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Pharmacokinetic and Pharmacodynamic Profiles of Danofloxacin Administered by Two Dosing Regimens in Calves Infected with *Mannheimia* (*Pasteurella*) *haemolytica*

Patxi Sarasola,[1] Peter Lees,[2] Fariborz Shojaee AliAbadi,[3]† Quintin A. McKellar,[4] William Donachie,[4] Kate A. Marr,[5] Simon J. Sunderland,[5]* and Tim G. Rowan[5]

Ondax Scientific, 20280 Hondarribia, Gipuzkoa, Spain[1]; Ministry of Jahad-e-Keshavarzi, Veterinary Organisation of I. R. Iran, Tehran, Iran[3]; and The Royal Veterinary College, Hawkshead Campus, North Mymms, Hatfield, Hertfordshire,[2] The Moredun Foundation, Pentlands Science Park, Bush Loan, Penicuik, Midlothian,[4] and Pfizer Global Research and Development, Sandwich, Kent,[5] United Kingdom

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The pharmacokinetics and pharmacodynamics of danofloxacin in calves with induced *Mannheimia* (*Pasteurella*) *haemolytica* pneumonia were evaluated. Calves received either saline as an intravenous (IV) bolus or danofloxacin (0.738 mg/kg of body weight) administered as either a single IV bolus or a 36-h continuous IV infusion. Blood samples and bronchial secretions were collected before and at predetermined times over 48 h following the start of treatment. Calves were assessed clinically throughout, and lung consolidation was assessed at necropsy. Bronchial secretions and lung tissue were cultured for *M. haemolytica*. Bolus administration of danofloxacin produced a high maximum drug concentration-to-MIC ratio (C_{max}:MIC) of 14.5 and a time period of 9.1 h when plasma danofloxacin concentrations exceeded the MIC ($T>$MIC). Following danofloxacin infusion, the C_{max}:MIC was low (2.3), with a long $T>$MIC (33.3 h). The area under the curve-to-MIC ratios were 43.3 and 49.1 for the bolus and infusion administrations, respectively. The single bolus of danofloxacin was more effective than the same dose administered by continuous infusion, as indicated by a significantly lower ($P < 0.05$) number of animals with *M. haemolytica* in bronchial secretions after treatment and lower rectal temperatures in the 24 h after the start of treatment. Thus, danofloxacin exhibited concentration-dependent antimicrobial activity in cattle with respiratory disease caused by *M. haemolytica*.

Danofloxacin is a fluoroquinolone antimicrobial drug with rapid bactericidal activity against a broad range of pathogens responsible for a number of disease syndromes of economic importance in the commercial rearing of livestock (8, 15). Since their introduction in the late 1980s, fluoroquinolones have been shown by a number of studies to exhibit concentration-dependent bactericidal activity, whereby the optimal effect is attained by the administration of high doses over a short period (4, 6, 14). This is a property shared by the aminoglycosides but is in contrast to the predominantly time-dependent bactericidal action shown by the β-lactam antibiotics (3), where the time that bacteria are exposed to antimicrobial concentrations exceeding the MIC ($T>$MIC) is the major determinant of efficacy. These different types of action have been confirmed for danofloxacin and amoxicillin in an in vitro pharmacodynamic model against *Actinobacillus pleuropneumoniae* (9).

The purpose of this study was to establish the pharmacokinetic and pharmacodynamic properties of danofloxacin in vivo by using an experimental model of calf pneumonia and to determine whether the concentration-dependent activity of danofloxacin in cattle operates under simulated clinical conditions. A fixed equal total dose of danofloxacin was administered either as a single intravenous (IV) bolus or by continuous infusion over a 36-h period, and the clinical and bacteriological outcomes in calves with induced infections of *Mannheimia* (*Pasteurella*) *haemolytica* were compared. The study was conducted in compliance with Good Clinical Practice guidelines (5), the analysis of samples was conducted in accordance with Good Laboratory Practice guidelines (16), and the husbandry of all animals was in compliance with the requirements of national legislation and local animal welfare guidelines. The study was conducted under veterinary supervision, with veterinary attention available at all times.

MATERIALS AND METHODS

Animals. Thirty-three male Friesian calves (approximately 11 to 13 weeks of age, with initial body weights of 66.5 to 106 kg) were enrolled in the study and inoculated with *M. haemolytica*. Prior to enrollment, the calves were free from preexisting medical or surgical conditions and had no history of previous respiratory disease. Following enrollment and prior to inoculation, the calves were allocated randomly to pens and to treatment groups by using an incomplete block design. The calves were housed in straw-bedded pens in a self-contained naturally ventilated calf rearing unit with a common airspace, but divided by solid partitions approximately 1.4-m high to prevent nasal contact between animals, and with 4.4 m^2 of floor space per calf. Water was supplied ad libitum, and the calves were maintained on an antibiotic-free concentrate diet following weaning at 6 to 7 weeks of age. On arrival at the study site, at approximately 1 week of age, each calf received an intramuscular injection (20 mg/kg of body weight) of long-acting oxytetracycline (Terramycin LA; Pfizer Ltd., Sandwich, United King-

* Corresponding author. Mailing address: Global Research and Development, Pfizer Ltd., Sandwich, Kent, CT13 9NJ United Kingdom. Phone: 44 1304 646617. Fax: 44 1304 651251. E-mail: simon_sunderland@sandwich.pfizer.com.

† Present address: The Royal Veterinary College, Hawkshead Campus, North Mymms, Hatfield, Hertfordshire, United Kingdom.

dom). There was no further antibiotic administration prior to enrollment and treatment with the test materials.

Inoculum. The inoculum was prepared by transferring a 1.0-ml aliquot of *M. haemolytica* type A1 (reference M7/2) into 9.0 ml of Oxoid nutrient broth no. 2. After incubation at 37°C for 16 to 18 h, the starter culture was inoculated into 290 ml of nutrient broth, shaken at 150 rpm, and incubated for 4 h to provide 300 ml of culture. Following incubation, the approximate viable count (CFU per milliliter) was determined by using a McFarland scale. Just prior to inoculation, the broth culture was diluted in 1-liter volumes of sterile phosphate-buffered saline (0.01 M, pH = 7.4, prewarmed to 37°C) to give an inoculum with an approximate viable count of 3.3×10^5 CFU/ml. The titer of the inoculum was confirmed pre- and postinoculation by culture on blood agar, following serial 10-fold dilutions, and the CFU per milliliter were calculated by multiplying the number of colonies by the relevant dilution factor.

Design. Animals selected for the study were each inoculated by endobronchial deposition (calves were conscious) over a period of approximately 1 min with 300 ml of the inoculum, representing an inoculum per animal of approximately 10^8 CFU of *M. haemolytica* type A1/calf (acceptable range was defined as 5×10^7 to 5×10^8 CFU/calf). A fiber-optic endoscope sterilized with ethylene oxide prior to the start of inoculation was inserted nasally and passed via the nasopharynx into the trachea. At the tracheal bifurcation, the endoscope was pushed approximately 10 cm into the principal bronchus where the inoculum was deposited.

Respiratory rates were assessed hourly from approximately 3 h after the inoculation of the first calf. When the respiratory rates of over 72% of the inoculated calves had doubled from those recorded immediately prior to inoculation, pretreatment samples (blood and bronchial secretion) were collected and the animals were administered their allocated treatment.

Prior to administration, the commercial danofloxacin 18% formulation (Advocin 180; Pfizer Ltd.) was diluted with 0.9% (wt/vol) sodium chloride to give solutions containing 6.0 and 0.75 mg of danofloxacin/ml for use as test materials. Sodium chloride (0.9% [wt/vol]) was also administered as a negative control.

Calves were randomly allocated to one of three treatments (11 animals per treatment): danofloxacin (6 mg/ml) administered as a single IV bolus injection at 0.738 mg/kg; danofloxacin (0.75 mg/ml) administered as a continuous IV infusion over 36 h to give a total infused dose of 0.645 mg/kg, following an initial small IV bolus of danofloxacin (6 mg/ml) at 0.093 mg/kg, to give a total dose of 0.738 mg of danofloxacin/kg; or saline at 0.123 ml/kg as a single IV bolus injection to give a dose volume equivalent to that of the danofloxacin bolus treatment. The dose regimen for the danofloxacin infusion was calculated by pharmacokinetic modeling of previously obtained plasma concentration-time data as the dose which would provide a steady-state concentration in plasma slightly exceeding the MIC for the *M. haemolytica* strain used in the respiratory infection model (MIC = 30 ng/ml) when infused continuously over a 36-h period. Time zero was defined individually for each calf as the time when treatment administration commenced. Injections were administered with one or two 10-ml disposable syringes via an IV catheter placed immediately prior to treatment or with two 1-ml disposable syringes via the indwelling catheter in animals to be infused. The infusion was delivered by an ambulatory infusion pump (CADD-PLUS model 5400; SIMS Deltec, Inc., Minneapolis, Minn.) attached to the calf's back with a specially designed pouch and harness. An 18-gauge indwelling catheter (Leader-Flex [PUR] Seldinger catheter; Vygon UK Ltd., Cirencester, United Kingdom) was placed in one jugular vein, with local anesthetic being used, and secured by cutaneous sutures. The pump was connected to the IV catheter in the jugular vein via an opaque sterile giving set (1.5-m length of tubing, dead volume of 1.3 ml; Becton Dickinson, Oxford, United Kingdom). The tubing was secured to the animal with sutures and tape and included several loops to relieve tension. Prior to connection of the pump, the initial single bolus was administered via the catheter, followed by administration of 5 to 10 ml of saline. The pump was then connected to the catheter, and the infusion was initiated. The pump was set to deliver the dose volume at a set rate for each animal such that the total dose was delivered over exactly 36 h. The body weight recorded for each animal prior to inoculation was used to determine the dosages administered and the infusion rate.

Clinical observations. Clinical observations for signs of bovine respiratory disease and measurement of rectal temperature were carried out immediately prior to inoculation, immediately prior to treatment administration at time zero, and at 4, 8, 12, 24, 36, and 48 h thereafter. The respiratory rate, rectal temperature, character of respiration, and general demeanor were assessed for each calf. The veterinarian making these observations was unaware of the treatment allocations of the animals receiving the bolus treatments (saline and danofloxacin), although the presence of the infusion apparatus made masking impossible for calves receiving the danofloxacin infusion.

Any calf which was recumbent and showed severe depression and/or signs of respiratory distress was immediately euthanatized on welfare grounds. All calves were euthanatized after the final sampling (48 h after the start of treatment). At necropsy, a score of the percentage of lung consolidation was estimated from the extent of visible consolidation, both dorsally and ventrally, as a percentage of the total lung surface area. The necropsies were performed by an experienced pathologist who was unaware of the treatment allocation. In addition, lung samples were collected at necropsy for determination of viable counts of *M. haemolytica*.

Sample collection and handling. Blood samples were collected immediately prior to treatment and at approximately 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h following single-bolus administration. For animals receiving the continuous infusion, blood samples were collected immediately before treatment and then approximately 15 and 30 min and 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 36.5, 37, 38, 42, and 48 h after the start of administration of the small loading bolus. Blood samples (5 ml) were collected by jugular venipuncture of the vein contralateral to that used for treatment administration, except for two samples (at 4 and 10 h) from one calf receiving the danofloxacin single bolus, where the ipsilateral vein was used due to sampling difficulties. Samples were collected using a 1-inch by 20-gauge sterile needle and were then deposited into blood tubes with heparin anticoagulant. The blood tubes were protected from light and were centrifuged at approximately $1{,}400 \times g$ for 10 min, and the supernatant was transferred by pipette into duplicate plastic tubes. Plasma samples were stored at approximately −20°C prior to assay. Measurement of concentrations of danofloxacin and its active metabolite *N*-desmethyldanofloxacin in plasma was performed by using a validated high-pressure liquid chromatography method with solid-phase extraction and fluorescence detection (20). The limit of quantification (LOQ) was 10 ng/ml.

Bronchial secretion samples were collected from each animal by using an established, well-tolerated method (13). Samples were collected following endotracheal intubation approximately 16 h before inoculation, immediately before treatment administration began, and at approximately 1, 3, 6, 12, 18, 24, 36, and 48 h thereafter for determination of viable counts of *M. haemolytica*. An absorbent cotton fabric plug, fixed to the tip of a solid flexible polyethylene rod, was placed inside a sterile disposable stomach tube (one per animal for each sample) and inserted into the trachea orally by using a laryngoscope and a gag. At the tracheal bifurcation, the rod was pushed approximately 10 cm beyond the end of the stomach tube and into the principal bronchus. After a maximum residence time in the bronchus of 2 min, the absorbent plug was withdrawn into the stomach tube and the device was removed from the animal. The absorptive cotton plug was placed in a disposable syringe, and the bronchial secretion was extracted into an inert plastic sample tube by manual pressure. Sample tubes were placed on ice and kept in the dark until transferred to the laboratory within 1 h for immediate processing to determine the viable bacterial cell counts.

Following euthanasia, tissue samples of approximately 0.5 g each were excised from eight standard sites in the lung and pooled to give four samples per calf. Each pooled sample was weighed, placed in a separate stomacher bag with 9.0 ml of peptone water, and homogenized.

M. haemolytica counts were performed for each bronchial secretion sample and each lung homogenate following serial 10-fold dilutions. Duplicate aliquots of each dilution were cultured overnight at 37°C on blood agar, and the mean colony count was used to determine the mean viable count of each sample. The four pooled lung samples per calf were counted separately, and an overall mean was calculated for each calf.

Data analysis. A logarithmic transformation (log [bacteria count + 1]) was applied to the *M. haemolytica* counts (in both bronchial secretion and lung tissue samples) prior to analysis. Bronchial secretion bacterial counts, respiration rates, and rectal temperatures were analyzed by using a repeated measurement model with the pretreatment value as a covariate. Lung lesion scores and lung tissue bacterial counts were analyzed by using a general linear model. A categorical analysis for repeated measurements was carried out separately for the clinical scores for respiration and demeanor. The proportion of animals completing the study in each treatment group (i.e., treatment successes) was calculated as the number of calves completing the study at 48 h multiplied by 100 and divided by the difference between the number of calves treated and the number of calves removed from the study for reasons not related to respiratory disease. The proportion of animals completing the study (treatment successes) and the proportion of animals with positive *M. haemolytica* counts in posttreatment bronchial secretions and in lung tissue samples were analyzed by using a logistic model. A priori contrasts were used to assess differences between treatments. The 5% level of significance ($P \leq 0.05$) was used to assess statistical differences for all tests.

Pharmacokinetic analysis. Pharmacokinetic analyses were performed by using WinNonlin version 1.1 (Scientific Consulting Inc., Cary, N.C.). For calves receiving danofloxacin by single bolus or infusion, plasma danofloxacin concentrations were used to determine the concentration in plasma at time zero (C_p^0); the

TABLE 1. Mean pharmacokinetic parameters for danofloxacin in cattle with respiratory disease following administration either as a single bolus or as a continuous infusion

Danofloxacin administration[a]	Pharmacokinetic parameter (mean [± SD])									
	C_p^0 (ng/ml)	$C_{max(obs)}$ (ng/ml)	$C_{max(obs)}$:MIC	AUC_{0-t} (ng · h/ml)	$AUC_{0-\infty}$ (ng · h/ml)	AUC:MIC	CL_b (ml/h · kg)	*T*>MIC (h)	λ_z (h^{-1})	$t_{1/2}$[b] (h)
Single bolus	589 (±86.7)	436 (±52.2)	14.5 (±1.7)	1,190 (±189)	1,298 (±195)	43.3 (±6.5)	578.2 (±71.3)	9.1 (±1.6)	0.16 (±0.02)	4.3
Continuous infusion	85.8 (±9.2)	69.0 (±5.8)	2.3 (±0.2)	1,412 (±166)	1,472 (±177)	49.1 (±5.9)	507.8 (±61.5)	33.3 (±8.3)	0.31 (±0.08)	2.3

[a] Eleven animals in each treatment, but two animals were withdrawn from the infusion treatment for welfare reasons and are only included in the calculation of C_p^0, $C_{max(obs)}$, and $C_{max(obs)}$:MIC.
[b] $t_{1/2}$ was calculated as a harmonic mean.

maximum observed concentration in plasma [$C_{max(obs)}$]; the total body clearance (CL_b), calculated as the dose administered divided by the area under the curve from time zero to infinity ($AUC_{0-\infty}$); and the terminal elimination rate constant (λ_z), calculated from regression analysis of log concentrations over time. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The linear trapezoidal rule was used to calculate the AUC_{0-t} (where *t* is the last time of measurable plasma concentrations). The MIC for the *M. haemolytica* strain used as the inoculum was 30 ng/ml; hence, the *T*>MIC was the time during which plasma danofloxacin concentrations exceeded 30 ng/ml. Plasma $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t was the last measurable plasma danofloxacin concentration. The ratio of AUC to MIC (AUC:MIC) was calculated as $AUC_{0-\infty}$/MIC. The plasma $C_{max(obs)}$:MIC was also calculated. All pharmacokinetic parameters and concentrations of danofloxacin were calculated for individual animals and are presented as means ± standard deviations (SD), except for $t_{1/2}$ values, which were calculated as harmonic means.

RESULTS

The mean pharmacokinetic values for danofloxacin in treated animals are presented in Table 1. Two calves receiving continuous-infusion treatment were not included in the calculation of mean pharmacokinetic parameters [except for C_p^0, $C_{max(obs)}$, and $C_{max(obs)}$:MIC], since they were withdrawn from the study on welfare grounds related to severe respiratory disease and the infusion was terminated prior to the 36-h assessment time. When danofloxacin was administered as a single IV bolus, peak plasma drug concentrations were obtained at the first sampling time point, 15 min after bolus administration, with a mean $C_{max(obs)}$ of 436 ng/ml and an extrapolated C_p^0 of 589 ng/ml (Fig. 1). Following rapid distribution, danofloxacin was eliminated, with an overall mean $t_{1/2}$ of 4.3 h.

The continuous IV infusion of danofloxacin was preceded by the administration of an initial small IV bolus to rapidly achieve the target steady-state plasma drug concentration of ≥30 ng/ml. Peak plasma danofloxacin concentrations were detected at the first time point, 15 min after administration of the small bolus, with a mean $C_{max(obs)}$ of 69.0 ng/ml and an extrapolated C_p^0 of 85.8 ng/ml. Danofloxacin concentrations declined rapidly until steady-state concentrations slightly higher than 30 ng/ml were achieved approximately 4 h after commencement of infusion and were maintained at this level over the 36-h infusion period with only minor fluctuations (Fig. 1). Following the end of the IV infusion at 36 h, the pump was disconnected and plasma danofloxacin concentrations declined, with a mean $t_{1/2}$ of 2.3 h.

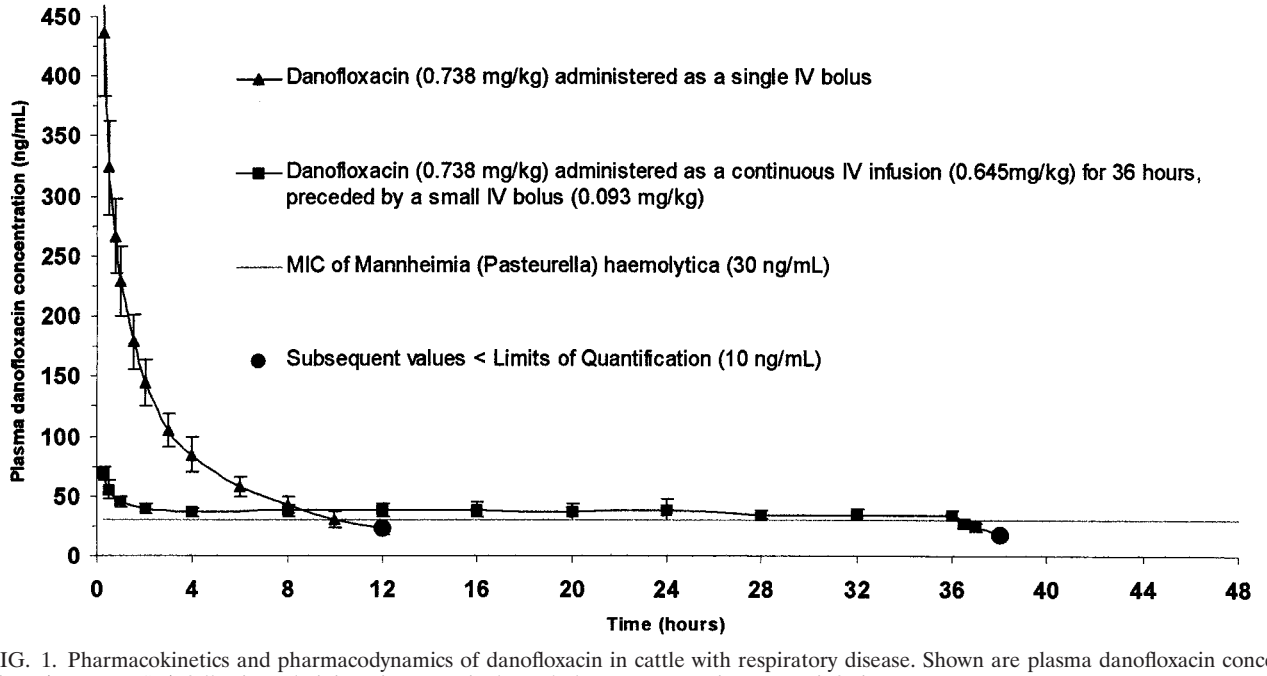

FIG. 1. Pharmacokinetics and pharmacodynamics of danofloxacin in cattle with respiratory disease. Shown are plasma danofloxacin concentrations (means ± SD) following administration as a single IV bolus or as a continuous IV infusion.

TABLE 2. Lung lesion scores and classification of animals by presence of *M. haemolytica* in posttreatment bronchial secretions and in lung tissue samples

Treatment[d]	Lung lesion score[a]	No. of animals with *M. haemolytica* present in:	
		Bronchial secretion[b]	Lung tissue
Saline	19.5	10 (90.9)	7 (63.6)
Danofloxacin (single bolus)	13.4	1 (9.1)	0 (0)[c]
Danofloxacin (continuous infusion)	13.5	5 (45.5)	2 (18.2)[c]

[a] Calculated as percentage of lung consolidation of the total lung surface area.
[b] *M. haemolytica* isolated from bronchial secretion samples at one or more posttreatment assessment times. Values were significantly different from each other ($P < 0.05$).
[c] Significantly different from the value obtained with the saline treatment ($P < 0.05$).
[d] Eleven animals were enrolled in each treatment group.

The administration of danofloxacin as a single IV bolus produced a high C_{max}:MIC of 14.5 and a relatively short $T>$MIC of 9.1 h (Table 1). In contrast, when danofloxacin was administered as an IV infusion over 36 h, a low C_{max}:MIC of 2.3 was obtained and steady-state plasma danofloxacin concentrations were maintained above the MIC for a prolonged period, resulting in a $T>$MIC of 33.3 h. The steady-state concentrations achieved were as intended, i.e., slightly in excess of the MIC for *M. haemolytica*, 30 ng/ml (Fig. 1). The plasma AUC:MIC values for the single IV bolus and the continuous-infusion danofloxacin treatments were similar, 43.3 and 49.1, respectively (Table 1). *N*-Desmethyldanofloxacin was not detected at measurable concentrations (LOQ = 20 to 50 ng/ml) at any time point in any animal in the study, and danofloxacin was not detected in any sample from a control animal.

The number of animals withdrawn from the study for welfare reasons due to severe respiratory disease was greater in the saline treatment group (5 of 11, 45.5%) than in either the danofloxacin single-bolus (0 of 11, 0%) or the danofloxacin continuous-infusion (2 of 11, 18.2%) treatment group, although the difference was only statistically significant ($P = 0.0037$) for the comparison between the saline and the danofloxacin single-bolus treatment groups. There were significant reductions in the number of animals with *M. haemolytica* in bronchial secretions ($P \leq 0.0173$) and in lung tissue samples ($P \leq 0.0266$) for each of the danofloxacin treatment groups compared with the saline treatment group (Table 2). In addition, the number of animals with *M. haemolytica* in bronchial secretions was significantly lower ($P = 0.0477$) for the danofloxacin single-bolus treatment group than for the danofloxacin continuous-infusion treatment group. There were no significant differences in the lung lesion scores between the treatment groups.

M. haemolytica was isolated from bronchial secretion samples in some of the animals in the saline treatment group at each assessment time after treatment and in the majority of postmortem lung tissue samples in these animals. In comparison, *M. haemolytica* was isolated only at a very low count from one animal at 48 h after administration of a single bolus of danofloxacin, and low counts were recovered from five animals in the continuous-infusion treatment group. Compared with the saline treatment, each of the danofloxacin treatments resulted in significantly lower *M. haemolytica* counts in bronchial secretions ($P \leq 0.0001$) collected from 3 to 48 h, inclusive, and in lung tissue samples ($P \leq 0.0053$); however, the differences between the results for the two danofloxacin treatment regimens were not significant (Table 3).

Animals treated with each of the danofloxacin regimens showed greater clinical improvements (character of respiration and general demeanor) than those in the saline control group (Tables 4 and 5), and animals treated with single-bolus administrations of danofloxacin tended to have greater clinical improvement than those treated with the continuous infusion. Compared with animals in the saline treatment group, the animals in both danofloxacin treatment groups showed a significant improvement in demeanor from 0 to 48 h ($P \leq 0.0097$); although the demeanor of the animals in the continuous-infusion treatment group was significantly different from that of the animals in the saline control group from 0 to 24 h ($P \leq 0.0151$), the demeanor of the animals in the single-bolus treatment group was not. However, for improvement in the character of respiration, the single-bolus treatment produced significantly better results than the saline treatment at 24 and 48 h ($P \leq 0.0416$). The difference between the results with continuous-infusion treatment and saline treatment was not significant. There were no significant differences in rectal temperatures between animals receiving the saline and continuous-infusion treatments; however, for those receiving the single-bolus treatment, rectal temperatures were significantly lower than for those receiving saline treatment ($P \leq 0.0167$) at 12 and 24 h and significantly lower ($P \leq 0.0075$) than for those receiving the infusion at 8, 12, and 24 h after the commencement of treatment.

Respiratory rates were generally lower in animals in either danofloxacin treatment group than in animals in the saline control group, except at the 36- and 48-h time points. The reduction in respiratory rate was greatest at 8 and 12 h for animals receiving the single bolus, but this result was not significantly different from that seen with animals receiving saline. At 36 and 48 h, the respiratory rates for animals receiving

TABLE 3. Geometric mean *M. haemolytica* counts in bronchial secretions and lung tissue samples

Treatment	*M. haemolytica* count (CFU/ml) in bronchial secretions at time (h)[a]:								*M. haemolytica* count in lung tissue (CFU/g)
	1	3	6	12	18	24	36	48	
Saline	2.0	1.2×10^3	1.6×10^5	4.4×10^5	1.6×10^7	1.8×10^7	1.3×10^6	2.3×10^7	1.9×10^4
Danofloxacin (single bolus)	0	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	6.1[b]	0[b]
Danofloxacin (continuous infusion)	0	0[b]	2.7[b]	0[b]	1.2[b]	0.8[b]	4.3[b]	9.1[b]	5.6[b]

[a] Treatment commenced at time zero.
[b] Significantly different from the value obtained with the saline treatment ($P < 0.05$).

TABLE 4. Frequency distribution of clinical signs (assessment of demeanor)

Treatment[a]	No. of calves in each assessment category at each time point after treatment[b]								
	0 h			24 h			48 h		
	Normal	Depressed	Recumbency/ withdrawn	Normal	Depressed	Recumbency/ withdrawn	Normal	Depressed	Recumbency/ withdrawn
Saline	3	8	0	5	3	3	6	0	5
Danofloxacin (single bolus)	5	6	0	11	0	0	11	0	0
Danofloxacin (continuous infusion)	1	8	2[c]	9	1	1	9	0	2

[a] The results for the saline treatment group were significantly different from those for the single-bolus treatment group from 0 to 48 h, but not from 0 to 24 h, and from those for the continuous-infusion treatment group from 0 to 24 h and from 0 to 48 h. Results for the two danofloxacin treatment groups were not significantly different over time.
[b] Each treatment group consisted of 11 calves.
[c] Significantly different from the result obtained with the single-bolus treatment group ($P < 0.05$).

single-bolus danofloxacin treatment were higher than for those receiving either saline or continuous-infusion treatments; however, at these time points, no animals had been withdrawn from the single-bolus treatment group, while four and five calves were withdrawn (at 36 and 48 h, respectively) from the saline treatment group and two calves were withdrawn from the continuous-infusion treatment group because they showed severe respiratory disease. Thus, the interpretation of data between treatments at these time points is difficult as only those calves remaining in the study were included for saline and danofloxacin infusion, so the results from the early time points (0 to 24 h) give a more accurate indication of the clinical response.

DISCUSSION

The pharmacokinetics of danofloxacin has been investigated in ruminant species including cattle (7, 8, 10), sheep (12), and goats (19). Following administration of danofloxacin, there is rapid distribution to the lungs (12) and high tissue concentrations are achieved in pneumonic lung, including areas of consolidation (1, 2). Danofloxacin has a broad range of activity against bacteria and mycoplasmas involved in bovine respiratory disease and is known to have a rapid bactericidal effect in vitro against *M. haemolytica* (15). The concentration-dependent killing profile is associated with a relatively prolonged postantibiotic effect (18).

The purpose of this study was to evaluate the pharmacokinetic and pharmacodynamic characteristics of danofloxacin in an in vivo model of *M. haemolytica* pneumonia in calves. This evaluation was carried out by comparing the clinical and bacteriological outcomes of two regimens with predetermined equal total doses of danofloxacin administered to calves with a respiratory infection, thus establishing whether or not danofloxacin exhibits a concentration-dependent bactericidal effect in cattle. The same total dose of danofloxacin (0.738 mg/kg) was administered either as a single IV bolus injection or as a prolonged continuous IV infusion. The single IV bolus of danofloxacin was predicted to give a high C_{max}:MIC and a short $T>$MIC, while the IV infusion of danofloxacin was predicted to give a low C_{max}:MIC and a long $T>$MIC.

As predicted, for those calves receiving the danofloxacin infusion treatment, concentrations in plasma were maintained above the MIC for the majority of the 36-h period ($T>$MIC = 33.3 h). In contrast, for those calves receiving the single bolus of danofloxacin, concentrations in plasma exceeded the MIC for less than 10 h posttreatment. The initial distribution of danofloxacin following the single IV bolus was rapid, and this was followed by an elimination phase which was monitored for 12 h postadministration, after which concentrations fell below the LOQ (Fig. 1). The graph shown in Fig. 1 suggests a multiexponential elimination curve with a terminal elimination phase slope evident after 2 to 3 h postadministration. For calves receiving danofloxacin by continuous infusion, the initial distribution following the loading bolus was also rapid and steady-state concentrations were achieved from 2 to 4 h after the first administration, with only minor fluctuations until the cessation of the infusion. The mean $AUC_{0-\infty}$ estimates for the continuous-infusion (1,472 ng · h/ml) and single-bolus (1,298 ng · h/ml) treatments represented only small extrapolations over the mean AUC_{0-t} estimates (1,412 ng · h/ml and 1,190 ng · h/ml, respectively), and the similarities in the estimates of $AUC_{0-\infty}$ for both routes confirm that the total doses administered were equivalent overall. Thus, the AUC:MIC estimates

TABLE 5. Frequency distribution of clinical signs (assessment of character of respiration)

Treatment	No. of calves in each assessment category at each time point after treatment[a]								
	0 h			24 h			48 h		
	Normal	Shallow/ abdominal effort	Dyspnea/ withdrawn	Normal	Shallow/ abdominal effort	Dyspnea/ withdrawn	Normal	Shallow/ abdominal effort	Dyspnea/ withdrawn
Saline	0	11	0	6	1	4	5	1	5
Danofloxacin (single bolus)	1	10	0	9	2	0[b]	10	1	0[b]
Danofloxacin (continuous infusion)	2	8	1	7	3	1	9	0	2

[a] Each treatment group consisted of 11 calves.
[b] Significantly different from the value obtained with the saline treatment ($P < 0.05$).

for each treatment regimen were also similar (49.07 for the infusion treatment compared with 43.27 for the bolus treatment), as were the values for CL_b for both treatments (507.8 and 578.2 ml/h · kg, respectively). However, the $t_{1/2}$ and λ_z values for the two regimens were different (2.3 h and 0.3052 h^{-1}, respectively, for the infusion treatment compared with 4.3 h and 0.1597 h^{-1} for the bolus treatment). These apparent differences in the $t_{1/2}$ may have resulted from differences in the number of time points used to estimate the terminal elimination rate constant in the bolus treatment group compared with that in the danofloxacin infusion group.

In this study, danofloxacin administered either as a single bolus or as a continuous infusion was significantly more effective in the treatment of *M. haemolytica* infection in calves than the control saline treatment. Overall, the administration of danofloxacin as a single bolus was more effective than administration of the same dose as a continuous infusion, as reflected in a higher percentage of animals successfully completing the study, significantly lower rectal temperatures over the initial 24-h period, and a significantly lower number of animals with *M. haemolytica* in bronchial secretions. This was in spite of the marginally lower AUC:MIC for danofloxacin following bolus administration.

These data establish that danofloxacin exhibits a concentration-dependent antimicrobial activity when administered to the target species, cattle, with respiratory disease caused by *M. haemolytica* under conditions that closely simulated field conditions. Therefore, these data suggest that maximum therapeutic benefits can be obtained with danofloxacin with the administration of high doses over short periods. In the present study, the C_{max}:MIC for the bolus regimen was 14.5. In a previous in vitro pharmacodynamic model, in which danofloxacin was shown to have concentration-dependent bactericidal action against *Actinobacillus pleuropneumoniae*, danofloxacin showed maximal bactericidal effect and there was no regrowth observed when the C_{max} was at least eight times the MIC (9).

The extent of protein binding of danofloxacin in plasma was not determined in the present study, and the concentrations and pharmacokinetic values presented correspond to total danofloxacin. However, the extent of protein binding of danofloxacin was determined in previous studies and can be described as reversible and relatively low, with values of approximately 49% in bovine plasma and 31 and 14% in bovine bronchial secretions and nasal secretions, respectively (7).

In addition, danofloxacin has been shown to achieve concentrations in lungs and in bronchial mucosa that are approximately fivefold and threefold higher, respectively, than that achieved in plasma (7). Therefore, the steady-state free-drug concentrations achieved in the target tissues (i.e., bronchi and lungs) during the present study would have largely exceeded the MICs for *M. haemolytica*. Thus, the concentration-dependent activity observed with danofloxacin can be considered as a real effect rather than the result of subtherapeutic concentrations in animals treated with danofloxacin administered as an IV infusion.

In addition to the correlation of increased efficacy with high C_{max}-to-MIC and AUC-to-MIC ratios, high C_{max}-to-MIC ratios have also been shown to minimize the potential for the development of resistance to fluoroquinolones (11). This characteristic has been established in several studies where the development of resistance to fluoroquinolones could be eliminated or drastically reduced when concentrations of the antimicrobial drug to which the bacteria were exposed exceeded the MIC by at least 8- to 10-fold (17).

The pharmacokinetic and pharmacodynamic evaluation of danofloxacin in this *M. haemolytica* pneumonia model in calves has demonstrated the concentration-dependent activity of this drug in cattle. The principle of a concentration-dependent approach to therapy has been used to select the commercial dose of danofloxacin in the 18% formulation as 6 mg/kg administered subcutaneously either once or, if clinically required, twice 48 h apart. This selection is based on relating the AUC:MIC and C_{max}:MIC results to recently determined MICs for field isolates of susceptible pathogens such as *M. haemolytica* (with MICs ranging from 0.015 to 2 μg/ml, an MIC at which 50% of isolates are inhibited of 0.06 μg/ml, and an MIC at which 90% of isolates are inhibited of 0.25 μg/ml [data not shown]). It is proposed that this concept will maximize the therapeutic characteristics of this potent molecule while minimizing the potential for the development of resistance and ensuring a high level of treatment compliance.

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