Duration of therapy with EXCEDE™ or Micotil® in a bovine respiratory disease challenge model

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Key Points

• EXCEDE™ (ceftiofur crystalline free acid) Sterile Suspension controlled bovine respiratory disease (BRD) for up to nine days after administration in a challenge model.
• EXCEDE was superior to Micotil® (tilmicosin) 300 Injection for lung lesion scores, respiratory scores, and rectal temperature.
• Concentrations of ceftiofur and related metabolites were above the efficacy threshold for BRD pathogens* (>0.2 µg/mL) for >7 days after EXCEDE administration.

Introduction

EXCEDE™ Sterile Suspension is a single-administration veterinary antimicrobial containing ceftiofur crystalline free acid, to be administered by a novel and patented subcutaneous ear route of administration (ERA). EXCEDE is active against the target BRD pathogens Mannheimia haemolytica, Pasteurella multocida and Haemophilus somnus.1

Pharmacokinetic data demonstrated that adequate concentrations of ceftiofur and related metabolites are present above the efficacy threshold (t>0.2, model)** in plasma for 183 hours or 7.6 days.2 The efficacy threshold represents a plasma concentration that is three to six times higher than the MIC90 values of target pathogens. The present study evaluated the duration of activity and clinical response when a single injection of EXCEDE was administered at predetermined times before intratracheal challenge with M. haemolytica compared with the positive control Micotil or a negative control (vehicle treatment). This study was conducted according to the VICH Good Clinical Practice Guidelines.3

Materials and Methods

Holstein calves (n=144; 76.7 ± 9.2 kg) were randomly assigned to groups challenged three, five, seven, or nine days after treatment (n=36/challenge group). On day 0, calves were randomly assigned to one of three treatments: negative control (vehicle treatment; n=6/group); EXCEDE at 6.6 mg ceftiofur equivalents (CE)/kg (3.0 mg CE/lb; 1.5 mL EXCEDE per 100 lb) body weight (BW) administered subcutaneously (SC) in the posterior aspect of the ear (n=15/group); or Micotil (10 mg tilmicosin/kg; n=15/group) administered SC within each day’s challenge group. The principal investigator remained blinded to treatments and performed daily clinical observations of each calf beginning the day after arrival. Six calves treated with

* The efficacy threshold is >0.2 µg/mL of ceftiofur and related metabolites; 0.2 µg/mL is three to six times higher than the MIC90 values for target BRD pathogens.
** Model represents the compartmental pharmacokinetic analysis.
EXCEDE were randomly selected from each challenge group for blood sample collection to determine ceftiofur plasma concentrations before treatment on day 0 and on the day of challenge. Additional blood samples were collected from the same calves and from the six negative controls and from six randomly selected Micotil-treated calves in each challenge group to determine serum haptoglobin concentrations. Haptoglobin has been identified as a major acute-phase protein in cattle.4,5,6 Serum samples were collected from all groups on day 1; and from different groups on the day before challenge; the day of challenge; and 1, 2, 3, 5, 7, and 9 days after challenge.

On the day before the challenge, the calves were transported to a feedlot, where they were wetted, branded, and received a clostridial vaccine, and housed together in a large pen overnight before being transported back to the original facility. The intrarachoreal challenge was administered as two separate challenges on the assigned day. The first challenge consisted of 10 mL of challenge material acidified to pH of 4.5 containing from 2.34 x 10^7 to 1.29 x 10^8 CFU of M. haemolytica/mL. The second challenge was administered four hours after the first, was not acidified, and consisted of 15 mL containing from 3.8 x 10^7 to 8.2 x 10^7 CFU of M. haemolytica/mL. New challenge material was prepared each day.

Daily evaluations for each group continued through nine days after challenge when all surviving calves were euthanized and lung lesions evaluated. Variables included lung lesion scores, respiratory scores, and rectal temperature up to nine days after challenge. Animals were also classified as normal/abnormal based on a composite clinical score. A normal composite score was defined as normal respiratory score, normal attitude score, and rectal temperature less than the calf’s mean pre-injection temperature +1.26°F.

**Results**

The results are summarized in Table 1. Cumulative mortality due to BRD was 0% for EXCEDE-treated, 3.3% for Micotil-treated, and 33% for negative controls. The mortality rate in the negative controls provided assurance that the challenge resulted in severe BRD. Mean lung lesion scores for calves challenged seven and nine days after treatment were 1.46% and 3.94% for EXCEDE-treated calves and 4.88% and 13.7% for Micotil-treated calves (P<0.05).

Across all challenge groups, significantly fewer EXCEDE-treated than Micotil-treated calves had clinically abnormal composite scores one, three, four, and five days after challenge (P<0.05). On days 1 to 7 after challenge, 49%, 23%, 53%, 43%, 62%, 43%, and 7% more Micotil-treated calves were determined to be clinically abnormal compared with EXCEDE-treated calves. The EXCEDE-treated calves had a significantly (P=0.035) smaller proportion of days following challenge that calves were scored as abnormal for respiration compared with the Micotil-treated group (22.0% vs. 34.6%, respectively). The Micotil-treated calves had a significantly (P=0.0007) smaller proportion of days following challenge, in calves scored as abnormal for respiration, compared with those in the control group (34.6% vs. 69.4%, respectively).

Across all challenge groups, mean rectal temperatures for the EXCEDE-treated group were lower than for negative controls through seven days after challenge (P<0.05) and were lower than the Micotil-treated group through three days after challenge (P<0.05).

Plasma concentrations of ceftiofur and related metabolites were 0.94 µg/mL at three days post-treatment, 0.62 µg/mL at five days, and 0.39 µg/mL at seven days. By nine days after treatment, plasma concentrations remained above the limit of quantitation (LOQ; 0.15 µg/mL) in four of six EXCEDE-treated calves, and the mean plasma concentration for those four calves was 0.18 µg/mL. These concentrations were all above the MIC (≤0.03 µg/mL) of the challenge organism.

Mean serum haptoglobin concentrations on day 1 were 25 mg/100 mL. After challenge, the serum haptoglobin concentrations increased for all treatment groups although the increase was higher numerically in the Micotil-treated and control calves than in the EXCEDE-treated calves. Peak serum haptoglobin concentrations were 70 to 97 mg/100 mL for the EXCEDE-treated groups, 121 to 207 mg/100 mL for the Micotil-treated groups, and 142 to 183 mg/100 mL for the control groups.

### Table 1. Results Of M. haemolytica Challenge 3, 5, 7, Or 9 Days After Treatment

<table>
<thead>
<tr>
<th></th>
<th>Challenge 3 Days After Treatment</th>
<th>Challenge 5 Days After Treatment</th>
<th>Challenge 7 Days After Treatment</th>
<th>Challenge 9 Days After Treatment</th>
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</thead>
<tbody>
<tr>
<td><strong>Number of Calves</strong></td>
<td></td>
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<tr>
<td>EXCEDE</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<tr>
<td>Micotil</td>
<td>15</td>
<td>15</td>
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<td>15</td>
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<tr>
<td>Negative Controls</td>
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<tr>
<td><strong>Back-Transformed Mean Lung Lesion Scores 9 Days after Challenge (%)</strong></td>
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<tr>
<td>EXCEDE</td>
<td>2.63</td>
<td>2.77</td>
<td>1.46*</td>
<td>3.94*</td>
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<tr>
<td>Micotil</td>
<td>4.59*</td>
<td>6.63</td>
<td>4.88*</td>
<td>13.7**</td>
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<td>Negative Controls</td>
<td>22.1</td>
<td>7.97</td>
<td>9.52</td>
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<td><strong>Calves with Abnormal Respiratory Scores (%)</strong></td>
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<td></td>
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<tr>
<td>EXCEDE</td>
<td>20</td>
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<td>Micotil</td>
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<td>Negative Controls</td>
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<tr>
<td><strong>Mean Serum Haptoglobin 48 Hours after Challenge (mg/100 mL)</strong></td>
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<td>EXCEDE</td>
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<td>Micotil</td>
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<td>207</td>
<td>121</td>
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<td>160</td>
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<td><strong>Mortality Due to BRD (%)</strong></td>
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<td></td>
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<td>Micotil</td>
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<td>0</td>
<td>0</td>
<td>6.7</td>
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<tr>
<td>Negative Controls</td>
<td>50</td>
<td>33</td>
<td>17</td>
<td>33</td>
</tr>
</tbody>
</table>

*a,b* Values within a column with different superscripts are significantly different (P<0.05).

*x,y* Values within a row with different superscripts are significantly different (P<0.05).
Discussion
A single dose of EXCEDE prevented BRD mortality even when a significant *M. haemolytica* challenge was administered up to nine days after treatment. In contrast, deaths occurred in the 3- and 9-day challenge groups treated with Micotil and in all of the control groups. The results of this challenge study demonstrate that a single dose of EXCEDE provides superior efficacy against a *M. haemolytica* challenge compared to Micotil. The EXCEDE-treated groups had fewer calves with abnormal composite scores at one, three, four, and five days after challenge compared to those treated with Micotil, and fewer abnormal calves for days 1 to 9 compared with negative controls. The Micotil-treated group had fewer abnormal calves than did the negative control group on days 2 to 9. The appetite score was not included in the clinical scores but also provided evidence that the EXCEDE-treated calves overcame the challenge more readily than did the Micotil-treated or negative control calves. Although the lung microbiology data were not analyzed statistically, *M. haemolytica* was isolated from more Micotil-treated than EXCEDE-treated calves in the 9-day challenge group.

The initial clinical study conducted to evaluate the efficacy of EXCEDE for the control of BRD allowed additional treatment to be administered the day after arrival processing. Given that plasma concentrations of ceftiofur and metabolites are still above 0.2 µg/mL at that time, it may not be necessary to administer additional treatments. In fact, additional treatments based on clinical signs alone are probably not warranted as long as plasma concentrations of ceftiofur and metabolites surpass the efficacy threshold of the target pathogens. Plasma concentrations of ceftiofur and related metabolites observed in the calves in this study are representative of concentrations observed in feeder cattle when EXCEDE was administered at the same dose at arrival processing, and were consistent with data from the pivotal pharmacokinetic study. Baseline (day 1) haptoglobin concentrations were similar for all groups and averaged 25 mg/100 mL. Peak serum haptoglobin concentrations were lowest for EXCEDE-treated groups, intermediate for the Micotil-treated groups, and highest for the control groups. Not only was the magnitude of increase in serum haptoglobin smaller for the EXCEDE-treated calves, the patterns of increase were different for the different groups. For the EXCEDE-treated calves, there was no further significant increase two days after challenge compared with the first day after challenge. In contrast, Micotil-treated cattle in all challenge groups had a higher serum haptoglobin concentration two days after challenge compared with the first day after challenge.

Conclusions
EXCEDE at 6.6 mg CE/kg SC in the middle one-third of the posterior aspect of the ear provided superior efficacy compared with Micotil in a BRD challenge model. These results confirm clinically that BRD can be controlled for >7 days after a single administration of EXCEDE, and support the efficacy threshold of >0.2 µg/mL for ceftiofur and related metabolites in plasma. Appropriate subcutaneous injection of EXCEDE in the middle one-third of the posterior aspect of the ear is essential for animal safety.

References
For subcutaneous injection in the middle third of the posterior aspect of the ear of beef and non-lactating dairy cattle.

CAUTION
Federal (USA) law restricts this drug to use by or on order of a licensed veterinarian.

DESCRIPTION
EXCEDE Sterile Suspension is a ready-to-use formulation that contains the crystalline free acid of ceftiofur, which is a broad spectrum cephalosporin antibiotic active against Gram-positive and Gram-negative bacteria including β-lactamase-producing strains.

Administration of ceftiofur to cattle as ceftiofur crystalline free acid provides effective concentrations of ceftiofur and desfuroylceftiofur, including the desfuroylceftiofur metabolite. The desfuroylceftiofur metabolite is a major metabolite of ceftiofur in cattle and is effective against a number of Gram-negative bacteria, including Escherichia coli and Salmonella typhimurium, or a single 6.6 mg CE/kg bolus dose of EXCEDE Sterile Suspension in the ear results in death and must be avoided.

Chemical name of ceftiofur crystalline free acid: 7-[2-(2-Amino-4-thiazolyl)-2-(methoxyimino)acetyl]amino]-3-[[(2R)-2-oxo-2-azabicyclol[4.2.0]oct-2-ene 2-carboxylic acid.

PHARMACOLOGY

CLINICAL PHARMACOLOGY
Cattle: Ceftiofur administered as either ceftiofur sodium (NAXCEL® brand of ceftiofur sterile suspension) or ceftiofur crystalline free acid (EXCEDE Sterile Suspension) is metabolized rapidly to desfuroylceftiofur, the primary metabolite.

administration of ceftiofur to cattle at a dosage of 3.0 mg CE/lb body weight (6.6 mg CE/kg) body weight. One steer was given a single 6.6 mg CE/kg bolus dose of EXCEDE Sterile Suspension per 100 lb body weight.

Adverse systemic effects, indicating that ceftiofur was well tolerated at 25 mg CE/lb/day for five consecutive days, as long as the injection was given subcutaneously. 

Conclusion:
Ceftiofur was well tolerated at 25 mg CE/lb/day for five consecutive days, as long as the injection was given subcutaneously. The biochemical and hematopoietic parameters indicated that ceftiofur was well tolerated at 25 mg CE/lb/day for five consecutive days, as long as the injection was given subcutaneously. The biochemical and hematopoietic parameters indicated that ceftiofur was well tolerated at 25 mg CE/lb/day for five consecutive days, as long as the injection was given subcutaneously.

CONTRAINDICATIONS
The use of EXCEDE Sterile Suspension is contraindicated in animals previously found to be hypersensitive to the drug.

W A R N I N G S
• Do not inject into the base of the ear. To avoid injury to the ear, remove the hair from the area of injection. 
• Do not inject into the entire ear.
• Do not inject the drug in the ear of the animal if the animal has indications of a blood vessel in close proximity (e.g., ear discharge, ear congestion, or ear swelling).
• Do not inject the drug in the ear of an animal that is being treated with another antibiotic.

Adverse systemic effects: The incidence of BRD in high-risk feedlot cattle in the 28-day period after arrival processing compared to negative controls. 

t>0.2, model (h) 183 ± 40.8

Pharmacokinetic Parameter Mean Value ± Standard Deviation

Table 2. Minimum Inhibitory Concentrations for Ceftiofur Against Clinical Isolates

Table 1. Pharmacokinetic parameters measured after a single SC administration of 3 mg CE/lb body weight (BW) (6.6 mg CE/kg) of EXCEDE Sterile Suspension in the posterior ear of cattle are presented in the following table:

Pharmacokinetic Parameter Mean Value ± Standard Deviation

t1/2 (h) 62.3 ± 13.5

≥ 4.2.0
t≥MIC (h) 103 ± 0.9

Cmax = maximum plasma concentration (in µg CE/mL)

Table 1. Pharmacokinetic parameters measured after a single SC administration of 3 mg CE/lb body weight (BW) (6.6 mg CE/kg) of EXCEDE Sterile Suspension in the posterior ear of cattle are presented in the following table:

tM ≥MIC = time of injection to the limit of quantitation of the assay (the 0.15 µg/mL level).

The discoloration was markedly reduced in size by the end of the study. Ears are inedible tissues in the US (9 CFR 301.2). 

Each mL of this ready-to-use sterile suspension contains ceftiofur crystalline free acid equivalent to 200 mg ceftiofur, sodium in normal feeder calves indicated that ceftiofur was well tolerated at 25 mg CE/lb/day for five consecutive days.

CAUTION
EXCEDE Sterile Suspension is contraindicated in treatment of BRD (shipping fever, pneumonia) associated with Mannheimia haemolytica, P. multocida and H. somnus. EXCEDE Sterile Suspension is also indicated for the control of respiratory disease in cattle which are at high risk of developing BRD associated with Mannheimia haemolytica, P. multocida and H. somnus.

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