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Pharmacokinetic/pharmacodynamic analysis of cephalothin after intramuscular administration in Thoroughbred horses

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A pharmacokinetic/pharmacodynamic (PK/PD) approach was used to determine a dosage regimen of cephalothin (CET) after intramuscular (IM) administration in horses. CET plasma concentrations were measured in eight horses after a single IM administration of 11 mg/kg bwt of CET. The data were modeled using a nonlinear mixed-effect model, and the probability of target attainment (PTA) of the PK/PD target was calculated for 5,000 horses generated by Monte Carlo simulations. IM administrations of CET at 11 mg/kg bwt q 8 hr and q 6 hr achieved a PTA of 90% against the MIC_{90} of S. zooepidemicus and S. aureus, respectively, and were considered to be effective dosage regimens. The total dose for the IM administration recommended in this study was lower than that for intravenous (IV) administration in previous studies.

Key words: *cephalosporins, gram-positive bacteria, intramuscular administration, pharmacokinetics*

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Intravenous (IV) administration of the first-generation cephalosporin antibiotic cephalothin (CET) at 22 mg/kg bwt q 6–8 hr is used as the first line of treatment for equine gram-positive infections [2, 3]. A pharmacokinetic/pharmacodynamic (PK/PD) analysis of IV administration has revealed that 22 mg/kg bwt q 8 hr and 22 mg/kg bwt q 4 hr regimens of CET are effective for the Minimum inhibitory concentration $(MIC)_{90}$ of *S. zooepidemicus* and *S. aureus*, respectively [6]. Since administration of CET q 4 hr is possible for hospitalized horses but is difficult for horses on farms, different administration methods are required as the first line of treatment to control *S. aureus* in horses.

Cephalosporins are time-dependent antimicrobials for which the appropriate PK/PD index is an $fT > MIC$ (the time during which free plasma concentrations are above MIC) of 40% for the dosing interval [4]. Since it has been found that the $fT > MIC$ of cefazoline in horses after intramuscular (IM) administration is significantly longer than for the same dose by IV administration, IM administration of CET is also expected to be more effective than IV administration [5]. IM administration of CET has been studied in horses [10], but a regional dosage regimen based on PK/PD has not been established. In this study, a PK/PD analysis of IM administration of CET in horses was conducted to optimize the dosage regimen.

Eight Thoroughbred horses (four stallions and four mares that were four to nine years of age) with body weights of 490–570 kg were used. In the experiment, 11 mg/kg bwt of CET (Coaxin injection 1 g, Chemix Inc., Yokohama, Japan) was dissolved in 25 m*l* sterile physiological saline and administered (IM) into the right lateral neck by bolus infusion (<30 sec). The dose was chosen according to a previous study [10]. Blood samples (10 m*l*) were collected at 0, 5, 10, 20, 30, and 45 min and 1, 2, 3, 4, 6, 8, and 12 hr. All blood samples were collected from the left jugular vein using a 16 G catheter (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) inserted under local anesthesia with 1 m*l* lidocaine (Xylocaine Injection Polyamp 0.5%, Sandoz Pharma, Tokyo, Japan). The 10 m*l* blood samples were collected in heparinized vacuum blood tubes (Venoject II, Terumo Corp., Tokyo, Japan) and immediately centrifuged at 1,500 × *g* for 10 min and stored at −20°C until they were analyzed. All experiments were approved by the Animal Care and Use Committee of the Equine Research Institute

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Plasma CET assays were performed with a liquid chromatography system (Nexera X2, Shimadzu Corp, Kyoto, Japan) connected to a mass spectrometer (QTRAP 4500, SCIEX Corp., Tokyo, Japan), using previously described methods [6]. The limit of quantification was 0.1 μg/m*l*. After aggregating the data collected from IM administration in the eight horses in this experiment with data from a previous experiment using 12 different horses with IV administration at a dose of 22 mg/kg [6], a pharmacokinetic analysis was conducted with commercially available software (Phoenix WinNonlin 8.3, Certara, Princeton, NJ, USA) using a nonlinear mixed-effect model (NLME) and Monte Carlo simulations (MCSs). A three-compartment structural model was selected based on the likelihood ratio test and Akaike information criterion (Fig. 1). The estimated parameters were the central (V1) and peripheral (V2 and V3) volumes of distribution, plasma clearance (CL), and inter-compartmental distribution clearances (CL2 and CL3). The absorption rate constant (Kabs) and bioavailability factor (F) were added to the model for IM administration. Interindividual variability was assumed to obey a log-normal distribution, and for a given structural parameter, the between-subject variability (BSV) was described using an exponential model. The residual model was an additive plus multiplicative (proportional) model. For fitting, the precision of the parameters was estimated using the Phoenix bootstrap tool (n=50 replicates). Statistical analysis of the *f*T > MIC for IM administration in this study and IV administrations in the previous studies was conducted using the Wilcoxon rank sum test [6]. Using this model and the free fraction value of 0.8 reported in a previous study [1], Free plasma concentrations after IM administration were generated by MCS for different dosing regimens in a virtual population of 5,000 horses using individual predictions. The *f*T > MIC was calculated over 24 hr after the first administration from concentration curves in the 5,000 horses. The *f*T > MIC for 40% of the dosing interval was used as the PK/PD target [4]. The probability of target attainment (PTA) for the PK/PD target in the 5,000 horses was calculated.

No neck pain, diarrhea, or other side effects were observed during experiments. The semilogarithmic plots of the disposition curves of CET after IM administration in each horse are shown in Fig. 2, and the pharmacokinetic parameters are shown in Table 1. The typical values for the half-life of absorption and bioavailability after IM administration were 0.65 hr and 99.7%, respectively. The *f*T > MIC values against *S. zooepidemicus* (0.12 mg/*l*) and *S. aureus* (0.5 mg/*l*) were significantly longer after a single 11 mg/kg IM administration (7.1 ± 2.6 hr and 4.7 ± 1.4 hr, respectively) than after a single 22 mg/kg IV administration (4.0 ± 1.0 hr and 2.5 ± 0.5 hr, respectively), which was estimated using previously reported data (P<0.01) [6]. The PTA values for the free drug concentration profiles obtained by MCS are shown in Fig. 3.

The MIC_{90} values of CET against *S. zooepidemicus* and *S. aureus* previously isolated from horses were 0.12 mg/*l* and 0.5 mg/*l*, respectively [7]. For antimicrobials, dosing regimens were considered to be effective when the PTA was 90% for the PK/PD target against the MIC_{90} of pathogens [5, 6, 9, 11]. In this study, IM administrations of CET at 11 mg/kg bwt q 8 hr and q 6 hr reached a PTA of 90% against

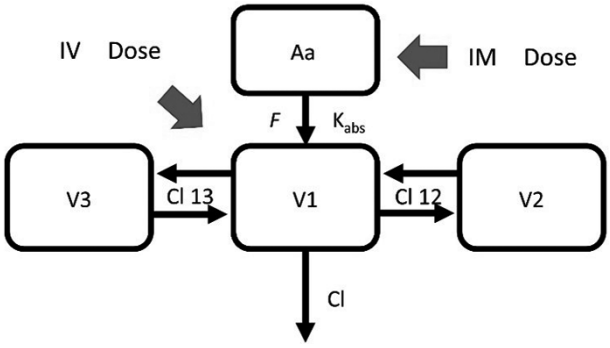

Fig. 1. A three-compartment model for intravenous (IV) and intramuscular (IM) administrations of cephalothin (CET).

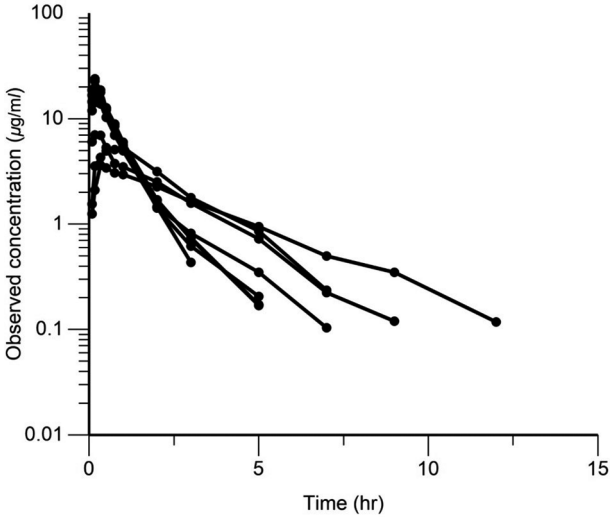

Fig. 2. Cephalothin (CET) concentrations after intramuscular (IM) administration of 11 mg/kg CET in eight Thoroughbred horses.

Table 1. Structural parameters and bootstrap estimates of typical (median) population primary and secondary parameters of cephalothin (CET) obtained from a three-compartment model (median, CV%, and 2.5% and 97.5% percentiles give the precision of typical value estimates) after intramuscular (IM) administrations at 11 mg/kg in six horses and intravenous (IV) administrations at 22 mg/kg in 12 horses

Primary structural parameters	Units	Typical values (median)	CV%	2.5%	97.5%
V	*l*/kg	0.083	8.1	0.071	0.099
V2	*l*/kg	0.060	16.5	0.040	0.082
V3	*l*/kg	0.054	71.1	0.039	0.057
CL	*l*/kg/hr	0.597	4.3	0.522	0.597
CL2	*l*/kg/hr	0.106	14.5	0.098	0.156
CL3	*l*/kg/hr	0.018	18.1	0.016	0.030
Kabs	1/hr	1.070	24.6	0.001	0.002
F	%	99.7	9.8	67.7	99.8
CMultStdev (residual, proportional, IV)	Scalar	0.117	9.5	0.081	0.125
stdev0 (residual, additive, IV)	*μg*/*l*	0.008	28.8	0.001	0.009
stdev1 (residual, additive, IM)	*μg*/*l*	0.109	50.0	0.002	0.109
Secondary parameters					
Absorption half-life	*H*	0.65	32.8	0.39	1.37
Vss (steady-state volume of distribution)	*l*/kg	0.143	5.7	0.135	0.170
MRT (mean residence time [IV])	*H*	0.24	7.5	0.24	0.30

V1, volume of distribution of central compartment; V2 and V3, volume of distribution of peripheral compartments; CL, plasma clearance; CL2 and CL3, distribution clearances; Kabs, absorption rate constant; F, bioavailability; CMultStdev, proportional component of residual error; stdev0 and stdev1, additive components of the residual; tv, typical value; Vss, steady-state volume of distribution; MRT, mean residence time.

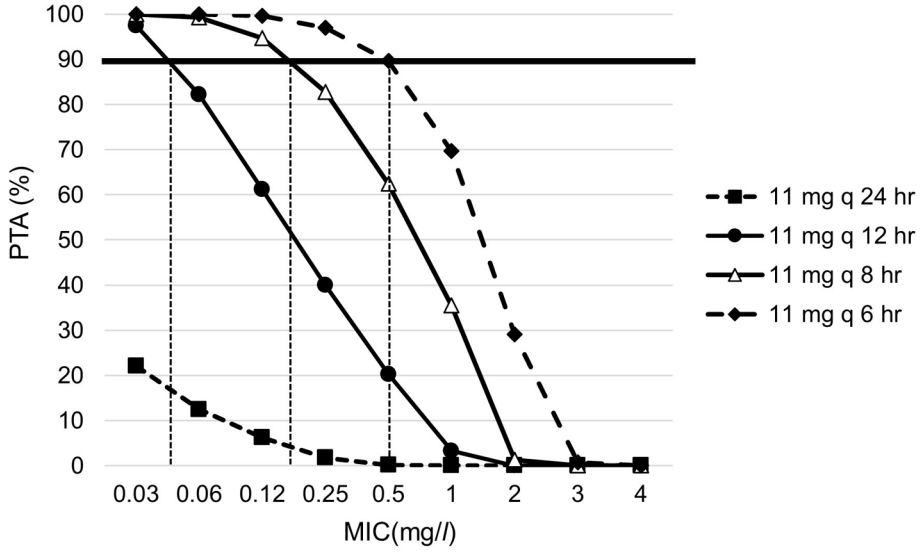

Fig. 3. Probability of target attainment (PTA) vs. minimum inhibitory concentration (MIC) (mg/*l*) of cephalothin for repeated intramuscular (IM) administration of 11 mg/kg bwt at different dosing intervals ranging from 6 to 24 hr. The pharmacokinetic/pharmacodynamic (PK/PD) index is the time the free plasma concentration exceeds the MIC for 40% of the dosing interval. Values were obtained from 5,000 simulated profiles of concentrations generated from a population model by Monte carlo simulation (MCS). PTA of 90% is indicated by the solid horizontal line, which is considered to be the target to achieve, and MIC values that correspond to a PTA of 90% are indicated by the vertical dotted lines.

the MIC_{90} of *S. zooepidemicus* (0.12 mg/*l*) and *S. aureus* (0.5 mg/*l*), respectively (Fig. 3). They were considered to be effective dosage regimens. In previous studies, IV administration of CET at 22 mg/kg q 8 hr, the same dosing interval as IM administration in this study, reached a PTA of 90% against the MIC_{90} of *S. zooepidemicus* [6]. For the MIC_{90} of *S. aureus*, the frequency of IM administration of CET (q 6 hr) for the effective dosage regimen in this study was a little lower than that for IV administration at 22 mg/kg q 4 hr in a previous study [5]. We expected a significant reduction of the injection frequency observed for IM administration of cefazoline [5], but this was not the case for IM administration of CET. The difference of pharmacokinetics between IM and IV administration is the bioavailability and absorption rate constant in IM administration. The bioavailability was almost 100% for both IM-administered CET in this study and cefazoline in the previous study, and there were no differences between CET and cefazoline [5]. Because the absorption half-life of CET after IM administration was shorter at 0.65 hr compared with the 1.25 hr for cefazoline, CET was rapidly absorbed from the muscle into plasma in horses [5]. Because of this rapid absorption from the muscle into plasma, the injection frequency of the effective dosage regimen for IM administration of CET was not decreased from that of IV administration, as slow absorption after IM administration leads to a long $fT > MIC$ [6]. We simulated IM administration at 22 mg/kg using an NLME model and the same injection frequency as for the 11 mg/kg regimen to control *S. zooepidemicus* and *S. aureus*. On the other hand, since the single IM dose (11 mg/kg) is half that of IV administration, a reduction in the total dose can be expected with IM administration. β-lactam appears to be less detrimental for horses than macrolides [7], but CET-associated diarrhea has been reported [8]. Hence, decreasing the total dose of CET by IM administration is expected to reduce the risk of diarrhea in horses. There is also an advantage in terms of the medical costs for owners.

In this study, the CET dosage regimens of 11 mg/kg bwt q 8 hr and q 6 hr for IM administration both achieved a PTA of 90% against the MIC_{90} of *S. zooepidemicus* and *S. aureus*, respectively. Although the injection frequency for IM administration was similar to that for IV administrations in previous studies, a reduction in the total dosage can be expected with IM administration.

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