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Pharmacokinetics of Ceftiofur Crystalline Free Acid in Male Rhesus Macaques (*Macaca mulatta*) after Subcutaneous Administration

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Trauma is a common sequela to agonistic social encounters in rhesus macaques (*Macaca mulatta*), and veterinarians often prescribe antibiotics as part of a balanced treatment plan. Long-acting, single-dose, injectable antibiotics for use in rhesus macaques are unavailable currently. Ceftiofur crystalline free acid (CCFA) is a long-acting, single-dose, injectable third-generation cephalosporin that provides at least 7 d of ceftiofur therapeutic plasma concentrations in swine (*Sus scrofa domesticus*). We hypothesized that CCFA would achieve similar therapeutic concentrations (≥0.2 μg/mL) in rhesus macaques. We describe the pharmacokinetic profile of CCFA in healthy, adult male rhesus macaques (*n* = 6) in this 2-period, 2-treatment crossover study of 5 and 20 mg/kg SC administered once. Plasma ceftiofur metabolite concentrations were determined prior to and for a maximum of 21 d after administration. Noncompartmental pharmacokinetic analysis was performed. The 5-mg dose achieved a maximal plasma concentration of 2.24 ± 0.525 μg/mL at 2.59 ± 1.63 h, an AUC of 46.9 ± 17.6 h/μg/mL, and a terminal elimination half-life of 56.5 ± 21.7 h; for the 20-mg/kg dose, these parameters were 9.18 ± 4.90 μg/mL at 1.82 ± 1.30 h, 331 ± 84.4 h/μg/mL, and 69.7 ± 8.86 h, respectively. No adverse effects were noted after either dose. Macaques maintained plasma ceftiofur concentrations of 0.2 μg/mL or greater for at least 2 d after 5 mg/kg SC and at least 7 d after 20 mg/kg SC.

Abbreviations: CCFA, ceftiofur crystalline free acid; C_{max}, maximal concentration; CNPRC, California National Primate Research Center; DCA, desfuroylceftiofuracetamide; MIC, minimal inhibitory concentration; t_{max}, time at maximal concentration.

Rhesus macaques (*Macaca mulatta*) are a highly social species that typically live in multimale, multifemale groups that use agonistic and affiliative encounters to maintain group hierarchy; however, when group stability is jeopardized, trauma is a potential sequela to these agonistic encounters.[1,2,8,21,22,32,34,37,47] The California National Primate Research Center (CNPRC; Davis, CA) houses approximately 4900 rhesus macaques, and more than 3400 are in outdoor social groups. At the CNPRC, the annual incidence of outdoor-housed animals presenting to the veterinary hospital for care due to traumas requiring sutures or amputation of digits ranges from approximately 5% to 9% annually.[32] Trauma cases often receive antibiotic therapy due to potential wound contamination and secondary bacterial infections.[32] Currently at the CNPRC, the antibiotic treatment most commonly used in these cases is the injectable, broad-spectrum, first-generation cephalosporin, cefazolin (West–Ward Pharmaceutical, Eatontown, NJ). Cefazolin is a β-lactam antibiotic that is reported to provide excellent coverage against gram-positive bacteria, variable to poor coverage for most gram-negative bacteria, and, with the exception of *Bacteroides fragilis*, covers most anaerobic bacteria as well.[39] Cefazolin is typically prescribed prophylactically in patients with traumatic wounds at 25 mg/kg IM twice daily for approximately 7 d.[10]

A long-acting, single-dose, injectable antibiotic formulation has many advantages for use in rhesus macaques, including reduced stress, discomfort, and risk to the animals or personnel from fewer clinical treatments. Ceftiofur crystalline free acid (CCFA) is a long-acting third-generation cephalosporin labeled for use in cattle, horses, and swine. A single dose provides therapeutic coverage for 1 wk or more in approved species.[48] Third-generation cephalosporins provide a wider spectrum of bacterial coverage than do first-generation cephalosporins against gram-positive, gram-negative, and anaerobic bacteria, including *Bacteroides* spp.[39] Like other cephalosporins, ceftiofur is a time-dependent, bactericidal antibiotic whose mechanism of action is through inhibition of bacterial cell wall synthesis.[39,48] CCFA, in its market swine formulation (Excede Sterile Suspension, 100 mg/mL; Zoetis, Madison, NJ), and its active metabolite, desfuroylceftiofuracetamide (DCA), have been shown to reach therapeutic plasma levels in swine within 1 h of administration, and remain above therapeutic plasma levels for an average of 8 d after a single intramuscular treatment.[48] CCFA has been licensed for intramuscular injection in swine and horses and subcutaneous injection in cattle,[39] and it has been explored as a potential therapeutic in a variety of other species, including livestock, fowl, reptiles, and exotic mammals (for example, *Cercocebus torquatus* [red-capped mangabey]).[3,4,11,15-17,20,25,27,29,31,35,41,46]

The primary objective of the current study was to describe the pharmacokinetics of 2 subcutaneous doses (5 and 20 mg/kg) of CCFA in rhesus macaques after a single injection. These data were then compared with each other and the published pharmacokinetic data available for swine at 5 mg/kg IM. Because the minimal therapeutic plasma concentration of ceftiofur in the rhesus macaque is unknown, we used that for swine (0.2 μg/mL) for comparison. This concentration represents the 90% minimal inhibitory concentration (MIC) for the swine respiratory pathogens *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*,

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Haemophilus parasuis, and *Streptococcus suis.* We hypothesized that CCFA at either 5 or 20 mg/kg SC would provide the therapeutic plasma concentrations established for swine (≥0.2 µg/mL) for at least 7 d in rhesus macaques. We chose to set the minimum for comparison at 7 d of therapeutic plasma concentration to ensure the time above MIC for rhesus macaques was at least comparable to the available swine data, given that the duration above MIC is the best predictor of clinical efficacy.[35,43] We chose the 5-mg/kg dose because it is the comparable dose established in swine; the 20-mg/kg dose was chosen to establish a range in which an appropriate and efficacious dose likely would fall. Both in vitro and in vivo studies in numerous veterinary species have used a threshold of 0.2 to 0.25 µg/mL to determine the clinical efficacy of ceftiofur and DCA against susceptible strains that would likely be analogous to macaque bacterial isolates of the same genus.[3,4,14-17,19,23,25,33,35,43] Therefore, we assessed the potential clinical use of this long-acting, single-dose cephalosporin formulation in the rhesus macaque.

Materials and Methods

Animals. This study involved 6 healthy, adult male rhesus macaques housed at the CNPRC (Davis, CA). At the start of each treatment period, body weight, body condition score, and age were collected. For the 2 treatment periods, body weight (mean ± 1 SD) was 11.9 ± 1.58 kg, with a median (range) of 11.9 (10.0 to 13.7) kg and 12.3 ± 1.59 kg with a median (range) of 12.9 (9.90 to 13.9) kg. Body condition scores according to a validated system[12] were 3.0 ± 0.3 with a median (range) of 3.0 (2.5 to 3.5) for the 2 treatment periods. Ages were 6.87 (± 0.66) y with a median (range) of 6.95 (6.16 to 7.93) y and 7.06 (± 0.66) y with a median (range) of 7.14 (6.35 to 8.12) y. All macaques were pair-housed intermittently (paired approximately from 0700 to 1500) with species-appropriate environmental enrichment, fed chow twice daily (LabDiet Monkey Diet 5047, Purina Laboratory, St Louis, MO), offered water free choice via automatic watering devices, and supplemented with fruits and vegetables biweekly. Approximately 3 wk prior to the start of the study, each macaque was acclimated and behaviorally conditioned to present for nonsedated, cageside cephalic venipuncture by trained personnel. A history, physical examination, CBC, and serum biochemical profile were performed on all macaques prior to entering the study, during the 10-wk washout period, and at the conclusion of the study. The current study was approved by the IACUC of the University of California, Davis. Animals were maintained in accordance with the USDA Animal Welfare Act and Regulations and the *Guide for the Care and Use of Laboratory Animals*.[5,6,30] The animal care and use program of the University of California, Davis is USDA-registered, maintains a Public Health Services Assurance,[36] and is fully accredited by AAALAC.

Study design and sample collection. A 2-period, 2-treatment crossover protocol was used to assess the pharmacokinetic parameters of 2 doses of CCFA. Over the study period, each macaque received a 5-mg/kg and a 20-mg/kg SC dose of CCFA, with a 10-wk washout period between the 2 treatment periods. Body weight was collected on each animal the day before dosing to ensure accurate dose calculations. CCFA, in its swine formulation,[48] was administered subcutaneously between the scapulae at a low dose of 5 mg/kg or a high dose of 20 mg/kg. Care was taken to ensure that CCFA was not injected directly into circulation. During the first treatment period, half of the macaques (n = 3) randomly received either the 5- or 20-mg/kg SC CCFA dose and then were crossed-over to the remaining dose for the second treatment period. Each macaque received a 10-wk washout period between the 2 doses. According to the manufacturer's dosing instructions, volumes greater than 2.0 mL were administered in 2 equal aliquots in 2 separate locations, approximately 7 cm apart, as a precaution to reduce the risk of potential injection-site reactions.[48] This precaution was necessary only in the high-dose group in animals weighing more than 10 kg. All injections were administered by using 22-gauge, 1.0-in. needles. Blood samples (5.0 mL per time point) were collected through nonsedated, cage-side venipuncture from the cephalic vein at 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 216, 264, 336, 432, and 504 h after the administration of CCFA into collection tubes containing sodium heparin (Becton Dickenson, Franklin Lakes, NJ) and immediately placed on ice until processed. All blood volumes collected during this study were in compliance with IACUC and CNPRC blood-collection guidelines of a maximum of 20% body weight per month. Blood samples were centrifuged at 3000 × g at 4 °C for 10 min; plasma was harvested and stored at –80 °C in microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA) until assayed.

Dose and route selection. Because an appropriate and efficacious dose for rhesus macaques is not yet established, we selected the 5-mg/kg dose on the basis of the single intramuscular dose approved in swine. Selection of a 20-mg/kg dose was then based on a 4-fold increase to establish a range in which the appropriate and efficacious dose for rhesus macaques likely would fall. Subcutaneous administration of CCFA in the rhesus macaque was chosen because 1) it has been deemed a safe and effective route of administration in cattle and several other species;[4,11,15,16,19,23,26,28,39,41,45] 2) the capacity for large injection volumes not permissible in swine; and 3) to prevent or limit potential adverse reactions. The swine formulation was chosen because it is less concentrated (100 mg/mL) than the cattle formulation and therefore was more conducive to administration to rhesus macaques. In addition, the decreased concentration may help to prevent or minimize injection-site reactions.

Daily observations. Typically, adverse effects of cephalosporins are not serious and occur at a low frequency; the most common adverse effects noted include injection-site reactions, gastrointestinal distress, and hypersensitivity reactions in subjects with known allergies to cephalosporins and other β-lactam drugs.[39,48] Trained personnel observed the macaques daily for 1 wk after CCFA administration specifically to document any injection-site reactions. In addition, stool quality was assessed daily for 1 wk by using an objective fecal score.[9] These observations accompanied routine daily health monitoring for subjective appetite, hydration, and stool quality throughout the treatment and washout periods.

Analysis of ceftiofur and DCA in plasma samples. Ceftiofur and DCA in plasma were quantitated by using a method similar to one previously described.[18] In this method, all residues of ceftiofur are converted into DCA prior to analysis, because DCA is more stable than ceftiofur. Ceftiofur and DCA metabolites were then extracted from plasma samples as desfuroylceftiofur. Plasma calibrators were prepared by diluting the DCA standard solutions with drug-free plasma collected from rhesus macaques to concentrations of 0.01, 0.025, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, 15, 20, 25 µg/mL. Calibration curves were prepared fresh for each quantitative assay. In addition, quality-control samples (at 3 concentrations within the standard curve) were included with each sample set, as an additional check of accuracy.

The response for DCA was linear and gave correlation coefficients (r^2) of 0.99 or better. The intraday, interday, analyst-to-analyst precision and accuracy of the assay were determined by assaying quality-control samples in replicates (n = 6) for DCA. Accuracy is reported as percentage nominal concentration, and precision is reported as percentage relative standard deviation (Table 1). All accuracy and precision data met FDA

Table 1. Accuracy and precision values for LC-MS/MS analysis of DCA in plasma from 6 rhesus macaques

	Intraday		Interday	
Concentration (µg/mL)	Accuracy (% nominal concentration)	Precision (% relative SD)	Accuracy (% nominal concentration)	Precision (% relative SD)
0.075	106.0	16.0	110.0	16.0
5.0	96.0	7.0	106.0	10.0
20.0	104.0	10.0	104.0	9.0

requirements for bioanalytical method validation.[44] The technique was optimized to provide a limit of quantitation of 0.01 µg/mL and a limit of detection of approximately 0.008 µg/mL for DCA.

Pharmacokinetic calculations. Pharmacokinetic analysis was performed on plasma ceftiofur concentrations by using compartmental and noncompartmental analysis by using commercially available software (Phoenix WinNonlin, version 6.2; Pharsight, Cary, NC). However, due to the poor fit of the data to compartmental models, noncompartmental analysis was used to determine pharmacokinetic parameters for both doses (5 and 20 mg/kg SC). The maximal measured plasma concentration (C_{max}) and time to maximal plasma concentration (t_{max}) were obtained directly from the plasma concentration data. The elimination rate constant was calculated by determination of the slope of the terminal portion of the plasma concentration compared with time curve and the plasma elimination half-life according to the formula (ln 2)/ the elimination rate constant. Areas under the curve from 0 to 504 h ($AUC_{0\text{ to }504}$) and from 0 h to infinity ($AUC_{0-\infty}$) were calculated by using the log-linear trapezoidal method. The $AUC_{0-\infty}$ % extrapolated was calculated by using the formula $[(AUC_{0-\infty} - AUC_{0\text{ to }504})/AUC_{0-\infty}] \times 100\%$. Pharmacokinetic parameters and plasma concentrations for ceftiofur are reported as individual, mean (± 1 SD), and median values.

Statistical analysis. Pharmacokinetic parameters (C_{max} [µg/mL], t_{max} [h], $AUC_{0-\infty}$ [µg/h/mL], and terminal elimination half-life [h]) for the 5- and 20-mg/kg SC doses were compared (Stata 13, StataCorp, College Station, TX) with those reported on the product insert for swine by using 1-sample *t* tests. The swine pharmacokinetic parameters were used as the basis of comparison because these parameters have been shown to exceed the MIC90 for the clinically relevant swine respiratory pathogens *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis*, and *Streptococcus suis*. A *P* value of less than 0.05 was considered significant.

Ceftiofur plasma concentrations for the 5- and 20-mg/kg SC doses were compared (Stata 13, StataCorp) at designated time points (0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 216, 264, 336, 432, and 504 h after administration) by using multilevel mixed-effects linear regression, with time point and dose as fixed effects, and animal ID as a random effect. Concentrations that were below the limit of detection (0.008 µg/mL) were assigned the value 0.007 µg/mL for data analysis.

For each time point, ceftiofur plasma concentrations for the 5- and 20-mg/kg SC doses were compared with the reported swine MIC90 (0.2 µg/mL) by using 1-sample *t* tests to test the 1-sided hypothesis that mean concentration exceeded 0.2 µg/mL.

Results

All macaques remained healthy throughout the study and no adverse effects were noted after subcutaneous administration of either 5 or 20 mg/kg CCFA. None of the physical evaluations or blood analyses at the conclusion of the study revealed any significant abnormal findings. No adverse effects, generally or specifically in the form of injection site reactions or gastrointestinal distress, were noted in any macaque, at any dose, over the course of the observation periods (data not shown).

Pharmacokinetic parameters for both doses are shown in Table 2. Mean ceftiofur and DCA concentrations for each dose (5 and 20 mg/kg SC) in rhesus macaques were significantly above the manufacturer-reported swine MIC90 of 0.2 µg/mL through 48 h and 7 d after administration, respectively ($P < 0.05$, Figure 1). Ceftiofur and DCA plasma concentrations differed significantly between the 5 mg/kg and 20 mg/kg SC doses from 0.5 h to 3 d after administration ($P < 0.05$, Figure 1). For both doses, C_{max} and t_{max} were significantly lower in rhesus macaques (subcutaneous administration) compared with swine (5 mg/kg IM; $P < 0.05$, Table 3).

Discussion

At the CNPRC, treatment with cefazolin consists of twice-daily intramuscular dosing for approximately 1 wk, a regimen that has potential disadvantages in terms of stress, discomfort, and risk to the animals or personnel from the frequency and duration of therapy. Currently, there are no published studies describing the pharmacokinetics of cefazolin in NHP. The treatment dose and frequency in NHP are based on anecdotal reports of therapeutic efficacy. Subsequent cephalosporin generations have better gram-negative and anaerobic coverage than do earlier formulations.[39] The current study describes 2 pharmacokinetic dose profiles of a potential long-acting, third-generation cephalosporin in rhesus macaques. We chose to study CCFA because of its extended spectrum relative to that of cefazolin and because of the potential for less frequent drug administration. We compared single subcutaneous injections of CCFA at 5 and 20 mg/kg in adult male rhesus macaques with each other and with the published pharmacokinetic parameters for swine (5 mg/kg IM). Minimal ceftiofur therapeutic plasma concentrations in rhesus macaques are unknown as yet; we therefore chose to use the published swine therapeutic plasma concentration of 0.2 µg/mL for comparison, given that determination of the MIC for rhesus macaque bacterial isolates was beyond the scope of the current study. An MIC threshold of 0.2 to 0.25 µg/mL has been used in vitro and in vivo to prove clinical efficacy in veterinary species for susceptible bacterial strains that are likely analogous to macaque isolates of the same genus.[3,4,14-17,19,23,25,33,35,43] In addition, because the efficacy of ceftiofur is time-dependent, maximizing the duration above the MIC threshold is the therapeutic goal and best predicts clinical efficacy.[35,43]

We recognize that a different route of administration was used in the swine study (intramuscular) as compared with the current study (subcutaneous). There are established differences in the extent and rate of absorption between the different routes of administration that ultimately can affect achievable plasma concentrations.[42] However, similar to that reported here, several studies in various species have reported therapeutic plasma concentrations after subcutaneous administration of

Table 2. Pharmacokinetic parameters after a single subcutaneous administration of 5 or 20 mg/kg CCFA to rhesus macaques (n = 6)

	5 mg/kg		20 mg/kg	
	Mean ± 1 SD	Median (range)	Mean ± 1 SD	Median (range)
t_{max} (h)	2.59 ± 1.63	3.00 (0.504–4.01)	1.82 ± 1.30	1.99 (0.504–4.01)
C_{max} (µg/mL)	2.24 ± 0.525	2.32 (1.26–2.82)	9.18 ± 4.90	7.71 (4.66–16.7)
λ_z (1/h)	0.015 ± 0.007	0.015 (0.008–0.024)	0.010 ± 0.001	0.010 (0.008–0.011)
$t_{1/2\,\lambda z}$ (h)[a]	56.5 ± 21.7	54.0 (33.1–88.3)	69.7 ± 8.86	67.4 (60.5–85.2)
$AUC_{0-\infty}$ (h/µg/mL)	46.9 ± 17.6	42.0 (30.5–70.6)	331 ± 84.4	329 (217–466)
$AUC_{0-\infty}$ % extrapolated	1.17 ± 0.866	1.15 (0.260–2.60)	0.850 ± 0.372	0.805 (0.309–1.31)
AUC_{0-504} (h/µg/mL)	46.5 ± 17.6	41.8 (30.2–69.8)	328 ± 82.3	326 (216–458)
V_z/F (L/kg)	9.59 ± 5.15	8.48 (4.69–19.4)	6.30 ± 1.29	6.18 (4.99–8.62)
Cl/F (mL/kg×h)	120 ± 42.0	125 (70.9–163)	63.8 ± 16.8	60.7 (43.0–91.9)

λ_z, terminal slope; $AUC_{0-\infty}$, area under the plasma concentration time curve extrapolated to infinity; AUC_{0-504}, area under the plasma concentration time curve from 0 to 504 h (21 d); Cl/F, clearance C_{max}, observed maximum plasma concentration; t_{max}, time to observed maximum plasma concentration; V_z/F, volume of distribution based on the terminal phase.
All values in this table were generated using noncompartmental analysis.
[a]Harmonic mean.

CCFA.[4,11,15,16,19,23,26,28,39,41,45] Furthermore, a study comparing the pharmacokinetic parameters for subcutaneous compared with intramuscular CCFA in horses showed minor route-associated differences in plasma concentrations administration that were unlikely to affect clinical efficacy.[23] Finally, differences in the plasma concentration profile might reflect interspecies differences with regard to absorption, distribution, metabolism, and excretion.

The low dose administered in the current study (5 mg/kg) was selected in light of the approved dose of 5 mg/kg in swine. The addition of the 20 mg/kg dose was intended to ensure that a safe and effective dose could be identified for use in rhesus macaques. There were no adverse effects noted with either the 5- or 20-mg/kg dose. In rhesus macaques, both CCFA doses achieved therapeutic plasma concentrations, as determined for swine, within the first 0.5 h after administration. As expected, the maximal plasma concentration was 4 times higher when 20 mg/kg was administered than was achieved with 5 mg/kg; in addition, the rate of absorption was faster when 20 mg/kg was used. Plasma concentrations remained above the therapeutic plasma concentration of 0.2 µg/mL longer after the high compared with low dose. Plasma AUC values increased in a disproportionate manner with increasing dose, indicating that ceftiofur may be subject to dose-dependent pharmacokinetics when administered subcutaneously at increased doses. A dose of 20 mg/kg increased the AUC to approximately 7 times that calculated for the 5-mg/kg dose. Although determining the metabolic profile of CCFA was beyond the scope of this study, this disproportionate increase in AUC values suggests the saturation of a specific metabolic pathway.

Contrary to our hypothesis that a SC dose of 5 mg/kg CCFA would provide therapeutic plasma concentrations in rhesus macaques, comparable to those in swine, for 7 d, therapeutic concentrations after this dose were maintained in rhesus macaques for only 2 d. That is, the 5-mg/kg SC dosage would require redosing every 2 d to provide rhesus macaques with long-term therapeutic coverage comparable to that of the same intramuscular dosage of CCFA in swine. The duration of coverage provided by CCFA at 5 mg/kg SC for 2 d is comparable to that of other long-term antibiotics already in use in rhesus macaques.[13] Therefore, CCFA at 5 mg/kg SC may not necessarily be an improvement, but it is a clinical alternative for consideration. This low dose of CCFA could be given, perhaps with repeated dosing, in cases in which prolonged long-term antibiotic coverage is unnecessary.

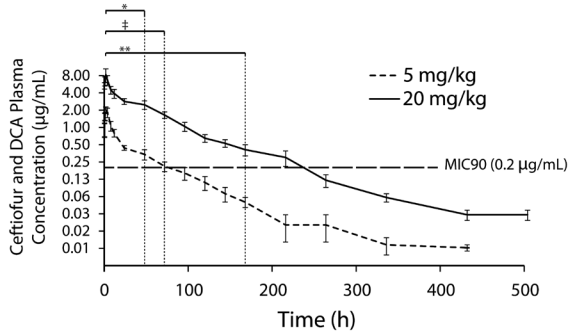

Figure 1. Semilog scale graph of mean ceftiofur and DCA concentrations (µg/mL) over 504 h (21 d) in rhesus macaques administered CCFA at a dose of either 5 mg/kg or 20 mg/kg via a single subcutaneous administration with manufacturer-reported MIC90 in swine (0.2 µg/mL). Error bars represent 1 SD above and below the data point. *, Time points at which ceftiofur and DCA concentrations after the 5-mg/kg SC dose were significantly ($P < 0.05$) above 0.2 µg/mL; **, ceftiofur and DCA concentrations after the 20-mg/kg SC dose were significantly ($P < 0.05$) above 0.2 µg/mL; ‡, ceftiofur and DCA concentrations differed significantly ($P < 0.05$) between doses.

A single dose of CCFA at 20 mg/kg SC likely represents a potential long-acting, third-generation cephalosporin for use in rhesus macaques for the medical management of traumatic wounds and other bacterial infections. Compared with dosage in swine, the 20-mg/kg dose provided statistically significant therapeutic plasma concentrations of ceftiofur for at least 7 d (likely 9 d or more; Figure 1). Results indicate that 20 mg/kg SC in rhesus macaques achieved a significantly higher maximal plasma concentration (greater than 2-fold) than was established in swine after the administration of a lower intramuscular dose (5 mg/kg). The maximal plasma concentration was achieved in significantly less time (about 10-fold faster) in rhesus macaques than in swine. The higher C_{max} and more rapid t_{max} both could be due to interspecies differences or reflect the route of administration. The half-life of the high dose in rhesus macaques was significantly longer than that previously published for swine, suggesting differences in metabolism or the rate of elimination between the 2 species. If the MIC are comparable between swine and rhesus macaque pathogens, these results support our hypothesis that a single injection of CCFA at 20 mg/kg SC in rhesus macaques is as good as, if not superior to, the published

Table 3. Comparison of CCFA pharmacokinetic parameters (mean ± 1 SD)

	Swine	Macaques		
	Dose, 5 mg/kg IM	Dose (mg/kg SC)	Value	*P*
C_{max} (µg/mL)	4.17 ± 0.92	5	2.24 ± 0.525	<0.01
		20	9.18 ± 4.90	0.05
t_{max} (h)	22.0 ± 12.2	5	2.59 ± 1.63	<0.01
		20	1.82 ± 1.30	<0.01
$AUC_{0-\infty}$ (h/µg/mL)	373 ± 56.1	5	46.9 ± 17.6	<0.01
		20	331 ± 84.4	0.28
$t_{1/2}$ (h)	49.6 ± 11.8	5	56.5 ± 21.7	0.47
		20	69.7 ± 8.86	<0.01

Swine data are from reference 48.

dose of 5 mg/kg IM in swine. Furthermore, pharmacokinetic results suggest that a single dose of CCFA at 20 mg/kg SC may be an improvement over currently available antibiotics used in rhesus macaques for the treatment of traumatic wounds and other bacterial infections.

To date, no antibiotic formulation or route of administration has been shown to provide therapeutic concentrations for 1 wk after a single injection in rhesus macaques. Currently, the only injectable antibiotic that does not require daily injections is procaine penicillin G, although florfenicol has been shown to reach therapeutic blood levels in rhesus macaques when dosed every 48 h.[13] Proven effective as a long-term, single-dose, injectable, third-generation cephalosporin in dogs and cats, cefovecin sodium requires increased dosages or frequency of administration in NHP to achieve the same MIC as those for canine pathogens, thus precluding cefovecin's use as a single-dose antibiotic in NHP.[7,38,40] As an alternative to conventional routes of antibiotic administration, antibiotic-impregnated polymethylmethacrylate beads have been used to treat osteomyelitis in NHP; these beads achieve high local antibiotic levels without repeated injections for cases in which tissue penetration is a concern.[32] However, our results suggest that CCFA at 20 mg/kg SC is a possible long-term treatment option for rhesus macaques.

A limitation of this study is the use of male animals only. We selected male macaques to reduce the number of animals required to establish statistically significant results and to follow the standard in the field of pharmacology at the time of study design. To confirm that our current results are not sex-specific, a study to establish the pharmacokinetic parameters of CCFA in healthy, adult female rhesus macaques is recommended to conform to the newly emerging standard for NIH-sponsored research and in the field of pharmacology. In addition, CCFA clearance was not evaluated and represents a potential future direction for investigation.

According to manufacturer recommendations, we divided the 20-mg/kg SC dose into 2 equal volumes, which were administered in separate sites at the same time, to keep injection volumes below 2 mL, as a precaution to minimize potential injection site reactions. However, the divided dose could potentially have differing pharmacokinetic parameters due to altered rates or extents of absorption of the multiple injection sites, thus potentially affecting clinical efficacy also. A similar study in horses showed that dividing doses to reduce the volume injected into a site had minor differences in pharmacokinetic parameters, which translated into little to no effect on clinical efficacy.[24] Because dividing the dose was necessary only in rhesus macaques weighing more than 10 kg and in view of the cross-over nature of this study, this practice likely presents only a minor concern regarding clinical efficacy in rhesus macaques, particularly given that the manufacturer-reported MIC90 threshold of 0.2 µg/mL for swine respiratory pathogens was achieved for at least 7 d in our macaques. Repeated administration of CCFA in other species increases the duration of coverage.[15]

The current study establishes that a single 20-mg/kg SC dose of CCFA in rhesus macaques is effective for 7 d, roughly comparable to the efficacy of a single 5-mg/kg IM injection in swine. To ensure that the pharmacokinetic data for the 20-mg/kg dose reported here is truly clinically effective in rhesus macaques, the necessary efficacy study is underway to establish an MIC of ceftiofur for common macaque bacterial isolates and to compare the standard course of cefazolin treatment with the presently established pharmacokinetics of CCFA at 20 mg/kg SC in rhesus macaques.

In summary, the present study established the pharmacokinetic profiles for subcutaneous doses of 5 and 20 mg/kg of CCFA in rhesus macaques and compared the parameters with those in a previously published report in swine (5 mg/kg IM). Our findings confirmed that CCFA at 5 and 20 mg/kg in rhesus macaques provided a similar pharmacokinetic profile and therapeutic plasma concentration (≥0.2 µg/mL) to that in swine for at least 2 and 7 d, respectively. Therefore, the use of the long-acting, single-dose, third generation, injectable cephalosporin, CCFA, may have far-reaching animal welfare and management implications in the field of medical primatology.

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