Short Communication

Pharmacokinetics and pharmacodynamics of cefazolin in healthy horses after intramuscular administration using Nonlinear Mixed Effect Modelling

Running title: Cefazolin PK/PD in horses

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Abstract

A pharmacokinetics/pharmacodynamics (PK/PD) approach was used to determine the best empirical dosage regimen of cefazolin (CEZ) after an intramuscular (IM) administration of CEZ in horses. Seven horses received a single IM or intravenous (IV) administration of CEZ of 5 mg/kg body weight (bwt) according to a crossover design. CEZ plasma concentrations were measured using LC-MS/MS. Plasma concentrations were modeled using nonlinear mixed-effect modeling followed by Monte Carlo simulations to establish a rational dosage regimen for CEZ. A 90% probability of target attainment (PTA) for a PK/PD target of a free serum plasma concentration exceeding MIC_{90} ($fT>_{MIC}$) for 40% of the dosing interval was set for selecting an effective IM and IV dosing regimen. The typical absorption rate constant and bioavailability after IM administration were 0.58/h and 95.4%, respectively. A CEZ dosage regimen of 5 mg/kg bwt q12h achieved therapeutic concentrations to control both *S. zooepidemicus* and *S. aureus*. The corresponding dosage regimens for IV administration for these horses were 5 mg/kg bwt q8h and q6h IV administration, respectively. For the same dose, the $fT>_{MIC}$ after IM administration was significantly longer than after IV administration of CEZ, and the IM route should be followed by clinicians for its simplicity and convenience.

KEYWORDS

Cefazolin, horse, pharmacokinetics, pharmacodynamics, IM dose

CEZ is a first-generation cephalosporin that has been investigated in horses (Sams & Ruoff, 1985; Donecker, Sams & Ashcraft, 1986) with a proposed standard dosage regimen of 10–22 mg/kg bwt q6–8h for intravenous (IV) administration (Davis & Papich, 2013). The pharmacokinetics (PK) of CEZ after IM administration in horses have been reported (Sams & Ruoff, 1985) but its dosage regimen was not established following pharmacokinetics/pharmacodynamics (PK/PD) principles. Cephalosporins, a class to which cefazolin belongs, are time-dependent antimicrobials, for which the appropriate PK/PD index is $fT>_{MIC}$ (the time during which free plasma concentrations are above the MIC) (Drusano, 2003). As IM administration may prolong $fT>_{MIC}$, it is expected to be more efficient than IV administration for a given total dose. Considering the corresponding IV PK has already been published, in this study, PK/PD analysis was conducted based on the PK of CEZ after IM administration to optimize the dosage regimen (Kuroda et al., 2020).

Seven healthy 2 to 8 year-old experimental thoroughbred horses (four males and three females) with body weights (bwts) 416–557 kg were used. The horses were kept in individual stalls during the experiment and had *ad libitum* access to grass, hay, and water. A randomized crossover design for IM or IV administration was followed by a two week washout period. The CEZ dose of (5 mg/kg bwt) was determined based on the summary of product characteristics of CEZ (cefazoline for injection 3 g, Kyoritsuseiyaku Corporation) for animals approved in Japan. CEZ was dissolved in 15 mL sterile physiological saline for both IM and IV and administered into the right lateral neck (IM) or into the right jugular vein through a short bolus infusion (<30 s).

Blood samples were collected at 0, 5, 10, 20, 30, and 45 min and then at 1, 2, 3, 4, 6, 8, and 12 h. All blood samples were collected from the left jugular vein using a 16G catheter (Becton Dickinson Company) that was inserted under local anesthesia of 1 mL lidocaine (Xylocaine

Injection Polyamp 0.5%, Aspen Japan); 10 mL blood samples were collected in heparinized vacuum blood collection tubes (Venoject 2, Terumo Corporation). The samples were immediately centrifuged at 1500 × g for 10 min, and the separated plasma samples were stored at −20°C until analysis.

The plasma CEZ assay was performed with a liquid chromatography system (Nexera X2, Shimadzu Corporation) connected to a mass spectrometer (QTRAP4500, SCIEX Corporation) using methods previously described (Kuroda et al., 2020). The limit of quantification was 0.01 μg/mL. Plasma pharmacokinetic analyses were conducted using a nonlinear mixed effect (NLME) model on commercially available software (Phoenix WinNonlin version 8.3, Certara), according to a model adapted from a previous study (Kuroda et al., 2020). A three-compartment structural model was selected based on the likelihood ratio test and Akaike information criterion. The estimated parameters were central (V1) and two peripheral (V2, V3) volumes of distribution, plasma clearance (CL), and inter-compartmental distribution clearances (CL2, CL3). The absorption rate constant (Kabs) and bioavailability factor (*F*) were added to the IV model for simultaneous IV and IM fitting. The statistical model describing inter-animal variability was exponential. A full OMEGA matrix was used to determine the random component: the between-subject variability associated with fixed pharmacokinetic parameters. All eta shrinkage values were <0.3. Given the experimental crossover design, the order of administration was considered with occasion as a covariate, and inter-occasion variability was included in the random component of the model. The residual model was an additive plus multiplicative (proportional) model. For the fitting, the precision of the parameters was estimated using the Phoenix bootstrap tool (n = 50 replicates). The Laplacian engine was used to maximize the likelihood, and data reported below the limit of quantitation (0.01 μg/mL) were treated as censored using Phoenix method 3 (M3).

93 The protein binding rate of CEZ was reported as 15.2% ± 8.5% in horses (Kuroda et al., 2020).
94 Using the developed model and reported free fraction, Monte Carlo simulations (MCS) were
95 run to generate free plasma concentrations in a virtual population of 5000 horses using
96 individual predictions or IPRED (eta was as estimated). Different scenarios were explored
97 corresponding to five different dosing intervals ranging from 4.8 to 24 h. From these 5000
98 curves, $fT>_{MIC}$ was calculated on day 3 after the first administration for a target of 40% of the
99 dosing interval (Drusano, 2003). The corresponding probability of the target attainment (PTA)
100 was calculated. To establish an empirical dosage regimen (i.e., without resorting to
101 antimicrobial susceptibility testing (AST)), the selected dose should cover at least 90% of the
102 simulated horses for the reported MIC_{90} of target pathogens (Rey et al., 2014; Toutain et al.,
103 2017; Kuroda et al., 2020). The MIC_{90} of CEZ against *S. zooepidemicus*, *S. aureus,* and *E. coli*
104 previously collected in horses were 0.12 mg/L, 0.12 mg/L, and 2.0 mg/L, respectively (Kuroda
105 et al., 2020).
106 No side effects including neck pain were observed during the experiment. Semilogarithmic
107 plots of the disposition curves of the CEZ in each horse are shown in Figure 1. From a
108 preliminary non-compartmental analysis conducted in the 7 horses, $fT>_{MIC}$ against *S.*
109 *zooepidemicus* and *S. aureus* were significantly longer after a single IM administration (10.0 h
110 ± 1.7 h and 6.8 h ± 1.3 h) than after an IV administration (6.1 h ± 1.2 h and 3.1 h ± 0.4 h)
111 (p<0.01). For NLME modelling, data were evenly distributed around the line of identity
112 between observed CEZ concentration and population predictions (PRED) and IPRED,
113 indicating no major bias in the population analysis (Figure 2). A visual predictive check
114 indicated that the simulated data were consistent with observed data (Figure 3). Bootstrap
115 estimates of typical values of the primary structural parameters of the model (thetas),
116 secondary parameters, and their associated coefficients of variation as a measure of the

precision of their estimation are given in Table 1. The typical values of the absorption rate constant and bioavailability after IM administration were 0.58 1/h and 95.4%, respectively, and other structural parameters, including clearance and distribution volume, were similar to those previously reported for IV data analysis (Kuroda et al., 2020). PTA for the free drug concentration profiles obtained by MCS for different MICs of CEZ in different regimens are shown in Figures 4. For IM administration, 5 mg/kg bwt q12h administration regimens were able to reach a PTA of 90% against the MIC_{90} of *S. zooepidemicus* (0.12 mg/L) and *S. aureus* (0.5 mg/L), respectively. The corresponding dosage regimen in IV administration was 5 mg/kg bwt q8h and q6h. Additionally, CEZ q8h 5 mg/kg IM administration achieved a PTA of 90% against *E. coli* (2.0 mg/L).

PK/PD considerations can help determine and optimize an efficient dosage regimen or, alternatively, determine, for a given dosage regimen, the corresponding MIC breakpoints for AST both in humans (Ambrose et al., 2007) and animals (Toutain, et al., 2017). In our previous publication on the IV data of the present trial, we reported that a CEZ dose of 10 mg/kg bwt q12h and q8h IV was required to reach a PTA of 90% against the MIC_{90} of *S. zooepidemicus* and *S. aureus*, respectively (Kuroda et al., 2020). The present study indicated that IM administration was more efficient than IV administration by significantly prolonging $fT>_{MIC}$ and that IM administration of 5 mg/kg twice a day is practical for veterinary clinicians and can reduce the total administered dose of CEZ compared to IV administration. For *E. coli* (MIC_{90}:2.0 mg/L), 5 mg/kg q8h IM administration was expected to be effective, and the daily dose was very small compared to the 20 mg/kg q6h IV administration previously reported to be effective for *E coli* (Kuroda et al., 2020). The Clinical and Laboratory Standards Institute (CLSI) reported a clinical breakpoint (CBP) for horses of <2.0 mg/L for susceptible organisms with a dosage regimen of 25 mg/kg q6h upon IV administration (CLSI, 2018). In this study, we

provided evidence that CBP can be achieved using only 5 mg q8h IM administration according to the VetCAST approach (Toutain et al., 2017). β-lactams appear to be less detrimental than tetracyclines or macrolides (McGorum & Pirie, 2010), but CEZ-associated diarrhea has been reported in horses (Nomura, Kuroda, Tamura, Muranaka and Niva, 2020). Hence, decreasing the total dose of CEZ by selecting the IM route of administration is expected to reduce the risk of diarrhea in horses.

Finally, our study indicated that a CEZ of only 5 mg/kg bwt q12h IM administration could attain therapeutic concentrations to control the MIC_{90} of *S. zooepidemicus* and *S. aureus*, respectively.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The authors disclose no conflict of interest.

Animal Welfare and Ethics Statement

This study was reviewed and approved by the Animal Care and Use Committee of Equine Research Institute, Japan Racing Association in accordance with ASPA (1986) legislation Protocol # 21-5.

Figure 1: Semilogarithmic spaghetti plots of the disposition curves of cefazolin 5 mg/kg bwt after a single IV (left) and IM (right) administration in seven horses.

Figure 2: Logarithmic plots of observed cefazolin plasma concentrations vs. individual predictions (IPRED) and population predictions (PRED) after IV (left plots) and IM (right plots) administrations.

Figure 3: Visual Predictive Check of a single dose of cefazolin 5 mg/kg bwt after IV (left) and IM (right) administration. The observed and predicted 10^{th} and 90^{th} percentiles are shown in solid red and black lines, respectively. The observed and predicted 50^{th} percentiles (median) are shown in red and black broken lines, respectively. Blue dots are individual raw data.

Figure 4: Probability of Target Attainment (PTA%) vs. MIC (mg/L) of cefazolin for repeated administration of cefazolin 5 mg/kg bwt upon IV (left) and IM (right) administration at different dosing intervals ranging from 4.8 to 24 h. The PK/PD index is the time the free plasma concentration is exceeding the MIC for 40% of the dosing interval. Values were obtained from 5000 simulated concentrations profiles generated from the population model by Monte Carlo simulations. PTA 90% is indicated by the solid horizontal blue line, which is considered as the target to achieve, and MIC that corresponds to PTA 90% are indicated by the vertical dotted blue lines.

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Table 1: Bootstrap estimates of typical (median) population primary and secondary parameters of CEZ after IV and IM administrations of CEZ in horses at 10 mg/kg as obtained from h a 3-compartment model (Legend: CV%, and 2.5% and 97.5% percentiles give the precision of estimates; for *F*, an ilogit transformation was used preventing estimate higher than 100%.).

Primary structural Parameters	Units	Median	CV%	2.50%	97.50%
tvV	L/kg	0.031	49.1	0.009	0.064
tvV2	L/kg	0.032	9.5	0.027	0.037
tvV3	L/kg	0.031	8.9	0.026	0.036
tvCL	L/kg/h	0.170	9.8	0.140	0.198
tvCL2	L/kg/h	0.148	18.8	0.100	0.191
tvCL3	L/kg/h	0.014	5.3	0.013	0.016
Kabs	1/h	0.58	8.8	0.49	0.67
F	%	95.4	4.2	86.0	99.3
tvCMultStdev0 (residual, proportional, IV)	Scalar	0.049	11.3	0.040	0.059
tvCMultStdev1 (residual, proportional, IM)	Scalar	0.174	13.3	0.127	0.204
stdev0 (residual, additive, IV)	µg/L	0.0098	20.5	0.0062	0.0136
stdev1 (residual, additive,IM)	µg/L	0.0150	86.1	0.0000002	0.0776
CEZ Secondary parameters					
Half_life_alpha	H	0.052	42.2	0.019	0.100
Half_life_Beta	H	0.341	11.9	0.283	0.422
Half_life_Gamma	H	1.653	11.3	1.374	2.071

Absorption_Half_life	H	1.19	9.0	1.04	1.42
Vss (steady-state volume of distribution)	L/kg	0.095	21.3	0.062	0.134
MRT (Mean residence time (IV))	H	0.56	13.5	0.43	0.70

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235 *V1: volume of distribution of central compartment; V2, V3: volume of distribution of*
236 *peripheral compartments; CL: plasma clearance; CL2, CL3: distribution clearances; Kabs:*
237 *absorption rate constant; F: bioavailability; CMultStdev0,1: proportional component of*
238 *residual error; stdev0,1: additive component of the residual; tv: typical value; Vss: steady-*
239 *state volume of distribution; MRT: mean residence time.*