_{max} - \frac{(E_{max} - E_0) \cdot C^N}{C^N + EC_{50}^N}$$

In the above formula, E indicates the effect of the antimicrobial agent and was measured as a $\log_{10}$ difference value of bacterial numbers before and after 24 h incubation with a PELF sample; E_0 and E_{max} are the changes in $\log_{10}$ difference values for bacterial counts between 0 and 24 h in the control sample and for the CEF containing samples, respectively. EC_{50} is the AUC_{0-24h}/MIC value that attained 50% of the E_{max}; C is the tested AUC_{0-24h}/MIC ratio; and N is the Hill coefficient.

The *ex vivo* antibacterial effects of CEF hydrochloride oily suspension after intramuscular administration were quantified into three levels: 1) bacteriostatic action (no change in bacterial count, E = 0), 2) bactericidal action (99.9% reduction in bacterial count, E = −3), and 3) bacterial elimination (99.99% reduction, E = −4). The dose was calculated by using the following formula:

$$Dose = \frac{CL \times (AUC/MIC)ex \times MIC}{F \times fu}$$

in which $(AUC_{0-24h}/MIC)_{ex}$ is the targeted endpoint for optimal efficacy; the MIC is the target pathogen; clearance rate (CL) is the daily clearance; *fu* is the free fraction of the drug in PELF (*fu* = 92% in this study); F is the bioavailability of CEF. CL/F is the clearance per day based on the bioavailability of CEF and obtained from the pharmacokinetic study.

To investigate the effect of different dosage regimens, the PD model describing bacterial growth rate as a function of CEF concentration was combined with the PK model, and simulations were performed with Mlxplore software (version-1.1.0; Lixoft, France).

Statistical analysis

Data are presented as mean ± SD and were analyzed by SPSS software (version 20; IBM, USA). Statistical significance was defined as a *p* value of 0.05 obtained by 1-way analysis of variance.

RESULTS

Establishment of HPLC method

The specificity of the detection method was good for DFC. There was no endogenous interference on chromatograms. The linear range of the standard curves of DFC was ranged from 0.1 to 50 μg/mL (R = 0.9988) in plasma and 0.1 to 50 μg/mL (R = 0.9997) in PELF. The LOD was 0.05 μg/mL, and the limit of quantification was 0.1 μg/mL in plasma and PELF. The mean recovery of DFC was > 80% in plasma and PELF. The relative standard deviations for intra-day and inter-day variation of DFC were below 8.0% in the plasma sample and PELF.

Pharmacokinetics of CEF hydrochloride oily suspension

The DFC concentrations in plasma and PELF vs. time curves after intramuscular administration of CEF hydrochloride oily suspension are illustrated in **Fig. 1**. After intramuscular dosing, the DFC (active metabolite) concentration in plasma and PELF in CEF hydrochloride oily suspension groups was best fitted with the non-compartmental model (**Table 1**). The C_{max}, $AUC_{0-\infty}$, and the elimination half-life time ($T_{1/2}$) for plasma were 3.69 ± 0.08 μg/mL, 112.65 ± 45.90 μg·h/mL, and 69.44 ± 9.02 h in healthy pigs and 3.42 ± 0.06 μg/mL, 100.43 ± 37.90 μg·h/mL, and 66.92 ± 9.66 h in infected pigs, respectively. The T_{max}, elimination rate constant (K_e), volume of distribution (V_d), CL, and mean residence time (MRT) in plasma were not significantly different between the healthy and infected groups. Significant differences in DFC concentrations were observed between plasma and PELF samples. The DFC concentration in PELF of healthy and infected pigs reached 24.02 ± 1.40 μg/mL and 23.79 ± 0.53 μg/mL at 2 h, respectively, which were higher than the MIC (2 μg/mL). The concentration of DFC in PELF slowly decreased to 20.73 ± 2.13 μg/mL at 12 h and 0.14 ± 0.02 μg/mL at 120 h in healthy pigs, and to 19.69 ± 0.60 μg/mL at 12 h and 0.13 ± 0.03 μg/mL at 120 h in infected pigs. It was noteworthy that the DFC concentration in PELF at 2 h
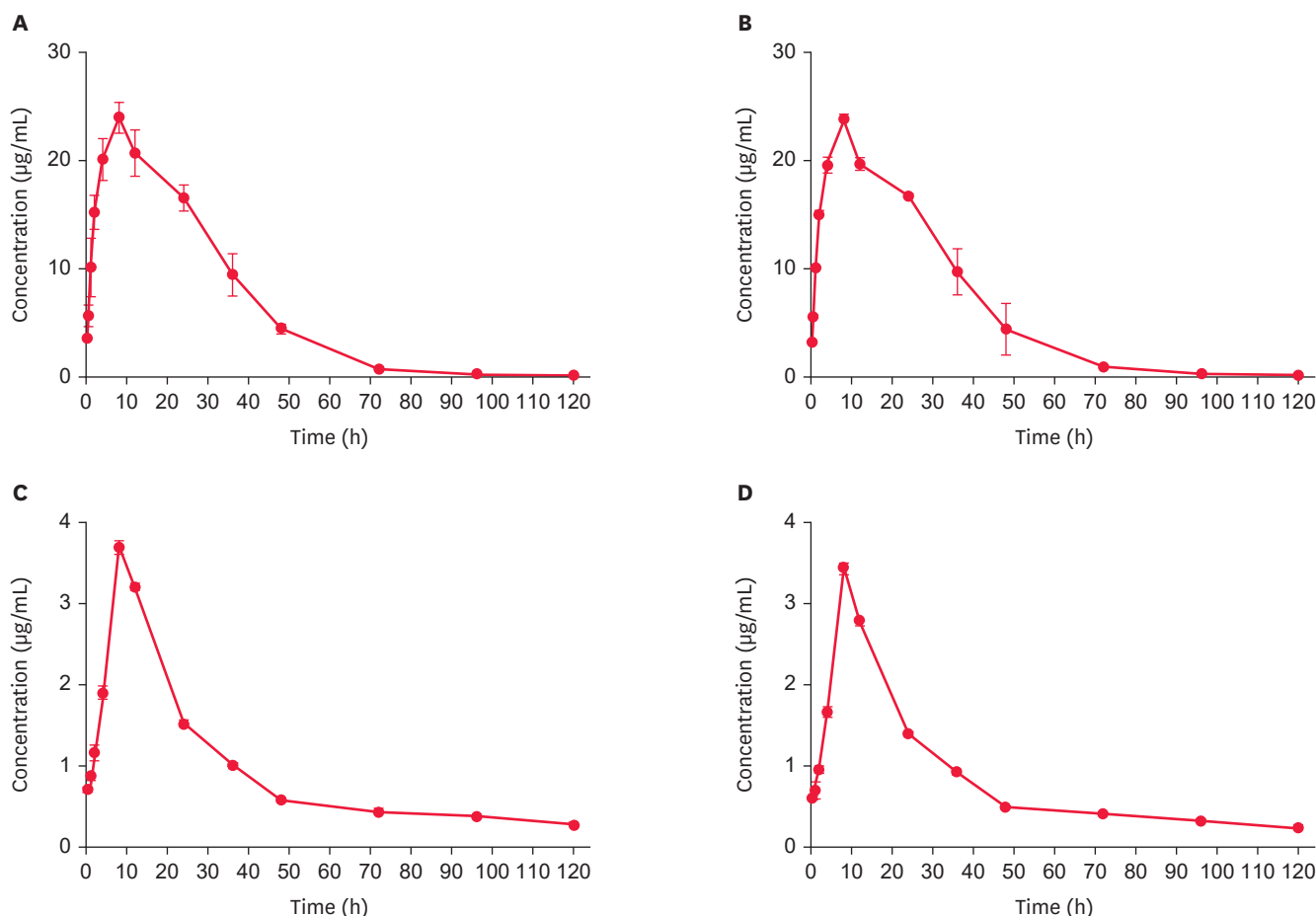
Fig. 1. Desfuroylceftiofur concentration vs. time curve of ceftiofur in plasma and PELF of healthy and infected pigs (mean ± SD, n = 6) (A) PELF of healthy pig group, (B) PELF of infected pig group, (C) plasma of healthy pig group, (D) plasma of infected pig group.
PELF, pulmonary epithelial lining fluid.

Table 1. Pharmacokinetic parameters of desfuroylceftiofur in plasma and PELF of healthy and infected pigs after intramuscular administration of CEF hydrochloride suspension (n = 6)

Parameters	Units	CEF hydrochloride oily suspension			
		Plasma		PELF	
		Healthy pigs	Infected pigs	Healthy pigs	Infected pigs
C_{max}	µg/mL	3.69 ± 0.08	3.42 ± 0.06	24.76 ± 0.92	33.04 ± 0.99*
AUC	µg·h/mL	112.65 ± 45.90	100.43 ± 37.90*	811.99 ± 54.70	735.85 ± 26.20**
T_{max}	h	8	8	8	8
$T_{1/2}$	h	69.44 ± 9.02	66.92 ± 9.66	13.16 ± 0.29	19.24 ± 1.32*
K_e	h^{-1}	0.01 ± 0.013	0.01 ± 0.002	0.05 ± 0.001	0.04 ± 0.017
V_d	L/kg	3.58 ± 0.51	3.88 ± 0.27	0.12 ± 0.011	0.12 ± 0.014
CL	L/h/kg	0.0357 ± 0.0006	0.0404 ± 0.0040	0.0062 ± 0.0004	0.0069 ± 0.001
MRT	h	34.21 ± 0.19	34.12 ± 0.20	23.47 ± 0.91	22.92 ± 2.99

Values are presented as mean ± SD.
PELF, pulmonary epithelial lining fluid; CEF, ceftiofur; C_{max}, maximal drug concentration; AUC, the area under the concentration-time curve; T_{max}, time to reach C_{max}; $T_{1/2}$, the elimination half-life; K_e, elimination rate constant; V_d, volume of distribution; CL, clearance rate; MRT, mean residence time.
*Infected group was significantly different from the healthy group ($P < 0.05$); **Infected group was significantly different from the healthy group ($P < 0.01$).

was 7.24-9.66 times that detected in plasma. The $AUC_{0-\infty}$ and $T_{1/2}$ of DFC in PELF were 811.99 ± 54.70 µg·h/mL and 13.16 ± 0.29 h in healthy pigs, and 735.85 ± 26.20 µg·h/mL and 19.24 ± 1.32 h in infected pigs, respectively. The T_{max}, K_e, V_d, CL, and MRT of DFC in PELF were not significantly different between healthy and infected pigs.

After intramuscular administration, DFC concentrations in PELF were significantly higher than those in plasma. The values for C_{max} and $AUC_{0-\infty}$ in PELF were obviously higher than those in plasma.

Antimicrobial susceptibility

The MIC and MBC distribution of CEF against 29 clinical strains of *S. suis* are shown in **Fig. 2**. The MIC values ranged from 0.06 to 32 μg/mL. The corresponding MIC_{50} and MIC_{90} were 0.25 and 32 μg/mL, respectively, suggesting that CEF displays a potent antibacterial effect against *S. suis*.

The pathogenic *S. suis cvcc* 607 strain with MIC equal to the MIC_{50} was chosen for investigating the antibacterial activity characteristics of CEF *in vitro* and *ex vivo*. The MIC and MBC values of CEF against *S. suis cvcc* 607 were 0.25 and 0.5 μg/mL in TSB broth and 0.25 and 0.5 μg/mL in PELF, respectively. The MBC/MIC ratios were both 2:1 in TSB broth and PELF, suggesting a relatively concentration-dependent tendency of CEF [21]. The MIC and MBC values of DFC against *S. suis cvcc* 607 were 0.125 and 0.5 μg/mL in TSB broth and 0.125 and 0.5 μg/mL in PELF, respectively. The MPC of CEF and DFC against *S. suis cvcc* 607 were 1 and 1 μg/mL, respectively. The PAE values of CEF for 1 and 2 h are shown in **Table 2**.

In vitro and *ex vivo* antimicrobial activity

In vitro time-killing curves of CEF against *S. suis cvcc* 607 are illustrated in **Fig. 3**. According to the profiles, CEF showed a concentration-dependent bactericidal effect as the increasing drug concentrations induced more swift and radical killing effects. When the concentration of CEF was 2×MIC (0.5 μg/mL), the bactericidal effect of CEF was observed. With an increasing concentration of CEF, there was an obvious inhibition of bacterial growth observed in a very short period. From this, it was suggested that the bactericidal activity was enhanced by the increase in drug concentration.
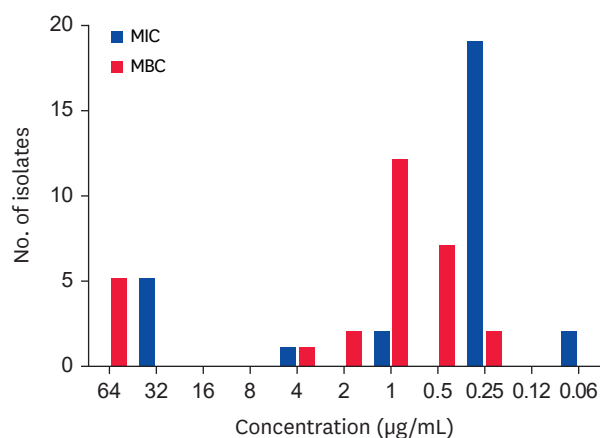
Fig. 2. MIC and MBC distribution of ceftiofur against 29 *S. suis* isolates.
MIC, minimum inhibitory concentration; MBC, minimal bactericidal concentration.

Table 2. The PAE of ceftiofur against *S. suis cvcc* 607

Drug concentration (μg/mL)	PAE (h)	
	Exposure of 1 h	Exposure of 2 h
0.25 (1 × MIC)	0.13	1.54
0.5 (2 × MIC)	0.45	1.80
1 (4 × MIC)	0.87	2.15

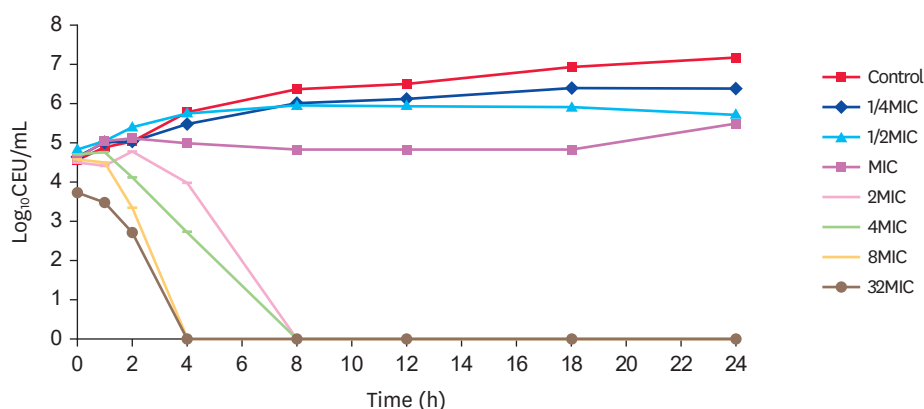PAE, post-antibiotic effect; MIC, minimum inhibitory concentration.

Fig. 3. *In vitro* killing curves of ceftiofur against *S. suis cvcc* 607 in TSB broth (n = 3). MIC, minimum inhibitory concentration.
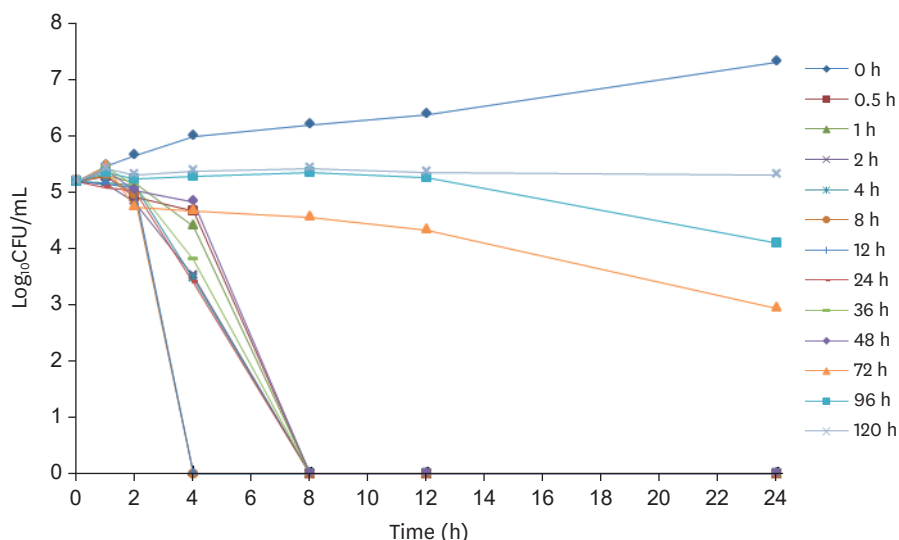

Fig. 4. *Ex vivo* killing curves of desfuroylceftiofur in pulmonary epithelial lining fluid against *S. suis cvcc* 607 (n = 3).

The PELF samples from six infected pigs after intramuscular administration of CEF hydrochloride oily suspension collected at different time points were used to determine *ex vivo* killing curves. In the healthy and diseased PELF, the concentration of DFC in PELF collected at 8 h was the highest, and then the concentration of the DFC was decreased with the increase of time. Therefore, its bactericidal effect against *S. suis cvcc* 607 was the strongest at 8 h (33.04 ± 0.99 μg/mL) according to **Fig. 4**. The *ex vivo* time-killing curve showed that the activity of DFC against *S. suis cvcc* 607 was concentration-dependent. When DFC concentrations were higher than the MIC (0.25 μg/mL), the bacteriostatic efficiency was gradually enhanced with an increase in DFC concentration.

PK-PD integration and modeling

The PK-PD indices of DFC against *S. suis cvcc* 607 were considered using the PK parameters and MIC data (**Table 3**). The mean AUC_{0-24h}/MIC and C_{max}/MIC ratios were 2943.40 ± 15.16 and 132.16 ± 0.24, respectively. The mean AUC_{0-24h}/MBC and C_{max}/MBC ratios were 1471.70 ± 7.58 and 66.08 ± 0.12, respectively. The mean values for AUC_{0-24h}/MPC and C_{max}/MPC were 735.85 ± 3.42 and 33.04 ± 0.11, respectively.

Table 3. Pharmacokinetic-pharmacodynamic integration parameters for desfuroylceftiofur in pulmonary epithelial lining fluid of infected pigs after intramuscular administration of a single dose of 5 mg/kg (n = 6)

Parameter	Values
AUC_{0-24h}/MIC	2,943.40 ± 15.16
AUC_{0-24h}/MBC	1,471.70 ± 7.58
AUC_{0-24h}/MPC	735.85 ± 3.42
C_{max}/MIC	132.16 ± 0.24
C_{max}/MBC	66.08 ± 0.12
C_{max}/MPC	33.04 ± 0.11

Values are presented as mean ± SD.
AUC_{0-24h}/MIC, the area under the curve by the minimum inhibitory concentration; AUC_{0-24h}/MBC, the area under the curve by the minimal bactericidal concentration; AUC_{0-24h}/MPC, the area under the curve of the ceftiofur by the mutant prevention concentration; C_{max}/MIC, the peak concentration by the minimum inhibitory concentration; C_{max}/MBC, the peak concentration by the minimal bactericidal concentration; C_{max}/MPC, the peak concentration by the mutant prevention concentration.

Table 4. The sigmoid E_{max} model of desfuroylceftiofur in pulmonary epithelial lining fluid of infected pigs

Parameter	Values
E_{max}	1.68 ± 0.32
E_0	−5.14 ± 0.19
EC_{50}	8.24 ± 1.36
N	4.83 ± 0.34
$E_{max}-E_0$	6.82 ± 0.87
$(AUC_{0-24h}/MIC)_{ex}E = 0$	6.54 ± 1.44
$(AUC_{0-24h}/MIC)_{ex}E = -3$	9.96 ± 1.62
$(AUC_{0-24h}/MIC)_{ex}E = -4$	11.49 ± 2.03

Values are presented as mean ± SD.
E_{max}, the maximum difference of antibacterial number logarithm; E_0, the difference after 24 h incubation in number antibacterial logarithm in control samples; EC_{50}, the pharmacokinetic-pharmacodynamic parameter value in the *ex vivo* study when the 50% maximal bactericidal effect is produced; N, the Hill coefficient, which is used to describe the slope of the pharmacokinetic-pharmacodynamic parameter value and the effect E linearization in the *ex vivo* study and to determine the S-shaped curve; $(AUC_{0-24h}/MIC)_{ex}E$, the difference in antibacterial number logarithm.

PK-PD modeling

The relationship between the antimicrobial efficacy and the *ex vivo* PK-PD parameters of the AUC_{0-24h}/MIC ratios was fitted by using the inhibitory sigmoid E_{max} model. The model parameters, including the Hill coefficient (N), E_0, E_{max}, and AUC_{0-24h}/MIC values for the three levels of growth inhibition arepresented in **Table 4**. The values of AUC_{0-24h}/MIC in PELF of infected pigs for bacteriostatic activity (E = 0), bactericidal activity (E = −3), and a virtual elimination effect (E = −4) were 6.54, 9.49, and 11.49 h, respectively.

Estimation of dose

Based on the distributions of CL/F, AUC_{0-24h}/MIC ratios for three levels of antibacterial effects derived from PK-PD modeling, a dosage regimen of 1.94 mg/kg every 72 h of CEF oily suspensions was suggested for bactericidal activity against *S. suis cvcc* 607. According to the AUC_{0-24h}/MIC ratio, a dosage regimen of 1.30 and 2.30 mg/kg was recommended to achieve the bacteriostatic activity and virtual eradication of bacteria.

Assessment of dose

Based on these figures, a dose of 1.30 mg/kg was not adequate to reduce the bacterial number, whereas a dose of 2.30 mg/kg might lead to a net reduction. Different dosage regimens for 3 days of treatment (1.30 mg/kg every 24 h, 1.94 mg/kg every 24 h, 2.30 mg/kg every 24 h, 1.94 mg/kg every 24 h, 1.94 mg/kg every 48 h, 1.94 mg/kg every 72 h, 1.94 mg/kg every 96 h, and 1.94 mg/kg every 120 h) (**Fig. 5**) were simulated. A dosage regimen of 1.94 mg/kg every 72 h should be sufficient to reach bactericidal activity.
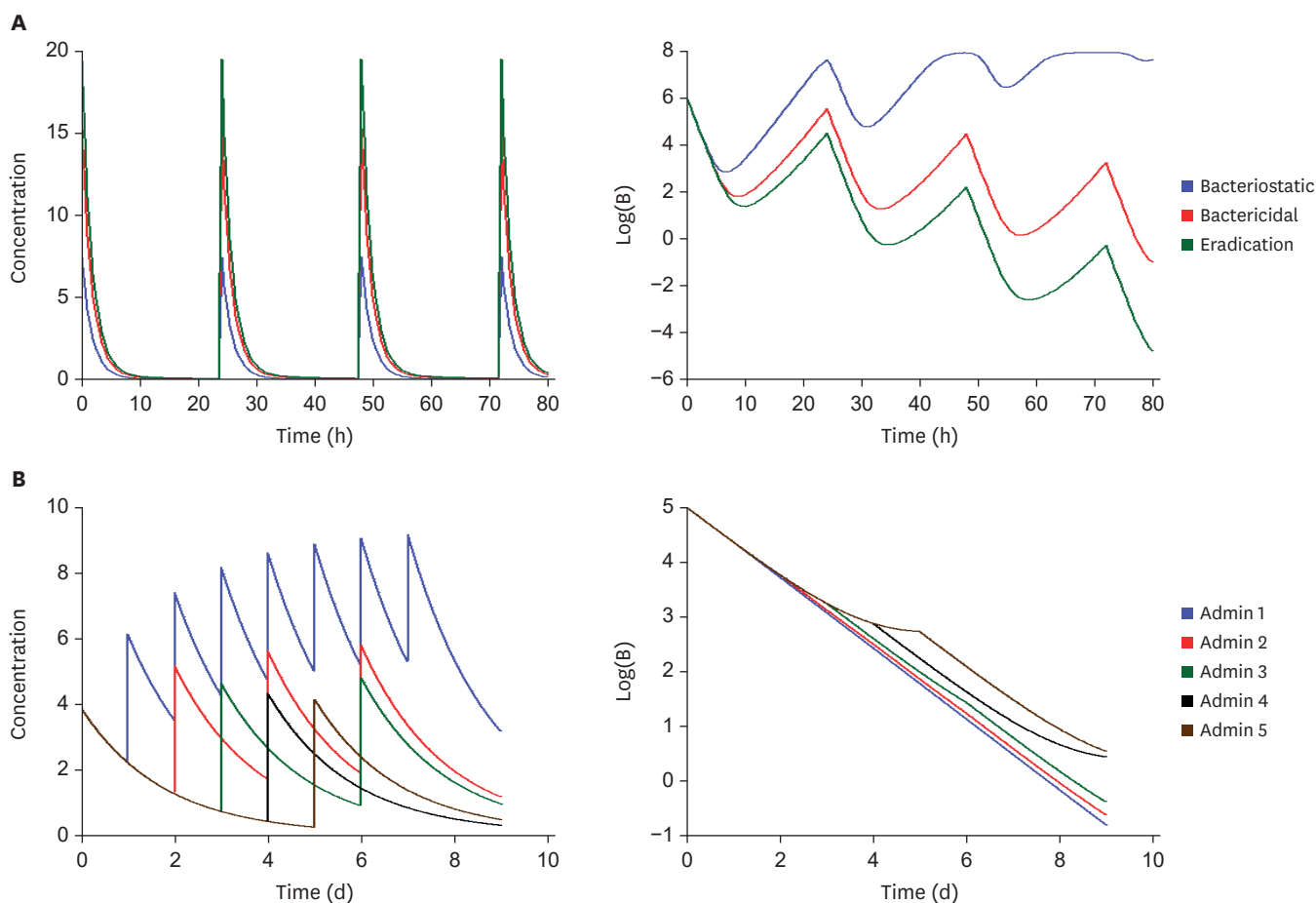
Fig. 5. The growth of bacteria under different schemes by Mlxplore simulation. (A) 1.30 mg/kg, 1.94 mg/kg, and 2.30 mg/kg every 24 h, (B) 1.94 mg/kg every 1, 2, 3, 4, and 5 day.

DISCUSSION

The MIC_{50} and MIC_{90} of CEF against *S. suis* were 0.25 and 32 μg/mL, respectively, suggesting that CEF has a potential antibacterial effect against the 29 clinical isolates. According to the MIC results, CEF is expected to be an ideal drug for the treatment of *S. suis* in pigs. In order to formulate a rational dosage regimen of our previously prepared CEF oily suspension, the *ex vivo* PK-PD relationship of CEF against swine *S. suis* was evaluated.

The MICs obtained for the TSB broth and PELF were not significantly different, indicating that the composition of the growth matrix does not affect antimicrobial susceptibility. The kill curve and PAE showed that CEF has bactericidal activity against *S. suis*, demonstrating that this antibiotic is concentration-dependent and has a certain PAE (0.13–2.15 h). In *in vitro* and *ex vivo* PD study, CEF resulted in a >4 log10 reduction in the viable bacterial count of *S. suis* after 24 h of exposure, with the viable counts typically reduced to lower than the LOD of the assay. According to the *in vitro* and *ex vivo* time-killing curve, a mixture of CEF and the metabolite of DFC in PELF displayed a concentration-dependent bactericidal effect with increasing drug concentrations induced more rapid and radical killing effects. As the mixture of CEF and DFC was found to be a concentration-dependent compound, the *ex vivo* AUC/MIC should be selected for PK-PD modeling, according to the results. The traditional

view is that cephalosporin is time-dependent, but the result of our study showed that it was concentration-dependent. Additionally, it has been reported that for drugs like the β-lactams, where efficacy has been correlated to the T > MIC, the best PK-PD index shifts toward AUC/MIC dependence as half-life increases [22]. Other results also showed that CEF had concentration-dependent characteristics against *Mannheimia haemolytica* and *P. multocida* [23]. This difference may be caused by differences within the target microorganism [13].

For the plasma PK study of healthy and infected pigs, the PK parameters of CEF (T_{max}, C_{max}, and AUC) obtained in this study were similar to the PK parameters in previous studies [24,25], which also treated pigs via intramuscular administration of CEF hydrochloride suspension. Therefore, T_{max}, C_{max}, and AUC seem to be in the range of values obtained previously [24,25]. Compared to plasma, the drug concentrations in the PELF of infected pigs with C_{max} and AUC_{0-24h} values of 33.04 ± 0.99 μg/mL and 735.85 ± 26.20 μg/h/mL were significantly higher. The large difference in DFC concentrations between these sample types may be due to a high amount of biliary excretion after intramuscular administration [26,27]. Most CEF was generally undetectable in plasma and rapidly metabolized into DFC in the body. Whether the PD of CEF or DFC was selected should be considered in the PK-PD modeling. It was reported that both CEF and DFC were highly active against *S. suis* [10].

In this study, the MIC of DFC (0.125 and 0.125μg/mL) against *S. suis cvcc* 607 in both and PELF was slightly lower than those of CEF (0.25 and 0.25μg/mL), while the MBC and MPC of DFC were the same as those of CEF, suggesting that DFC has equal or slightly stronger activity than CEF. In fact, CEF and DFC were simultaneously present in the PELF; the higher MIC of CEF was selected in the PK-PD modeling in order to ensure satisfactory effects from the formulated dosage regimes.

PK-PD modeling was used to determine the rational dosage regimen of DFC for swine *S. suis* therapy. For the PK-PD modeling, the PK parameters for free DFC in PELF were integrated with the MIC data (*in vitro* and *ex vivo*) using *S. suis cvcc* 607 as a typical pathogenic strain of *S. suis*. According to PK of infected pigs and the PD parameters, the single doses required to reach bacteriostatic, bactericidal, and eradication levels were 1.30, 1.94, and 2.30 mg/kg, respectively. After simulating different dosage regimens by Mlxplore simulation, a dosage regimen of 1.94 mg/kg every 72 h could be sufficient to reach bactericidal activity and provide satisfactory therapeutic effects.

In conclusion, the objective of this study was to formulate a dosage regimen for intramuscular administration of our previously prepared CEF hydrochloride oily suspension that would be sufficient for the treatment of pigs infected with *S. suis*. Based on the PK analysis and *in vitro* and *ex vivo* PD studies in PELF, a dosage regimen was designed. The dosage regimen was simulated using an E_{max} model. A dosage regimen of 1.94 mg/kg every 72 h could be sufficient to reach bactericidal activity. The calculated recommended dose could assist in achieving more precise administration and ensuring the treatment effectiveness of our previously prepared CEF hydrochloride oily suspension against *S. suis* infections. However, the suggested dose regimens should be validated in clinical practice.

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