Pharmacokinetics of ceftiofur hydrochloride in pigs infected with porcine reproductive and respiratory syndrome virus

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Objectives: To compare the pharmacokinetic profile of ceftiofur hydrochloride (ceftiofur) administered intramuscularly at 3 mg/kg body weight (BW) in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) versus clinically healthy pigs.

Methods: Sixteen 3- to 4-week-old PRRSV-negative pigs were randomly assigned to two groups (A and B), with eight pigs per group. Pigs in Group A were uninfected controls and pigs in Group B were intranasally challenged with a PRRSV isolate of Thai origin. Pigs in both groups were intramuscularly administered ceftiofur at 3 mg/kg BW at 7 days post-infection. Blood samples were serially collected up to 72 h post-injection. Plasma was analysed for ceftiofur and its related metabolites using HPLC. Pharmacokinetic parameters of ceftiofur were calculated based on non-compartmental analysis.

Results: Pharmacokinetic parameters of ceftiofur revealed statistically significant differences ($P < 0.01$) in maximum concentration ($C_{\text{max}}$), AUC, volume of distribution at the terminal phase over bioavailability ($V_z/F$), clearance over bioavailability ($CL/F$) and the terminal half-life ($t_{1/2z}$) between Groups A and B. PRRSV-infected pigs had a $V_z/F$ and $CL/F$ of ceftiofur significantly higher than in the non-infected pigs (116% increase in $V_z/F$, 234% increase in $CL/F$). The $C_{\text{max}}$ and AUC of the infected pigs decreased by 54% and 70%, respectively, compared with the non-infected pigs. The $t_{1/2z}$ of the infected pigs and the non-infected pigs was 13.1 and 21.0 h, respectively.

Conclusions: The pharmacokinetic profile of ceftiofur is altered in PRRSV-infected pigs due to the decreased plasma ceftiofur concentration compared with clinically healthy pigs.

Keywords: PRRSV, pharmacokinetic profile, PK

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important pathogen affecting swine production worldwide primarily due to reproductive failure in breeding stock and respiratory tract infection in pigs from the nursery until finishing. Losses in the nursery phase are associated with the porcine reproductive disease complex of which PRRSV is a component. Immunosuppression by PRRSV leads to secondary bacterial infections including Streptococcus suis, Haemophilus parasuis and Pasteurella multocida, etc.1,2 PRRSV infection in the breeding herd is controlled through herd stabilization including the acclimatization of replacement gilts. PRRSV infection in the nursery through the finishing phases is, in part, controlled by minimizing secondary bacterial infections through antimicrobial therapy.3

Ceftiofur is a broad-spectrum third-generation cephalosporin with activity against both Gram-positive and Gram-negative bacteria including β-lactamase-producing strains and anaerobes. Its antibacterial activity results from the inhibition of mucopeptide synthesis in the bacterial cell wall similar to other cephalosporins.1 Ceftiofur is generally undetectable in plasma as it is rapidly metabolized to desfuroylceftiofur (DFC) and furoic acid in the body.4 The DFC is either reversibly bound to macromolecules in plasma and tissue or further dimerized, forming disulfide products.5,6 Bound DFC is detected in plasma conjugated with protein, glutathione and cysteine.6,7

Ceftiofur hydrochloride (ceftiofur) has been approved for treatment against respiratory diseases in many animals.1,3,8–10 The recommended dosage regimen of ceftiofur for the treatment of swine respiratory disease is 3–5 mg/kg body weight (BW)
administered intramuscularly once daily for 3–5 consecutive days. Several field investigations have reported varying degrees of success when ceftiofur or other cephalosporins are administered to control secondary bacterial infections in PRRSV-infected pigs (personal observation, D. Nilubol). Some causes for these variations may be infections with resistant pathogens or a sub-optimal therapy regimen. In addition, the disposition kinetics of ceftiofur in PRRSV-infected pigs may differ from that of healthy pigs for several reasons, including variations in vascular permeability and/or changes in drug–protein interactions. An alteration in the ceftiofur pharmacokinetics may result in drug toxicity or loss of efficacy due either to overdose of the drug or to an insufficient concentration of the drug in plasma. Although the pharmacokinetics of ceftiofur have been widely investigated in healthy pigs, information on the pharmacokinetics of ceftiofur in PRRSV-infected pigs has not yet been reported. The objectives of this study were to investigate the pharmacokinetic profile of ceftiofur administered intramuscularly at 3 mg/kg BW in PRRSV-infected pigs and to compare the ceftiofur pharmacokinetic profile in PRRSV-infected pigs with that in non-infected pigs.

Materials and methods

Chemicals and reagents

Ceftiofur hydrochloride (Excenel® RTU Sterile Suspension, Pfizer Animal Health, Canada) was used as received. Cefotaxime, an internal standard, was a gift from Siam Pharmaceutical (Bangkok, Thailand). Solvents used for the mobile phase were of HPLC grade (Labscan, Bangkok, Thailand). All other chemicals and solvents used for sample preparation were of analytical grade. Oasis hydrophilic–lipophilic balance (HLB) solid-phase extraction (SPE) columns (60 mg/3 mL) were purchased from Waters (Bangkok, Thailand).

Experimental animals and study design

All study procedures and animal care activities were conducted at the Large Animal Experiment Facility in accordance with the guidelines and approval of the Committee on Animal Care and Use, Faculty of Veterinary Science, Chulalongkorn University (approval number 21/2549).

Sixteen 3- to 4-week-old PRRSV-negative pigs were procured from a PRRSV-negative herd. Pigs were randomly assigned, based on stratification by weight, age and gender, into two treatment groups. Group A consisted of control pigs (n = 8), while pigs in Group B were infected with a Thai strain of PRRSV (n = 8). The two groups were housed in two rooms each containing two pens of four pigs each. At 0 days post-infection, 2 mL of normal saline solution was administered intranasally to the pigs in Group A (1 mL/nostril), and pigs in Group B were intranasally infected with 2 mL of a Thai PRRSV isolate (10^18 TCID50/mL, 1 mL/nostril). The pigs were monitored daily for clinical disease associated with PRRSV. At 7 days post-infection, each pig received a single intramuscular injection of ceftiofur hydrochloride at a dosage of 3 mg/kg BW.

Blood sample collection

Blood samples (5 mL each) were collected into heparinized tubes at 0, 0.25, 0.50, 1, 4, 8, 16, 24, 36, 48, 60 and 72 h post-injection. Blood samples were centrifuged at 3000 rpm for 15 min. The plasma was collected and stored at −20°C until analysis was performed.

Determination of ceftiofur and DFC-related metabolites

Sample preparation. Samples were prepared according to the method developed by De Baere et al.6 Briefly, 50 μL of 100 mg/L cefotaxime (internal standard) was added to a 0.5 mL aliquot of plasma consisting of DFC associated with plasma protein or other sulphur-containing compounds. An aliquot of 7 mL of 0.4% (v/v) diithioerythritol in borate buffer solution was added to cleave bound DFC and yield free DFC. The free DFC was derivatized with 1.5 mL of iodoacetamide solution to form the more stable compound, desufurylcefuroxime acetylamine (DFA). The derivatized sample was transferred to an SPE column (Oasis HLB SPE column, 60 mg/3 mL) and was further eluted with 5% (v/v) acetic acid in acetonitrile. The eluate was dried under a gentle stream of nitrogen at ambient temperature. The dry sample was reconstituted in 200 μL of 0.01 M ammonium acetate and was then subjected to HPLC analysis.

Liquid chromatography analysis. The concentration of ceftiofur in plasma, as measured by the combination of free and bound DFC, was analysed in the form of DFA on an automated HPLC system (Shimadzu Corporation, Kyoto, Japan) using a validated method modified from Jaglan et al. Briefly, chromatographic separation was achieved using a Biobasic-C8 reverse phase column (4.6 x 100 mm, 5 μm; Thermo Electron Corporation, USA). The column temperature was maintained at 30°C. The autosample was conditioned at ambient temperature and the injection volume was 20 μL. A detector was set at a wavelength of 254 nm. Gradient elution was performed by using a mobile phase composed of 0.01 M ammonium acetate in water (mobile phase A, pH 5) and 0.6% (v/v) methanol in water (mobile phase B). The linear gradient elution started at: 0 min, 0% mobile phase B; 25.0 min, 29% mobile phase B; 30.0 min, 39% mobile phase B; 35.0 min, 100% mobile phase B; and 35.1 min, 0% mobile phase B. The flow rate was set at 1 mL/min. The retention times of DFA and cefotaxime were ~18.2 and 21.4 min, respectively, with a total run time of 40 min. Calibration curve equations were obtained by fitting peak area ratios (peak area of DFA to cefotaxime) versus ceftiofur concentration using a weighted (1/x) least square linear regression. The calibration curve for the assay was linear over the range of 0.1–20 mg/L, r^2 > 0.98. The lower limit of quantification (LLOQ) of the analytical method was 0.1 mg/L. The concentration value less than LLOQ was set as 0.5 x LLOQ in the data analyses. The within-day coefficient of variation (%CV) at 0.3, 10 and 18 mg/L was 7.58%, 11.7% and 0.82%, respectively. The mean ceftiofur recovery relative to the internal standard was 85%, 89% and 80%, respectively. All samples were analysed within 4 weeks of collection.

Pharmacokinetic analysis

The plasma concentration data of ceftiofur and its metabolites were subjected to non-compartmental analysis based on the statistical moment theory. Data at each time point were logarithmically transformed prior to the analysis. All pharmacokinetic calculations were performed by using linear modelling software (WinNonlin Version 3.2, Pharsight, Mountain View, CA, USA). The relevant pharmacokinetic parameters included the area under the concentration–time curve (AUC), the area under the first moment curve (AUMC), mean residence time (MRT), maximum concentration after dosing (C max), time to achieve maximum concentration after dosing (T max), terminal elimination half-life (t 1/2), terminal slope (b), extravascular
clearance (CL/F) and extravascular volume of distribution during the terminal phase (Vₜ/F).

Statistical analysis
Pharmacokinetic parameters were expressed as geometric means ± SD except t₁/₂, λ₂, Vₜ/F and CL/F values, which were expressed as harmonic means ± SD. The difference in pharmacokinetic parameters between non-infected pigs and PRRSV-infected pigs was determined using a Student’s t-test or a Mann–Whitney U-test (for Tₘax). All pharmacokinetic parameters except for Tₘax, t₁/₂, λ₂, Vₜ/F and CL/F were logarithmically transformed prior to data analysis. Probability values (P values) <0.05 were considered to be significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 14.0.

Results
Animal study
Non-infected pigs (Group A, no PRRSV challenge) remained clinically healthy throughout the study. PRRSV-infected pigs displayed clinical signs associated with PRRSV-induced respiratory diseases including fever and dyspnoea. Two PRRSV-infected pigs died prematurely due to pneumonia before receiving ceftiofur. Data from these pigs were excluded from the analysis.

Pharmacokinetic analysis of ceftiofur hydrochloride
A comparison of the mean plasma concentration versus time profiles of ceftiofur in non-infected and PRRSV-infected pigs is shown in Figure 1. The average plasma ceftiofur concentration of PRRSV-infected pigs was significantly lower than that of non-infected pigs. The pharmacokinetic parameters with statistically significant differences between Groups A and B (P < 0.01) were Cₘax, AUC, AUMC, MRT, Vₜ/F, CL/F, t₁/₂, and λ₂ (Table 1). The geometric mean values for the infected pigs were 6.03 mg/L for Cₘax, 50.4 h·mg/L for AUC, 841 h²·mg/L for AUMC and 16.7 h for MRT. The geometric mean values for the non-infected pigs were 12.9 mg/L for Cₘax, 168 h·mg/L for AUC, 4535 h²·mg/L for AUMC and 27.0 h for MRT. The harmonic mean values for the infected pigs were 1.14 L/kg for Vₜ/F, 0.058 L/h/kg for CL/F and 0.05 h⁻¹ for λ₂. The harmonic mean values for the non-infected pigs were 0.527 L/kg for Vₜ/F, 0.017 L/h/kg for CL/F and 0.032 h⁻¹ for λ₂. The harmonic mean elimination half-life (t₁/₂) for PRRSV-infected pigs was 13.1 h and for the non-infected pigs it was 21.0 h. The Tₘax values were not significantly different between the two groups (P > 0.05).

Discussion
Based on the regimen used in this study, the plasma pharmacokinetics of ceftiofur in healthy pigs are in agreement with those reported elsewhere. However, the ceftiofur pharmacokinetics in PRRSV-infected pigs were significantly different from those in healthy pigs (Table 1). PRRSV-infected pigs had a 234% higher CL/F, a 116% increase in Vₜ/F and a shorter half-life compared with the non-infected pigs. These results suggested that PRRSV infection caused an increase in the plasma drug elimination rate resulting in a decreased total concentration of ceftiofur in the infected pigs, as observed by a 70% decrease in AUC.

Ceftiofur is a highly protein-bound drug that is eliminated unchanged by the liver (through bile) and kidney (via glomerular filtration). It has been shown that protein binding extends the biological half-life of ceftiofur due to the protection of the β-lactam ring from cleavages and the reduction of the drug elimination rate by glomerular filtration. In animals with lung inflammation, the plasma albumin level often dramatically decreases, resulting in an increase in the fraction of the free drug. Free DFC is easily eliminated via glomerular filtration resulting in increased ceftiofur clearance; consequently, a decrease in plasma ceftiofur concentration and a shortened ceftiofur half-life were observed. Therefore, the increased ceftiofur clearance in PRRSV-infected pigs may be due to a decrease in plasma–protein binding.

Figure 1. Plasma concentration and time profiles of DFC-related metabolites in PRRSV-infected pigs (filled circles) and non-infected pigs (open squares) compared with the MIC values for 90% of S. suis, H. parasuis and P. multocida (broken line).
MRT0– due to an increase in netic parameters might suggest a satisfactory clinical outcome supports the observed failure of ceftiofur in the treatment of infection site. In other words, a pathophysiological approach alveolar membrane resulting in a lower drug concentration at the sacs. This increased thickness inhibits drug transport across the interstitial pneumonia resulting in the thickening of alveolar

high drug concentration in the lung should provide a satisfactory clinical target tissue in the treatment of swine respiratory dis-

an increase in ceftiofur concentration in the lung, which is the

against

Table 1. Average pharmacokinetic parameters of ceftiofur hydrochloride in non-infected and PRRSV-infected pigs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-infected pigs</th>
<th>PRRSV-infected pigs</th>
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<tbody>
<tr>
<td></td>
<td>mean (n = 8)</td>
<td>95% confidence intervals</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>12.9</td>
<td>10.6–15.8</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (h·mg/L)</td>
<td>168</td>
<td>133–211</td>
</tr>
<tr>
<td>$\text{AUMC}_{0-\infty}$ (h²·mg/L)</td>
<td>4535</td>
<td>3336–6166</td>
</tr>
<tr>
<td>MRT$_{0-\infty}$ (h)</td>
<td>27.0</td>
<td>22.6–32.4</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.5</td>
<td>0.306–0.693</td>
</tr>
<tr>
<td>$\lambda_z$ (1/h)</td>
<td>0.032</td>
<td>0.025–0.038</td>
</tr>
<tr>
<td>$t_{1/2z}$ (h)</td>
<td>21.0</td>
<td>17.5–24.4</td>
</tr>
<tr>
<td>$V/F$ (L/kg)</td>
<td>0.527</td>
<td>0.38–0.673</td>
</tr>
<tr>
<td>$CL/F$ (L/h/kg)</td>
<td>0.017</td>
<td>0.014–0.021</td>
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In addition, the decreased plasma–protein binding resulted in an increase in the ceftiofur volume of distribution since more free ceftiofur is available for tissue deposition. The distribution of ceftiofur in pig tissues is predominantly in the kidneys, followed by the injection site, lung, liver, fat and muscle. The increased $V/F$ value observed in PRRSV-infected pigs implied an increase in ceftiofur concentration in the lung, which is the clinical target tissue in the treatment of swine respiratory diseases. Regardless of the possibility of drug toxicity, the expected high drug concentration in the lung should provide a satisfactory outcome. However, animals with PRRSV infection often exhibit interstitial pneumonia resulting in the thickening of alveolar sacs. This increased thickness inhibits drug transport across the alveolar membrane resulting in a lower drug concentration at the infection site. In other words, a pathophysiological approach supports the observed failure of ceftiofur in the treatment of PRRSV-infected pigs, although interpretation of pharmacokinetic parameters might suggest a satisfactory clinical outcome due to an increase in $V/F$ and local drug concentration in the lung.

Antimicrobial activities of ceftiofur are time-dependent. In order to achieve the maximum efficacy, an average unbound plasma drug concentration above the pathogen’s MIC$_{90}$ for at least 50% to 70% of the dosing interval is critical. Ceftiofur is reported to show 65% protein binding with an MIC$_{90}$ for S. suis, H. parasuis and P. multocida of ~0.25, 0.06 and 0.03 mg/L, respectively. Thus, the total plasma concentration of ceftiofur should be in the range of 0.08–0.71 mg/L and be sustained for at least 12–17 h in a once-daily regimen. As illustrated in Figure 1, the plasma concentration of ceftiofur exceeded the MIC$_{90}$ for the pathogens for at least 24 h. Therefore, the dosing regimen used in the current study may be sufficient for treatment against H. parasuis, P. multocida and S. suis.

However, inconsistency in the therapeutic action of ceftiofur could be observed for some S. suis strains in PRRSV-infected pigs. The MIC range for S. suis is ~0.03–1 mg/L. Based on 65% protein binding, the plasma concentration required to inhibit the growth of S. suis should be in the range of 0.08–2.86 mg/L and be sustained for at least 12–17 h. The result of this study demonstrated that the plasma concentration of ceftiofur would not cover the entire MIC range for S. suis for the required interval. In fact, the plasma concentration of ceftiofur above the MIC range for S. suis was ~17% of the required dosing interval. Therefore, it is reasonable to suggest that reducing the dosing interval might improve outcomes in animals with PRRSV infection.

It should also be noted that protein binding of ceftiofur is redox-sensitive. The in vitro assessment of plasma protein binding may overestimate the actual percentage bound in vivo. The ceftiofur pharmacokinetic–pharmacodynamic data and their clinical outcomes should be used to justify the optimum therapeutic regimen. Further clinical studies to examine the relationship between the ratio of drug exposure to MICs of diverse strains of bacterial pathogens and therapeutic response are needed.

Conclusions

In this study, the ceftiofur pharmacokinetic parameters in healthy pigs were similar to those previously reported. A significant change in ceftiofur pharmacokinetic parameters was found in PRRSV-infected pigs. PRRSV-infected pigs demonstrated higher values of $V/F$ and $CL/F$, but lower values of AUC, AUMC, MRT and $t_{1/2z}$ compared with non-infected pigs. This indicates that plasma pharmacokinetics of ceftiofur are altered by a disease–drug interaction, specifically when pigs were infected with PRRSV. A dosage regimen adjustment may be indicated for ceftiofur hydrochloride for effective treatment against some secondary bacterial pathogens in PRRSV-infected pigs.

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Ceftiofur PK in PRRSV-infected pigs

Transparency declarations

None to declare.

References