Pulmonary pharmacokinetics of desfuroylceftiofur acetamide after nebulisation or intramuscular administration of ceftiofur sodium to weanling foals

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Summary

Reasons for performing study: Administration of ceftiofur sodium via nebulisation 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 nebulisation or i.m. administration of ceftiofur sodium and to determine if nebulisation of ceftiofur sodium induces airway inflammation.
Study design: Randomised experimental study.
Methods: Six weanling foals received ceftiofur sodium (2.2 mg/kg bwt daily for 5 doses) by the i.m. route and 6 foals received the same dose by nebulisation. 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 nebulisation had significantly lower peak concentrations (0.15 ± 0.12 vs. 6.15 ± 0.75 mg/l) and area under the curve (1.26 ± 0.96 vs. 37.63 ± 4.01 mg•h/l) in plasma compared with those receiving the drug by the i.m. route. In contrast, foals receiving ceftiofur sodium via nebulisation had significantly higher peak concentrations (4.52 ± 2.91 vs. 0.73 ± 0.73 mg/l) and area under the curve (24.14 ± 14.09 vs. 5.91 ± 3.28 mg•h/l) in PELF compared with those receiving the drug by the i.m. route. Cell concentration and differential cell count in bronchoalveolar lavage fluid of foals nebulised with ceftiofur sodium were not significantly different from those of foals nebulised with saline.
Conclusions: Administration of ceftiofur sodium via nebulisation is well tolerated and DCA concentrations in PELF remain above the minimum inhibitory concentration of the drug required to inhibit the growth of 90% of *Streptococcus zooepidemicus* for approximately 24 h after administration. Nebulised ceftiofur sodium warrants further investigation for the treatment of bacterial infections of the lower respiratory tract in horses.

Keywords: horse; foal; ceftiofur sodium; nebulisation; aerosol; pneumonia

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 USA [1]. It is likely, however, that the true incidence of infection is much higher and that many cases of infection go unrecognised. 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* ssp. *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 third-generation cephalosporin approved for the treatment of lower respiratory tract infections caused by susceptible strains of *S. zooepidemicus* in horses. Once administered parenterally, ceftiofur is rapidly metabolised 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]. Nebulisation of antimicrobial agents has been proposed as a method to increase drug concentrations in the lungs while minimising systemic concentrations and potential toxicity. In calves inoculated intrabronchially with *Mannheimia haemolytica*, nebulised ceftiofur sodium was more effective at preventing mortality than i.m. administration of the same drug [9].

As a basis for this study, we hypothesised that nebulised ceftiofur sodium would achieve higher concentrations of desfuroylceftiofur acetamide (DCA) in PELF than i.m. administration. The objectives of this study were to determine and compare concentrations of DCA in plasma and PELF of foals after nebulisation or i.m. administration of ceftiofur sodium and to determine if nebulisation of ceftiofur sodium induces airway inflammation in weanling foals.

Materials and methods

Animals and experimental design

Twelve healthy weanling foals aged 4–6 months, ranging in weight from 163–257 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. Ceftiofur sodium (Naxcel Sterile Suspension)[a] was reconstituted in sterile water to a concentration of 50 g/l and administered within 24 h of reconstitution at a dose of 2.2 mg/kg bwt once a day for 5 days (5 doses) to all foals. Foals were randomly assigned to one of 2 treatment groups. Six foals were administered ceftiofur sodium by nebulisation using a commercially available mask and nebuliser specifically designed for use in foals (Flexineb)[b]. The same 6 foals received the same volume of saline by i.m. injection in the neck muscles using a 2.5 cm 20 gauge needle. The other 6 foals received ceftiofur sodium by i.m. injection in the neck muscles and were nebulised with the same volume of 0.9% NaCl. Particle size of nebulised ceftiofur sodium was verified using laser diffraction (Spraytec)[c]. Blood samples were obtained from a catheter placed in a jugular vein at 0, 15, 30, 60 and 90 min, and 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 $500 \times \boldsymbol{g}$ for 10 min

and plasma was stored at -80°C 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 i.v. administration of xylazine hydrochloride (0.5 mg/kg bwt) and butorphanol tartrate (0.04 mg/kg bwt) 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 4 aliquots of 60 ml physiological 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 $200 \times \textbf{\textit{g}}$ for 10 min and supernatant fluid was frozen at -80°C until assayed.

Total nucleated cell count in BAL fluid was determined from an aliquot of the sample obtained 2 h after the fifth dose of ceftiofur sodium by use of an automated cell counter (Cellometer Auto T4)[e]. The remainder of the aliquot was cytocentrifuged (Shandon Cytospin)[f] for 2 min 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 analysed for concentrations of ceftiofur, desfuroylceftiofur and related metabolites by reduction and derivatisation to DCA using a validated ultrahigh pressure liquid chromatography tandem mass spectometry detection assay performed as described previously [10]. The lower limits of quantification of the assay were 0.01 mg/l for plasma and 0.0002 mg/l for BAL fluid supernatant, respectively. Intrarun 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 desfuroylceftiofur acetamide concentrations in PELF

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 vs. time data were analysed based on noncompartmental pharmacokinetics using computer software (PK Solutions 2.0)[i]. The rate constant of the terminal phase (λ_z) was determined by linear regression of the logarithmic plasma concentration vs. time curve using a minimum of 3 data points. Half-life of the terminal phase ($t_{1/2\lambda z}$) was calculated as ln $2/\lambda_z$. 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} is 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 nebulised ceftiofur sodium relative to i.m. was calculated as $AUC_{nebulised}/AUC_{IM}$. The accumulation factor was calculated as $C_{last-Dose5}/C_{last-Dose1}$, where $C_{last-Dose5}$ is the last quantifiable plasma concentration after Dose 5 and $C_{last-Dose1}$ is the last quantifiable plasma concentration after Dose 1.

Data analysis

Normality and equality of variance of the data were 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 (nebulised vs. i.m.), dose (first vs. fifth) and the interactions between route of administration and dose on plasma pharmacokinetic variables were assessed using a 2-way ANOVA with repeated measures on one 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 (nebulised vs. i.m.) 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 nebulised ceftiofur sodium at a concentration of 50 g/l was 5.205 μm with 89.4% of the particles being <10 μm. In preliminary experiments, diluting ceftiofur sodium to a concentration of 25 g/l did not affect particle size (data not shown). Quantifiable DCA concentrations were present in the plasma at all time points in foals administered i.m. ceftiofur sodium. In contrast, DCA concentrations were below the lower limit of quantification (<0.010 mg/l) in plasma at the 24 h time point after Dose 1 in 4 of 6 foals administered ceftiofur sodium via nebulisation. A single foal in the nebulisation group had plasma DCA concentrations below the limit of detection 6–24 h after Dose 1. The accumulation factor in plasma after administration of 5 doses of ceftiofur sodium was 1.74 ± 0.24 for i.m. administration and 2.27 ± 1.78 for nebulisation. Terminal half-life, maximum concentration of DCA (C_{max}), C_{min}, AUC and MRT were significantly higher after Dose 5 than after Dose 1 (Table 1). In contrast, time to maximum concentration was significantly shorter after Dose 5 than after Dose 1. Foals that received ceftiofur sodium via nebulisation had significantly lower plasma C_{max}, C_{min} and AUC compared with those administered the drug by the i.m. route (Table 1). In contrast, MRT was significantly longer for the nebulised route (Table 1). The systemic bioavailability of nebulised ceftiofur sodium relative to i.m. administration was 2.7 ± 1.8 %. Nebulisation resulted in plasma DCA concentrations below the minimum inhibitory concentration (MIC) required to inhibit the growth of 90% of organisms (MIC_{90}) of the drug against *S. zooepidemicus* (0.12 mg/l) for most of the dosing interval (Fig 1).

Quantifiable DCA concentrations were present in PELF at all sampled time points after both i.m. administration and nebulisation of ceftiofur sodium. Compared with the administration of ceftiofur sodium via the i.m. route, nebulisation of the drug resulted in significantly higher PELF C_{max} and AUC (Table 2). In contrast, terminal half-life and MRT in PELF were significantly shorter after nebulisation than after i.m. administration (Table 2). Average concentrations of DCA in PELF were higher after nebulisation than after i.m. administration and above the MIC_{90} of the drug against *S. zooepidemicus* for 24 h after administration by nebulisation (Fig 2). Cell concentration and differential cell count of foals nebulised with ceftiofur sodium were not significantly different from those of foals administered ceftiofur sodium i.m. and nebulised with 0.9% NaCl (Table 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 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 mature 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 microbiological 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 MIC_{90} for the entire dosing interval,

TABLE 1: Pharmacokinetic variables (mean ± s.d. unless otherwise specified*) for desfuroylceftiofur acetamide (DCA) in the plasma of foals after administration of the first and fifth dose of ceftiofur sodium (2.2 mg/kg bwt) by the i.m. (n = 6) or nebulised route (n = 6)

| Variable | Dose | Route | | P | | |
		i.m.	Nebulised	Dose	Route	Dose × route
λ_z (1/h)	1	0.097 ± 0.005^1	0.091 ± 0.026^1	<0.001	0.2	0.4
	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	0.09	0.2
	5	9.0 ± 0.8^2	11.3 ± 2.6^2			
C_{max} (mg/l)	1	$4.49 \pm 0.68^{a,1}$	$0.08 \pm 0.05^{b,1}$	<0.001	<0.001	0.08
	5	$6.15 \pm 0.75^{a,2}$	$0.15 \pm 0.12^{b,2}$			
C_{min} (mg/l)	1	$0.23 \pm 0.03^{a,1}$	<0.01 (<0.01–0.02)*^{b,1}	<0.001	<0.001	0.2
	5	$0.40 \pm 0.04^{a,2}$	$0.02 \pm 0.01^{b,2}$			
T_{max} (h)*	1	$1.25 (1.0–2.0)^1$	$1.5 (1.0–8.0)^1$	0.009	0.6	0.07
	5	$1.0 (1.0–1.5)^2$	$0.75 (0.25–1.0)^2$			
MRT (h)	1	$8.2 \pm 0.4^{a,1}$	$11.2 \pm 3.0^{b,1}$	<0.001	0.01	0.2
	5	$10.2 \pm 0.8^{a,2}$	$14.7 \pm 3.6^{b,2}$			
AUC_{0-t} (mg•h/l)	1	$29.31 \pm 3.14^{a,1}$	$0.64 \pm 0.52^{b,1}$	<0.001	<0.001	0.1
	5	$37.63 \pm 4.01^{a,2}$	$1.26 \pm 0.96^{b,2}$			
AUC_∞ (mg•h/l)	1	$31.70 \pm 3.38^{a,1}$	$0.82 \pm 0.55^{b,1}$	<0.001	<0.001	0.2
	5	$42.85 \pm 4.10^{a,2}$	$1.56 \pm 1.03^{b,2}$			

*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 vs. time curve from time 0 to the last quantifiable time point. AUC_∞ = area under the concentration vs. time curve extrapolated to infinity. [a,b]Different superscript letters within a given row indicate significant differences (P<0.05) between i.m and nebulised 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.

consistent with clinical efficacy arising from once daily dosing [15]. Hence, measurement of DCA is preferred for dose optimisation based on the 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 homogenisation 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
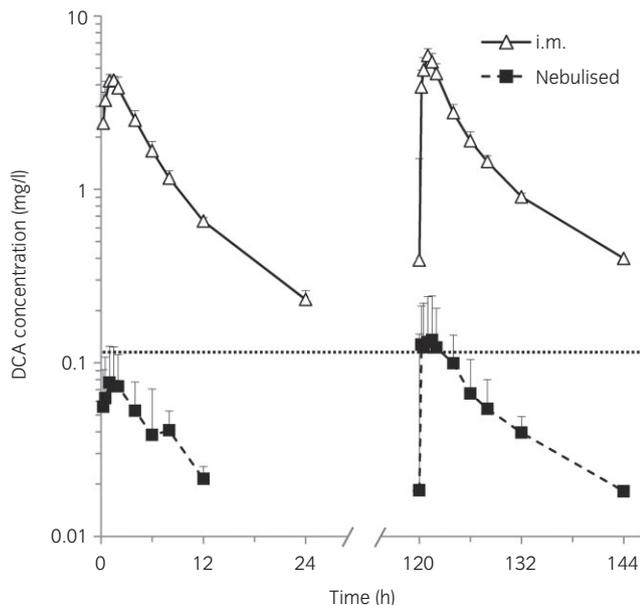

Fig 1: Mean (± s.d.) desfuroylceftiofur acetamide (DCA) concentrations in the plasma of foals after administration of the first and fifth dose of ceftiofur sodium (2.2 mg/kg bwt) by the i.m. (n = 6) or nebulised route (n = 6). The dotted horizontal line represents the minimum inhibitory concentration required to inhibit the growth of 90% of *Streptococcus equi* ssp.*zooepidemicus* (0.12 mg/l).

TABLE 2: Pharmacokinetic variables (mean ± s.d. unless otherwise specified*) for desfuroylceftiofur acetamide (DCA) in the pulmonary epithelial lining fluid (PELF) of foals after administration of ceftiofur sodium (2.2 mg/kg bwt) for 5 days by the i.m. (n = 6) or nebulised route (n = 6)

| Variable | Route | | P |
	i.m.	Nebulised	
λ_z (1/h)	0.078 ± 0.025	0.135 ± 0.051	0.03
$t_{1/2\lambda z}$ (h)	9.5 ± 2.4	5.8 ± 2.2	0.02
C_{max} (mg/l)	0.73 ± 0.73	4.52 ± 2.91	0.01
C_{min} (mg/l)	0.09 ± 0.02	0.16 ± 0.11	0.2
T_{max} (h)*	2.0 (2.0–2.0)	2.0 (2.0–2.0)	1.0
MRT (h)	13.4 ± 4.1	7.0 ± 3.7	0.02
AUC_{24h} (mg•h/l)	5.91 ± 3.28	24.14 ± 14.09	0.009
AUC_∞ (mg•h/l)	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 concentration; MRT = mean residence time; AUC_{24h} = area under the concentration vs. time curve for the first 24 after administration. AUC_∞ = area under the concentration vs. time curve extrapolated to infinity.

Fig 2: Mean (± s.d.) desfuroylceftiofur acetamide (DCA) concentrations in the pulmonary epithelial lining fluid of foals after administration of 5 doses of ceftiofur sodium (2.2 mg/kg bwt) by the i.m. (n = 6) or nebulised route (n = 6). The dotted horizontal line represents the minimum inhibitory concentration required to inhibit the growth of 90% of *Streptococcus equi* ssp. *zooepidemicus* (0.12 mg/l).
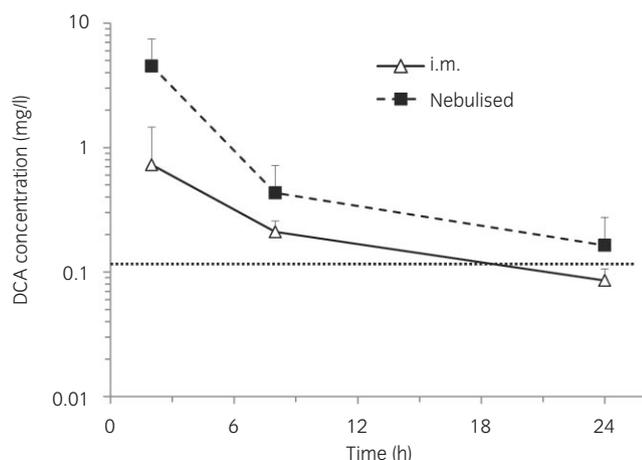
influenced the results of the present study because concentrations of DCA in equine bronchoalveolar cells are negligible and considerably below concentrations measured in PELF [10]. The plasma C_{max} obtained after i.m. administration of the first dose of ceftiofur sodium in the present study (4.49 ± 0.68 mg/l) was almost identical to that achieved after administration of the same dose of ceftiofur sodium to mature horses (4.46 ± 0.93 mg/l) [4]. Despite almost identical plasma concentrations, peak DCA concentration of lung tissue homogenate in the aforementioned study (1.40 ± 0.36 mg/l) [4] was considerably higher than peak PELF concentration in this study (0.73 ± 0.73 mg/l), suggesting that DCA concentrations in lung tissue homogenates overestimate PELF concentrations. In the present study, concentrations of DCA in PELF remained above the MIC_{90} of *S. zooepidemicus* (0.12 mg/l), *Pasteurella* spp. (<0.03 mg/l), and *Actinobacillus* spp. (<0.03 mg/l) [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 nebulised ceftiofur sodium in horses with bronchopneumonia. Peak concentrations of DCA in PELF achieved after i.m. 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 weanling foals (0.46 ± 0.03 mg/l) [10].

In healthy horses, nebulisation of 20 ml of the commercially available i.v. gentamicin sulfate solution (diluted to 50 g/l) using an ultrasonic nebuliser resulted in bronchial lavage fluid concentrations approximately 12 times higher than concentrations achieved by i.v. administration at a dose of 6.6 mg/kg bwt [20]. However, the major limitation to the use of aerosolised 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 nebulisation resulted in concentrations of DCA in PELF significantly (approximately 6-fold) higher than those obtained after administration by the i.m. 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.

Nebulisation of ceftiofur sodium resulted in low systemic bioavailability of DCA with plasma concentrations well-below therapeutic levels. Low systemic bioavailability after nebulisation of ceftiofur sodium has also been documented in calves [21]. Therefore, nebulisation of ceftiofur sodium increases the amount of drug that is present within the respiratory secretions while minimising the amount of drug in plasma, thereby decreasing the risk of systemic adverse effects. In an experimental model of *Escherichia coli* pneumonia in mechanically ventilated piglets, nebulisation 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 nebulisation than after i.v. 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, nebulisation may be more appropriate if used as an adjunct to systemic administration.

Use of systemic formulations of drugs for nebulisation can lead to exposure to potentially irritant substances, toxic additives and inappropriate pH or osmolality ranges. In the present study nebulisation 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 nebulised with ceftiofur sodium were not significantly different from those of foals nebulised with saline and were within established reference ranges for foals and mature horses [23–25]. Similarly, once daily nebulisation 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 nebuliser. 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 current 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 g/l or ceftiofur concentrations of 25 g/l produced the optimal combinations of particle size and aerosol density when using a medical ultrasonic nebuliser when compared with more concentrated solutions [29]. In the present study, dilution of the ceftiofur sodium concentration to 25 g/l did not improve particle size. Differences between the 2 studies likely relates to the type of nebuliser used. Nebulisation of a 50 g/l solution instead of 25 g/l offers the advantage of decreasing the time of administration in half.

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

Authors' declaration of interests

G.S. Grover and D.A. Merritt are employees of Zoetis.

Ethical animal research

The study was approved by the Animal Care and Use Committee at the University of Georgia.

TABLE 3: Cell concentration and differential cell count (mean ± s.d.) in bronchoalveolar fluid of foals after administration of ceftiofur sodium (2.2 mg/kg bwt) for 5 days by the i.m. (n = 6)* or nebulised route (n = 6)

	Route		
Variable	i.m.*	Nebulised	P
Cell concentration (× 10^6/l)	1054 ± 264	820 ± 346	0.2
Macrophages (%)	60.3 ± 8.6	64.9 ± 3.0	0.3
Lymphocytes (%)	38.4 ± 4 8	32.8 ± 4.2	0.2
Neutrophils (%)	1.3 ± 1.6	2.3 ± 2.8	0.5
Eosinophils (%)	0	0	NA
Mast cells (%)	0	0	NA

*Foals administered ceftiofur sodium i.m. were nebulised with 0.9% NaCl.

Manufacturers' addresses

[a]Zoetis, Madison, New Jersey, USA.
[b]Nortev Ltd, Galway, Ireland.
[c]Malvern Instrument Limited, Malvern, Worcestershire, UK.
[d]Jorgenson Laboratories, Loveland, Colorado, USA.
[e]Nexcelom Bioscience, Lawrence, Massachusetts, USA.
[f]Thermo Fisher Pittsburg, Pennsylvania, USA.
[g]Fisher Scientific, Pittsburg, Pennsylvania, USA.
[h]Biochain, Hayward, California, USA.
[i]Summit Research Services, Montrose, Colorado, USA.

References

1. Cohen, N.D. (1994) Causes of and farm management factors associated with disease and death in foals. *J. Am. Vet. Med. Ass.* **204**, 1644-1651.
2. Hoffman, A.M., Viel, L., Juniper, E. and Prescott, J.F. (1993) Clinical and endoscopic study to estimate the incidence of distal respiratory tract infection in thoroughbred foals on Ontario breeding farms. *Am. J. Vet. Res.* **54**, 1602-1607.
3. Hoffman, A.M., Viel, L. and Prescott, J.F. (1993) Microbiologic changes during antimicrobial treatment and rate of relapse of distal respiratory tract infections in foals. *Am. J. Vet. Res.* **54**, 1608-1614.
4. Jaglan, P.S., Roof, R.D., Yein, F.S., Arnold, T.S., Brown, S.A. and Gilbertson, T.J. (1994) Concentration of ceftiofur metabolites in the plasma and lungs of horses following intramuscular treatment. *J. Vet. Pharmacol. Ther.* **17**, 24-30.
5. Salmon, S.A., Watts, J.L. and Yancey, R.J., Jr (1996) *In vitro* activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J. Vet. Diagn. Invest.* **8**, 332-336.
6. Meyer, S., Giguère, S., Rodriguez, R., Zielinski, R.J., Grover, G.S. and Brown, S.A. (2009) Pharmacokinetics of intravenous ceftiofur sodium and concentration in body fluids of foals. *J. Vet. Pharmacol. Ther.* **32**, 309-316.
7. Valcke, Y., Pauwels, R. and Van der Straeten, M. (1990) Pharmacokinetics of antibiotics in the lungs. *Eur. Respir. J.* **3**, 715-722.
8. Drusano, G.L. (2005) Infection site concentrations: their therapeutic importance and the macrolide and macrolide-like class of antibiotics. *Pharmacotherapy* **25**, 150S-158S.
9. Sustronck, B., Deprez, P., Muylle, E., Vermeersch, H., Vandenbossche, G. and Remon, J.P. (1995) Evaluation of the nebulisation of sodium ceftiofur in the treatment of experimental *Pasteurella haemolytica* bronchopneumonia in calves. *Res. Vet. Sci.* **59**, 267-271.
10. Credille, B.C., Giguère, S., Berghaus, L.J., Burton, A.J., Sturgill, T.L., Grover, G.S., Donecker, J.M. and Brown, S.A. (2012) Plasma and pulmonary disposition of ceftiofur and its metabolites after intramuscular administration of ceftiofur crystalline free acid in weanling foals. *J. Vet. Pharmacol. Ther.* **35**, 259-264.
11. Rennard, S.I., Basset, G., Lecossier, D., O'Donnell, K.M., Pinkston, P., Martin, P.G. and Crystal, R.G. (1986) Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J. Appl. Physiol.* **60**, 532-538.
12. Auckenthaler, R. (2002) Pharmacokinetics and pharmacodynamics of oral beta-lactam antibiotics as a two-dimensional approach to their efficacy. *J. Antimicrob. Chemother.* **50**, *Suppl.*, 13-17.
13. Liu, P., Muller, M. and Derendorf, H. (2002) Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. *Int. J. Antimicrob. Agents* **19**, 285-290.
14. Clarke, C.R., Brown, S.A., Streeter, R.N., Clarke, J.M., Hamlow, P.J., Callahan, J.K., Hubbard, V.L., Speedy, A.K. and Burrows, G.E. (1996) Penetration of parenterally administered ceftiofur into sterile vs. *Pasteurella haemolytica*-infected tissue chambers in cattle. *J. Vet. Pharmacol. Ther.* **19**, 376-381.
15. Brown, S.A., Jaglan, P.S. and Banting, A. (1991) Ceftiofur sodium: disposition, protein-binding, metabolism, and residue profile in various species. *Acta Vet. Scand., Suppl.* **87**, 97-99.
16. Nix, D.E., Goodwin, S.D., Peloquin, C.A., Rotella, D.L. and Schentag, J.J. (1991) Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob. Agents Chemother.* **35**, 1947-1952.
17. Mouton, J.W., Theuretzbacher, U., Craig, W.A., Tulkens, P.M., Derendorf, H. and Cars, O. (2008) Tissue concentrations: do we ever learn? *J. Antimicrob. Chemother.* **61**, 235-237.
18. Kiem, S. and Schentag, J.J. (2008) Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrob. Agents Chemother.* **52**, 24-36.
19. Bade, D.J., Sibert, G.J., Hallberg, J., Portis, E.S., Boucher, J. and Bryson, L. (2009) Ceftiofur susceptibility of *Streptococcus equi* subsp *zooepidemicus* isolated from horses in North America between 1989 and 2008. *Vet. Ther.* **10**, E1-E7.
20. McKenzie, H.C., III and Murray, M.J. (2000) Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration of gentamicin to horses. *Am. J. Vet. Res.* **61**, 1185-1190.
21. Vermeersch, H., Vandenbossche, G., Remon, J.P., Samyn, W., Vandendriessche, K., Sustronck, B., Muylle, E. and Deprez, P. (1996) Pharmacokinetics of nebulized sodium ceftiofur in calves. *J. Vet. Pharmacol. Ther.* **19**, 152-154.
22. Goldstein, I., Wallet, F., Nicolas-Robin, A., Ferrari, F., Marquette, C.H. and Rouby, J.J. (2002) Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. *Am. J. Respir. Crit. Care Med.* **166**, 1375-1381.
23. Balson, G.A., Smith, G.D. and Yager, J.A. (1997) Immunophenotypic analysis of foal bronchoalveolar lavage lymphocytes. *Vet. Microbiol.* **56**, 237-246.
24. Flaminio, M.J., Rush, B.R., Davis, E.G., Hennessy, K., Shuman, W. and Wilkerson, M.J. (2000) Characterization of peripheral blood and pulmonary leukocyte function in healthy foals. *Vet. Immunol. Immunopathol.* **73**, 267-285.
25. Sweeney, C.R., Rossier, Y., Ziemer, E.L. and Lindborg, S. (1992) Effects of lung site and fluid volume on results of bronchoalveolar lavage fluid analysis in horses. *Am. J. Vet. Res.* **53**, 1376-1379.
26. McKenzie, H.C., III and Murray, M.J. (2004) Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days. *Am. J. Vet. Res.* **65**, 173-178.
27. Art, T., Ramery, E. and Fraipont, A.L.P. (2010) Pulmonary function, airway cytology and bronchoalveolar lavage fluid drug concentration after aerosol administration of cefquinome to horses. *Equine Vet. Educ.* **22**, 473-479.
28. Diot, P., Dequin, P.F., Rivoire, B., Gagnadoux, F., Faurisson, F., Diot, E., Boissinot, E., Le, P.A., Palmer, L. and Lemarie, E. (2001) Aerosols and anti-infectious agents. *J. Aerosol Med.* **14**, 55-64.
29. McKenzie, H.C. (2003) Characterization of antimicrobial aerosols for administration to horses. *Vet. Ther.* **4**, 110-119.