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

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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 (Cmax/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 Cmax/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 to have 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 m² 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-
would provide a steady-state concentration in plasma slightly exceeding the MIC eliciting of previously obtained plasma concentration-time data as the dose which regimen for the dano oxacin infusion was calculated by pharmacokinetic model-

flcin), although the presence of the infusion apparatus made masking impossible and at 4, 8, 12, 24, 36, and 48 h thereafter. The respiratory rate, rectal temper-
delivered over exactly 36 h. The body weight recorded for each animal prior to connection of the pump, the initial single bolus was administered via the animal with sutures and tape and included several loops to relieve tension. Prior cutaneous sutures. The pump was connected to the IV catheter in the jugular designed pouch and harness. An 18-gauge indwelling catheter (Leader-Flex delivered by an ambulatory infusion pump (CADD-PLUS model 5400; SIMS zer Ltd.) was diluted with 0.9% (wt/vol) sodium chloride to give solutions containing 6.0 and 0.75 mg of dano-

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 × 10^6 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 10^6 CFU of *M. haemolytica* type A1/calf (acceptable range was defined as 5 × 10^5 to 5 × 10^6 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 approxi-

mately 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 inocu-

Aortic perfusion was established, well-tolerated method (13). Samples were collected following endo-
tracheal 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 absor-

Lung samples were used to determine the concentration in plasma at time zero (p

prior contrasts were used to assess differences between treatments. The 5% level of significance (P < 0.05) was used to determine statistical significance for all comparisons. The planned post hoc tests were Bonferroni. The data were analyzed with a general linear model. A categorical analysis of variance with repeated measures was performed to assess the effects of treatments over time and to assess the interactions between treatments and time. Post hoc comparisons were made using the Tukey-Kramer method.

Pharmacokinetic analysis. Pharmacokinetic analyses were performed by using WinNonlin version 1.1 (Scientific Consulting Inc., Cary, N.C.). For calves receiving dano-

Inoculum. A 1.0 ml aliquot of *M. haemolytica* type A1 (reference M7/2) into 9.0 ml of Oxoid nutrient broth no. 2. After inoculation at 37°C for 16 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 inoculation, the approximate viable count (CFU per milli-

flcin), although the presence of the infusion apparatus made masking impossible for calves receiving the dano-

Any calf which was recumbent and showed severe depression and/or signs of respiratory distress was immediately euthanized on welfare grounds. All calves were euthanized after the final sampling (48 h after the start of the treatment trials. At necropsy, the score of the percent 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 pa-

clot, the infection was considered treated when clinical signs abated and the animal was healthy at the end of the study. A treatment cure was defined as a 100% reduction in the CFU of *M. haemolytica* on blood agar. Each calf was manually restrained in a specially designed cage, with the head free to move; a standard face mask was applied with one eye left open to allow observation of the animal. A stop watch was started when the face mask was applied and stopped once the animal was restrained and all clinical signs of infection were absent. The time of treatment cure was recorded for each animal.

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 temper-

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 propor-

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maximum observed concentration in plasma \( C_{\text{max(obs)}} \); the total body clearance (\( \text{CLb} \)), calculated as the dose administered divided by the area under the curve from time zero to infinity (\( \text{AUC}_{0\rightarrow\infty} \)); and the terminal elimination rate constant (\( \lambda_z \)), 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 \( \text{AUC}_{0\rightarrow\infty} \) (where \( t \) is the last time of measurable plasma concentrations). The MIC for the \( M. \) haemolytica strain used as the inoculum was 30 \( \text{ng/ml} \); hence, the \( T>MIC \) was the time during which plasma danofoxacin concentrations exceeded 30 \( \text{ng/ml} \). Plasma \( \text{AUC}_{0\rightarrow\infty} \) was calculated as \( \text{AUC}_{0\rightarrow\infty} + C_t/\lambda_z \), where \( C_t \) was the last measurable plasma danofoxacin concentration. The ratio of \( \text{AUC} \) to MIC (\( \text{AUC}/\text{MIC} \)) was calculated as \( \text{AUC}_{0\rightarrow\infty}/\text{MIC} \). The plasma \( C_{\text{max(obs)}}/\text{MIC} \) was also calculated. All pharmacokinetic parameters and concentrations of danofoxacin 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 danofoxacin 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 \( C_p^{\text{obs}} \), \( C_{\text{max(obs)}}^{\text{obs}} \), and \( C_{\text{max(obs)}}/\text{MIC} \)], 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 danofoxacin 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 \( C_{\text{max(obs)}} \) of 436 \( \text{ng/ml} \) and an extrapolated \( C_p \) of 589 \( \text{ng/ml} \) (Fig. 1). Following rapid distribution, danofoxacin was eliminated, with an overall mean \( t_{1/2} \) of 4.3 h.

The continuous IV infusion of danofoxacin was preceded by the administration of an initial small IV bolus to rapidly achieve the target steady-state plasma drug concentration of \( \geq 30 \text{ ng/ml} \). Peak plasma danofoxacin concentrations were detected at the first time point, 15 min after administration of the small bolus, with a mean \( C_{\text{max(obs)}} \) of 69.0 \( \text{ng/ml} \) and an extrapolated \( C_p \) of 85.8 \( \text{ng/ml} \). Danofoxacin concentrations declined rapidly until steady-state concentrations slightly higher than 30 \( \text{ng/ml} \) 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 danofoxacin concentrations declined, with a mean \( t_{1/2} \) of 2.3 h.

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

<table>
<thead>
<tr>
<th>Danofloxacin administration*</th>
<th>Pharmacokinetic parameter (mean [± SD])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C_0 ) (ng/ml)</td>
</tr>
<tr>
<td>Single bolus</td>
<td>589 (±86.7)</td>
</tr>
<tr>
<td>Continuous infusion</td>
<td>85.8 (±9.2)</td>
</tr>
</tbody>
</table>

* 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 \( C_p^{\text{obs}} \), \( C_{\text{max(obs)}}^{\text{obs}} \), and \( C_{\text{max(obs)}}/\text{MIC} \). \( t_{1/2} \) was calculated as a harmonic mean.

![FIG. 1. Pharmacokinetics and pharmacodynamics of danofoxacin in cattle with respiratory disease. Shown are plasma danofoxacin concentrations (means ± SD) following administration as a single IV bolus or as a continuous IV infusion.](http://aac.asm.org/)

- **Danofloxacin (0.738 mg/kg) administered as a single IV bolus**
- **Danofloxacin (0.738 mg/kg) administered as a continuous IV infusion (0.845mg/kg) for 36 hours, preceded by a small IV bolus (0.093 mg/kg)**
- **MIC of Mannheimia (Pasteurella) haemolytica (30 ng/mL)**
- **Subsequent values < Limits of Quantification (10 ng/mL)**

**FIG. 1.** Pharmacokinetics and pharmacodynamics of danofoxacin in cattle with respiratory disease. Shown are plasma danofoxacin concentrations (means ± SD) following administration as a single IV bolus or as a continuous IV infusion.
The administration of danofloxacin as a single IV bolus produced a high C_{max} to 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} to 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-Desmethyldanofoxacin 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 ≤ 0.0173) and in lung tissue samples (P ≤ 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 ≤ 0.0001) collected from 3 to 48 h, inclusive, and in lung tissue samples (P ≤ 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 ≤ 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 ≤ 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 ≤ 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 ≤ 0.0167) at 12 and 24 h and significantly lower (P ≤ 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 2. Lung lesion scores and classification of animals by presence of M. haemolytica in posttreatment bronchial secretions and in lung tissue samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lung lesion score</th>
<th>No. of animals with M. haemolytica present in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bronchial secretions</td>
</tr>
<tr>
<td>Saline</td>
<td>19.5</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Danofloxacin (single bolus)</td>
<td>13.4</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Danofloxacin (continuous infusion)</td>
<td>13.5</td>
<td>5 (45.5)</td>
</tr>
</tbody>
</table>

*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.

### Table 3. Geometric mean M. haemolytica counts in bronchial secretions and lung tissue samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>M. haemolytica count (CFU/ml) in bronchial secretions at time (h)*</th>
<th>M. haemolytica count in lung tissue (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Saline</td>
<td>2.0</td>
<td>1.2 × 10^3</td>
</tr>
<tr>
<td>Danofloxacin (single bolus)</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>Danofloxacin (continuous infusion)</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

*a* Treatment commenced at time zero.  
*b* Significantly different from the value obtained with the saline treatment (P < 0.05).
single-dose danofoxacin 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-dose 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 danofoxacin 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 danofoxacin has been investigated in ruminant species including cattle (7, 8, 10), sheep (12), and goats (19). Following administration of danofoxacin, there is rapid distribution to the lungs (12) and high tissue concentrations are achieved in pneumonic lung, including areas of consolidation (1, 2). Danofoxacin 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 danofoxacin 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 danofoxacin administered to calves with a respiratory infection, thus establishing whether or not danofoxacin exhibits a concentration-dependent bactericidal effect in cattle. The same total dose of danofoxacin (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 danofoxacin was predicted to give a high C_{\text{max}:\text{MIC}} and a short T>\text{MIC}, while the IV infusion of danofoxacin was predicted to give a low C_{\text{max}:\text{MIC}} and a long T>\text{MIC}.

As predicted, for those calves receiving the danofoxacin infusion treatment, concentrations in plasma were maintained above the MIC for the majority of the 36-h period (T>\text{MIC} = 33.3 h). In contrast, for those calves receiving the single bolus of danofoxacin, concentrations in plasma exceeded the MIC for less than 10 h posttreatment. The initial distribution of danofoxacin 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 multipexponential elimination curve with a terminal elimination phase slope evident after 2 to 3 h postadministration. For calves receiving danofoxacin 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_{\text{bolus}:\text{MIC}} 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_{\text{bolus}} estimates (1,412 ng·h/ml and 1,190 ng·h/ml, respectively), and the similarities in the estimates of AUC_{\text{bolus}} for both routes confirm that the total doses administered were equivalent overall. Thus, the AUC:MIC estimates

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal</th>
<th>Depressed</th>
<th>Recumbency/withdrawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Danofoxacin (single bolus)</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Danofoxacin (continuous infusion)</td>
<td>1</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

* Each treatment group consisted of 11 calves.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal</th>
<th>Shallow/abdominal effort</th>
<th>Dyspnea/withdrawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Danofoxacin (single bolus)</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Danofoxacin (continuous infusion)</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

* Each treatment group consisted of 11 calves.

* 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 CLb for both treatments (507.8 and 578.2 ml/h·kg, respectively). However, the t1/2 and λ1 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 t1/2 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 Cmax/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 Cmax 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 Cmax-to-MIC and AUC-to-MIC ratios, high Cmax-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 Cmax/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.

**ACKNOWLEDGMENTS**

We acknowledge the contribution made by the scientific and laboratory staff involved in the study whose expertise and professionalism ensured its successful conduct and completion.

**REFERENCES**


