

Single subcutaneous dosing of cefovecin in rhesus monkeys (*Macaca mulatta*): a pharmacokinetic study

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Cefovecin is a third-generation cephalosporin approved for antibacterial treatment with a 14-day dosing interval in dogs and cats. This antibiotic may also be useful for zoo and wildlife veterinary medicine, because of its broad spectrum and long duration of activity. The aim of the study was to determine whether cefovecin is a suitable antibiotic to prevent skin wound infection in rhesus monkeys. Therefore, the pharmacokinetics (PK) of cefovecin after a single subcutaneous injection at 8 mg/kg bodyweight in four rhesus monkeys (*Macaca mulatta*) and sensitivity of bacterial isolates from fresh skin wounds were determined. After administration, blood, urine, and feces were collected, and concentrations of cefovecin were determined. Further, the minimum inhibitory concentrations (MIC) for bacteria isolated from fresh skin wounds of monkeys during a health control program were determined. The mean maximum plasma concentration (C_{\max}) of cefovecin was 78 $\mu\text{g}/\text{mL}$ and was achieved after 57 min. The mean apparent long elimination half-life ($t_{1/2}$) was 6.6 h and excretion occurred mainly via urine. The MIC for the majority of the bacteria examined was $>100 \mu\text{g}/\text{mL}$. The PK of cefovecin in rhesus monkeys is substantially different than for dogs and cats. Cefovecin rapidly reached C_{\max} which however was lower than most of the MIC levels and with a very short $t_{1/2}$. Therefore, cefovecin is not recommended for treating skin wounds in rhesus monkeys.

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INTRODUCTION

Following single-dose subcutaneous (s.c.) injection, the third-generation cephalosporin cefovecin has been shown to have antibacterial activity in transudate in cats and dogs, a high bioavailability, and a very long half-life ($t_{1/2}$) (Stegemann *et al.*, 2006b,c) indicating a treatment interval of 14 days. This antibiotic has a broad spectrum of activity against Gram-positive and Gram-negative bacteria and is therefore indicated for the treatment of many forms of bacterial infections in veterinary practice in cats and dogs (Stegemann *et al.*, 2006a).

Because of the long $t_{1/2}$ found in dogs and cats, cefovecin would appear to be an attractive antibiotic for wildlife and zoo veterinarians. Rhesus monkeys (*Macaca mulatta*) are prime candidates for long-acting antibiotics to treat bacterial infection as they are difficult to handle for daily injections, and notorious for trying to avoid medicated food. Furthermore, the formal hierarchy of a colony of rhesus monkeys involves social conflicts and aggression regularly occurs, leading to severe wounding.

Separating an animal from a group for treatment can worsen these social conflicts, particularly in debilitated individuals. Use of a long-acting antibiotic that only needs to be administered once every 14 days would greatly reduce unnatural social disturbance, and the need to handle and restrain monkeys, thereby decreasing the stress. Therefore, cefovecin might be a good candidate for antibiotic treatment.

However, the pharmacokinetics (PK) of cefovecin in macaques has not yet been determined. In this study, we determined the PK of cefovecin after a single s.c. injection in rhesus monkeys to assess whether cefovecin could be used as an effective treatment to prevent infection.

MATERIALS AND METHODS

Drug administration

Cefovecin sodium (Convenia[®]; Pfizer Animal Health, Capelle a/d IJssel, the Netherlands) is an extended-spectrum injectable

third-generation cephalosporin approved for s.c. injection in dogs and cats. Within 1 h prior to administration, vials containing lyophilized cefovecin (as the sodium salt) were reconstituted in diluent to a final concentration of 80 mg/mL. To ensure consistency, cefovecin from the same batch was used in all trials. Each animal was given one single injection s.c. at the lateral trunk at 8 mg/kg bodyweight.

Animals

Four clinically healthy, socially housed adult outbred rhesus monkeys (*Macaca mulatta*) were included in this study, $n = 2$ males and $n = 2$ females, age 8.5 years (range: 5.3–15.0 years) and bodyweight 6.9 kg (range: 4.6–9.5 kg). The animals were of Indian origin, and were housed at the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands) in-house breeding colony. Based on regular clinical examinations, as well as clinical chemical and hematological studies, the animals were declared healthy. Animals were selected for a uniform nutritional status and body condition [score three (Clingerman & Summers, 2005)].

Injured animals in the BPRC breeding colony, consisting of animals with ages ranging from parturition to over 30 years, were used to isolate bacteria from fresh skin wounds to assess the minimum inhibitory concentrations (MIC).

Housing and care

The protocol of this study was approved by the Animal Experiment Committee of the BPRC, and was conducted in accordance with Dutch law and international ethical and scientific standards and guidelines. The animals were fed with commercial monkey pellets supplemented with fruit and vegetables, and drinking water was available *ad libitum*. As a result of the frequent sedation with fasting for blood sampling, the animals that were included in PK-study received nutritional supplementation by stomach tube once daily during the first 3 days during anesthesia. All animals were housed socially in stainless steel wire cages. As for two animals, urine and feces had to be collected, individual housing was required and a divider separated the cages. As soon as possible, the animals were socially housed again. During the course of the study, animals were checked at least once daily for appetite, general behavior, stool consistency, and local side-effects of the administration of cefovecin.

The injured animals were part of family groups, with both indoor and outdoor accommodation, and the monkeys were able to move freely between the two environments. The monkeys' environmental enrichment was optimized with a complex system of fixed and swinging branches, ropes, and nets. The bedding of the inside enclosure was sawdust, outside sand.

Experimental design

A study was designed, which involved a single s.c. dosing of cefovecin at 8 mg/kg bodyweight in all four animals. One blood sample ($T = 0$) was collected prior to dosing. Blood samples were

collected regularly after administration. For two animals (one male and one female), blood samples were collected at 30 min, 1, 2, 4, 8, 12, 24, 48, 72, and 120 h postdosing. For the other two animals (animal id: 95005 and R02092), analysis focussed on the period directly following administration. Blood samples were collected at 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h postdosing. Additionally, feces and urine was collected from these two animals to investigate the excretion pattern of cefovecin in rhesus monkeys. Urine and fecal samples prior to administration and voided over a 6-, 12-, 24-, 48-, and 72-h period were collected. Blood sampling was performed under ketamine sedation (10 mg/kg bodyweight). Before administration of the drug, the bodyweight of the animals was determined. On each subsequent sedation for blood sampling, the bodyweight was noted and the injection site of the cefovecin was examined for local adverse effects to the injected antibiotic.

Bacteria were isolated from fresh skin wounds of rhesus monkeys in the BPRC breeding colony during a health control program using a swab, and MIC was determined for the various bacteria. Between December 2009 and March 2010 (90 days), 13 animals were sampled. From two individuals, several swabs were taken as they were presented with multiple wounds.

Sample collection

Two milliliter blood samples were collected from the *vena femoralis* into lithium heparin tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) and mixed by inversion. Plasma was separated by centrifugation and frozen in polypropylene tubes within 1 h of collection and stored upright below -20°C until assayed.

Urine and fecal samples were collected with a collection tray underneath their home cage. Urine samples were collected, filtered, and stored below -20°C until analyzed. Fecal samples (each consisting of 5 g) were stored below -20°C until analyzed.

Analysis of cefovecin

Samples were analyzed by high-performance liquid chromatography (HPLC) with a UV detector as previously described (Stegemann *et al.*, 2006b,c) and modified (Thuesen *et al.*, 2009).

Pharmacokinetic and biometrical analyses

Peak areas were determined using the software EMPOWER PRO (Waters, Empower software; Waters Corporation, Milford, MA, USA). PK parameters were ascertained by noncompartmental analysis of time and concentration data using Microsoft Excel 2007 professional (Microsoft Corporation, Redmond, WA, USA) as previously described (Thuesen *et al.*, 2009).

Isolation of bacteria and MIC determination

Bacteria were isolated from fresh skin wounds to determine MIC. The wound was sampled by rolling the tip of the swab (Copan Italia S.p.a., Brescia, Italy) for one full rotation over the injured area. The swab was immediately placed in charcoal transport

medium, inoculated on to the surface of Columbia Agar with 5% Sheep Blood plates (Becton Dickinson GmbH, Heidelberg, Germany), and examined after 24 and 48 h incubation at 35 °C. BioMérieux's API® identification products (bioMérieux b.v., Boxtel, The Netherlands) were used for identification of Gram-positive and Gram-negative bacteria. Each identified bacterial isolate was suspended in sterile 0.9% NaCl (sterile saline). Different cefovecin concentrations were prepared in sterile saline. In 96-well plates (Greiner Bio-One b.v., Alphen aan den Rijn, The Netherlands), 50 µL of a given bacterial suspension (1 McFarland) was mixed with 50 µL of a cefovecin solution, ensuring that each bacterium was exposed to increasing cefovecin concentrations (0–100 µg/mL). The plates were incubated at 35 °C for 24 h, whereafter 10 µL from each well was inoculated on to Columbia Agar with 5% Sheep Blood to determine viability of bacteria. They were incubated at 35 °C and examined after 24 h. All procedures were performed according to the routine procedures used in human hospitals. Bacteria that formed visible colonies after 24 h were defined as not sensitive to the dose of antibiotic used. Bacteria that failed to grow were described as sensitive to the dose of antibiotic used.

RESULTS

All animals remained in good health, and no significant bodyweight changes occurred during this study. No adverse reactions were observed following administration of cefovecin.

Concentration of cefovecin in plasma

Limited animal-to-animal variation was observed for maximum plasma concentration (C_{max}) values as well as the time required to achieve C_{max} . The average plasma concentration of the four animals is depicted in Fig. 1. Maximum plasma concentration of 79 ± 5.7 µg/mL was observed after approximately 57 ± 8.6 min (T_{max}), and rapidly declined thereafter. Mean $t_{1/2}$ was calculated as 6.6 ± 1.0 h. The levels returned to below the detection limit (<0.030 µg/mL) in <48 h after dosing in two animals, and between 48 h (1.34 and 1.72 µg/mL) and 72 h for the other two animals. The mean values for PK parameters are shown in Table 1.

Excretion of cefovecin in urine and feces

To assess whether rapid decrease of cefovecin concentration in blood resulted from renal excretion, urine samples were analyzed in two animals at different time points. High concentrations of cefovecin were found in urine shortly after drug administration. Those levels were considerable higher compared to the plasma levels (Fig. 2a, b) and peaked later. In animal R02092 (Fig. 2b), it peaked at 6 h (307 µg/mL), and in the animal 95005 (Fig. 2a) at 12 h (249 µg/mL) post dosing. Forty-eight hours after dosing, the concentrations returned to below the detection limit. As fecal excretion accounted up to 25% of total elimination in cats (Stegemann *et al.*, 2006c), feces were analyzed. However, no

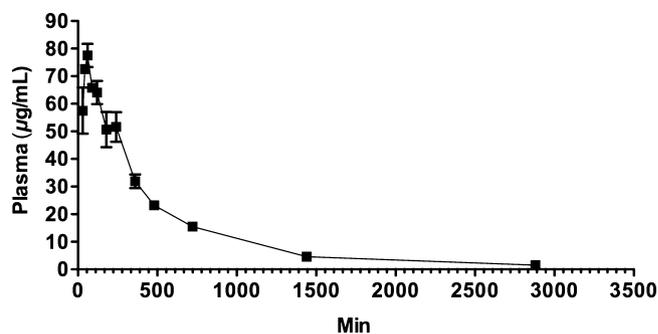


Fig. 1. Mean average cefovecin concentration in plasma following a single s.c. dose at 8 mg/kg bodyweight ($n = 4$). Error bars indicate the standard deviation.

Table 1. Pharmacokinetic parameters for cefovecin in plasma following a s.c. dose at 8 mg/kg bodyweight in rhesus monkeys ($n = 4$)

	Units	Average \pm SD
$t_{1/2}$	Hours	6.6 ± 1.0
Cl	mL·min/kg	0.21 ± 0.024
Vd	L/kg	0.12 ± 0.029
AUC	µg·min/mL	$39 \ 367 \pm 4728$
AUC	µg·h/mL	656 ± 79
T_{max}	Minutes	57 ± 8.6
C_{max}	µg/mL	79 ± 5.7

Vd, volume of distribution.

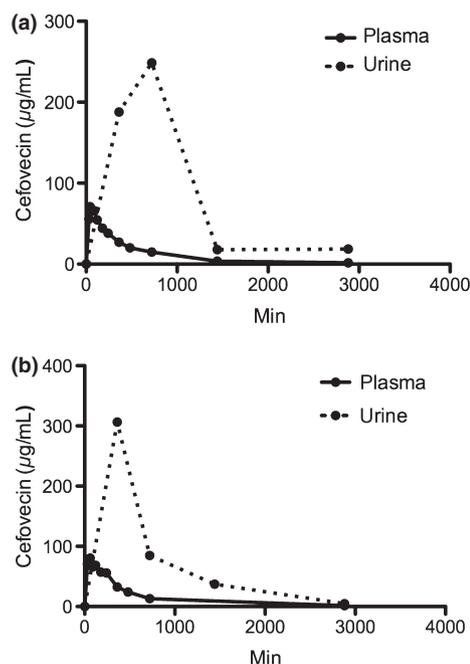


Fig. 2. (a) Concentrations of cefovecin in plasma and urine following a single s.c. dose at 8 mg/kg bodyweight (Animal id: 95005).

(b) Concentrations of cefovecin in plasma and urine following a single s.c. dose at 8 mg/kg bodyweight (Animal id: R02092).

cefovecin was detected in fecal samples. Urine and fecal analysis demonstrated that cefovecin was excreted very rapidly from the body via urine, suggesting the drug is not metabolized and is

principally excreted via the kidneys. We computed an order of magnitude of the renal clearance [Cl (renal)] to explain the short half-life of cefovecin in these animals. Using a urine output at 50 mL/kg/day (National Research Council 2003), the Cl (renal) was calculated to be 0.22 mL/h/kg which is comparable to the plasma clearance (Cl) (Fig. 3).

Isolated bacteria and MIC

Seventeen bacterial sp. were isolated from fresh skin wounds in rhesus monkeys. The most abundantly present was *Staphylococcus aureus* (35%) (Table 2).

The MIC values of cefovecin against *Acinetobacter lwoffii*, *Enterococcus durans*, and *Leuconostoc* spp. were 100, 50, and 2 µg/mL, respectively. For all other isolates, cefovecin was not bactericidal for concentrations up to 100 µg/mL (Table 2).

DISCUSSION

Because of long $t_{1/2}$, cefovecin is an attractive antibiotic for wildlife and zoo veterinarians. Cefovecin is empirically used off-label in various animal species (Thuesen *et al.*, 2009; Bertelsen *et al.*, 2010; García-Párraga *et al.*, 2010a,b; Montesinos *et al.*, 2010). However, without extensive knowledge of the PK profiles, this can lead to treatment failure, toxicity, or the development of antibiotic resistance. In the present study, the PK profile of cefovecin in rhesus monkeys was determined. The outcome of PK showed large dissimilarities when compared to those for dogs and cats. After cefovecin was administered s.c., it rapidly achieved C_{max} ; however, C_{max} was considerably lower than reported for domestic carnivores (Stegemann *et al.*, 2006b,c). Clearance for cefovecin was considerably higher, and $t_{1/2}$ was shorter than those described for dogs and cats (Stegemann *et al.*, 2006b,c). The observed differences can have several explanations including differences in renal excretion, urine pH, and protein binding. In dogs and cats, cefovecin was found to extensively bind to plasma proteins (>99.5% for cats and >96% in dogs) (Stegemann *et al.*, 2006b,c). Although the protein binding of cefovecin in rhesus monkeys was not determined, one could speculate that protein binding in this species is less effective when compared to dogs and cats. Another explanation might be differences in the distribution of cefovecin in the deep

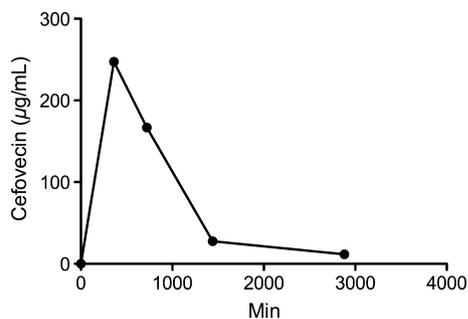


Fig. 3. Computed renal clearance ($n = 2$) using an estimated urine output of 50 mL/kg/day.

Table 2. The different bacterial strains isolated and determined MIC

Pathogen group (no. of isolates tested)	MIC
<i>Acinetobacter lwoffii</i> ($n = 2$)	100 µg/mL
<i>Aerococcus urinae</i> ($n = 1$)	NS
<i>Aerococcus viridans</i> ($n = 4$)	NS
<i>Enterococcus durans</i> ($n = 1$)	50 µg/mL
<i>Enterococcus faecium</i> ($n = 1$)	NS
<i>Leuconostoc</i> spp. ($n = 1$)	2 µg/mL
<i>Ochromobacterium anthropi</i> ($n = 1$)	NS
<i>Pasteurella pneumotropica</i> ($n = 2$)	NS
<i>Pasteurella</i> spp. ($n = 1$)	NS
<i>Proteus vulgaris</i> ($n = 1$)	NS
<i>Pseudomonas fluorescens</i> ($n = 1$)	NS
<i>Staphylococcus aureus</i> ($n = 12$)	NS
<i>Staphylococcus xylosum</i> ($n = 1$)	NS
<i>Streptococcus agalactiae</i> ($n = 1$)	NS
<i>Streptococcus bovis</i> ($n = 2$)	NS
<i>Streptococcus dysgalactiae</i> ($n = 1$)	NS
<i>Streptococcus equines</i> ($n = 1$)	NS

NS = not sensitive to cefovecin concentration up to 100 µg/mL.

compartment. The curves of plasma concentration of cefovecin in dogs and cats suggest that a three-compartment model is invoked, although a noncompartment analysis was applied in the literature (Stegemann *et al.*, 2006b,c). The influence of protein binding and volume of distribution on Cl and $t_{1/2}$ is however debatable (Toutain & Bousquet-Mélou, 2004a,b). As the estimated Cl (renal) is equal to the Cl, we can assume that all or most of the dose is eliminated in urine (Toutain & Bousquet-Mélou, 2004a,b) and most within 24 h (Fig. 3).

The C_{max} of cefovecin was determined in rhesus monkeys to be 79 µg/mL, while the average MIC was >100 µg/mL. In addition, we observed that the majority of isolated bacteria from wounds did not show growth inhibition in the presence of cefovecin at all. When C_{max} of antibiotics is lower than the bacterial MIC value, use will most likely not inhibit bacterial growth and even potentially lead to antibiotic resistance. We present here, the first documented report of limited antibacterial activity of cefovecin of bacteria isolated from wounds of socially housed rhesus macaques. This observation was surprising and alarming, but is consistent with bacterial resistance to broad-spectrum antibiotics as described earlier in captive lion tamarins (Lilenbaum *et al.*, 2006). The source of the animal's wound flora will be on the surface and deep layers of skin, the saliva and oral mucosa, the gastrointestinal tract, or in environmental bacteria. A detailed history of the use of antimicrobial drugs in these subjects did not reveal previous exposure to cefovecin and could therefore not justify the high degree of bacterial resistance.

Care should be taken to use preventive antibiotics in general. The isolated bacterial sp. (e.g. *S. aureus*) will not cause disease or infection under normal circumstances as resident bacterial species on an intact skin layer, in absence of predisposing factors for skin disease and in not-immunocompromised animals. However, once the bacteria gain entry into the body through breaks in the skin, they can cause infection, depending greatly on the immune status of the animal. In addition, in humans, micro-organisms can be identified in the deep tissue of all chronic wounds, although their role and

the impact of the micro-organisms on wound longevity are unclear. Besides, in fresh skin wounds, the first objective is to provide wound healing and not resolution of the infection. The use of preventive antibiotics can be avoided when topical antimicrobial preparations are used. These preparations include both antibacterials (e.g., silver sulfadiazine) and antiseptics such as chlorhexidine and povidone-iodine (Howell-Jones *et al.*, 2005; College voor zorgverzekeringen 2010a).

The assessed $t_{1/2}$ is inconsistent with the elimination period observed for dogs and cats, and more consistent with third-generation cephalosporins used in humans (College voor zorgverzekeringen 2010b) and would support a phylogenetic pattern, stated also by our unpublished data from a single Geoffroy's spider monkey (*Ateles geoffroyi*) which showed rapid excretion with complete elimination by 48 h. A possible means of prolonging the $t_{1/2}$ could be achieved by the simultaneous administration of probenecid, as in humans probenecid impairs the clearance of selected cephalosporins by blocking tubular secretion (Foord, 1976; Verhagen *et al.*, 1994). It has also been hypothesized that probenecid may affect tissue distribution of cephalosporins. The concurrent administration of probenecid may result in increased C_{max} , area under the curve (AUC), and $t_{1/2}$ of cefovecin (Foord, 1976; Welling *et al.*, 1979; Verhagen *et al.*, 1994; Garton *et al.*, 1997; Thuesen *et al.*, 2009) and should therefore be further examined.

In conclusion, an antibiotic with a long $t_{1/2}$ would make the treatment of wounded rhesus monkeys less invasive and therefore preferred over the use of antibiotics with a short $t_{1/2}$. Given the extended $t_{1/2}$ of cefovecin in small pets, this might be a good candidate. Our results show however that the $t_{1/2}$ of cefovecin in rhesus monkeys is much shorter than observed in dogs and cats; therefore, the advantage of cefovecin is reduced. In addition, C_{max} of cefovecin in rhesus monkeys is lower than the MIC values of the most common bacteria isolated from skin wounds and therefore we conclude that cefovecin should not be recommended for the treatment of skin lesions in rhesus monkeys.

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