Efficacy of florfenicol for treatment of naturally occurring infectious bovine keratoconjunctivitis

John A. Angelos, MS, DVM, DACVIM; Erica L. Dueger, DVM, DACVIM; Lisle W. George, DVM, PhD, DACVIM; Tracey K. Carrier, DVM, MPVM; Judy E. Mihalyi, BS; Sallie B. Cosgrove, MS, DVM; John C. Johnson, DVM, MS

RUMINANTS

Objective—To determine the efficacy of florfenicol for treatment of calves with naturally occurring infectious bovine keratoconjunctivitis (IBK).

Design—Randomized controlled field trial.

Animals—63 beef calves and 80 dairy calves between 4 and 12 months of age.

Procedure—Calves were randomly assigned to 1 of 3 treatment groups. Calves in the SC treatment group received a single dose of florfenicol (40 mg/kg [18.2 mg/lb] of body weight), SC, on day 0. Calves in the IM treatment group received florfenicol (20 mg/kg [9.1 mg/lb]), IM, on days 0 and 2. Calves in the control group received injections of saline solution (0.9% NaCl), IM, on days 0 and 2. Calves were reevaluated every other day for 20 days after treatment.

Results—Corneal ulcers healed by day 20 in 48 of 49 (98%) calves treated with florfenicol IM, 39 of 42 (93%) calves treated with florfenicol SC, and 33 of 52 (63%) control calves.

Conclusions and Clinical Relevance—Florfenicol administered SC (1 dose) or IM (2 doses 48 hours apart) was effective for treatment of calves with naturally occurring IBK. (*J Am Vet Med Assoc* 2000;216: 62–64)

Florfenicol, a derivative of chloramphenicol, is approved for treatment of nonlactating cattle with respiratory tract disease associated with *Pasteurella haemolytica*, *P multocida*, or *Haemophilus somnus* infection. However, florfenicol is also active against a variety of gram-positive and gram-negative bacteria, including *Streptococcus dysgalactiae*, *S uberis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp, and *Pasteurella* spp.[1] The volume of distribution of florfenicol following IV administration to calves ranges from 0.65 to 0.80 L/kg,[2] and florfenicol concentrations in CSF are approximately 46% of the corresponding plasma concentrations.[3] These data indicate that the drug has high lipid solubility and low ionic partitioning, suggesting that it may be extensively distributed to ocular tissues and may, therefore, be beneficial for treatment of **infectious bovine keratoconjunctivitis (IBK)**. In a related study,[4] corneal ulcers in calves experimentally infected with *Moraxella bovis* that were treated with florfenicol healed at a fourfold greater rate, compared with ulcers in untreated control calves. However, calves in that study were housed indoors in artificially lighted, mechanically ventilated stalls for as long as a month and were exposed to UV light prior to inoculation with a single dose of a highly concentrated suspension of *M bovis*. Responses of these calves to florfenicol treatment could differ from those of cattle under field conditions. Therefore, the purpose of the study reported here was to determine the efficacy of florfenicol for treatment of calves with naturally occurring IBK.

From the Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616 (Angelos, Dueger, George, Carrier, Mihalyi); Schering-Plough Animal Health Corp, 1095 Morris Ave, Union, NJ 07083 (Cosgrove); and Schering-Plough Animal Health Corp, PO Box 3113, Omaha, NE 68103 (Johnson). Dr. Carrier's present address is Third Avenue Animal Medical Center, 1280 3rd Ave, Chula Vista, CA 91911.

Supported by Schering-Plough Animal Health Corp.

Presented at the American College of Veterinary Internal Medicine Forum, May, 1998; the American Association of Bovine Practitioners Annual Convention, September, 1998; and the Nuflor Symposium held in conjunction with the World Buiatrics Congress, July, 1998.

The authors thank J. Michael Connor, Matthew L. Sween, and Dr. Neil Willits for technical assistance.

Materials and Methods

Calves—Calves from 4 farms in northern California were used in the study. Farms included the University of California's **Sierra Foothills Field Station (FS)**, the university's **feedlot (FL)**, a **commercial cow-calf ranch (CR)**, and a **commercial dairy (CD)**. The terrain consisted of flat dry lot or Sierra foothill range pasture, and farms ranged from 0.7 to 175 m above sea level. All farms had a high prevalence of IBK. Calves at the FS and CR were maintained on irrigated mixed grass pasture; calves at the FL and CD were penned in corrals on dry lots and fed alfalfa hay and concentrate consisting of barley, cracked corn, and protein-mineral supplements. The FS, FL, and CR herds were composed of predominantly Angus or Angus-Hereford crossbred calves; the CD herd consisted of Holstein calves. Calves in the FL herd were vaccinated with a commercial *M bovis* vaccine prior to enrollment in the study, but calves in the other 3 herds were not vaccinated against *M bovis*. The study commenced during June (FS and FL) and July (CR and CD) 1997.

Experimental procedure—Calves in each herd were examined twice weekly for corneal ulceration, lacrimation, blepharospasm, and photophobia. Eyes suspected to have corneal ulcers were stained with fluorescein dye,[a] and a **corneal ulcer score (CUS)** of 0, 1, or 2 was assigned on the basis of widest diameter of the ulcer. The diameter of corneal ulcers was measured by holding a ruler next to the eye. A CUS of 0 indicated that an ulcer was not seen, a score of 1 indicated an ulcer with widest diameter ≤ 5 mm, and a score of 2 indicated an ulcer with widest diameter > 5 mm. Ulcerated eyes were photographed for subsequent determination of the corneal ulcer **surface area measurement (SAM)** as described elsewhere,[5] except that a ninefold magnification of the projected images was used. To minimize iatrogenic infections, plastic aprons, obstetrical sleeves, and rubber gloves were worn when examining calves, and garments were rinsed in 1% chlorhexidine solution[b] after each calf was examined.

Calves with a CUS of 1 or 2 were enrolled in the study, with the day of enrollment designated day 0. Calves were then randomly assigned to 1 of 3 treatment groups, using a prospective randomized blocked design based on day-0 CUS. Unique randomization schedules were used for each farm. Calves in the SC treatment group received a single dose of florfenicol[c] (40 mg/kg [18.2 mg/lb]), SC, on day 0. Calves in the IM treatment group received florfenicol (20 mg/kg [9.1 mg/lb]), IM, on days 0 and 2. Calves in the control group received injections of saline solution (0.9% NaCl[d]), IM, on days 0 and 2. The volume of saline injected was equivalent to the volume of florfenicol that would have been given at a dose of 20 mg/kg.

After enrollment, calves were reexamined and a CUS was assigned and a SAM obtained every 48 hours for 20 days or until the corneal ulcer healed (ie, CUS = 0) or perforated. All examinations were performed by 3 veterinarians who were not aware of treatment group assignments. Treatment was considered successful in calves in which the ulcer healed on or before day 20. Treatment was considered unsuccessful in calves with ulcers remaining on day 20 and in calves that developed perforated ulcers. Calves in which treatment was unsuccessful were treated with oxytetracycline[e] (20 mg/kg [9.1 mg/lb], IM). Ulcers that developed in the opposite eye during the study were not included in the study. Withdrawal times of 28 and 90 days prior to slaughter were observed for calves treated with florfenicol IM and SC, respectively.

Bacteriologic studies—Ocular secretions consisting of a mixture of tears and mucus were collected on day 0 from eyes with suspected corneal ulcers prior to the application of fluorescein dye strips. Secretions were collected by lightly rubbing a sterile applicator in the superior and inferior conjunctival cul-de-sacs, and then directly inoculating the secretions onto 5% sheep or cow blood agar plates. Samples from enrolled calves were subsequently processed for isolation of *M bovis*. Inoculated plates were chilled until transported to the laboratory, where they were further streaked for isolation. Plates were incubated at 35 C for 48 hours and then examined for bacterial colonies with morphology characteristic of *M bovis*. Suspect colonies were selected and subcultured until pure. Identity of the colonies was then determined by use of biochemical criteria.[6] Isolates identified as *M bovis* were suspended in skim milk-glycerol media and stored frozen at –80 C. For determination of the **minimum inhibitory concentration (MIC)** of florfenicol, frozen *M bovis* isolates were thawed, inoculated onto 5% bovine blood agar plates, and incubated for 24 hours at 35 C. Colonies were selected, inoculated onto blood agar plates, and incubated for an additional 24 hours. Bacteria were then harvested and suspended in sterile saline solution. Minimum inhibitory concentrations of florfenicol were then determined using the agar dilution method, following guidelines of the National Committee for Clinical Laboratory Standards.[7]

Data analyses—For all analyses, the square root of the original planimetric measurement of corneal ulcer surface area was determined and used to represent SAM. Individual daily CUS and corneal ulcer surface areas were averaged for each calf for each week of the study (week 1 = days 2, 4, and 6; week 2 = days 8, 10, 12, and 14; week 3 = days 16, 18 and 20). Weekly mean values for each calf were then averaged to obtain weekly mean values for each treatment group. For ulcers that healed prior to day 20, values of 0 were used for CUS and surface area for all subsequent observation days. For ulcers that perforated, a final ulcer surface area was measured on the day of perforation, and data on CUS and surface area for subsequent observation days were considered missing. Weekly mean CUS and surface area were not computed for any calf with missing values, and these calves were not included in calculations of weekly mean treatment group values during weeks when values were missing.

Weekly mean CUS and SAM were compared among treatment groups by use of the Kruskal-Wallis test. If a significant difference among treatment groups was identified, the Mann-Whitney test was used for pairwise comparisons to determine which treatment groups were significantly different.

Healing time was defined as the time from enrollment (day 0) to the time of ulcer healing (CUS = 0). For calves in which treatment was not successful (ie, persistent ulcers on day 20 or perforated ulcers), the healing time was defined as 20 days.

A Cox regression model was used to compare corneal ulcer healing rates (rate at which SAM decreased over time) between treatment groups. For this model, dummy variables representing the treatment groups (IM and SC) were created, and a regression coefficient (β) was determined. The hazard ratio (exponentiated β) determined the relative risk of healing in the 2 treatment groups compared with the control group. In this analysis, calves that developed corneal perforations and, therefore, had treatment failure prior to day 20 were considered to have nonhealed ulcers on day 20. Covariates included in the final model were the square root of the corneal ulcer surface area on day 0 and treatment group. For all analyses, a value of $P < 0.05$ was considered significant.

Results

One hundred forty-three calves were enrolled in the study. There were 80 calves from the CD, 46 from the FS, 14 from the CR, and 3 from the FL. Calves weighed between 125.9 and 364.5 kg (277 and 802 lb) and were between 4 and 12 months of age. Twenty-five were male and 118 were female. There were 80 Holstein, 15 Angus, 6 Hereford, 5 Beefmaster, 3 Limousin, 1 Charolais, and 33 mixed-breed beef calves. The 3 calves from the FL had been vaccinated against *M bovis* infection prior to enrollment. Two of these calves were assigned to the IM treatment group, and 1 was assigned to the control group.

Corneal ulcers healed by day 20 in 48 of 49 (98%) calves treated with florfenicol IM, 39 of 42 (93%) calves treated with florfenicol SC, and 33 of 52 (63%) control calves. Corneal ulcers perforated in 8 control calves and 1 calf treated with florfenicol SC. On day 0, mean CUS and mean corneal ulcer surface area for the 3 groups were similar (**Tables 1 and 2**), but during weeks 1 and 2, mean CUS and mean SAM for calves treated with florfenicol IM or SC were significantly less than values for control calves. During week 3, mean CUS and mean SAM were not significantly different among groups. Results were unchanged when data for the 3 calves vaccinated against *M bovis* were excluded

Table 1—Corneal ulcer sores for calves before (week 0) and after treatment with florfenicol IM or SC and for control calves

	Group		
Week	Control	IM	SC
0	1.17 ± 0.38 (52)	1.18 ± 0.39 (49)	1.17 ± 0.38 (42)
1	0.98 ± 0.82 (52)	0.49 ± 0.69* (49)	0.45 ± 0.55* (41)
2	0.70 ± 0.89 (47)	0.20 ± 0.53* (49)	0.13 ± 0.43* (41)
3	0.49 ± 0.81 (43)	0.06 ± 0.21 (49)	0.10 ± 0.44 (41)

*Significantly ($P < 0.05$) less than value for control calves.
Data are given as mean ± SD; numbers in parentheses indicate number of calves.

Table 2—Corneal ulcer surface area in calves before (week 0) and after treatment with florfenicol IM or SC and in control calves

Week	Group		
	Control	IM	SC
0	0.98 ± 0.83 (50)	1.07 ± 1.09 (48)	0.88 ± 0.78 (40)
1	1.12 ± 1.24 (52)	0.53 ± 0.97* (49)	0.38 ± 0.60* (41)
2	0.92 ± 1.31 (47)	0.20 ± 0.56* (49)	0.13 ± 0.51* (41)
3	0.47 ± 0.89 (43)	0.05 ± 0.17 (49)	0.07 ± 0.33 (41)

Data represent square root of the surface area in centimeters. A day-0 photograph was not taken for 5 calves; thus, a day-0 surface area measurement could not be determined.
See Table 1 for key.

from the analysis. Median healing time for calves treated with florfenicol IM or SC (median, 4 days; range, 2 to 20 days) was significantly shorter than for control calves (median, 9 days; range, 2 to 20 days). None of the calves had signs of inflammation or abscessation at the injection sites.

Covariates included in the final Cox regression model were the square root of the corneal ulcer surface area on day 0 and treatment group. Regression coefficients were 1.2, 0.94, and –0.75 for the IM, SC, and day-0 SAM variables, respectively, and standard errors were 0.25, 0.25, and 0.14, respectively. The hazard ratios (exponentiated β) indicated that after adjustment for initial ulcer size, corneal ulcer healing rates were 3.3 and 2.6 times greater for calves in the IM and SC groups, respectively, compared with control calves ($P < 0.001$).

Moraxella bovis was isolated from ocular secretions of 98 of 143 calves. The 24-hour MIC of florfenicol for these isolates ranged from 0.125 to 0.5 µg/ml. The minimum concentration of florfenicol that would inhibit growth of 50% of the isolates and the minimum concentration that would inhibit growth of 90% of the isolates were both 0.5 µg/ml.

Discussion

In the study reported here, IM and SC administration of florfenicol were equally effective for treatment of IBK in calves. Calves treated with florfenicol had lower CUS and smaller corneal ulcer surface areas 1 and 2 weeks after treatment, compared with control calves; healing times of ulcers for calves treated with florfenicol IM or SC were shorter than those for control calves.

A previous study[8] of calves with IBK that were treated with oxytetracycline suggested that the corneal epithelium may regenerate at a constant rate following clearance of an *M bovis* infection. Therefore, elimination of *M bovis* infection is considered important for achieving resolution of IBK. Florfenicol[4] and oxytetracycline[9] have both been shown to eliminate *M bovis* in calves with IBK; however, the comparative efficacy of these 2 antibiotics remains unknown. Florfenicol may be an effective therapeutic alternative to oxytetracycline for the treatment of IBK in the event that *M bovis* develops tetracycline resistance or in cattle from areas where anaplasmosis is endemic and oxytetracycline use must be restricted.

At this time, florfenicol is not approved by the US Food and Drug Administration for treatment of IBK in cattle. Therefore, this use of florfenicol is considered extralabel, and florfenicol could be used for this purpose only if all of the criteria established under the Animal Medicinal Drug Use Clarification Act for extralabel drug use are met. For this study, FDA approval was granted for the experimental use of florfenicol for treatment of IBK in calves and for use at the higher SC dosage. Currently, oxytetracycline is approved by the FDA for the treatment of IBK in cattle.[10]

In another study,[11] corneal ulcer healing times were compared among treatment groups by use of a Cox regression model. We also elected to use this robust, nonparametric analytical method because the corneal ulcer surface area data were not normally distributed. The model also provided a way for comparing time-to-response data and for including right-censored healing time data. The number of recrudescent corneal ulcers was not determined in the study reported here, because calves were commingled, there was no fly control, and calves were treated for a maximum of 48 hours. Inclusion of the numbers of ulcers that recurred because of reinfection after tissue antibiotic concentrations were depleted would have confounded the statistical analyses.

[a]Fluor-I-Strip, Ayerst Laboratories Inc, Philadelphia, Pa.
[b]Nolvasan, Fort Dodge Laboratories, Ames, Iowa.
[c]Nuflor, Schering-Plough Animal Health Corp, Union, NJ.
[d]0.9% Sodium chloride injection USP, Baxter Healthcare Corp, Deerfield, Ill.
[e]LA-200, Pfizer Inc, New York, NY.

References

1. Lavy E, Ziv G, Soback S, et al. Clinical pharmacology of florfenicol in lactating goats. *Acta Vet Scand* 1991;87(suppl): 133–136.
2. Varma KJ, Adams PE, Powers TE, et al. Pharmacokinetics of florfenicol in veal calves. *J Vet Pharmacol Ther* 1986;9:412–425.
3. de Craene BA, Deprez P, D'Haese E, et al. Pharmacokinetics of florfenicol in cerebrospinal fluid and plasma of calves. *Antimicrob Agents Chemother* 1997;41:1991–1995.
4. 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.
5. Daigneault J, George LW. Topically applied benzathine cloxacillin for treatment of experimentally induced infectious bovine keratoconjunctivitis. *Am J Vet Res* 1990;51:376–380.
6. Weyant RS, Wayne Moss C, Weaver RE, et al. *Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria.* 2nd ed. Baltimore: The Williams & Wilkins Co, 1996; 392–393.
7. Jorgensen JH, Ferraro MJ, Craig WA, et al. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Vol 17. Wayne, Pa: NCCLS, 1997;6–10.
8. George L, Mihalyi J, Edmondson A, et al. Topically applied furazolidone or parenterally administered oxytetracycline for the treatment of infectious bovine keratoconjunctivitis. *J Am Vet Med Assoc* 1988;192:1415–1422.
9. 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.
10. Arrioja-Dechert A, ed. *Compendium of veterinary products*. 5th ed. Port Huron, Mich: Adrian J. Bayley, 1999.
11. Edmondson AJ, George LW, Farver TB. Survival analysis for evaluation of corneal ulcer healing times in calves with naturally acquired infectious bovine keratoconjunctivitis. *Am J Vet Res* 1989; 50:838–844.