PHARMACOKINETICS OF CEFTIOFUR CRYSTALLINE FREE ACID IN ADULT HORSES AFTER WEEKLY INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION AND DISPOSITION OF CEFTIOFUR SODIUM AFTER NEBULIZATION AND INTRAMUSCULAR ADMINISTRATION TO WEANLING FOALS

by

LISA EMILY FULTZ

(Under the Direction of Steeve Giguère)

ABSTRACT

The goal of the studies presented herein was to investigate the pharmacokinetic behavior of two formulations of ceftiofur (ceftiofur crystalline free acid (CCFA) and ceftiofur sodium) in an effort to provide data that could expand therapeutic applications.

First, a study of plasma and pulmonary pharmacokinetics of CCFA in adult horses supported the administration of CCFA every 7 days if prolonged administration is clinically warranted after the initial 2 doses recommended by the manufacturer.

Second, the plasma concentration versus time profile of ceftiofur and metabolites after subcutaneous administration of CCFA to adult horses was similar to that observed after intramuscular administration. However, visible injection site swellings were more severe after subcutaneous administration.

Lastly, nebulization of ceftiofur sodium to weanling foals was well tolerated and resulted in significantly higher drug concentrations in pulmonary epithelial lining fluid (PELF) compared to administration of the same dose intramuscularly.

INDEX WORDS: Ceftiofur crystalline free acid, Ceftiofur sodium, Equine bronchopneumonia, Pharmacokinetics, Nebulization, Aerosol

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CHAPTER 1

INTRODUCTION

Bacterial pneumonia is an important cause of morbidity and mortality in both adult horses and foals. The Gram-positive organism *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is the most frequently isolated organism in both age groups of horses. Ceftiofur is a broad-spectrum third generation cephalosporin that has documented efficacy against *S. zooepidemicus* and a variety of other bacterial pathogens commonly isolated from the respiratory tract of horses. In the United States, ceftiofur is approved by the Food and Drug Administration for the treatment of lower respiratory tract infection caused by susceptible strains of *S. zooepidemicus* in two commercial forms: 1) ceftiofur crystalline free acid (CCFA), the long-acting depot formulation that is formulated in cottonseed oil, and 2) ceftiofur sodium, the aqueous form. Once administered parenterally in either form, ceftiofur it is rapidly metabolized into desfuroylceftiofur (DCA). Current labeling for the use of CCFA in horses states that 2 intramuscular doses must be administered 4 days apart. This regimen is designed to provide approximately 10 days of therapeutic coverage. While a 10-day treatment course is appropriate for the treatment of most mild to moderate respiratory infections, it will not be sufficient for the long term treatment of horses with severe lung consolidation or for horses with pleuropneumonia. There is currently no information available to help practitioners determine what would be an appropriate dosing interval for long-term administration of CCFA.

The most common adverse reaction after intramuscular injection of CCFA to horses is a self-limiting injection site swelling (edema) at a frequency rate of 3.6% [1]. In some horses, the injection site reaction can be quite large and very painful. Administration of CCFA by the subcutaneous route may minimize injection site irritation and maximize ease of administration. There is currently no

information available in adult horses concerning how the subcutaneous route of administration of CCFA may alter the pharmacokinetics or if adverse reactions could occur.

Nebulization of antimicrobial agents has been proposed as a method to increase drug concentrations in the lungs. However, nebulization of antimicrobial agents formulated for IV use can lead to exposure to potentially irritant or toxic additives and inappropriate pH or osmolality ranges. Ceftiofur sodium is commonly nebulized to horses with bronchopneumonia despite the complete lack of knowledge of the safety or efficacy of this practice. The studies reported herein were undertaken to determine the proper dosing interval for CCFA when long term therapy is required and to investigate alternative routes of administration for both CCFA and ceftiofur sodium with a particular focus on drug concentrations in the respiratory tract. This thesis reports the results of 3 studies.

The objectives and hypothesis of the first study (Chapter 3) are:

1- To determine steady-state plasma and PELF concentrations of desfuroylceftiofur acetamide (DCA) after repeated weekly IM administration of CCFA to adult horses.

2- To document the frequency, size, and duration of injection site reactions after repeated administration of CCFA.

Our *hypothesis* is that that weekly administration of CCFA to adult horses will be well tolerated and result in plasma and PELF concentrations above the MIC_{90} for *S. zooepidemicus* (0.12 μg/mL) for the entire dosing interval.

The objectives and hypothesis of the second study (Chapter 4) are:

1- To compare the pharmacokinetic profile of CCFA administered by the subcutaneous (SC) route to that achieved after administration by the intramuscular (IM) route.

2- To compare the size of visible injection site swellings after administration by the 2 routes of administration.

Our *hypothesis* is that that SC administration of CCFA to adult horses will be well tolerated and result in therapeutic plasma concentrations.

The objectives and hypothesis of the third study (Chapter 5) are:

1- To determine and compare concentrations of DCA in plasma and pulmonary epithelial lining fluid (PELF) of foals after nebulization or IM administration of ceftiofur sodium.

2- To determine if nebulization of ceftiofur sodium induces airway inflammation in weanling foals.

Our *hypothesis* is that that nebulization of ceftiofur sodium will be well tolerated and result in higher DCA concentrations in PELF compared to those achieved after IM administration.

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CHAPTER 2

LITERATURE REVIEW

SECTION I: *STREPTOCOCCUS EQUI* SUBSPECIES *ZOOEPIDEMICUS* AND ITS ROLE IN BACTERIAL PNEUMONIA OF ADULTS AND FOALS

Bronchopneumonia is the term commonly utilized to describe bacterial infection of the lower respiratory tract of horses [1-3]. Risk factors for the development of bronchopneumonia in adult horses include long-distance transport, recent viral infection, racing within 48 hours prior to the onset of clinical signs, and aspiration following esophageal obstruction [1]. *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is a beta hemolytic streptococcus that normally colonizes the pharyngeal tonsils and is the most common bacterial pathogen isolated from adult horses with bronchopneumonia [4-6]. Immunophenotyping of the gene for the variable surface M-like protein (SzP) confirms the high rate of migration of genetically identical strains of *S. zooepidemicus* from the pharyngeal tonsil to the trachea in horses undergoing long-distance transport [7]. Likewise, phenotypically identical strains of *S. zooepidemicus* have been concurrently isolated from the nasopharynx of healthy horses and trans-tracheal aspirate samples from pneumonic donkeys and horses [8], thereby suggesting a role of the upper respiratory tract commensal bacteria as pathogens in acquired bronchopneumonia. Direct inoculation of *S. zooepidemicus* into the equine lower airway is able to produce lesions that resemble the lesions observed in naturally-occurring transport-associated equine pneumonia in as little as 3 hours post-inoculation [2]. The lesions of experimentally-induced [2] and naturally-occurring [3] *S. zooepidemicus* pneumonia range from serous, hemorrhagic pneumonia to multiple foci of coagulative necrosis, depending on duration of infection time. Acute necrotic lacunar tonsillitis, a

component of some upper respiratory viral infections, is considered an important contributing factor for the development of pneumonia in these studies [2,3].

Beyond the role as a bacterial pathogen of respiratory importance, *S. zooepidemicus* is also a common pathogen in other localized infections in horses. In a recent surveillance study, *S. zooepidemicus* was the most common isolate overall and was the most commonly isolated bacteria in samples submitted from acute and chronic wounds, uterine, guttural pouch, tracheal, eye, and post-procedural orthopedic infections [6].

A variety of other bacteria can be isolated concurrently with *S. zooepidemicus* or as sole bacterial pathogens in naturally-occurring cases of bronchopneumonia in horses. In a subset of clinical cases of bronchopneumonia in Australia, 91% of culture-positive cases had multiple bacteria isolated from tracheal samples, with an average of seven bacterial species isolated [9]. Although *S. zooepidemicus* is often the predominant isolate in tracheal samples obtained from pneumonic horses (present in 82% of culture-positive horses in one study) [9] other commonly isolated bacteria include *Actinobacillus suis and A. equuli* [6], *Pasteurella* spp. [6, 9], and *E. coli* [9]. Obligate anaerobes such as *Bacteroides* spp., *Fusobacterium necrophorum*, and *Peptostreptococcus anaerobius* are also commonly isolated from tracheobronchial aspirates from horses with severe bronchopneumonia or pleuropneumonia [10]. Similar to the pathogenesis of *S. zooepidemicus* pneumonia, DNA hybridization data and phenotypic characteristics support that the pharyngeal tonsillar area is the most likely source of bacterial infection of the lower respiratory tract with obligate and facultative anaerobic species (such as *Eubacterium fosser* and *Bacteriodes heparinolyticus)* [11].

The prognosis for equine bronchopneumonia has varied considerably between studies; however, survival and return to racing are both heavily influenced by time to detection and early institution of antimicrobial therapy [9, 12, 13]. The mortality rate in case studies ranges from 43% to 96% [1], with 100% mortality reported in horses for which clinical signs persisted greater than

24 hours prior to intervention in a single study [9]. A case series on 17 racehorses revealed that 65% successfully returned to racing following treatment of pleuropneumonia. In that study, *Streptococcus* spp. were the primary pathogens (61% of cases) and *Klebsiella pneumoniae* was the second most common pathogen (16.6% of cases) [12]. A similar return to racing performance (61%) was documented in an earlier study of thoroughbred racehorses afflicted with pleuropneumonia [13].

A prospective study of over 2000 foals in Texas identified pneumonia as the single most common cause of death in neonates and weanlings [14]. Foals aged 1 to 8 months old diagnosed with bacterial pneumonia share many of these same pathogens with adult horses. In clinically-affected foals with signs of undifferentiated distal respiratory tract disease, *S. zooepidemicus* was the most commonly isolated pathogen, present in 88% of bronchial lavage samples [15]. Aside from *S. zooepidemicus*, a variety of Gram negative organisms such as *Actinobacillus* spp., *Pasteurella* spp. *Bordetella bronchiseptica* and Enterobacteriaceae are also isolated in samples from pneumonic weanlings [15]. There has been limited study on the prognosis for general bronchopneumonia in weanlings, with most reports focusing on *Rhodococcus equi*, a common cause of bronchopneumonia in this age group. A retrospective study investigating the relationship of mixed bacterial infections with concurrent *R. equi* pneumonia revealed that no particular microbial subset (including Gram-positive bacteria, beta-hemolytic streptococci, Gram-negative bacteria, enteric Gram-negative bacteria, nonenteric Gram-negative bacteria, and fungi) was significantly associated with outcome, with survival ranging from 50%-71% [16].

SECTION II: COMPARATIVE PHARMACOKINETICS OF CCFA AND CEFTIOUR SODIUM IN HORSES

Ceftiofur

Ceftiofur is approved for the treatment of lower respiratory tract infections caused by *S. zooepidemicus* in weanling foals and adult horses. Ceftiofur is an aminothiazole-containing third-

generation cephalosporin within the beta-lactam antibiotic class. Substitutions at the third and seventh carbon atoms of the beta-lactam ring define the differences in activity of ceftiofur and other third generation cephalosporins when compared to the first, second and fourth generation cephalosporins [17]. Ceftiofur exerts its inhibitory effect by interfering with bacterial cell wall synthesis due to its covalent binding to the penicillin-binding proteins, which are essential for synthesis of the bacterial wall [18]. Ceftiofur and other third generation cephalosporins have enhanced hydrolytic stability to many of the beta-lactamases due to an oxyimino side chain [17]. Ceftiofur was developed for veterinary medicine and is approved in numerous species including horses, cattle, swine, dogs, sheep, poultry, and goats. When ceftiofur is administered, either as the sodium salt or as free acid, it dissociates into the ceftiofur anion and the positively charged counter ion as it goes into solution. Although the salt and free acid are different in solid form, they become the identical ceftiofur anion in solution [19]. Therefore, any subsequent metabolism and/or disposition should be identical.

The metabolism of ceftiofur is similar in all animal species studied and is characterized by rapid cleavage of the thioester bond to the active metabolite desfuroylceftiofur [20]. Ceftiofur, administered to horses as either ceftiofur sodium (Naxcel®), ceftiofur hydrochloride (Excenel®) or ceftiofur crystalline free acid (CCFA) (Excede®), is rapidly metabolized to desfuroylceftiofur, the primary metabolite which retains antimicrobial activity. When plasma concentrations are determined in the laboratory, the compound desfurylceftiofur acetamide (DCA) is assayed, which represents the sum of the parent molecule of ceftiofur and the biologically active metabolites. [21]

Currently, only ceftiofur sodium and ceftiofur crystalline free acid are approved for administration to horses in the United States. There are multiple pharmacokinetic studies in horses for both of these FDA-approved formulations of ceftiofur. A review of these studies is included in this section, as is a comparison of the pharmacokinetics of CCFA and ceftiofur sodium in horses. When comparing the depot formulation of ceftiofur (CCFA) to the aqueous formulation (ceftiofur

sodium) there are differences in some of the pharmacokinetic parameters, including absorption, distribution, metabolism and elimination.

Ceftiofur crystalline free acid

A depot medication is an injection, usually subcutaneous or intramuscular, of a pharmacological agent which releases its active compound in a consistent way over a long period of time. Depot injections are usually either solid or oil-based and may only be available as certain forms, such as decanoate salts or esters. Forming a salt or ester with a drug increases the affinity for fatty tissue and creates a more long-acting injectable form of a drug [22]. Depot formulations of medications are an attractive treatment option in veterinary medicine as the decreased dosing interval can increase owner and patient compliance [23] and provide more consistent serum concentrations over longer time periods compared to aqueous formulations of the same drug [24-26].

Ceftiofur Crystalline Free Acid (CCFA; Excede®) was approved by the United States Food and Drug Administration in 2010 for the treatment of lower respiratory tract infection in horses caused by susceptible bacterial strains of *S. zooepidemicus* with a minimum inhibitory concentration of less than or equal to 0.25 μg/mL. CCFA also has *in vitro* activity against *Pasteurella spp., Actinobacillus* spp., streptococci, *Mannheimia hemolytica, Histophilus somni*, and some Enterobacteriaceae [19] which are also common causes of equine or ruminant pneumonia. CCFA is the longest-acting depot formulation of ceftiofur that is commercially available. CCFA is marketed with caprylic/ capric triglyceride in a cottonseed oil based suspension. Caprylic/capric triglyceride oils are medium-chain triglycerides (eight-carbon saturated fatty or octanoic acid) whose relatively short chain facilitates penetration of lipid cell wall components.

To arrive at the labeled dose in horses, a series of pharmacokinetic studies were performed. CCFA was well-absorbed following intramuscular injection with an absolute bioavailability of 100%

when compared to IV ceftiofur sodium. In a dose-proportionality study using a variety of doses, the ideal dosing of the depot formulation of ceftiofur was determined to be a series of two intramuscular injections given 96 hours apart with concentrations maintained above 0.2 μg/dl for 6 days after the second injection (for a total of 10 days antimicrobial coverage) [27].

Comparative pharmacokinetics of CCFA and ceftiofur sodium

Absorption

Absorption of ceftiofur sodium after intramuscular administration to adult horses is rapid with a time to maximum plasma concentration (T_{max}) of 1.25 ± 0.46 h [21]. In adult horses, intramuscular and subcutaneous administration of ceftiofur sodium results in similar pharmacokinetic profiles and peak concentrations [28].

The pharmacokinetic variables of particular interest in treating equine pneumonia are time to peak concentration, peak concentration and terminal half-life [29]. The pharmacodynamic parameter best correlated with outcome for beta-lactam antimicrobial agents is the amount of time that drug concentrations remain above the MIC [29] of the target pathogen, which is typically ≤ 0.12 μg/ml for *S. zooepidemicus* [30-32]. The plasma concentrations versus time profiles of DCA in adult horses are similar after administration of ceftiofur sodium by various parenteral routes (intravenously, intramuscularly, and subcutaneously) [28]. When ceftiofur sodium is given intramuscularly at the recommended dose of 2.2 mg/kg every 24 hours for 10 consecutive days the mean peak concentration is 4.27 ± 0.26 μg/dl with a T_{max} of 1 hour. In contrast, after intramuscular administration of CCFA at the recommended dose of 6.6 mg/kg, the C_{max} is lower (0.89 ± 0.08 μg/mL) and T_{max} is much longer (median 25.5 hours; range 16.7-34.3 hours). At the recommended dose, either formulation would result in plasma concentrations above the MIC_{90} of *S. zooepidemicus* for the entire dosing interval.

Many depot medications exhibit “flip-flop kinetics”. In pharmacokinetics, the “flip-flop” phenomenon occurs when a drug is released at a sustained slow rate instead of immediate release, causing the absorption constant (k_a) to be much slower than the elimination constant (k_e). The apparent difference shifts the slope of the plasma log concentration versus time curve so the apparent part of k_e appears much smaller than it would be if the drug was administered intravenously or using an immediate-release formulation. The downward portion of the curve becomes a reflection of actual k_a while the upward part of the curve is the actual representation of k_e [22]. In a simplified sense, a "flip-flop" exists when rate of absorption is the rate-limiting step in the sequential and overlapping processes of drug absorption, distribution and elimination.

Consistent with ‘flip-flop’ pharmacokinetics, the terminal half-life after administration of CCFA is approximately four times longer than that the true elimination half-life of DCA observed after intravenous administration of ceftiofur sodium [27]. A variety of other factors can affect absorption, such as the site and route of administration, or potential irritation from the drug or vehicle. The site of administration may influence the pharmacokinetic parameters of the CCFA. In a series of studies investigating the pharmacokinetics of CCFA in different age groups of horses (neonates, weanlings and adults) it was determined that weanling foals had a significantly higher C_{max} and $AUC_{0\rightarrow\infty}$ compared to adult horses along with similar half-life and mean residence time (MRT); this suggested a higher bioavailability of CCFA following intramuscular administration to weanlings [24]. Neonates were given subcutaneous doses and had an even higher C_{max} but shorter terminal half-life compared to the weanling group [33]. Since the route of administration was different it is impossible to determine if such differences were due to age or route of administration. Subcutaneous administration was shown to be an effective and well-tolerated route in equine neonates [33]. The subcutaneous route has not been studied in adult horses.

Another factor that can influence absorption is the possible effect of tissue trauma. The most common adverse reaction following intramuscular injection of CCFA in horses is a self-

limiting injection site swelling [25, 34] at a frequency rate of 4% [34]. In an effort to decrease the potential irritating effects of large volumes of cottonseed oil, one study investigated the effects of dividing the dose of CCFA in half and administering equal volumes on either side of the adult horse's cervical musculature. Splitting the dose equally did not result in clinically important changes in pharmacokinetic variables (half-life, C_{max}, AUC, or T_{max}). Interestingly, the plasma concentration of DCA at the conclusion of the treatment period (day 10) was significantly higher in the divided dose group [25].

Distribution

In horses, ceftiofur and its metabolites distribute to the following tissues and body fluids at concentrations above the MIC for most primary target pathogens for at least a portion of the dosing interval: pulmonary epithelial lining fluid [24], lung tissue [21], synovial cavities [35, 36], muscle tissue [37], endometrium [26], colostrum [38] and urine [36]. The distribution of DCA to the CSF [36], placental tissue [38], and foals in-utero [38] is considered insufficient for target pathogens when administered systemically.

The ability of antimicrobial agents to reach the target site is a key determinant of clinical outcome. A study in weanling foals investigated the concentrations of DCA in both the pulmonary epithelial lining fluid (PELF) and bronchoalveolar cells of horses to better characterize the distribution of DCA to various sub-components of the lungs after administration of a single dose of CCFA [24]. Intracellular concentrations were very low and well below the MIC of common intracellular or extracellular bacterial pathogens of horses. However, DCA concentrations in pulmonary epithelial lining fluid were within the therapeutic range but for a shorter duration compared to plasma (107 versus 185 hours above 0.2 μg/ml) [24]. The distribution of ceftiofur and its metabolites has not been investigated in PELF of adult horses.

While plasma concentrations can serve as a driving force for penetration to the site of infection, the actual drug concentration–time profile in tissues may vary substantially from that in plasma. The rate and extent of drug distribution can be influenced by ceftiofur's molecular charge and size, lipid solubility, extent of plasma protein binding, and by blood flow at the site of infection [39]. The ability for ceftiofur to penetrate infected tissue following systemic CCFA administration was investigated in a cattle model following implantation of tissue chambers infected with *Mannheimia hemolytica* in a remote muscle location [40]. In general, DCA concentrations were highest in plasma, higher in infected tissue chamber fluid compared to non-infected tissue chamber fluid, and lowest in lung tissue. This pattern mimicked that found after intravenous administration of ceftiofur sodium to calves in a similar tissue chamber model [41]. Importantly, DCA concentrations remained above the FDA-mandated therapeutic threshold of 0.2 μg/mL for plasma and chamber fluid and 0.2 μg/g for lung tissue for at least 7 days post-treatment [40].

The distribution of an antibiotic and its metabolites into a particular tissue compartment may vary when pathologic or biological processes alter the organ or its function; however, there is little evidence in the current veterinary literature to support this for ceftiofur. The pharmacokinetics of DCA in pregnant mares with induced placentitis [38] is similar to that of non-pregnant mares [26]. However, DCA is undetectable in amniotic fluid and foal serum and far below the MIC of *S. zooepidemicus* in a variety of other placental and foal tissues both in normal mares and after induction of placentitis [38].

Metabolism

Ceftiofur is rapidly metabolized to desfuroylceftiofur and furoic acid after parenteral administration [42]. The metabolism of ceftiofur has been studied extensively in cattle [42], swine [43], sheep and rats [17, 42] but not specifically in horses. Ceftiofur, unlike many other late-generation cephalosporins, is highly metabolized. The half-life of ceftiofur is less than 10 minutes

due to rapid cleavage of the thioester bond. Desfuroylceftiofur binds to cysteine moieties due to an exposed sulfhydryl group, a phenomenon that allows the formation of reversible disulfide bonds. These bonds are responsible for the reversible covalent binding to plasma and tissue proteins that extend the biological half- life of desfuroylceftiofur and other biologically active metabolites. Desfuroylceftiofur and the parent molecule ceftiofur have comparable spectrums of activity against the primary bacterial pathogens of interest in horses, with the exception of staphylococci species, for which ceftiofur displays increased activity compared to desfuroylceftiofur [30].

Molecular modeling was performed to better predict metabolism of ceftiofur *in vivo*. The surface area and volume of DCA is significantly different from ceftiofur and the other metabolites, allowing exclusive access to particular enzymes that are responsible for a large part of the observed activity of ceftiofur as a drug. These chemical models suggested that DCA and DCA-dimers have electron-deficient areas on their structures that make each of them capable of reacting with glutathione and nucleobases in DNA [44]. Such molecular characteristics suggest the potential for ceftiofur and its metabolites to induce potentially harmful oxidative stress and DNA-damage in the host [44]. In general, antimicrobial activity is lost once the beta lactam ring is metabolized. Based on molecular modeling, the majority of ceftiofur is expected to be excreted as a DCA-dimer with the beta lactam rings intact [44].

Elimination

Little information is available on the elimination of cephalosporins in animals; much of the data in the literature is based on extrapolation from human data. Since ceftiofur is a veterinary-exclusive cephalosporin there is a paucity of data available. The mechanisms of elimination of CCFA in horses have not been documented. However, when a similar depot compound (ceftiofur hydrochloride) was administered to pigs, greater than 83% of the dose was recovered (> 68% in urine; > 13% in feces) [43]. Recovery of ceftiofur metabolites in cattle and rats after a parenteral

dose of ceftiofur sodium was similar to that in pigs (60% in urine; 30% in bile/feces) [42]. The stability of excreted residues is controversial; they may retain antimicrobial activity that could influence the susceptibility pattern of environmental bacteria [45], while others suggest such metabolites are inactive likely due to mechanisms of degradation present in feces and urine [17].

The kidney is the primary excretory organ for ceftiofur metabolites. Cephalosporin antibiotics, such as cefazolin and cefalotin have been shown to inhibit the activity of cytochrome *c* oxidase in renal mitochondria and may cause nephrotoxicity [46]. Although the exact mechanism was not identified, hepatorenal changes were documented when calves were given high doses of ceftiofur sodium (10 mg/kg IM) for 3 consecutive days, as evidenced by significant elevations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine at the 3rd day and at the 1st, 2nd week post-injection [47]. The clinical significance of these changes is unknown but these findings suggest considerable activity of ceftiofur and its metabolites in the primary organs of excretion. Overall, the pharmacokinetics of the depot formulation of ceftiofur (CCFA) and ceftiofur sodium vary between each other and amongst themselves when given via different routes or to different age groups. These differences may ultimately influence the pharmacodynamics and clinical outcome of individual cases.

SECTION III: CEFTIOFUR: SAFETY, EFFICACY, SUSCEPTIBILITY AND RESISTANCE

Safety

The safety of ceftiofur sodium was demonstrated in healthy horses after intramuscular injection at up to five times the labeled dose (11 mg/kg) for up to three times the recommended duration of treatment (30 days). The only abnormalities noted were slight increases in the numbers of circulating neutrophils, increases in plasma concentration of fibrinogen, and increases in muscle enzymes aspartate transaminase and creatine kinase; the source of the leukogram changes

(injection site inflammation versus colonic inflammation secondary to a change in microbial flora) was not determined [48].

A review of adult horses with a presumptive diagnosis of antimicrobial-induced colitis failed to find an association between administration of ceftiofur and development of diarrhea/colitis [49]. In a study of efficacy of ceftiofur sodium, none of the 28 horses administered the drug for up to 12 days developed diarrhea or injection site pain or swelling [50].

Based on the $AUC_{0\rightarrow\infty,}$ the exposure to DCA from two doses of CCFA four days apart is markedly less than exposure to DCA from 10 daily doses of intramuscular ceftiofur sodium. These data suggest that target MIC's may be reached using the depot formulation with the benefit of decreased circulating concentrations of antibiotic which could decrease the risk of adverse reactions such as antimicrobial-induced colitis [27]. When 2 doses of CCFA were administered to 278 horses with clinical signs of respiratory disease, only 25 (9%) developed transiently soft manure, with no horses developing clinical colitis. The other most common adverse event was local inflammation at the injection site (4% of CCFA treated horses) [34].

Efficacy

The efficacy of ceftiofur sodium as therapy for lower respiratory tract infection in horses was demonstrated using a daily dose of 2.2 mg/kg for a maximum of 12 days and compared to an FDA-approved positive control (ampicillin IM). Clinical improvement (92.9%) and complete recovery (78.6%) was superior to the positive control, suggesting that ceftiofur is an effective therapy for respiratory tract infections [50]. In a separate study, 28 of 30 horses (93%) treated with ceftiofur sodium showed clinical improvement and 2 (7%) were classified as failures. In contrast, only 6 of 30 controls (20%) improved and 24 (80%) were classified as failures. Clinical improvement was defined as near disappearance of all clinical signs or full recovery on the last day of treatment [19].

The efficacy of the two-dose regimen of the depot formulation of ceftiofur (CCFA) was demonstrated in cases of confirmed, naturally-occurring *S. zooepidemicus* pneumonia as 67% of the 136 treated horses achieved clinical cure compared to only 32% of the 57 non-treated control horses [34]. The efficacy of local administration of ceftiofur sodium for the treatment of septic arthritis was studied using a controlled in-vivo subcutaneous tissue cage model infected with *Staphylococcus aureus*. Although intra-articular therapy with 150 mg of ceftiofur sodium decreased the amount of viable bacteria, the bacteria were not eliminated [37]. A similar study has not been performed using CCFA.

Susceptibility and Resistance

Based on breakpoints established by the veterinary subcommittee of the Clinical and Laboratory Standard Institute (CLSI), isolates of *S. zooepidemicus* from the respiratory tract of horses are considered susceptible to ceftiofur when their MIC is <0.25 μg/mL. There are no CLSI breakpoints for *S. zooepidemicus* cultured from other sites or for other bacterial pathogens of horses.

In vitro susceptibility testing is performed with ceftiofur whereas the drug present *in vivo* after systemic administration of ceftiofur is mainly desfuroylceftiofur. For the majority of equine isolates, desfuroylceftiofur has comparable activity to its parent molecule, ceftiofur. In a study of 539 veterinary isolates, ceftiofur and desfuroylceftiofur demonstrated similar *in vitro* activity against the common Gram negative isolates, including *Actinobacillus* spp., *Pasteurella* spp., *Salmonella* spp., and *E. coli.* However, a marked difference exists in the MIC_{90} of ceftiofur compared to the main metabolite desfuroylceftiofur for staphylococci, with the MIC_{90} for staphylococci being approximately 4-fold higher for desfuroylceftiofur (4.0-8.0 μg/mL) compared to ceftiofur [30]. This difference is clinically important, making the systemic route suboptimal for the treatment of infections caused by staphylococci with ceftiofur. This suggests that, if a route of administration

could bypass the metabolism of ceftiofur into desfuroylceftiofur (such as could occur via aerosol administration) the concentrations of ceftiofur could possibly reach the target MIC_{90} for staphylococci. Once derivitized to desfuroylceftiofur acetamide (DCA) via the addition of acetamide, the sum of ceftiofur, desfuroylceftiofur is determined in total, witht the relative contribution of each biolgically active metabolite impossible to determine [21].

S. zooepidemicus isolates have demonstrated no change in antimicrobial susceptibility in North America (representing 21 states and 3 Canadian locations) since 1989 [32], in Europe since 2002 [31], nor in western Canada over a 5 year submission period [6]. The MIC_{90} for *S. zooepidemicus* isolated from lower respiratory tract isolates remained 0.12 μg/mL in isolates grouped into time periods ranging from 1989 to 2007, resulting in 97.3% of isolates classified as susceptible to ceftiofur [32]. A similarly high level of susceptibility to ceftiofur (99%) was found amongst *S. zooepidemicus* samples obtained from 1998-2003 in western Canada [6].

A variety of other equine veterinary pathogens demonstrate varying levels of susceptibility to ceftiofur and its metabolites. *Pseudomonas aeruginosa* is an opportunistic pathogen that can colonize equine lungs during bronchopneumonia and is the primary target of nebulized anti-microbial therapy in humans with cystic fibrosis [51]. *In vitro* testing of ceftiofur against *Pseudomonas aeruginosa* culture from horses revealed that all 14 of the equine isolates were classified as resistant to ceftiofur using a disk diffusion technique [52].

Anaerobic bacteria can play an important role in equine bronchopneumonia, particularly if treatment is delayed beyond 24 hours of recognition of clinical signs [9, 11]. Approximately one third of severe cases of bronchopneumonia in adult horses will have an anaerobic isolate [1]. Specifically, *Bacteriodes* spp., *Fusobacterium necrophorum* and *Peptostreptococcus anaerobius* account for most equine anaerobic isolates. While 100% of equine isolates of *F. necrophorum* and *P. anaerobius* were susceptible to ceftiofur, only 38% of *B. fragilis* species were susceptible, with an $MIC_{90} \geq 16$ μg/mL [10].

A variety of pathogens are important in foal sepsis and foal pneumonia. Based on MIC data and known plasma concentrations it was suggested that ceftiofur sodium administered IV at a dose of 5 mg/kg q 12 h to neonatal foals would result in plasma concentrations sufficient to target approximately 77% of all blood culture isolates and 87% of Gram negative isolates from ill neonatal foals [37]. Specifically, at least 90% of *E. coli*, *Klebsiella* spp., *Pasteurella* spp., and *β hemolytic* streptococci isolates were susceptible, while only 88% and 73% of *Enterobacter* spp. and *Salmonella* spp. were susceptible, respectively [36].

Mechanisms for both intrinsic and acquired β- lactam resistance to ceftiofur exist, including: inaccessibility of the drugs to their target, target alterations, and/or inactivation of the drugs by beta-lactamases. Particularly important are plasmid-encoded AmpC-type CMY and CTX-M beta-lactamases via transposons/integrons [53]. In horses, the influence that ceftiofur metabolites present in the environment have on microflora has not been determined, but exposure to urine from cephalosporin-treated cattle has been shown to drive cephalosporin resistance in soil-borne *E. coli* [45].

Overall, strong evidence for the administration of ceftiofur influencing resistance patterns in equine-origin bacterial isolates is absent. However, there was an increased risk of the finding nosocomial methicillin-resistant *Staphylococcus aureus* in equine nasal swabs after administration of ceftiofur during hospitalization in one survey study [53]. Since the relationship between the administration of ceftiofur to horses and the development of bacterial resistance is controversial, rational administration of ceftiofur should be guided by culture and susceptibility data when possible.

SECTION IV: AEROSOL THERAPY WITH CEFTIOFUR SODIUM

The justification for use of aerosolized antimicrobials in humans suffering from respiratory infections include an enormous absorptive surface area across a permeable membrane,

reproducible absorption kinetics, low enzyme environment, little drug degradation, avoidance of hepatic first-pass metabolism, and direct, higher pulmonary drug deposition [54]. In particular, reducing systemic exposure to antimicrobials has the potential benefit of reducing disruption of flora within the equine gastrointestinal tract [55].

The potential negative aspects to administration of antimicrobials via inhalation include the lack of data on the effect of disease on pulmonary dynamics, lack of 'preservative free' formulations of veterinary-specific antimicrobials such as ceftiofur, unknown local adverse effects, and the lack of guidelines for administration of most drugs [54].

The 2010 consensus on use of aerosolized antimicrobial agents in humans reveals that many of human diseases that necessitate nebulization (including cystic fibrosis, ventilator-acquired pneumonia, *Pneumocystis jiroveci* pneumonia, and bronchiectasis) are not as applicable to veterinary medicine, making extrapolation of research performed in people difficult [54]. Two of the primary human indications for the aerosolization of cephalosporins, include bronchiectasis and hospital-acquired pneumonia.

Regional mismatching of blood flow in the lungs of horses with heaves was documented using nuclear scintigraphy [56], suggesting that variations in blood flow exist with pulmonary disease. Therefore, nebulization has been investigated as a method of bypassing systemic circulation and delivering antimicrobials directly to the pulmonary parenchyma [57].

In horses, investigations utilizing aerosolized antimicrobials have been limited but suggest they may have a role in the treatment of bacterial pneumonia. The aerosol administration of gentamicin reached drug concentrations in PELF approximately twelve times greater than what was achieved after a similar intravenous dose [58] while no accumulation or respiratory inflammation was noted following 7 consecutive days of aerosol therapy with gentamicin to adult horses [59].

No studies have previously investigated the safety or pharmacokinetics of ceftiofur sodium administered via nebulization in horses. Furthermore, the concentration of ceftiofur and its metabolites in PELF has not been determined after systemic or aerosol administration of ceftiofur sodium in horses. However, DCA concentrations in PELF have been published in weanling foals after administration of CCFA [24] and DCA concentrations in lung tissue homogenate have been reported in adults after intramuscular administration of ceftiofur sodium [21].

In one study in horses, the pharmacokinetics and effect on pulmonary function and cytological characteristics of PELF were examined for cefquinome, a fourth generation cephalosporin approved for use in several animal species in Europe. At a dose of 225 mg per inhalation (equal to approximately 40-50% the systemic dose), the mean cefquinome concentration present within the PELF was 0.44 ± 0.30 μg/ml after 5 daily administrations. Although this mean concentration is above the MIC for many common respiratory equine pathogens, the time above MIC was not reported. In addition, the concentrations were reported without correction for dilution by PELF and a high level of inter-individual variation was noted. No effect on pulmonary function or cytologic characteristics of BAL cells was noted [60].

The efficacy of nebulization of ceftiofur sodium was compared to the same dose administered intramuscularly (1.0 mg/kg) after experimental infection of calves with *Mannheimia haemolytica*. After a single administration of aerosolized ceftiofur, drug activity in bronchial secretions was above the MIC of *Mannheimia haemolytica* (0.078 μg/mL) for 18-24 hours with negligible systemic absorption. The calves that received nebulized ceftiofur sodium had significantly lower rectal temperatures and mortality rates compared to calves treated by the intramuscular route. *Mannheimia haemolytica* was not isolated from the BAL of any of the calves receiving aerosolized ceftiofur at any time point [61].

Overall , bronchopneumonia is an important disease in both adult horses and weanlings. The in-vitro and in-vivo efficacy of both FDA- approved formulations in horses (ceftiofur sodium

and CCFA) justify the label indication for treatment of bacterial lower respiratory tract infections with *S. zooepidemicus*. There are knowledge gaps for certain methods of administration and dosing protocols for each formulation, thereby warranting pharmacokinetic studies to provide data to potentially expand the therapeutic role of ceftiofur in horses.

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CHAPTER 3

PLASMA AND PULMONARY PHARMACOKINETICS OF DESFUROLCEFTIOFUR ACETAMIDE AFTER WEEKLY ADMINISTRATION OF CEFTIOFUR CRYSTALLINE FREE ACID TO ADULT HORSES [1]

[1] Fultz L, Giguère S, Berghaus L, Davis J. 2014. *Equine Veterinary Journal*. 46(2):252-255. Reprinted here with permission of the publisher.

ABSTRACT

REASONS FOR PERFORMING STUDY: Current labeling for the use of ceftiofur crystalline free acid (CCFA) in horses states that 2 intramuscular (IM) doses must be administered 4 days apart to provide 10 days of therapeutic coverage. A 10-day treatment regimen is not sufficient for the long-term treatment of horses with severe lung consolidation or pleuropneumonia caused by susceptible bacteria. There is currently no data to guide an appropriate dosing interval when a longer treatment regimen is warranted.

OBJECTIVES: To determine steady-state plasma and pulmonary epithelial lining fluid (PELF) concentrations of desfuroylceftiofur acetamide (DCA) after weekly IM administration of CCFA to adult horses.

STUDY DESIGN: Prospective study.

METHODS: Seven adult horses received IM CCFA at a dose of 6.6 mg/kg on day 0, day 4, and every 7 days thereafter for 3 additional doses. Concentrations of DCA in plasma and PELF were measured at various time intervals.

RESULTS: After weekly IM administration, mean (± SD) steady state peak DCA concentration in plasma (2.87 ± 1.50 μg/mL) was significantly higher than that in PELF (0.84 ± 0.53 μg/mL). Mean terminal half-lives in plasma (77.5 ± 17.5 h) and PELF (92.8 ± 59.0 h) were not significantly different. Concentrations of DCA in plasma and PELF remained in the therapeutic range for the entire dosing interval.

CONCLUSIONS: After the initial 2-dose regimen 4 days apart, weekly IM administration of CCFA was well tolerated and resulted in plasma and PELF DCA concentrations above the MIC_{90} of common lower respiratory tract pathogens of horses.

POTENTIAL RELEVANCE: Weekly administration of CCFA would appear appropriate when a treatment regimen longer than 10 days is warranted based on clinical signs and disease severity. Additional studies will be necessary to assess the efficacy and safety of this dosage regimen in a clinical setting.

INTRODUCTION

Ceftiofur crystalline free acid (CCFA) is approved by the United States Food and Drug Administration Center for Veterinary Medicine for treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*). Ceftiofur, administered as either ceftiofur sodium or CCFA, is rapidly metabolized to desfuroylceftiofur [1]. The *in vitro* activity of desfuroylceftiofur against common Gram-negative pathogens and streptococci is almost identical to that of ceftiofur [2]. Current labeling for the use of CCFA in horses states that 2 intramuscular (IM) doses must be administered 4 days apart. This regimen is designed to provide concentrations of ceftiofur and desfuroylceftiofur metabolites in plasma above the minimum inhibitory concentration that inhibits growth of at least 90% (MIC_{90}) of *S. zooepidemicus* isolates for approximately 10 days [1, 3].

A 10-day treatment regimen is appropriate for the treatment of most mild to moderate respiratory infections. However, it is not sufficient for the long-term treatment of horses with severe lung consolidation or for horses with pleuropneumonia caused by susceptible bacteria. Horses with severe pulmonary disease may require weeks and in some cases months of antimicrobial therapy for complete resolution of lung lesions [4, 5]. There is currently no published pharmacokinetic data to guide an appropriate dosing interval for long-term administration of CCFA.

While drug concentration in plasma is clearly a driving force for penetration to the site of infection, the actual drug-concentration versus time profile at a peripheral site of infection may be quite different from that of plasma. For pulmonary infections caused by extracellular bacteria such as *S. zooepidemicus*, concentrations of antimicrobials in the extracellular or interstitial space within the lungs would provide additional relevant information. Measurement of drug concentration in pulmonary epithelial lining fluid (PELF) collected by bronchoalveolar lavage is a widely used method to estimate antimicrobial concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections caused by extracellular pathogens [6, 7].

The primary objective of the present study was to determine steady-state plasma and PELF concentrations of desfuroylceftiofur acetamide (DCA) after repeated weekly IM administrations of CCFA to adult horses. A secondary objective was to document the frequency, size, and duration of injection site reactions after repeated administration of CCFA. We hypothesized that weekly administration of CCFA to adult horses would be well tolerated and result in plasma and PELF concentrations above the MIC_{90} for *S. zooepidemicus* (0.12 μg/mL) for the entire dosing interval.

MATERIALS AND METHODS

Animals

Seven adult non-pregnant mares (7-17 years of age) ranging in weight from 471 to 675 kg were used in the study. The animals were considered healthy on the basis of history, physical examination, complete blood count, and plasma biochemical profile. The animals were housed in individual stalls for the first 24 h after administration of the drug and in a group paddock for the rest of the study. The study was approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Study design and sample collection

Ceftiofur crystalline free acid, in its market formulation[a] was administered to each horse at a total dose of 6.6 mg/kg of body weight (total volume ranging between 15.5-22.0 mL) on days 0 (dose 1), 4 (dose 2), 11 (dose 3), 18 (dose 4), and 25 (dose 5) for a total of 5 doses. For each injection, half of the calculated volume was administered IM on 1 side of the neck and the other half on the other side of the neck. All injections were performed using an 18 gauge 1.5 inch needle.

Blood samples were obtained by jugular venipuncture and collected in 10-mL heparinized tubes[b] 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 96 h after administration of the first dose. Blood was also collected 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 96, and 168 h after administration of doses 2 and 5, and just

before injection of doses 3 and 4. Blood samples were centrifuged at 500 × g for 10 min, and plasma was stored at −80 °C until assayed.

Bronchoalveolar (BAL) lavage was performed 24 and 96 h after administration of dose 1; 24, 96, and 168 h after administration of dose 2; and 4, 24, 96, and 168 h after administration of dose 5. Horses were sedated by intravenous administration of xylazine hydrochloride (0.5 mg/kg)[c] and butorphanol tartrate (0.04 mg/kg)[d] prior to BAL fluid collection. A 10-mm diameter, 2.4-m BAL catheter[e] was passed via nasal approach until wedged into a bronchus. The lavage solution consisted of four aliquots of 60 mL physiologic saline (0.9% NaCl) solution infused and aspirated immediately. Volume of BAL fluid was measured using a graduated cylinder. The fluid was centrifuged at 200 × g for 10 minutes and supernatant fluid was frozen at -80ºC until assayed.

Measurement and analyses of injection site reactions

The sites of injection were monitored daily for visible swelling. When injection site swelling was detected, the horizontal and vertical diameters of each site of swelling were measured using Vernier calipers. For each side of the neck, the surface area of each visible swelling was estimated by multiplying the horizontal diameter by the vertical diameter. On a given day, the total surface area was obtained by adding the surface areas from swellings on each side of the neck. The number of days a given area of swelling at an injection site was detectable was also recorded.

Analysis of desfuroylceftiofur acetamide (DCA) concentrations in plasma and pulmonary epithelial lining fluid (PELF)

Ceftiofur and desfuroylceftiofur metabolites in plasma and BAL fluid samples and standards were derivitized to desfuroylceftiofur acetamide (DCA) and then subjected to solid phase extraction using a previously described and validated method [8, 9]. Drug concentrations in plasma were determined using HPLC with ultraviolet detection at 266 nm[f] as previously described [9]. Standards

were prepared by spiking blank equine plasma with ceftiofur sodium reference standard[g] dissolved in methanol and diluted in water. Standard curves for DCA ranged from 0.125 to 16 µg /mL and were linear on each day of analysis (R^2 > 0.99). Limits of detection and quantification of DCA in plasma were 0.0625 µg /mL and 0.125 µg /mL, respectively.

Measurement of DCA concentrations in BAL fluid was performed using a sample volume of 5 mL with a validated ultra-high pressure liquid chromatography tandem mass spectrometry[h] detection assay as described previously [10]. The mobile phase was modified slightly to run a 1.5-min gradient separation from 5% to 45% organic mobile phase consisting of 95 / 5 acetonitrile (ACN) / 0.3% formic acid in water. The aqueous mobile phase consisted of 95 / 5 0.3% formic acid in water / ACN. The column was flushed with 95% organic mobile phase for 2.5 min and allowed to equilibrate for 1 min prior to the next sample injection. The retention time of DCA under these conditions was approximately 0.83 min. Standards were prepared by spiking saline with ceftiofur sodium reference standard dissolved in methanol and diluted in water. Ceftiofur-d3, dissolved in methanol and 0.1M ammonium acetate (50:50 v/v), was used as the internal standard at a concentration of 0.2 µg/mL. Standard curves for DCA in BAL were linear on each day of analysis (R^2 > 0.99). The limit of detection and quantification of DCA in BAL fluid was 0.000125 µg /mL.

Calculation of DCA concentrations in Pulmonary Epithelial Lining Fluid

Estimation of the volume of PELF was determined by urea dilution method [11]. Urea nitrogen concentrations in plasma ($Urea_{PLASMA}$) and in BAL fluid ($Urea_{BAL}$) were determined in 96-well plates by use of a commercial kit.[i] Plasma samples (5 µL) were assayed in duplicate and compared to a standard (50 mg/dL). BAL fluid was assayed in duplicate using a sample volume of 50 µL and compared against a standard (5 mg/dL). For both plasma and BAL fluid, absorbance was read at 520 nm by use of a spectrophotometer.[j] The volume of PELF (V_{PELF}) in BAL fluid was derived from the following equation: $V_{PELF} = V_{BAL} \times (Urea_{BAL}/Urea_{PLASMA})$, where V_{BAL} is the volume of

recovered BAL fluid. The concentration of DCA in PELF (DCA_{PELF}) was derived from the following relationship: $DCA_{PELF} = DCA_{BAL} \times (V_{BAL}/V_{PELF})$, where DCA_{BAL} is the measured concentration of DCA in BAL fluid.

Pharmacokinetic analysis

For each horse, plasma and PELF DCA concentration versus time data was analyzed based on noncompartmental pharmacokinetics using computer software.[k] The rate constant of the terminal phase (λ_z) was determined by linear regression of the terminal phase of the logarithmic plasma concentration versus time curve using a minimum of 3 data points. Half-life of the terminal phase ($t_{½\lambda z}$) was calculated as ln 2 divided by λ_z. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using C_{min}/λ_z, where C_{min} was the final measurable DCA concentration. Mean residence time (MRT) was calculated as: AUMC/AUC, where AUMC is the area under the first moment of the concentration–time curve.

Statistical analysis

Normality of the data and equality of variances were assessed using Shapiro-Wilk's and Levene's tests, respectively. Variables that did not meet the assumptions for parametric testing were log- or rank-transformed prior to analysis. A one-way repeated measures ANOVA was used to assess the potential effect of dose (dose 1, dose 2, and dose 5) on each measured and calculated plasma pharmacokinetic variable. A 2-way repeated measure ANOVA was use to assess the potential effects of dose (dose 2 versus dose 5) and type of sample (plasma versus PELF) and the interactions between dose and type of sample on each measured and calculated pharmacokinetic variable. When applicable, multiple pairwise comparisons were performed using the method of Holm–Sidak. Differences were considered significant at $P < 0.05$.

RESULTS

After IM administration of the first dose of CCFA, DCA had a $t_{1/2\lambda z}$ in plasma of 56.3 ± 13.4 h, a C_{max} of 2.28 ± 1.37 μg/mL, and an $AUC_{0-\infty}$ of 134.5 ± 22.78 μg · h/mL. The AUC for the first 7 days after administration of dose 5 (AUC_{7days}) was significantly (P = 0.03) greater than the $AUC_{0-\infty}$ after dose 1 and the AUC_{7days} of dose 2 and dose 5 were not significantly different (P = 0.711), indicating that steady state DCA concentration were reached. Plasma $t_{1/2\lambda z}$ and MRT were significantly longer after administration of dose 5 than after administration of dose 1 (Table 3.1). In contrast, C_{max} and T_{max} were not significantly different between doses (Table 3.1). Concentrations of DCA in plasma were ≥ 0.22 μg/mL at all times sampled (Figure 3.1).

Concentrations of DCA in PELF were considerably lower than in plasma (Figure 3.1). Concentrations of DCA in BAL fluid 7 days after administration of dose 5 (day 32) were below the limit of quantification of the assay in 3 of 7 horses. Therefore, mean (± SD) DCA concentrations in PELF on day 32 were calculated from 4 horses. C_{max} and AUC_{7days} were significantly lower in PELF than in plasma (Table 3.1). In contrast, $t_{1/2\lambda z}$, MRT, and T_{max} were not significantly different between plasma and PELF (Table 3.1).

Injection site reactions, characterized by mild soft non-painful edema, were observed in 5 of 35 injections. Three of the 5 injection site swellings (surface areas of 11.8 to 19.6 cm^2) occurred in the same horse after doses 1, 2, and 4. The largest of the injection site swellings (surface area of 49.4 cm^2) occurred in a horse that had a single reaction after dose 2. The largest injection site swelling reached its maximal surface area 48 hours after injection and was visible for 48 hours. The last injection site swelling (surface area of 22.8 cm^2) occurred after dose 3 in a single horse. No horses had a visible injection site reaction to dose 5. All 5 injection site swellings detected were only present on 1 side of the neck. No other adverse reactions were noted during or after the study period.

DISCUSSION

A safe antimicrobial agent providing high and sustained drug concentrations in plasma and PELF would be a useful addition to currently available antimicrobial agents for the long term treatment of severe bronchopneumonia or pleuropneumonia in horses. The present study demonstrates that, after the recommended initial 2-dose regimen, weekly administration of CCFA is sufficient to maintain therapeutic concentrations of DCA in both plasma and PELF for bacteria with a MIC < 0.12 μg/mL.

The plasma and PELF concentration versus time profile of DCA after IM administration of CCFA in the present study illustrates a long-acting antimicrobial formulation absorbed slowly from extravascular injection sites. The slow absorption rate controls the apparent drug elimination rate and, as a result, the elimination $t_{1/2}$ in plasma (53.6 ± 13.4 to 77.5 ± 17.5 h) in the present study is considerably longer than the true elimination half-life of DCA observed after IM administration of ceftiofur sodium to adult horses (3.2 h) [1]. The pharmacokinetic variables obtained after IM administration of dose 1 and dose 2 of CCFA in the present study are similar to those reported in adult horses in previous studies [12-14].

The optimal dosing of an antimicrobial agent is determined by both the pharmacokinetics and pharmacodynamics of the drug. Currently, most pharmacokinetic/pharmacodynamic models rely on plasma concentrations and MIC. The most important factor determining the efficacy of β-lactam antimicrobials such as ceftiofur is the duration of time that plasma concentrations of the drug exceed the MIC of a given pathogen [15, 16] In the present study, plasma concentrations of DCA remained above 0.22 μg/mL for the entire study. This concentration is above the MIC_{90} of *S. zooepidemicus* (0.12 μg/mL), *Pasteurella* spp. (< 0.03 μg/mL), and *Actinobacillus* spp. (< 0.03 μg/mL) [1, 3].

Because most infections occur in tissues rather than in plasma, the ability of antimicrobial agents to reach the target site is a key determinant of clinical outcome [16]. Only the free

(unbound) fraction of the drug in interstitial fluids at the target site is responsible for therapeutic success. Protein free desfuroylceftiofur accounts for only about 10 % of the total desfuroylceftiofur metabolites found in plasma obtained from adult cattle. However, protein binding of desfuroylceftiofur is reversible and protein bound desfuroylceftiofur acts as a reservoir for release of active drug at the site of infection [17]. While drug concentration in plasma is clearly a driving force for penetration to the site of infection, the actual drug-concentration versus time profile in tissues may be quite different from that of in plasma. The rate and extent of drug penetration into most sites outside the vascular space are also determined by the drug's molecular charge and size, lipid solubility, extent of plasma protein binding, and by blood flow at the site of infection [18]. In some tissues such as the bronchial epithelium, antimicrobial drug diffusion to the site of infection (bronchial secretions and PELF) is further restricted by tight junctions between cells [19]. Peak DCA concentrations in PELF achieved in the present study were similar to those achieved after administration of a single dose to weanling foals [10]. Weekly IM administration of CCFA in the present study resulted in DCA concentrations in PELF above or very close to the MIC_{90} of *S. zooepidemicus* for approximately 7 days post-injection. Concentrations of DCA in PELF on day 32 could not be calculated for 3 horses. Failure to quantify DCA in BAL fluid could have been due to low drug concentrations in PELF, to a particularly dilute BAL sample that contained less respiratory secretions, or to a combination of both. Concentrations of urea in BAL fluid were unusually low in these 3 samples (data not shown), suggesting that poor sample quality (i.e. particularly dilute sample) contributed, at least in part, to the failure to quantify DCA.

In a recent study, self-limiting injection site swelling was reported in 10 of 278 (3.6%) horses treated with the labeled 2-dose regimen of CCFA [20]. However, it was unknown if horses that develop injection site swellings are more likely to have a similar or more severe reactions after repeated administrations. Only 3 horses developed mild injection site swellings in the present study. One horse had multiple injection site reactions after doses 1, 2 and 4, but not after dose 5.

The other 2 horses developed a mild swelling after only 1 of the 5 doses (dose 2 or 3). Interestingly, the swelling was only present on one side of the neck at a given time despite administration of the same volume on both sides. The lack of worsening in the severity of injection site swelling with repeated administration and the fact that most injection site swellings were unilateral go against the theory of an allergic or acquired hypersensitivity reaction to the drug or its vehicle. Colitis as a result of alteration of the gastrointestinal microflora is a feared complication of antimicrobial therapy in horses. In one study, the rate of transient diarrhea in CCFA-treated horses (25 of 278 or 9%) was not significantly different (P = 0.832) from that of placebo-treated horses (7 of 95 or 7%), indicating that diarrhea is an unlikely complication of administration CCFA at the labeled dosage [20]. Diarrhea was not observed in the horses enrolled in the present study but additional studies involving a larger population of horses would be required to determine if repeated weekly administration of CCFA is more likely to cause diarrhea than the labeled dosage regimen. The potential negative effects of antimicrobials on the equine digestive tract might be potentiated by long-acting depot formulations of antibiotics due to the inability to cease the antimicrobial effects if an adverse response occurs.

In conclusion, after the initial 2-dose regimen 4 days apart, weekly IM administration of CCFA was well tolerated and resulted in plasma and PELF DCA concentrations above the MIC_{90} of common lower respiratory tract pathogens of horses. Weekly administration of CCFA would appear appropriate when severe bronchopneumonia caused by susceptible microorganisms warrant therapy for longer than 10 days.

FOOTNOTES

[a]Excede® Sterile Suspension, Zoetis, Madison, NJ, USA

[b]Tyco Healthcare Group, Mansfield, MA, USA

[c]AnaSed®, Akorn, Decatur, IL, USA

[d]Torbugesic®, Zoetis, Kalamazoo, MI, USA

[e]Jorgenson Laboratories, Loveland, CO, USA

[f]Alliance HPLC; Waters Corporation, Milford, MA, USA

[g]United States Pharmacopeia; Rockville, MD, USA

[h]Xevo TQD, Waters Corp., Milford, MA, USA

[i]Biochain Urea Assay Kit, Hayward, CA, USA

[j]Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc., Winooski, VT, USA

[k]PK Solutions 2.0, Summit Research Services, Montrose, CO , USA

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Table 3.1. Pharmacokinetic variables (mean ± SD unless otherwise specified*) for DCA in plasma and PELF of 7 adult horses after IM administration of ceftiofur crystalline free acid (6.6 mg/kg of body weight) on days 0 (dose 1), 4 (dose 2), 11 (dose 3), 18 (dose 4) and 25 (dose 5).

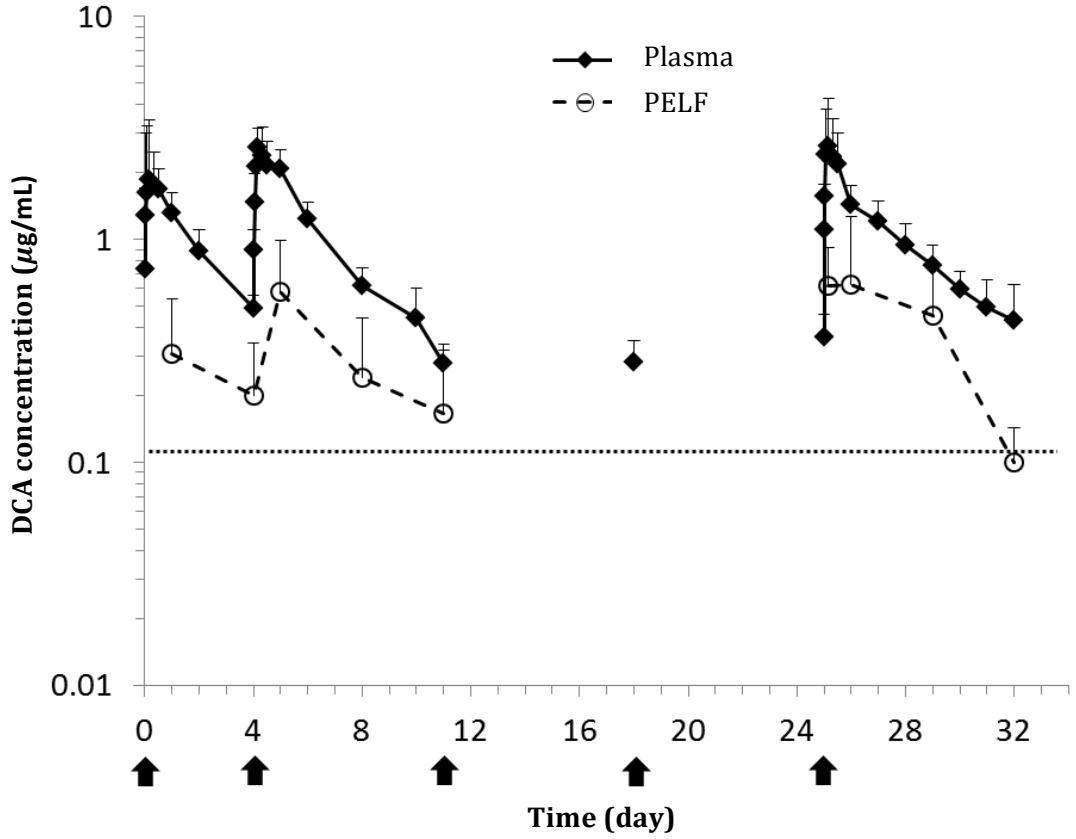
Variable	Dose	Plasma	PELF
λ_z (1/h)	Dose 1	0.013 ± 0.002[1]	N/A
	Dose 2	0.011 ± 0.0015 [1,2,a]	0.012 ± 0.008 [1,a]
	Dose 5	0.009 ± 0.002[2,a]	0.020 ± 0.027 [1,a]
$t_{1/2\lambda z}$ (h)	Dose 1	53.6 ± 13.4[1]	N/A
	Dose 2	63.2 ± 9.2[1, 2, a]	77.9 ± 45.7[1, a]
	Dose 5	77.5 ± 17.5[2, a]	92.8 ± 59.0 [1, a]
C_{max} (µg/mL)	Dose 1	2.28 ± 1.37[1]	N/A
	Dose 2	2.76 ± 0.59[1,a]	0.53 ± 0.40[1,b]
	Dose 5	2.87± 1.50[1,a]	0.84 ± 0.53[1,b]
$C_{7\ days}$ (µg/mL)	Dose 1	NA	N/A
	Dose 2	0.28 ± 0.06[1,a]	0.17 ± 0.15[1,b]
	Dose 5	0.43 ± 0.19[1,a]	0.10 ± 0.04[1,b]
T_{max} (h)*	Dose 1	8 (4-12) [1]	N/A
	Dose 2	4 (4-24) [1,a]	N/A
	Dose 5	8 (2-12) [1,a]	4 (4-96) [1,a]
MRT (h)	Dose 1	76.4 ± 20.5[1]	N/A
	Dose 2	80.54 ± 9.49[1, 2,a]	151.6 ± 102.2[1, a]
	Dose 5	111.1 ± 38.3 [2, a]	120.8 ± 91.9[1, a]
$AUC_{7\ days}$ (µg · h/mL)	Dose 1	NA	N/A
	Dose 2	169.66 ± 28.0[1, a]	49.0 ± 38.2[1, b]
	Dose 5	166.77 ± 27.5[1, a]	63.1 ± 36.4[1, b]

*Median and range. N/A =Not applicable

λ_z = rate constant of the terminal phase; $t_{1/2\lambda z}$ = half-life of the terminal phase; C_{max} = Maximum concentration of DCA; $C_{7\ days}$ = Concentration of DCA 7 days after administration; T_{max} = Time to maximum concentrations; MRT = Mean residence time; $AUC_{7\ days}$ = Area under the concentration versus time curve for the first 7days after administration.

[a,b,c] Different superscript letters within a given row indicate significant differences ($P < 0.05$) between plasma and PELF for a given dose. [1,2] Different superscript numbers within a given column indicate significant differences ($P < 0.05$) between doses (dose 1, dose 2, and dose 3) for a given sample. When at least one letter or number is common the difference is not statistically significant.

Figure 3.1. Mean (± SD) DCA concentrations in plasma and pulmonary epithelial lining fluid of 7 adult horses after IM administration of ceftiofur crystalline free acid (6.6 mg/kg of body weight) on days 0, 4, 11, 18 and 25 (solid arrows). The dotted horizontal line represents the MIC_{90} of *S. zooepidemicus* (0.12 µg/mL).



CHAPTER 4

COMPARATIVE PHARMACOKINETICS OF DESFUROLCEFTIOFUR ACETAMIDE AFTER INTRAMUSCULAR VERSUS SUBCUTANEOUS ADMINISTRATION OF CEFTIOFUR CRYSTALLINE FREE ACID TO ADULT HORSES [2]

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[1] Fultz L, Giguère S, Berghaus L, Davis J. 2013. *Journal of Veterinary Pharmacology and Therapeutics.* 36(3):309-312.

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ABSTRACT

REASONS FOR PERFORMING STUDY: Current labeling for the use of ceftiofur crystalline free acid (CCFA) in horses states that 2 intramuscular (IM) doses must be administered 4 days apart to provide 10 days of therapeutic coverage. Subcutaneous administration might minimize injection site irritation and facilitate administration. However there is currently no pharmacokinetic or safety data to support this route of administration in adult horses.

OBJECTIVES: To compare the pharmacokinetic profile of CCFA administered by the SC route to that achieved after administration by the IM route. In addition we sought to compare the size of visible injection site swellings after administration by the 2 routes of administration..

STUDY DESIGN: Prospective study.

METHODS: Six adult horses received SC CCFA at a dose of 6.6 mg/kg on day 0, and day 4, while another six horses received IM CCFA at the same dose and interval. Concentrations of DCA in plasma were measured using HPLC tandem mass spectrometry at various time intervals and compared.

RESULTS: Plasma concentrations of DCA 4 days after administration of CCFA were significantly higher after SC than after IM administration while AUC for the first 24 h after administration of each dose was significantly higher after IM than after SC administration. All other pharmacokinetic parameters were not significantly different between routes of administration. Concentrations of DCA in plasma remained in the therapeutic range for the entire dosing interval. The injection site reactions were significantly larger following subcutaneous administration.

CONCLUSIONS: Subcutaneous administration of CCFA using a 2-dose regimen 4 days apart resulted in plasma DCA concentrations above the MIC_{90} of common lower respiratory tract pathogens of horses.

POTENTIAL RELEVANCE: Subcutaneous administration of CCFA is a viable means to achieve necessary plasma concentrations of DCA for at least 11 days. However, SC administration may be limited by the significantly larger injection site reactions.

INTRODUCTION

Ceftiofur crystalline free acid (CCFA) is approved by the U.S. Food and Drug Administration Center for Veterinary Medicine for treatment of lower respiratory tract infections in horses caused by susceptible strains of *Streptococcus equi* subspecies *zooepidemicus*. Ceftiofur, administered as either ceftiofur sodium or CCFA, is rapidly metabolized to desfuroylceftiofur [1]. The *in vitro* activity of desfuroylceftiofur against common Gram-negative pathogens and streptococci is almost identical to that of ceftiofur [2]. Current labeling for the use of CCFA in horses states that 2 intramuscular (IM) doses must be administered 4 days apart. This regimen is designed to provide concentrations of ceftiofur and desfuroylceftiofur related metabolites in plasma above the therapeutic target of 0.2 μg/mL for a minimum of 10 days from the beginning of treatment. This threshold of 0.2 μg/mL is above the minimum inhibitory concentration that inhibits growth of at least 90% (MIC_{90}) of *S. equi* subspecies *zooepidemicus* isolates [1, 3].

To allow slow and sustained release of the drug, CCFA is in a caprylic/capric triglyceride and cottonseed oil based suspension. An oil-based suspension is more likely to be irritating to surrounding muscles than the sterile aqueous solution used for the ceftiofur sodium formulation. In a recent study, self-limiting injection site swelling was reported in 10 of 278 (3.6%) horses treated with IM CCFA [4]. In some horses, the IM injection site reaction can be quite large and very painful. In a recent study, administration of CCFA to newborn foals by the subcutaneous (SC) route did not result in swelling or pain at the site of injection [5]. Administration of CCFA to adult horses by the SC route might minimize injection site irritation and facilitate administration. However, because the entire dosage regimen depends on only 2 administrations, alterations in the pharmacokinetic profile as a result giving the dose SC could affect efficacy.

The main objective of the present study was to compare the pharmacokinetic profile of CCFA administered by the SC route to that achieved after administration by the IM route. In addition we sought to compare the size of visible injection site swellings after administration by the

2 routes of administration. We hypothesized that SC administration of CCFA to adult horses will be well tolerated and result in therapeutic plasma concentrations.

MATERIALS AND METHODS

Animals

Twelve barren adult mares (7-17 years of age) ranging in weight from 471 to 675 kg were used in the study. The animals were considered healthy on the basis of history, physical examination, complete blood count, and plasma biochemical profile. The animals were housed in individual stalls for the first 24 h after administration of the drug and in a group paddock for the rest of the study. The study was approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Study design and sample collection

Horses were randomly assigned into 2 treatment groups. Ceftiofur crystalline free acid[a] was administered to each horse at a total dose of 6.6 mg/kg of body weight on day 0 and again on day 4. Six horses received half of the calculated volume SC on each side of the neck. Six other horses received half of the calculated volume IM on each side of the neck. The volume of drug administered at each injection site ranged from 7.8 to 11.1 mL. All injections were performed using an 18 gauge 1.5 inch needle. Blood samples (10 ml) were obtained by jugular venipuncture 0, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after administration of the drug on day 0 and on day 4. Additionally, blood samples were collected on day 8 and on day 10 of the study. Blood samples were centrifuged and plasma was stored at -80°C until analysis.

Injection site monitoring

The sites of injection were monitored daily for visible swelling by a non-blinded investigator. When injection site swelling was detected, the horizontal and vertical diameters of each site of swelling were measured using Vernier calipers. For each side of the neck, the surface area of each visible swelling was estimated by multiplying the horizontal diameter by the vertical diameter. On a given day, the total surface area was obtained by adding the surface areas from swellings on each side of the neck. The number of days a given area of swelling at an injection site was detectable was also recorded.

Calculation of DCA concentrations

Ceftiofur and desfuroylceftiofur metabolites in plasma samples and standards were derivitized to desfuroylceftiofur acetamide (DCA) and then subjected to solid phase extraction using a previously described and validated method [6, 7].. Drug concentrations were then determined using HPLC with ultraviolet detection at 266 nm[b] as previously described [7]. Standards were prepared by spiking blank equine plasma with CS reference standard[c] dissolved in methanol and diluted in water. Standard curves for DCA ranged from 0.125 to 16 µg /mL and were linear on each day of analysis ($R^2 > 0.99$). Limits of detection (LOD) and quantification (LOQ) of DCA were 0.0625 µg /mL and 0.125 µg /mL, respectively.

Pharmacokinetic analysis

For each horse, plasma concentration versus time data was analyzed based on noncompartmental pharmacokinetics using computer software[d]. The rate constant of the terminal phase (λ_z) was determined by linear regression of the terminal phase of the logarithmic plasma concentration versus time curve using a minimum of 3 data points. Half-life of the terminal phase ($t_{½\lambda z}$) was calculated as ln 2 divided by λ_z. The area under the concentration-time curve (AUC) was

calculated using the trapezoidal rule, with extrapolation to infinity using C_{min}/λ_z, where C_{min} was the final measurable DCA concentration. Mean residence time (MRT) was calculated as: AUMC/AUC.

Statistical analysis

Normality and equality of variance of the data was assessed by use of the Shapiro-Wilk's and Levene's tests, respectively. Pharmacokinetic and injection site parameters were compared between the 2 groups using the Student t-test for normally distributed data or the Mann-Whitney U test for data that was not normally distributed. A value of $P < 0.05$ was considered significant.

RESULTS

After SC administration of 2 doses of CCFA, DCA had a $t_{1/2\lambda z}$ of 66.2 ± 30.6 h and an $AUC_{0-\infty}$ of 303.8 ± 67.82 (Table 4.1). Peak plasma concentration (C_{max}) of 2.13 ± 0.41μg/mL was achieved 18 h (range 2 to 48 h) after administration of the second dose (Table 4.1). Plasma concentrations of DCA 4 days after administration of CCFA were significantly higher after SC than after IM administration. AUC for the first 24 h after administration of each dose was significantly higher after IM than after SC administration (Table 4.1). All other pharmacokinetic parameters were not significantly different between routes of administration (Table 4.1). All samples were above the therapeutic target of 0.2 μg/mL (Figure 4.1).

Injection site reactions, characterized by soft non-painful edema, were observed in 5 of 6 horses in the SC administration group and in 2 of 6 horses in the IM group (Table 4.2). Administration of CCFA by the SC route resulted in significantly higher maximum lesion area after both doses, and a significantly longer lesion duration after administration of the second dose compared to administration by the IM route (Table 4.2). The maximum area of the lesions obtained after SC administration of dose 2 was significantly ($P = 0.043$) higher than those obtained after SC administration of dose 1. The maximum area of the lesions obtained after IM administration of

dose 2 was not significantly different (P = 1.00) from those obtained after IM administration of dose 1. The injection site reactions were no longer visible by day 5 after CCFA administration. No other adverse reactions were noted during or after the study period.

DISCUSSION

The plasma concentration versus time profile of CCFA observed for both intramuscular and subcutaneous administration in the present study was typical of a long-acting formulation that is slowly absorbed from an extravascular site. The slow absorption rate controls the apparent drug elimination rate. The pharmacokinetic variables obtained after IM administration of CCFA in the present study were comparable to those reported in adult horses in previous studies [8, 9]. With the exception of significantly higher DCA concentrations 4 days after administration of dose 1, pharmacokinetic variables obtained after SC administration of CCFA did not differ significantly from those obtained after IM administration.

The most important factor determining the efficacy of β-lactam antimicrobials such as ceftiofur is the amount of time that serum concentrations exceed the MIC of a given pathogen [10]. In the present study, plasma concentrations remained above the therapeutic target of 0.2 μg/mL for the entire 10 day period, regardless of the route of administration. This threshold of 0.2 μg/mL is above the minimum inhibitory concentration that inhibits growth of at least 90% of *S. equi* subspecies *zooepidemicus* (0.12 μg/mL), *Pasteurella* spp. (< 0.03 μg/mL), and *Actinobacillus* spp. (< 0.03 μg/mL) [1, 3]. Therefore, it is unlikely that the minor differences in plasma concentrations between the IM and SC routes observed in the present study would affect efficacy.

However, SC administration of CCFA to adult horses in the present study resulted in significantly larger areas of visible swelling than those observed after administration of the same dose by the IM route. The present study was limited to evaluation of visible external swelling and the amount of muscle damage was not investigated. Visible swellings were observed in 5 of 6

horses in the SC administration group and in 2 of 6 horses in the IM group. Although, this difference was not statistically significant, our study was underpowered to detect such differences. Our results differ from those obtained in neonatal foals in which SC administration of CCFA did not result in detectable injection site reactions [5], The reasons for this discrepancy are unknown.

In conclusion, administration of CCFA to adult horses by the SC route resulted in therapeutic drug concentrations. However, the clinical utility of the SC route of administration might be limited by the significantly larger external swellings compared to those obtained after IM administration.

FOOTNOTES

[a]Excede® CCFA, Pfizer Animal Health, Kalamazoo, MI, USA

[b]Alliance HPLC, Waters Corporation, Milford, MA, USA

[c]United States Pharmacopeia, Rockville, MD, USA

[d]PK Solutions 2.0, Summit Research Services, Montrose, CO

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Table 4.1. Pharmacokinetic variables (mean ± SD unless otherwise specified*) in adult horses given 2 doses of ceftiofur crystalline free acid (6.6 mg/kg) 4 days apart by the SC (n=6) or by the IM (n=6) routes.

Dose/ Variable	Route of administration		*P*-value
	SC	IM	
Dose 1			
$AUC_{0\text{-}24\ h}$ (μg · h/mL)	22.60 ± 13.53	38.37 ± 15.40	0.040
$AUC_{0\text{-day }4}$ (μg · h/mL)	85.32 ± 10.67	98.73 ± 22.34	0.394
C_{max} (μg/mL)	1.41 ± 0.29	2.37 ± 1.48	0.132
$C_{4\ days}$ (μg/mL)	0.69 ± 0.18	0.51 ± 0.06	0.036
T_{max} (h)	18 (2-96)*	8 (4-12)*	0.240
Dose 2			
$AUC_{day\ 4\text{-}day5}$ (μg · h/mL)	39.52 ± 14.31	53.10 ± 12.00	0.035
$AUC_{day\ 4\text{-}day\ 10}$ (μg · h/mL)	165.3 ± 17.22	175.8 ± 24.97	0.420
$AUC_{day\ 4\text{-}\infty}$ (μg · h/mL)	218.5 ± 58.32	202.0 ± 30.49	0.937
C_{max} (μg/mL)	2.13 ± 0.41	2.81 ± 0.63	0.052
$C_{10\ days}$ (μg/mL)	0.50 ± 0.17	0.47 ± 0.15	0.182
T_{max} (h)	18 (2-48)*	6 (4-24)*	0.240
λ_z (1/h)	0.012 ± 0.005	0.011 ± 0.002	0.633
$t_{1/2\lambda z}$ (h)	66.2 ± 30.6	63.4 ± 10.0	0.818
MRT (h)	99.2 ± 36.8	80.2 ± 10.34	0.310
Total			
$AUC_{0\text{-}\infty}$ (μg · h/mL)	303.8 ± 67.82	300.4 ± 32.92	0.913

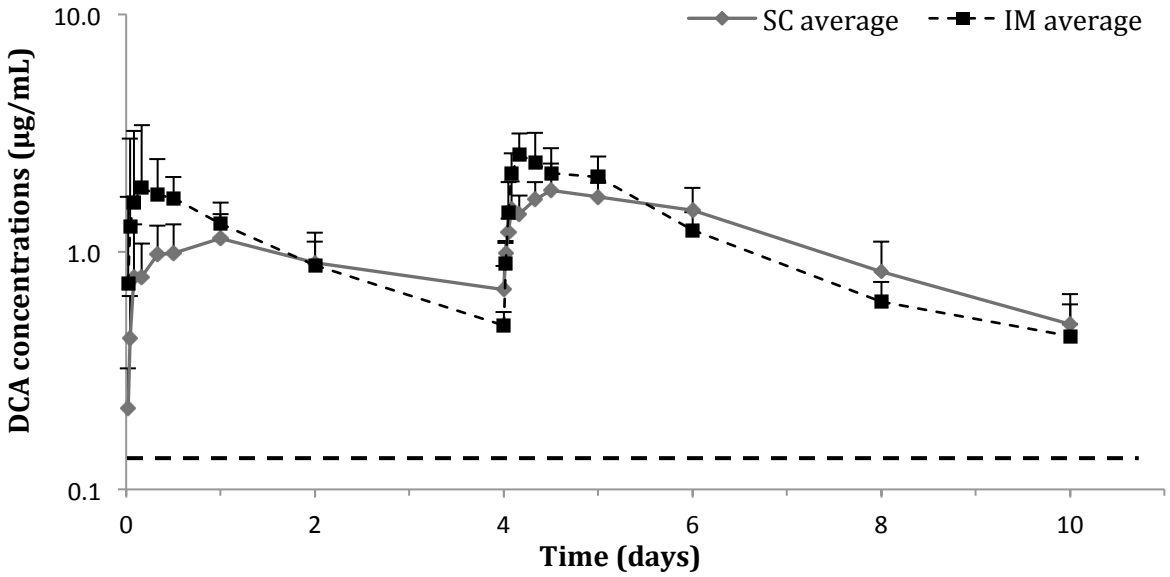
*Median and range.

$AUC_{0\text{-}24\ h}$ = Area under the plasma concentration versus time curve for the first 4 days; $AUC_{0\text{-day }4}$ = Area under the plasma concentration versus time curve for the first 4 days; C_{max} = Maximum plasma concentration of DCA; $C_{4\ days}$ = Plasma concentration of DCA on day 4 prior to administration of the second dose; T_{max} = Time to maximum plasma concentrations; $AUC_{day\ 4\text{-}day5}$ = Area under the plasma concentration versus time curve for the first 24 h after the second dose; $AUC_{day\ 4\text{-}day\ 10}$ = Area under the plasma concentration versus time curve for the second dose; $AUC_{day\ 4\text{-}\infty}$ = Area under the plasma concentration versus time curve for the second dose extrapolated to infinity; $C_{10\ days}$ = Plasma concentration of DCA on day 10; λ_z = rate constant of the terminal phase; $t_{1/2\lambda z}$ = half-life of the terminal phase; MRT = Mean residence time; $AUC_{0\text{-}\infty}$ = Area under the plasma concentration versus time curve for both doses extrapolated to infinity.

Table 4.2. Injection site external swellings (median and range) in adult horses given 2 doses of ceftiofur crystalline free acid (6.6 mg/kg) 4 days apart by the SC (n=6) or by the IM (n=6) routes.

Dose/ Variable	Route of administration		*P*-value
	SC	IM	
Dose 1			
Maximum area (cm^2)	83 (0-174)	0 (0-12)	0.015
Time to maximum area (days)	1 (0.5-4)	0 (0-4)	0.101
Lesion duration (days)	1 (0.5-2)	0 (0-3)	0.132
Horses with lesions (n)	5	1	0.080
Dose 2			
Maximum area (cm^2)	276 (0-308)	0 (0-49)	0.026
Time to maximum area (days)	1 (0.5-2)	0 (0-2)	0.394
Lesion duration (days)	4 (0.5-4)	0 (0-1)	0.041
Horses with lesions (n)	5	2	0.242

Figure 4.1. Mean (SD) plasma concentrations of DCA as measured by UPLC-MS/MS in adult horses given 2 doses of ceftiofur crystalline free acid (6.6 mg/kg) 4 days apart by the SC (n=6) or by the IM (n=6) routes. The horizontal dotted line represents the therapeutic target of 0.2 µg/mL.



CHAPTER 5

PULMONARY PHARMACOKINETICS OF DESFUROLCEFTIOFUR ACETAMIDE AFTER NEBULIZATION OR INTRAMUSCULAR ADMINISTRATION OF CEFTIOFUR SODIUM TO WEANLING FOALS [3]

[3]Fultz, L., Giguère, S., Berghaus, L. Grover, S., and D. Merritt. Accepted for publication in *Equine Veterinary Journal*, 6/24/2014.

ABSTRACT

REASONS FOR PERFORMING STUDY: Administration of ceftiofur sodium via nebulization has been recommended for the treatment of bronchopneumonia in horses despite the lack of pharmacokinetic and safety data.

OBJECTIVES: To compare concentrations of desfuroylceftiofur acetamide (DCA) in plasma and pulmonary epithelial lining fluid (PELF) of foals after nebulization or IM administration of ceftiofur sodium and to determine if nebulization of ceftiofur sodium induces airway inflammation.

STUDY DESIGN: Randomized experimental study.

Methods: Six weanling foals received ceftiofur sodium (2.2 mg/kg daily for 5 doses) by the intramuscular (IM) route and 6 foals received the same dose by nebulization. Concentrations of DCA in plasma and PELF were measured after doses 1 and 5, and differential cell counts were performed on bronchoalveolar lavage samples obtained after dose 5.

RESULTS: Foals receiving ceftiofur sodium via nebulization had significantly lower peak concentrations (C_{max} = 0.15 ± 0.12 vs 6.15 ± 0.75 μg/mL) and area under the curve (AUC = 1.26 ± 0.96 vs 37.63 ± 4.01 μg•h/mL) in plasma compared to those receiving the drug by the IM route. In contrast, foals receiving ceftiofur sodium via nebulization had significantly higher C_{max} (4.52 ± 2.91 vs 0.73 ± 0.73 μg/mL) and AUC (24.14 ± 14.09 vs 5.91 ± 3.28 μg•h/mL) in PELF compared to those receiving the drug by the IM route. Cell concentration and differential cell count in bronchoalveolar lavage fluid of foals nebulized with ceftiofur sodium were not significantly different from those of foals nebulized with saline.

CONCLUSIONS: Administration of ceftiofur sodium via nebulization is well tolerated and DCA concentrations in PELF remain above the MIC_{90} of the drug against *Streptococcus zooepidemicus* for approximately 24 h after administration.

POTENTIAL RELEVANCE: Nebulized ceftiofur sodium warrants further investigation for the treatment of bacterial infections of the lower respiratory tract in horses.

INTRODUCTION

Pneumonia is the leading cause of morbidity and mortality in foals in the USA [1]. The mortality rate has been estimated to be about 6% across the United States [1]. It is likely, however, that the true incidence of infection is much higher and that many cases of infection go unrecognized. Indeed, careful weekly physical examination of more than 200 Thoroughbred foals on 10 farms demonstrated an average morbidity from bacterial infection of the distal respiratory tract of 82% [2]. *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is by far the most common bacterial pathogen isolated from pneumonia in older foals [3]. A variety of other bacterial pathogens such as *Rhodococcus equi*, *Klebsiella* spp., *Escherichia coli, Pasteurella* spp., *Bordetella bronchiseptica, and Actinobacillus* spp. may be isolated as primary pathogens, or may occur in association with *S. zooepidemicus* [3].

Ceftiofur sodium is a 3rd generation cephalosporin approved for the treatment of lower respiratory tract infections caused by susceptible strains of *S. zooepidemicus* in horses. Once administered parenterally, ceftiofur it is rapidly metabolized into desfuroylceftiofur [4]. The *in vitro* activity of desfuroylceftiofur against common Gram-negative pathogens and streptococci is almost identical to that of ceftiofur [5]. With the exception of *R. equi*, ceftiofur exerts good *in vitro* activity against all the pathogens associated with pneumonia in foals [6]. The outcome of respiratory tract infection is more closely associated with antimicrobial drug concentrations within the airways than with concentrations in serum [7]. Measurement of drug concentration in pulmonary epithelial lining fluid (PELF) is a widely used method to estimate antimicrobial concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections caused by extracellular pathogens [8]. Nebulization of antimicrobial agents has been proposed as a method to increase drug concentrations in the lungs while minimizing systemic concentrations and potential toxicity. In calves inoculated intrabronchially with *Mannheimia haemolytica*, nebulized ceftiofur

sodium was more effective at preventing mortality than intramuscular administration of the same drug [9].

As a basis for this study, we hypothesized that nebulized ceftiofur sodium will achieve higher concentrations of desfuroylceftiofur acetamide (DCA) in PELF than intramuscular (IM) administration. The objectives of this study were to determine and compare concentrations of DCA in plasma and PELF of foals after nebulization or IM administration of ceftiofur sodium and to determine if nebulization of ceftiofur sodium induces airway inflammation in weanling foals.

MATERIALS AND METHODS

Animals and experimental design

Twelve healthy 4-6 month-old weanling foals ranging in weight from 163 to 257 kg were used in the study. Foals were considered healthy on the basis of a thorough physical examination, complete blood count, and biochemical profile. The animals were housed in individual stalls during periods of sample collection and in a group paddock for the remainder of the study. The study was approved by the Institutional Animal Care and Use Committee of the University of Georgia. Ceftiofur sodium[a] was reconstituted in sterile water to a concentration of 50 mg/mL and administered within 24 h of reconstitution at a dose of 2.2 mg/kg once a day for 5 days (5 doses) to all foals. Foals were randomly assigned to 1 of 2 treatment groups. Six foals were administered ceftiofur sodium by nebulization using a commercially available mask and nebulizer specifically designed for use in foals.[b] The same 6 foals received the same volume of saline by IM injection in the neck muscles using a 1 inch 20 gauge needle. Six other foals received ceftiofur sodium by IM injection in the neck muscles and were nebulized with the same volume of 0.9% NaCl. Particle size of nebulized ceftiofur sodium was verified using laser diffraction.[c] Blood samples were obtained from a catheter placed in a jugular vein at 0, 15, 30, 60, 90 min; 2, 3, 4, 6, 8, 12, and 24 h after administration of the first and last dose of the drug and placed in 8 mL collection tubes containing EDTA. Blood samples were

centrifuged at 500 × g for 10 minutes and plasma was stored at -80ºC until assayed. Bronchoalveolar (BAL) fluid was collected at 2, 8, and 24 h after administration of the last dose of the drug.

Bronchoalveolar lavage

Foals were sedated by intravenous administration of xylazine hydrochloride (0.5 mg/kg) and butorphanol tartrate (0.04 mg/kg) prior to BAL fluid collection. A 10-mm diameter, 2.4-m BAL catheter[d] was passed via nasal approach until wedged into a bronchus. The lavage solution consisted of four aliquots of 60 mL physiologic saline (0.9% NaCl) solution infused and aspirated immediately. The volume of BAL fluid was measured using a graduated cylinder. The BAL fluid was centrifuged at 200 × g for 10 minutes and supernatant fluid was frozen at -80ºC until assayed.

Total nucleated cell count in BAL fluid was determined from an aliquot of the sample obtained 2h after the fifth dose of ceftiofur sodium by use of an automated cell counter.[e] The remainder of the aliquot was cytocentrifuged[f] for 2 minutes onto a microscope slide for cytological examination. Preparations were stained with a Diff-Quick kit[g] according to the manufacturer's directions and a manual differential count of 200 cells was performed.

Concentration of DCA

Samples were analyzed for concentrations of ceftiofur, desfuroylceftiofur, and related metabolites by reduction and derivatization to DCA using a validated ultra-high pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) detection assay performed as described previously [10]. The lower limits of quantifications (LLOQ) of the assay were 0.01 μg/mL for plasma and 0.0002 μg/mL for BAL fluid supernatant, respectively. Intra-run bias of standards, including samples at the lower limit of quantification, ranged from -7.7 to 10.1%. The inter-run coefficient of variation ranged from 1.6 to 9.1%.

Calculation of DCA concentrations in Pulmonary Epithelial Lining Fluid

Estimation of the volume of PELF was determined by urea dilution method [11]. Urea nitrogen concentrations in BAL fluid ($Urea_{BAL}$) and concurrent plasma samples ($Urea_{PLASMA}$) were determined by use of a commercial quantitative colorimetric kit.[h] The volume of PELF (V_{PELF}) in BAL fluid was derived from the following equation: $V_{PELF} = V_{BAL} \times (Urea_{BAL}/Urea_{PLASMA})$, where V_{BAL} is the volume of recovered BAL fluid. The concentration of DCA in PELF (DCA_{PELF}) was derived from the following relationship: $DCA_{PELF} = DCA_{BAL} \times (V_{BAL}/V_{PELF})$, where DCA_{BAL} is the measured concentration of DCA in BAL fluid supernatant.

Pharmacokinetic analysis

For each foal, plasma and PELF concentration versus time data was analyzed based on noncompartmental pharmacokinetics using computer software.[i] The rate constant of the terminal phase (λ_z) was determined by linear regression of the logarithmic plasma concentration versus time curve using a minimum of 3 data points. Half-life of the terminal phase ($t_{½\lambda z}$) was calculated as ln 2 divided by λ_z. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using C_{min}/λ_z, where C_{min} was the final measurable DCA concentration. Mean residence time (MRT) was calculated as: AUMC/AUC, where AUMC is the area under the first moment of the concentration–time curve. Systemic bioavailability of nebulized ceftiofur sodium relative to IM was calculated as $AUC_{nebulized}/AUC_{IM}$. The accumulation factor was calculated as $C_{last\text{-}dose5}/C_{last\text{-}dose1}$, where $C_{last\text{-}dose5}$ is the last quantifiable plasma concentration after dose 5 and $C_{last\text{-}dose1}$ is the last quantifiable plasma concentration after dose 1.

Statistical analysis

Normality and equality of variance of the data was assessed by use of the Shapiro-Wilk and Levene tests, respectively. Data that were not normally distributed were log or rank transformed.

The effects of route of administration (nebulized vs IM), dose (first versus fifth) and the interactions between route of administration and dose on plasma pharmacokinetic variables were assessed using a two-way ANOVA with repeated measures on 1 factor (dose). When appropriate, multiple pairwise comparisons were performed using the Holm-Sidak method. Comparison of PELF pharmacokinetic variables and BAL cytology results between the 2 experimental groups (nebulized versus IM) were done using the Student t-test or the Mann Whitney U test. A value of $P < 0.05$ was considered significant.

RESULTS

No adverse effects were noted during the course of the study. The median particle size of nebulized ceftiofur sodium at a concentration of 50 mg/mL was 5.205 μm with 89.4 % of the particles being < 10 μm. In preliminary experiments, diluting ceftiofur sodium to a concentration of 25 mg/mL did not affect particle size (data not shown). Quantifiable DCA concentrations were present in the plasma at all time-points in foals administered IM ceftiofur sodium. In contrast, DCA concentrations were below the LLOQ (< 0.010 μg/mL) in plasma at the 24 hour time point after dose 1 in 4 of 6 foals administered ceftiofur sodium via nebulization. A single foal in the nebulization group had plasma DCA concentrations below the limit of detection from 6 to 24 h after dose 1. The accumulation factor in plasma after administration of 5 doses of ceftiofur sodium was 1.74 ± 0.24 for IM administration and 2.27 ± 1.78 for nebulization. Terminal half-life, C_{max}, C_{min}, AUC and MRT were significantly higher after dose 5 than after dose 1 (Table 5.1). In contrast, T_{max} was significantly shorter after dose 5 than after dose 1. Foals that received ceftiofur sodium via nebulization had significantly lower plasma C_{max}, C_{min}, and AUC compared to those administered the drug by the IM route (Table 5.1). In contrast, MRT was significantly longer for the nebulized route (Table 5.1). The systemic bioavailability of nebulized ceftiofur sodium relative to IM administration

was 2.7 ± 1.8 %. Nebulization resulted in plasma DCA concentrations below the MIC_{90} of the drug against *S. zooepidemicus* (0.12 μg/mL) for most of the dosing interval (Figure 5.1).

Quantifiable DCA concentrations were present in PELF at all sampled time points after both IM administration and nebulization of ceftiofur sodium. Compared to the administration of ceftiofur sodium via the IM route, nebulization of the drug resulted in significantly higher PELF C_{max} and AUC (Table 5.2). In contrast terminal half-life and MRT in PELF were significantly shorter after nebulization than after IM administration (Table 5.2). Average concentrations of DCA in PELF were higher after nebulization than after IM administration and above the MIC_{90} of the drug against *S. zooepidemicus* for 24 h after administration by nebulization (Figure 5.2). Cell concentration and differential cell count of foals nebulized with ceftiofur sodium were not significantly different from those of foals administered ceftiofur sodium IM and nebulized with 0.9% NaCl (Table 5.3).

DISCUSSION

The optimal dosing of an antimicrobial agent is determined by both the pharmacokinetics and pharmacodynamics of the drug. Currently, most pharmacokinetic/pharmacodynamic models rely on plasma concentrations and MIC. The most important factor determining the efficacy of β-lactam antimicrobials such as ceftiofur is the duration of time that plasma concentrations of the drug exceed the MIC of a given pathogen [12, 13]. However, only the free (unbound) fraction of the drug in interstitial fluids at the target site is responsible for therapeutic success. Free desfuroylceftiofur accounts for only about 10 % of the total desfuroylceftiofur related metabolites found in plasma obtained from adult cattle. However, protein binding of desfuroylceftiofur is reversible and protein bound desfuroylceftiofur acts as a reservoir for release of active drug at the site of infection [14]. This phenomenon may explain the apparent discrepancy between documented clinical efficacy of ceftiofur after once a day dosing in cattle and pharmacokinetic evaluation based on microbiologic assay which suggests rapid disappearance of the drug. The

discrepancy is resolved if efficacy is compared with measurement of DCA regardless of protein binding. In the latter case, plasma concentrations remain above the MIC90 for the entire dosing interval, consistent with clinical efficacy arising from once daily dosing [15]. Hence, measurement of DCA is preferred for dose optimization based on pharmacokinetic/pharmacodynamic relationship.

Because most infections occur in tissues rather than in plasma, the ability of antimicrobial agents to reach the target site is a key determinant of clinical outcome [13]. For pulmonary infections caused by extracellular bacteria such as S. zooepidemicus, concentrations of antimicrobial agents in the extracellular or interstitial space within the lungs would provide additional relevant information. While it is common practice to measure drug concentration in tissue homogenates, the homogenization procedure disrupts cell membranes and produces a suspension containing both intracellular and extracellular components [16]. This typically results in unreliable estimation of antimicrobial drug concentrations in the extracellular environment [16, 17].

Measurement of drug concentration in PELF collected by BAL is the most widely used method to estimate antimicrobial concentrations at the site of infection for antimicrobial agents intended to treat lower respiratory tract infections caused by extracellular bacterial pathogens in people [8, 18]. Damage to cells during BAL collection or processing may lead to drug leakage from the cells and overestimation of true PELF concentration for drugs that concentrate intracellularly [18]. This is unlikely to have influenced the results of the present study because concentrations of DCA in equine bronchoalveolar cells are negligible and considerably below concentrations measured in PELF [10]. The plasma Cmax obtained after IM administration of the first dose of ceftiofur sodium in the present study (4.49 ± 0.68 μg/mL) was almost identical to that achieved after administration of the same dose of ceftiofur sodium to adult horses (4.46 ± 0.93 μg/mL) [4]. Despite almost identical plasma concentrations, peak DCA concentration of lung tissue homogenate

in the aforementioned study (1.40 ± 0.36 μg/mL) [4] was considerably higher than peak PELF concentration in this study (0.73 ± 0.73 μg/mL), suggesting that DCA concentrations in lung tissue homogenates overestimate PELF concentrations. In the present study, concentrations of DCA in PELF remained above the MIC90 of S. zooepidemicus (0.12 μg/mL), Pasteurella spp. (< 0.03 μg/mL), and Actinobacillus spp. (< 0.03 μg/mL) [4, 19] for most of the 24 h dosing interval regardless of the route of administration. Treatment of infections caused by microorganisms with higher MICs might require more frequent administration. Additional studies will be required to determine the optimal dose and dosing interval of nebulized ceftiofur sodium in horses with bronchopneumonia. Peak concentrations of DCA in PELF achieved after IM administration of ceftiofur sodium in this study were slightly higher than those achieved after administration of a single dose of ceftiofur crystalline free acid to foals weanling foals (0.46 ± 0.03 μg/mL) [10].

In healthy horses, nebulization of 20 mL of the commercially available IV gentamicin sulfate solution (diluted to 50 mg/mL) using an ultrasonic nebulizer resulted in bronchial lavage fluid concentrations approximately 12 times higher than concentrations achieved by IV administration at a dose of 6.6 mg/kg [20]. However, the major limitation to the use of aerosolized gentamicin in horses is its lack of activity against S. zooepidemicus, the most common bacterial pathogen of the equine respiratory tract. In the present study, daily administration of ceftiofur sodium via nebulization resulted in concentrations of DCA in PELF significantly (approximately 6-fold) higher than those obtained after administration by the IM route. Maximum concentrations of DCA in PELF were observed 2 h post-administration, regardless of the route. However, the accuracy of reported pharmacokinetic variables in PELF was limited by the small number of BAL samples obtained.

Nebulization of ceftiofur sodium resulted in low systemic bioavailability of DCA with plasma concentrations well-below therapeutic levels. Low systemic bioavailability after nebulization of ceftiofur sodium has also been documented in calves [21]. Therefore, nebulization of ceftiofur sodium increases the amount of drug that is present within the respiratory secretions while

minimizing the amount of drug in plasma, thereby decreasing the risk of systemic adverse effects. In an experimental model of Escherichia coli of pneumonia in mechanically ventilated piglets, nebulization of amikacin was found to be more effective than systemic administration and even poorly ventilated and consolidated areas of the lungs contained higher antimicrobial drug concentrations after nebulization than after IV administration [22] Nevertheless, the administration of antimicrobial agents by inhalation alone may not be sufficient in patients with severe parenchymal involvement or substantial consolidation. In these cases, nebulization may be more appropriate as an adjunct to systemic administration.

Use of systemic formulations of drugs for nebulization can lead to exposure to potentially irritant substances, toxic additives and inappropriate pH or osmolality ranges. In the present study nebulization with the formulation of ceftiofur sodium commercially available for systemic use did not result in clinical signs of respiratory disease or airway inflammation as assessed by cytological examination of BAL fluid. The BAL cell concentrations and differential cell counts of foals nebulized with ceftiofur sodium were not significantly different from those of foals nebulized with saline and were within established reference ranges for foals and adult horses [23-25]. Similarly, once daily nebulization of gentamicin or cefquinome to healthy horses for 5-7 consecutive days did not result in pulmonary inflammation [26, 27]. Antimicrobial delivery by inhalation is greatly influenced by the product formulation and type of nebulizer. The pattern of deposition of aerosol particles in the airway is influenced by characteristic of the patients (inspiratory flow, tidal volume, respiratory rate, breathing pattern etc.) and by the size of the aerosol particles. Particles > 10 μm are typically filtered in the nose and nasopharynx, particles of 5-10 μm generally reach the larger airways, and particles of 1-5 μm reach the periphery of the lungs [28]. In the present study, approximately 50% of the particles were 1-5 μm with 89.4 % of the particles being < 10 μm. In one study, the particle size distribution and particle density of gentamicin sulfate and ceftiofur sodium aerosols were affected by the antimicrobial concentration of the solution [29]. Gentamicin concentrations of 50

mg/mL or ceftiofur concentrations of 25 mg/mL produced the optimal combinations of particle size and aerosol density when using a medical ultrasonic nebulizer when compared to more concentrated solutions [29]. In the present study, dilution of the ceftiofur sodium concentration to 25 mg/mL did not improve particle size. Differences between the 2 studies likely relates to the type of nebulizer used. Nebulization of a 50 mg/ml solution instead of 25 mg/ml offers the advantage of decreasing the time of administration in half.

In conclusion, administration of ceftiofur sodium via nebulization is well tolerated and results in significantly higher drug concentrations in PELF when compared to administration of the same dose by the IM route. Nebulized ceftiofur sodium warrants further investigation for the treatment of susceptible bacterial infections of the lower respiratory tract in horses.

FOOTNOTES

[a]Naxcel® Sterile Suspension, Zoetis, Madison, NJ, USA

[b]Flexineb, Nortev Ltd, Galway, Ireland

[c]Spraytec, Malvern Instrument Limited, Malvern, Worcestershire, UK

[d]Jorgenson laboratories, Loveland, CO, USA

[e]Cellometer Auto T4, Nexcelom Bioscience, Lawrence, MA, USA.

[f]Shandon Cytospin, Thermo Fisher Pittsburg, PA

[g]Diff-Quick stain kit, Fisher Scientific, Pittsburg, PA

[j]Biochain Urea Assay Kit, Hayward, CA, USA

[l]PK Solutions 2.0, Summit Research Services, Montrose, CO , USA

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Table 5.1. Pharmacokinetic variables (mean ± SD unless otherwise specified*) for DCA in the plasma of foals after administration of the first and fifth dose of ceftiofur sodium (2.2 mg/kg of body weight) by the intramuscular (n=6) or nebulized route (n=6).

Variable	Dose	Route		*P* value		
		IM	Nebulized	dose	route	dose × route
λ_z (1/h)	1	0.097 ± 0.005[1]	0.091 ± 0.026[1]	< 0.001	0.245	0.373
	5	0.077 ± 0.007[2]	0.064 ± 0.014[2]			
$t_{1/2\lambda z}$ (h)	1	7.2 ± 0.365[1]	8.1 ± 2.0[1]	< 0.001	0.094	0.154
	5	9.0 ± 0.8[2]	11.3 ± 2.6[2]			
C_{max} (mg/mL)	1	4.49 ± 0.68[a,1]	0.08 ± 0.05[b,1]	< 0.001	< 0.001	0.076
	5	6.15 ± 0.75[a,2]	0.15 ± 0.12[b,2]			
C_{min}(mg/mL)	1	0.23 ± 0.03[a,1]	<0.01 (<0.01–0.02)*[b,1]	< 0.001	< 0.001	0.178
	5	0.40 ± 0.04[a,2]	0.02 ± 0.01[b,2]			
T_{max} (h)*	1	1.25 (1.0 – 2.0)[1]	1.5 (1.0-8.0)[1]	0.009	0.577	0.070
	5	1.0 (1.0 – 1.5)[2]	0.75 (0.25 – 1.0)[2]			
MRT (h)	1	8.2 ± 0.4[a,1]	11.2 ± 3.0[b,1]	< 0.001	0.014	0.193
	5	10.2 ± 0.8[a,2]	14.7 ± 3.6[b,2]			
AUC_{0-t} (mg•h/mL)	1	29.31 ± 3.14[a,1]	0.64 ± 0.52[b,1]	< 0.001	< 0.001	0.128
	5	37.63 ± 4.01[a,2]	1.26 ± 0.96[b,2]			
AUC_{∞} (mg•h/mL)	1	31.70 ± 3.38[a,1]	0.82 ± 0.55[b,1]	< 0.001	< 0.001	0.154
	5	42.85 ± 4.10[a,2]	1.56 ± 1.03[b,2]			

*Median and range. λ_z = rate constant of the terminal phase; $t_{1/2\lambda z}$ = half-life of the terminal phase; C_{max} = Maximum concentration of DCA; C_{min}= Minimum concentration of DCA; T_{max} = Time to maximum concentrations; MRT = Mean residence time; AUC_{0-t} = Area under the concentration versus time curve from time 0 to the last quantifiable time point. AUC_{∞} = Area under the concentration versus time curve extrapolated to infinity.

[a,b] Different superscript letters within a given row indicate significant differences ($P < 0.05$) between IM and nebulized for a given dose. [1,2] Different superscript numbers within a given column indicate significant differences ($P < 0.05$) between doses (dose 1 and dose 5) for a given route of administration.

Table 5.2. Pharmacokinetic variables (mean ± SD unless otherwise specified*) for DCA in the PELF of foals after administration of ceftiofur sodium (2.2 mg/kg of body weight) for 5 days by the intramuscular (n=6) or nebulized route (n=6).

Variable	Route		*P* value
	IM	Nebulized	
λ_z (1/h)	0.078 ± 0.025	0.135 ± 0.051	0.034
$t_{1/2\lambda z}$ (h)	9.5 ± 2.4	5.8 ± 2.2	0.020
C_{max} (µg/mL)	0.73 ± 0.73	4.52 ± 2.91	0.011
C_{min} (µg/mL)	0.09 ± 0.02	0.16 ± 0.11	0.188
T_{max} (h)*	2.0 (2.0 – 2.0)	2.0 (2.0 – 2.0)	1.00
MRT (h)	13. 4 ± 4.1	7.0 ± 3.7	0.019
AUC_{24h} (µg•h/mL)	5.91 ± 3.28	24.14 ± 14.09	0.009
AUC_{∞} (µg•h/mL)	7.05 ± 3.15	25.75 ± 13.77	0.004

*Median and range. λ_z = rate constant of the terminal phase; $t_{1/2\lambda z}$ = half-life of the terminal phase; C_{max} = Maximum concentration of DCA; C_{min}= Minimum concentration of DCA; T_{max} = Time to maximum concentrations; MRT = Mean residence time; AUC_{24h} = Area under the concentration versus time curve for the first 24 after administration. AUC_{∞} = Area under the concentration versus time curve extrapolated to infinity.

Table 5.3. Cell concentration and differential cell count (mean ± SD) in bronchoalveolar fluid of foals after administration of ceftiofur sodium (2.2 mg/kg of body weight) for 5 days by the intramuscular (n=6)* or nebulized route (n=6).

Variable	**Route**		***P* value**
	IM*	Nebulized	
Cell concentration (/µL)	1,054 ± 264	820 ± 346	0.219
Macrophages (%)	60.3 ± 8.6	64.9 ±3.0	0.282
Lymphocytes (%)	38.4± 4 8	32.8 ± 4.2	0.211
Neutrophils (%)	1.3 ± 1.6	2.3 ± 2.8	0.489
Eosinophils (%)	0	0	NA
Mast cells (%)	0	0	NA

*Foals administered ceftiofur sodium IM were nebulized with 0.9% NaCl.

Figure 5.1. Mean (± SD) DCA concentrations in the plasma of foals after administration of the first and fifth dose of ceftiofur sodium (2.2 mg/kg of body weight) by the intramuscular (n=6) or nebulized route (n=6). The dotted horizontal line represents the MIC_{90} of *S. zooepidemicus* (0.12 µg/mL).

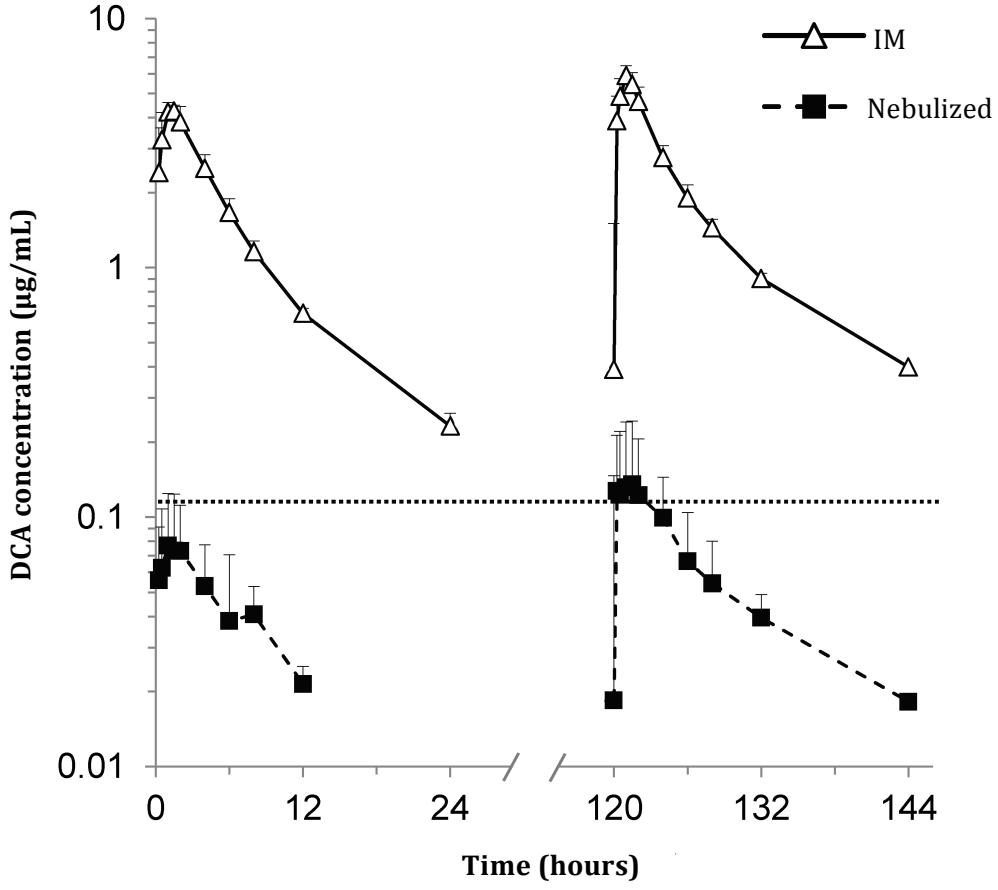
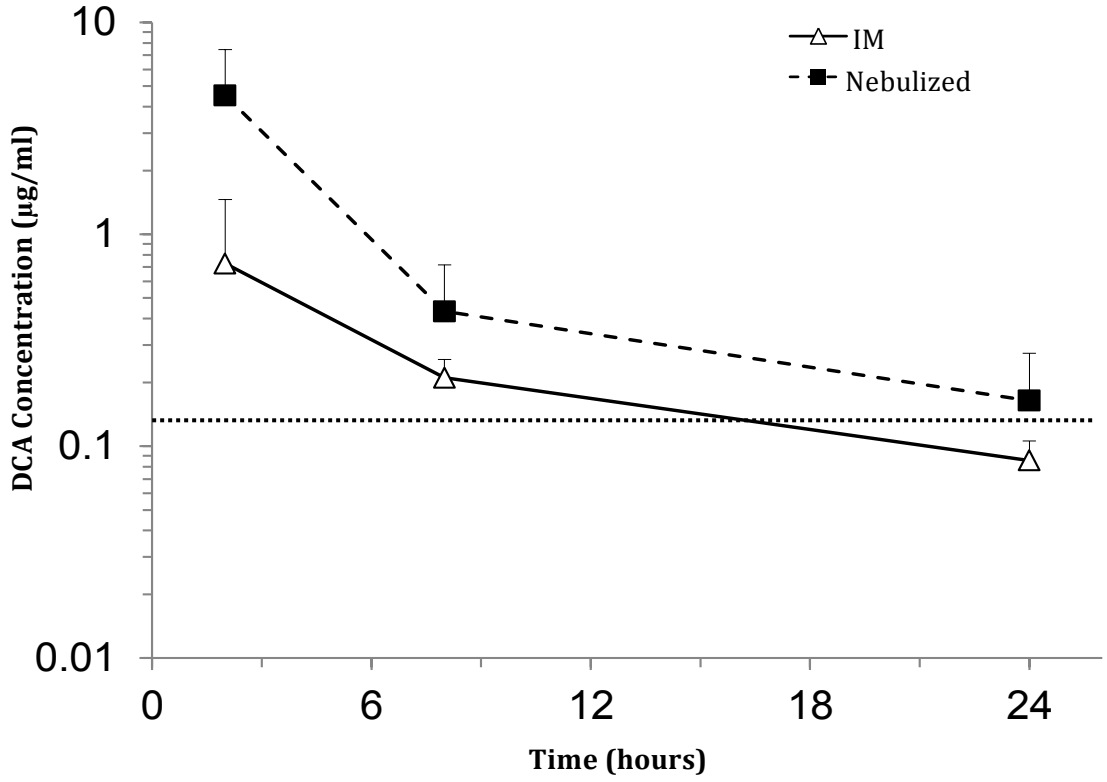


Figure 5.2. Mean (± SD) DCA concentrations in the PELF of foals after administration of five doses of ceftiofur sodium (2.2 mg/kg of body weight) by the intramuscular (n=6) or nebulized route (n=6). The dotted horizontal line represents the MIC_{90} of *S. zooepidemicus* (0.12 µg/mL).



CHAPTER 6

CONCLUSION

Ceftiofur crystalline free acid (CCFA) and ceftiofur sodium are two commercially-available formulations of ceftiofur, a broad spectrum third generation cephalosporin antimicrobial, that have been studied extensively, particularly in the context of application for equine bacterial pneumonia. Following parenteral administration at the labeled doses, the primary metabolite of ceftiofur (desfuroylceftiofur; DCA) has been shown to maintain concentrations in both plasma and pulmonary epithelial lining fluid (PELF) above the mean inhibitory concentration (MIC) for at least 50% of the dosing interval for the most common bacterial isolates in equine bacterial pneumonia including: *Streptococcus equi* subspecies *zooepidemicus, Actinobacillus spp., Pasteurella spp., Bacteriodes* spp*., Fusobacterium necrophorum* and *Peptostreptococcus anaerobius*. The overall goal of the studies reported herein were to determine the proper dosing interval for CCFA when long term therapy is required and to investigate alternative routes of administration for both CCFA and ceftiofur sodium beyond the current labeling, with a particular focus on drug concentrations in the respiratory tract.

The first study aimed to determine steady-state plasma and PELF concentrations of desfuroylceftiofur acetamide (DCA) after repeated weekly IM administrations of CCFA to adult horses. The data demonstrated that, after the initial 2-dose regimen 4 days apart, weekly IM administration of CCFA was well tolerated and resulted in plasma and PELF DCA concentrations above the MIC_{90} of common lower respiratory tract pathogens of horses for the entire dosing interval. Given this data, an extended course of therapy with CCFA using weekly intramuscular administration could be clinically applied in cases requiring greater than the labeled 10 days of therapy with CCFA, such as moderate to severe cases of bronchopneumonia.

The second study aimed to compare the pharmacokinetic profile of CCFA administered by the SC route to that achieved after administration by the IM route. In addition we sought to compare the size of visible injection site swellings after administration by the 2 routes of administration. Subcutaneous and intramuscular administration at the labelled dose and frequency of CCFA (6.6 mg/kg q 96 hours for 2 doses) resulted in similar plasma concentrations versus time profiles. After subcutaneous administration, plasma concentrations of DCA were above the MIC_{90} of common lower respiratory tract pathogens of horses for the entire course of therapy. However, the clinical utility of the SC route of administration might be limited by the significantly larger external swellings compared to those obtained after IM administration.

The third study evaluated the pharmacokinetics of ceftiofur sodium when administered via aerosol administration (nebulization) to horses. One group of weanling foals received ceftiofur sodium intramuscularly (2.2 mg/kg) once daily for 5 consecutive doses and was nebulized with saline, while another group was administered ceftiofur sodium via nebulization and given saline IM. Ceftiofur sodium administered via nebulization was only 2.7 ± 1.8 % bioavailable, resulting in low systemic exposure to the antimicrobial. However, in the target organ (the lungs) the concentrations of DCA in PELF were significantly higher in the foals that received nebulized ceftiofur sodium compared to intramuscular ceftiofur sodium for the duration of the dosing interval. In nebulized foals, the concentration of DCA in PELF remained above the MIC_{90} of *S. zooepidemicus* for approximately 24 hours after each daily dose and no signs of inflammation were evident upon analysis of bronchoalveolar fluid following the 5^{th} consecutive dose. Therefore, the nebulization of ceftiofur sodium warrants further investigation for the treatment of bacterial infections of the lower respiratory tract in horses.

In conclusion, the studies reported herein have provided important clinical information regarding the use of CCFA and ceftiofur sodium in adult horses and weanling foals. A dosing interval was established for situations when prolonged therapy with CCFA is warranted. In

addition, the SC route of administration of CCFA was found to result in therapeutic concentrations but caused larger and longer-lasting injection site reactions compared to IM administration. Finally, nebulization of ceftiofur sodium did not result in airway inflammation and provided DCA concentrations in PELF above the MIC of susceptible microorganisms for approximately 24 hours. Additional studies will be required to assess the safety of these dosing regimens and routes of administration in a clinical setting.