Efficacy of a long-acting formulation of ceftiofur crystalline-free acid for the treatment of naturally occurring infectious bovine keratoconjunctivitis

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Objective—To evaluate the efficacy of ceftiofur crystalline-free acid (CCFA) administered into the posterior aspect of an ear for treatment of corneal ulceration associated with naturally occurring infectious bovine keratoconjunctivitis (IBK).

Animals—78 beef calves located at Sierra Foothills Field Station (SFS) and 52 calves located at a commercial dairy (CD). All calves were from 3 to 9 months old.

Procedure—At each site, calves were randomly allocated to 1 of 2 treatment groups by use of a block design determined by corneal ulcer size. A single dose of CCFA (6.6 mg of ceftiofur equivalents/kg, SC) was administered into the posterior aspect of a pinna. A second group of calves received a single dose of vehicle (0.03 mL/kg, SC; controls). Corneal ulcers were photographed, and clinical signs were assessed in calves every 3 to 4 days for 21 days.

Results—A positive treatment effect was detected at SFS. Results at the CD were inconclusive because ulcer healing occurred rapidly in control and CCFA-treated calves. At SFS, treatment with CCFA resulted in shorter mean healing times, smaller corneal ulcer surface area measurements, amelioration of ocular discharge and photophobia, and a 50% increase in the percentage of calves healed by day 14. After adjustment for initial corneal ulcer size, treatment with CCFA resulted in a 4-fold increase in the odds of corneal ulcer healing by day 14, compared with controls.

Conclusions and Clinical Relevance—A single dose of CCFA administered into the posterior aspect of a pinna had a positive treatment effect against naturally occurring IBK in calves with corneal ulcerations. (*Am J Vet Res* 2004;65:1185–1188)

Ceftiofur crystalline-free acid (CCFA), the free-acid form of ceftiofur in a sterile oil suspension, has been developed as a single-dose treatment for nonlactating cattle with respiratory disease associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*.[1] As a broad-spectrum cephalosporin, ceftiofur has in vitro activity against a variety of pathogenic gram-negative bacteria.[2] Results of 1 study[1] indicate that concentrations of ceftiofur and desfuroylceftiofur metabolites in plasma remain > 0.2 µg/mL for more than 7 days when administered SC in the posterior aspect of the pinna. This route of administration into the inedible tissue of the pinna was chosen because of reduced risk for tissue residue and lower potential for injection-site trimming at slaughter. The purpose of the study reported here was to evaluate the efficacy of CCFA administered into the posterior aspect of a pinna for treatment of corneal ulceration associated with naturally occurring **infectious bovine keratoconjunctivitis (IBK)**.

Materials and Methods

Calves—The experimental protocol was performed with the approval of the University of California Animal Care and Use Committee. This study was completed under an established, investigational new drug application provided by the FDA's Center for Veterinary Medicine. The study was performed at 2 separate field sites in northern California from May 23 to August 13, 2002. Angus-Hereford crossbred beef calves were enrolled from a group of 100 calves from 3 to 9 months of age and weighing 135 to 255 kg at the University of California's **Sierra Foothills Field Station (SFS)** in Browns Valley, Calif. Holstein dairy calves were enrolled from a group of 150 calves from 3 to 9 months of age and weighing 130 to 293 kg at a **commercial dairy (CD)** in Wheatland, Calif. Calves at the SFS were maintained on irrigated mixed-grass pasture; calves at the CD were penned in corrals on flat dry lots and fed alfalfa hay and concentrate consisting of barley, cracked corn, and protein and mineral supplements. Calves had not been treated with antimicrobials for at least 14 days before enrollment and not been vaccinated against *Movaxella bovis*.

Experimental procedure—Calves in each herd were examined twice weekly during the 9-week enrollment period by 1 of 4 veterinarians for corneal ulceration, lacrimation, photophobia, and blepharospasm. Eyes suspected of

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having naturally occurring corneal ulcers were stained with fluorescein dye, and a **corneal ulcer score (CUS)** of 0, 1, 2, or 3 was determined on the basis of the following criteria: no lesion (score 0), corneal ulcer ≤ 5 mm in diameter (score 1), corneal ulcer > 5 mm in diameter (score 2), and perforating ulcer (score 3). Eyes with corneal ulcers were photographed for subsequent determination of corneal ulcer **surface area measurement (SAM)** in square centimeters, as previously described.[3] Plastic aprons, boots, and obstetric gloves were worn and disinfected in 1% chlorhexidine solution after each calf was examined to minimize iatrogenic infections.

Calves with a CUS of 1 or 2 were enrolled in the study (day 0) and randomly allocated to 1 of 2 treatment groups blocked by the CUS at day 0. Separate randomization schedules were used for each farm. Calves with bilateral ulcers were allocated to groups on the basis of the CUS in the worst affected eye; subsequent observations were determined on the basis of the enrolled eye only. Calves in the CCFA treatment group (n = 64) received a single dose of CCFA (6.6 mg of **ceftiofur equivalents** [**CE**]/kg, SC) administered into the posterior aspect of a pinna on day 0. Calves in the control group (n = 66) received a single dose of vehicle (a mixture of a purified coconut oil extract[a] and cottonseed oil that were manufactured in the same proportion as the CCFA suspension; 0.03 mL/kg, SC) administered in the same manner on day 0. Calves were weighed on a precalibrated scale at each site to determine correct dosages; scale accuracy was checked daily before calves were weighed.

Following enrollment, calves were reexamined to evaluate clinical signs (lacrimation, photophobia, and blepharospasm), CUS, and SAM on days 3 or 4, 7, 10 or 11, 14, 17 or 18, and 21 or until the ulcer had perforated. All examinations were performed by 1 of 4 veterinarians who were unaware of treatment-group allocations. Treatment was considered successful in calves in which the ulcer healed on or before day 21. Treatment was considered unsuccessful in calves with continuous ulcers through day 21 and in calves that developed perforated corneas on or before day 21. Oxytetracycline (20 mg/kg, IM) was administered to any calves for which treatment was unsuccessful. Ulcers that developed in the opposite eye were not included in the observations. The CCFA-treated calves were retained for 51 days to comply with a 21-day withdrawal time for CCFA and a 30-day observation period; control calves were observed for a 30-day period following study completion.

Bacteriologic studies—Before application of fluorescein dye on day 0, ocular secretions were collected from any eyes with suspected corneal ulcers by lightly rubbing a sterile applicator swab in the superior and inferior conjunctival fornices. Swabs were then immediately inoculated onto 5% sheep blood agar plates. Samples from enrolled calves were subsequently processed for isolation of *M bovis*. Inoculated plates were chilled during transport to the laboratory, streaked for isolation, and incubated at 35°C for 24 hours. Bacterial colonies with morphology characteristic of *M bovis* were selected, subcultured until pure, and positively identified by colony morphology and biochemical criteria.[4] Isolates identified as *M bovis* were suspended in trypticase soy broth medium[b] supplemented with 10% glycerol solution and stored frozen at –70°C until tested. Growth from freshly prepared boiled blood chocolate agar plates that were incubated at 37°C in 5% CO_2 overnight was used as inocula for **minimum inhibitory concentration (MIC)** testing. The MICs were determined by use of a commercially available broth microdilution system[c] that conformed to the guidelines of the NCCLS broth microdilution method. Because of the fastidious nature of *M bovis*, a modification of the NCCLS methodology was required and 10% fetal bovine serum was added as a supplement to the broth.[5]

Statistical analyses—All corneal ulcer SAMs were analyzed after square-root transformation and reported as the square root of SAM. Ulcers were considered healed on the first day that a CUS of 0 was observed. For ulcers that healed before day 21, values of 0 were used for CUS and SAM for analysis of all subsequent observation days. The SAM data were analyzed by use of a repeated-measures general linear model. Logistic models for ulcer healing by day 7 or 14 were determined. The percentage of calves with lacrimation, photophobia, or blepharospasm was compared by use of the Mantel-Haenszel χ^2 test for each of the following time intervals: day 0, days 3 to 7 (week 1), days 10 to 14 (week 2), and days 17 to 21 (week 3). Means of continuous variables (healing time, days with discharge, and day 0 SAM) were analyzed by use of the unpaired 2-tailed Student *t* test, with a Bonferroni adjustment for multiple comparisons where appropriate. All discrete numerical data were analyzed by use of the Mantel-Haenszel χ^2 test. For all statistical analyses, a value of $P < 0.05$ was used to reject the null hypothesis.

Results

Results were stratified by study site. Seventy-eight female Angus-Hereford crossbred calves (38 CCFA-treated and 40 control calves) were enrolled at SFS and 52 female Holstein calves (26 CCFA-treated and 26 control calves) were enrolled at the CD during the 9-week enrollment period. The percentage of calves at each site with healed corneal ulcers was determined (**Table 1**). At SFS, these percentages represented a 50% and 29% increase in healing on days 14 and 21 in CCFA-treated calves, compared with control calves. Mean ± SE healing times were also significantly ($P = 0.029$) lower in CCFA-treated calves (8.6 ± 1.0 days), compared with control calves (12.2 ± 1.2 days) at SFS. Five calves at SFS had a CUS of 3 before day 21; 2 control calves had ulcers that perforated on day 18, and 3 CCFA-treated calves had perforating ulcers on days 7, 11, and 18. No significant differences in percentage of calves with healed corneal ulcers or mean healing times were observed at the CD; 90% of dairy calves in both treatment groups had healed ulcers 7 days after treatment.

Mean ± SE day 0 SAM was significantly ($P = 0.004$) lower in CD calves (0.17 ± 0.02), compared with SFS calves (0.28 ± 0.03). Results of a repeated mea-

Table 1—Number (percentage) of calves at Sierra Foothills Field Station (SFS) and a commercial dairy (CD) with healed corneal ulcers after receiving a single dose of ceftiofur crystalline-free acid (CCFA; 6.6 mg of ceftiofur equivalents [CE]/kg, SC) or vehicle (controls; 0.03 mL/kg, SC) for treatment of infectious bovine keratoconjunctivitis.

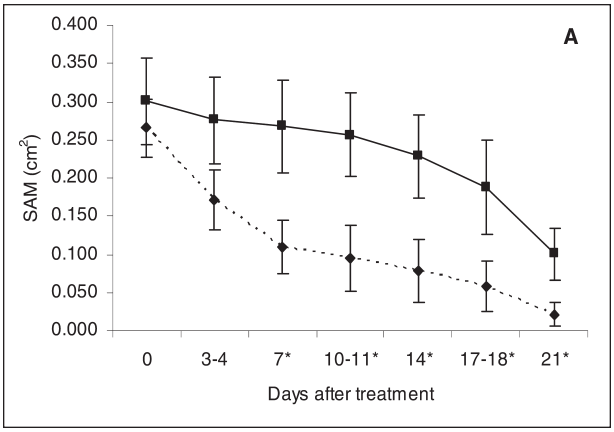
	SFS beef calves			CD calves		
Days after treatment	CCFA No. (%)	Control No. (%)	*P* value	CCFA No. (%)	Control No. (%)	*P* value
7	27 (71)	19 (48)	0.036	23 (88)	24 (92)	0.641
14	31 (86)	23 (58)	0.006	25 (96)	24 (92)	0.556
21	32 (91)	27 (71)	0.028	26 (100)	26 (100)	—

At the CD, n = 26 for each treatment group. At SFS, 38, 36, and 35 calves were observed on days 7, 14, and 21, respectively, in the CCFA-treated group and 40, 40, and 38 calves were observed, respectively, in the control group. Attrition resulted from perforating ulcers.– = Not applicable.

sures general linear model indicated that for calves at SFS, the mean corneal ulcer SAM was significantly (P = 0.026) smaller in CCFA-treated calves than in control-group calves from days 7 through 21 (**Figure 1**). No significant differences between treatment groups for SAM were observed at the CD. Additionally, results of a logistic regression model indicated that after adjustment for SAM on day 0, CCFA-treated calves at SFS were 4.8 and 4.1 times more likely than control calves to have a healed ulcer by day 7 or 14, respectively (**Table 2**).

During week 2 after treatment at SFS, 8 (22.2%) and 6 (16.7%) CCFA-treated calves had ocular discharge or photophobia, respectively, compared with 24 (60%) and 17 (42%) control calves with each clinical sign, and this difference was significant ($P < 0.01$). In addition, the mean number of days with ocular discharge was also significantly (P = 0.001) lower in CCFA-treated calves (0.36 ± 0.12) than control calves (1.0 ± 0.14) at SFS. No significant differences between groups were detected for blepharospasm at SFS or for any clinical signs at the CD.

On day 0, *M bovis* was isolated from the ocular secretions of 22.3% of all calves, with no significant difference between calves allocated to either CCFA-treated or control groups at the SFS or CD. However, significantly fewer calves at SFS (11.5%) had *M bovis* isolates, compared with calves at the CD (38.5%; **Table 3**). Hemolytic gram-negative cocci were identified in 49.2% of all bacteriologic cultures of ocular secretions, with no significant difference between study sites. Neither isolation of *M bovis* nor the gram-negative cocci were significant in determining the healing status in logistic models. Twenty-six *M bovis* iso-



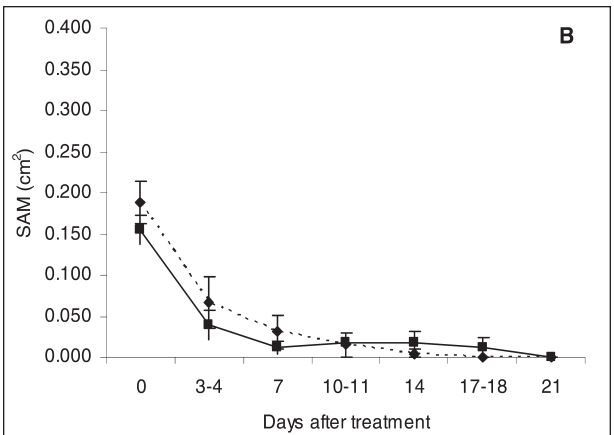


Figure 1—Mean corneal ulcer surface area measurements (SAMs) in beef calves at Sierra Foothills Field Station (A) or calves at a commercial dairy (B) receiving a single dose of ceftiofur crystalline-free acid (6.6 mg of ceftiofur equivalents/kg, SC; dashed line) or vehicle (controls; 0.03 mL/kg, SC; solid line) for treatment of infectious bovine keratoconjunctivitis. Error bars indicate SE. *Significantly ($P < 0.05$) different from values for control calves.

Table 2—Odds ratios (ORs) for corneal ulcer healing by day 7 or 14 in beef calves at SFS after receiving a single dose of CCFA (6.6 mg of CE/kg, SC) for treatment of infectious bovine keratoconjunctivitis.

Dependent variable	Independent variable	β	SE	OR	*P* value
Healed by day 7	Day 0 SAM	–8.6	2.8	0.01	0.001
	CCFA	1.6	0.6	4.8	0.010
	Constant	2.4	0.6	11.4	0.001
Healed by day 14	Day 0 SAM	–3.8	1.3	0.02	0.001
	CCFA	1.4	0.6	4.1	0.020
	Constant	2.0	0.5	7.5	0.001

β = Regression coefficient. Constant = Regression intercept. Day 0 SAM = Square root of ulcer surface area measurement obtained on day 0.
See Table 1 for remainder of key.

Table 3—Number (percentage) of bacterial isolates identified on bacteriologic culture of ocular secretions from calves with corneal ulcers at SFS or at a CD obtained before receiving a single dose of CCFA (6.6 mg of CE/kg, SC) or vehicle (controls; 0.03 mL/kg, SC) for treatment of infectious bovine keratoconjunctivitis.

	SFS beef calves (n = 78)	CD calves (52)
Ocular culture isolates	**No. (%)**	**No. (%)**
No isolation	29 (37.2)	12 (23.1)
Moraxella bovis	9 (11.5)*	20 (38.5)
Gram-negative cocci	41 (52.6)	23 (44.2)
M bovis and gram-negative cocci	1 (1.3)	3 (5.8)

*Significantly ($P < 0.001$) different from CD site.
See Table 1 for remainder of key.

Table 4—Minimal inhibitory concentration (MIC) distribution for 8 antimicrobial agents against 26 bacterial isolates of *M bovis* identified on bacteriologic culture of ocular secretions from calves with corneal ulcers at SFS or at a CD obtained before receiving a single dose of CCFA (6.6 mg of CE/kg, SC) or vehicle (controls; 0.03 mL/kg, SC) for treatment of infectious bovine keratoconjunctivitis.

	No. of isolates that yielded each MIC value (µg/mL)					
Antimicrobial	**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.50**	**1.0**
Ceftiofur	4	7	13*†	2	0	0
Cefquinome	1	2	2	14*	7†	0
Enrofloxacin	16*	9†	1	0	0	0
Florfenicol	0	0	8	12*	6†	0
Gentamicin[a]	0	0	26*†	0	0	0
Penicillin[a]	0	0	2	15*	8†	1
Tetracycline[a]	0	0	2	13*	11†	0
TMS[b]	0	1	5	19*†	0	1

[a]Test limit of detection ≤ 0.12 µg/mL. [b]Test limit of detection ≤ 0.06. TMS = Trimethoprim-sulfamethoxazole. * = The MIC value that completely inhibited 50% of the strains tested. † = The MIC value that completely inhibited 90% of the strains tested.
See Table 1 for remainder of key.

lates had adequate growth from frozen stocks to determine the MIC for 8 antimicrobials, including ceftiofur (**Table 4**).

Discussion

Administration of a single dose of CCFA (6.6 mg of CE/kg, SC) into the posterior aspect of a pinna was effective for the treatment of naturally occurring IBK in beef calves at SFS. Treatment with ceftiofur had no measurable therapeutic effect in the dairy calves in which ulcer healing occurred equally rapidly in both control and CCFA-treated groups. At SFS, treatment with CCFA resulted in shorter mean healing times, lower corneal ulcer SAM, and a 50% increase in the percentage of calves with healed corneal ulcers by day 14. Antimicrobial treatment with CCFA also resulted in a 4- to 5-fold increase in the odds of an ulcer healing by day 14 or 7, respectively, compared with control calves after adjustment for initial corneal ulcer size. The CCFA-treated calves at SFS also had significant amelioration of photophobia and ocular discharge during week 2 after treatment, compared with control calves.

In the study reported here, failure to indicate efficacy of CCFA in the treatment of IBK at the CD site was most likely attributable to differences in severity and course of disease at the 2 sites and to intervals between observations. Corneal ulcers in dairy calves were significantly smaller at enrollment, compared with those in calves at SFS, and 90% of ulcers in dairy calves were healed by day 7 in CCFA-treated and control calves. This rapid healing of small ulcers combined with a protocol that evaluated calves only twice a week made it difficult to detect differences between treatment groups at the CD. Many ulcers at SFS were already well established at the onset of the trial, whereas at the CD, IBK developed late in the summer, and thus calves were often enrolled into both CCFA-treated and control groups with small ulcers that may have resolved spontaneously. Differences in virulence of the causative organism, rates of secondary infection (eg, bacterial, mycoplasmal, viral, or chlamydial), innate immunity, calf management, and other environmental risk factors (eg, flies, solar irradiation, and mechanical trauma from seed awns) may also account for the difference in disease course observed in control calves at each site.

Because conjunctival flora are often different from corneal pathogens, corneal biopsy and bacteriologic culture of corneal ulcers may have provided further insight into the bacterial population present in corneal ulcers. However, in the study reported here, corneal ulcer healing was used as the primary end point to determine efficacy; bacteriologic culture of the conjunctiva and MIC results were included for comparison to results of previous studies and were not important for efficacy results.

The auricular drug administration method used in this study has been found to be effective in the treatment and control of naturally occurring bovine respiratory disease.[1] Florfenicol[6,7] and oxytetracyline[8] have previously been found to be effective in the treatment of IBK in controlled trials. However, the comparative efficacy of these antimicrobials, compared with CCFA, has not been established. Ceftiofur crystalline-free acid may be an alternative to oxytetracycline treatment of IBK in areas in which tetracycline use is restricted because of the presence of anaplasmosis. Efficacy against IBK may serve as an additional benefit to the use of CCFA for the treatment of bovine respiratory disease in feedlot calves.

[a]Miglyol 812 caprylic-capric triglyceride, Condea Vista Co, Cranford, NJ.
[b]Trypticase soy broth, Difco Laboratories, Livonia, Mich.
[c]Trek Diagnostic Systems Inc, Westlake, Ohio.

References

1. Hibbard B, Robb EJ, Chester ST Jr, et al. Dose determination and confirmation for ceftiofur crystalline-free acid administration in the posterior aspect of the ear for control and treatment of bovine respiratory disease. *Vet Ther* 2002;3:22–30.
2. Salmon SA, Watt JL, Case CA, et al. In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J Vet Diagn Invest* 1996; 8:332–336.
3. Daigneault J, George LW. Topically applied benzathine cloxacillin for treatment of experimentally induced infectious bovine keratoconjunctivitis. *Am J Vet Res* 1990;51:376–380.
4. Weyant RS, Wayne Moss C, Weaver RE, et al. *Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria.* Baltimore: The Williams & Wilkins Co, 1996.
5. Shryock TR, White DW, Werner CS. Antimicrobial sensitivity of *Moraxella bovis*. *Vet Microbiol* 1998;61:305–309.
6. Dueger EL, Angelos JA, Cosgrove S, et al. Efficacy of florfenicol in the treatment of experimentally induced infectious bovine keratoconjunctivitis. *Am J Vet Res* 1999;60:960–964.
7. Angelos JA, Dueger EL, George LW, et al. Efficacy of florfenicol for treatment of naturally occurring infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 2000;216:62–64.
8. Smith JA, George LW. Treatment of acute ocular *Moraxella bovis* infections in calves with a parenterally administered long-acting oxytetracycline formulation. *Am J Vet Res* 1985;46:804–807.