Efficacy of Intramammary Infusion of Ceftiofur Hydrochloride at Drying Off for Treatment and Prevention of Bovine Mastitis during the Nonlactating Period*

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CLINICAL RELEVANCE

This study evaluated the efficacy of intramammary infusion of ceftiofur hydrochloride for the treatment of intramammary infections present at the last milking of lactation and for prevention of new intramammary infections during the nonlactating period. Cows were randomly assigned to five treatment groups (untreated negative control, 125, 250, and 500 mg of ceftiofur, and a positive control group receiving 300 mg cephapirin benzathine). A dose of 125 mg of ceftiofur per mammary quarter was effective for treatment of existing infections present at the time of milk cessation, but only the 500-mg dose of ceftiofur per mammary quarter was effective both for treatment of existing intramammary infections at the time of milk cessation and for prevention of new intramammary infections during the nonlactating period.

INTRODUCTION

The nonlactating period of dairy cows, more commonly referred to as the *dry period*, is a dynamic time when mammary glands undergo transition both from and to a state of active milk synthesis. The early dry period is associated with an abrupt cessation of milk removal; engorgement of the udder cisternal spaces, ducts, and alveoli with milk constituents; marked changes in milk composition; and regression of secretory tissue.[1] Near calving, mammary glands again undergo marked transition characterized by rapid differentiation of mammary secretory tissue; intense mammary growth; synthesis and secretion of proteins, fat, and carbohydrates; and accumulation of colostrum.[1]

*Sponsored by Pfizer Animal Health Veterinary Medicine Research & Development, Kalamazoo, MI.

The importance of the dry period in the control of mastitis and health of dairy cows in the subsequent lactation has been well documented and recognized for more than 50 years. A classic study by Neave and coworkers[2] published in 1950 demonstrated that udders were markedly susceptible to new intramammary infections (IMI) during the early dry period. The rate of new IMI during the first 21 days of the dry period was more than six times higher than the rate observed during the previous lactation. This was verified in several subsequent studies reviewed recently by Leslie and Dingwell.[3] Since the report by Neave and coworkers,[2] more recent studies have shown that udders are also highly susceptible to new IMI near calving. Many infections that occurred during the dry period resulted in clinical mastitis in the subsequent lactation period, particularly during early lactation.[4–9] Thus, in the absence of effective mastitis prevention and control measures at the time of milk cessation, more mammary quarters will be infected at calving than at drying off.

Most dairy advisers recommend that all mammary quarters of all cows be infused with antibiotics after the last milking of lactation.

Since the early work by Neave and coworkers,[2] procedures have been developed to control infections during the dry period. Intramammary antibiotic therapy following the last milking of lactation, commonly referred to as *dry cow therapy*, is practiced routinely on many dairy farms throughout the world. Most dairy advisers recommend that all mammary quarters of all cows be infused with antibiotics approved for use in dry cows after the last milking of lactation. The objectives of dry cow therapy are twofold: to eliminate infections present during late lactation and to prevent new infections during the early dry period when mammary glands are highly susceptible to new IMI.

Ceftiofur hydrochloride is a broad-spectrum third-generation cephalosporin antibiotic for veterinary use active against both gram-positive and gram-negative bacteria. Ceftiofur inhibits bacterial cell wall synthesis by interfering with enzymes essential for peptidoglycan synthesis, which results in lysis of the bacterial cell and accounts for the bactericidal nature of this antibiotic.[10] Consequently, ceftiofur should be effective against a wide range of contagious and environmental mastitis pathogens. Indeed, Salmon and associates[11] demonstrated that ceftiofur and its major metabolite, desfuroylceftiofur, had low minimum inhibitory concentration (MIC) values for such mastitis pathogens as *Staphylococcus aureus,* coagulase-negative *Staphylococcus* spp (CNS), *Streptococcus agalactiae, Streptococcus dysgalactiae* subsp *dysgalactiae, Streptococcus uberis*, and *Escherichia coli.* Recent studies by Oliver et al[12,13] demonstrated that intramammary infusion of 125 mg of ceftiofur hydrochloride into mammary quarters of lactating cows with subclinical and clinical mastitis was effective in eliminating infections caused by several different mastitis pathogens. In a pilot efficacy study, 500 mg of ceftiofur hydrochloride infused per mammary quarter following the last milking of lactation was effective in the treatment of existing IMI and for the prevention of new IMI during the dry period.[14] The objective of the present study was to evaluate the efficacy of a single intramammary infusion of several con-

centrations of ceftiofur for the treatment of existing IMI at the end of lactation and for prevention of new IMI during the dry period.

MATERIALS AND METHODS

Study Design

This multiple-location, randomized block design study was conducted at 21 private dairy farms in the United States. Licensed veterinarians with experience in bovine mastitis and dairy practice functioned as principal investigators at each site. The objective of the study was to compare the efficacy of a single intramammary infusion of 125, 250, or 500 mg ceftiofur equivalents from ceftiofur hydrochloride in an oil-based formulation (Pfizer Animal Health) or a positive control containing 300 mg cephapirin benzathine (Cefa-Dri, Fort Dodge Animal Health) with an untreated negative control for treatment of existing IMI present at the last milking of lactation and for prevention of new IMI that occur during the dry period. The study was conducted and analyzed in accordance with US FDA Center for Veterinary Medicine and Good Clinical Practices guidelines to support FDA registration of ceftiofur as a dry cow therapy.[15,16]

Herds

Eligible herds had a history of mastitis caused by staphylococci and/or streptococci and routinely used dry cow antibiotic therapy. Herds had to be large enough to provide a sufficient number of dry cows that met inclusion criteria to fill the designated blocks. Herds with a high incidence of damaged teats or teat ends, poor recordkeeping, or poor sanitation were not used.

Enrollment

Healthy Holstein dairy cows of all parities were eligible for enrollment as they approached dry-off. The principal investigator at each location obtained somatic cell count (SCC) data from the last Dairy Herd Improvement Association test or equivalent. Only cows with an SCC of 400,000 cells/ml or greater or a linear SCC score of 5 or greater were eligible for enrollment. Cows were excluded from the study if they had clinical mastitis or severe teat lesions at the time of dry cow therapy, were currently being treated for any concurrent illness, had received systemic or intramammary antiinfectious or antiinflammatory treatments for any reason within 30 days before enrollment, or had an expected dry period of less than 45 days or more than 80 days.

Blocking and Randomization

The study was conducted as a randomized complete block design. Within each herd, cows were blocked by lactation, with first through third lactation cows blocked together and fourth and greater lactation cows blocked together. Within each block of five cows, treatments were assigned randomly using a sponsor generated predetermined randomization schedule.

Treatment Groups

Five treatments were evaluated:

- Untreated negative control: n = 84 cows
- 125 mg of ceftiofur: n = 81 cows
- 250 mg of ceftiofur: n = 90 cows
- 500 mg of ceftiofur: n = 86 cows
- 300 mg cephapirin benzathine: n = 90 cows

Except for the untreated negative control group, mammary quarters of cows were treated with one 10-ml disposable syringe containing the assigned antibiotic per mammary quarter after the last milking of lactation. Immediately before treatment, teats were cleaned thoroughly and dried with individual disposable paper towels and teat ends were sanitized with swabs containing 70% isopropyl alcohol. Treatment

administrators used the full insertion technique in which the entire length of the cannula was inserted into the teat through the streak canal.

Milk Sampling and Testing

Milk samples were collected from each mammary quarter of each cow for microbiologic evaluation at the last milking of lactation immediately before treatment and 3 and 5 days after calving. The principal investigator or trained designee obtained a single sample containing at least 5 ml of foremilk from each mammary quarter of each cow enrolled in the study. Samples were collected aseptically before milking using standard procedures described by Hogan et al.[17] Before sample collection, teats were dipped in a premilking teat disinfectant, cleaned thoroughly, and dried with individual disposable paper towels and teat ends were sanitized with swabs containing 70% isopropyl alcohol. Samples obtained at the last milking of lactation (pretreatment milk samples) were used to identify mammary quarters infected at the time of drying off before treatment. Three and 5 days after calving, foremilk samples were collected aseptically from each mammary quarter of cows. Samples obtained at 3 and 5 days after calving (posttreatment samples) were used to establish the cure–failure rate for each treatment. The day each cow was milked for the first time after calving was considered day 0.

Samples were stored frozen until the cow completed the study. Samples were shipped in overnight freezer packs to the Milk Microbiology Laboratory at the Veterinary Teaching and Research Center, University of California, Davis, Tulare, California. Milk samples were examined following procedures recommended by the National Mastitis Council.[17] Briefly, an aliquot (10 µl) of foremilk from each mammary quarter was plated onto one quadrant of a trypticase soy agar plate supplemented with 5% washed bovine erythrocytes. Plates were incubated at 37°C, and bacterial growth was observed at 24-hour intervals for 2 days. Bacteria on primary culture medium were identified tentatively according to colony morphologic features, hemolytic characteristics, and catalase test. Isolates identified presumptively as *Staphylococcus* spp were tested for coagulase by the tube coagulase method. Isolates identified presumptively as *Streptococcus* spp were evaluated for growth by esculin hydrolysis and cAMP reaction. Streptococcal organisms were identified to the species level using the API 20 Strep system (bioMerieux, Hazelwood, MI). Gram-negative isolates were evaluated to the species level using the API 20E system (bioMerieux).

Definitions of *Cure*

A mammary quarter was considered microbiologically cured if bacteria identified in the pretreatment milk sample obtained at the last milking of lactation were absent from both samples obtained at 3 and 5 days after calving. Microbiologic cure of existing subclinical infection at the time of dry-off was used to decide which of the three ceftiofur doses or cephapirin cured IMI at a rate significantly greater than in untreated control cows.

A treatment was judged effective for prevention of new IMI during the dry period if both milk samples obtained after calving from an uninfected mammary quarter at the time of drying off were still negative for mastitis pathogens. Successful prevention of new IMI during the dry period consisted of a significantly greater prevention rate for antibiotic-treated mammary quarters than for untreated mammary quarters. Differences in the prevention rate for each treatment were determined. The percentage of mammary quarters that were uninfected after calving, regardless of infection status at the last milking of lactation, was also determined. This required that both

milk samples obtained after calving were microbiologically negative regardless of pretreatment infection status.

Statistical Analysis

The study was designed to enroll a sufficient number of animals to achieve 80% power for detecting 17% improvement using a one-sided test with a 5% significance level. Cows from the 21 herds that met inclusion criteria were blocked in groups of five based on lactation number and entered into the study. Parity groups were design factors and were not included in the analysis model. The proportion of mammary quarters cured was analyzed using the GLIMMIX macro of SAS (SAS Institute, Cary, NC).[18] Using the logit link with the binomial error, treatment was the fixed effect and herds, herds × treatment, and cows (herd × treatment) were random effects. For dose determination purposes, the four doses of 0, 125, 250, and 500 mg of ceftiofur per mammary quarter were used. The selected dose was the lowest dose that was significantly better than the untreated negative control at a one-sided $\alpha = .05$ level; and it and all higher doses were not significantly worse than the highest dose (500 mg) at a one-sided $\alpha = .10$ level.

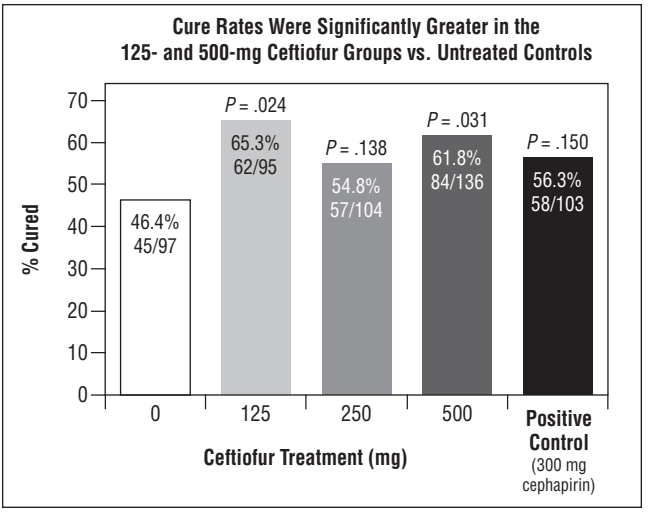

Figure 1. *Mammary quarter microbiologic cure rates after intramammary infusion of ceftiofur hydrochloride at the last milking of lactation.*

RESULTS

From the 21 participating herds, 431 cows and 1,708 mammary quarters were enrolled in this study. Approximately 85% and 15% of cows were in their first to third lactations and fourth or greater lactations, respectively. Since 61 mammary quarters had two pathogens isolated at dry-off, this increased the total number of quarters eligible for analysis to 1,769. Of these, 535 quarters had pathogens isolated in pretreatment samples that were used for microbiologic cure analysis. A total of 1,165 quarters with no growth in pretreatment samples was used for analysis of prevention of new infections during the dry period.

Ceftiofur hydrochloride as an intramammary infusion was well tolerated. No adverse formulation-related events were noted in any of the cows in this study that received intramammary infusion of ceftiofur after the last milking of lactation.

In milk samples obtained at the last milking of lactation before treatment, CNS were isolated most frequently (62.6% of isolates), followed by *S. aureus* (22.1%), environmental *Streptococcus* spp (2.8%), *Corynebacterium* spp (1.7%), and gram-negative mastitis pathogens (1.5%). *S. agalactiae* was not found in any of the herds participating in this study.

Results of microbiologic cure rates for the five treatment groups are shown in Figure 1. A mammary quarter was considered microbiologically cured if bacteria identified in the pre-

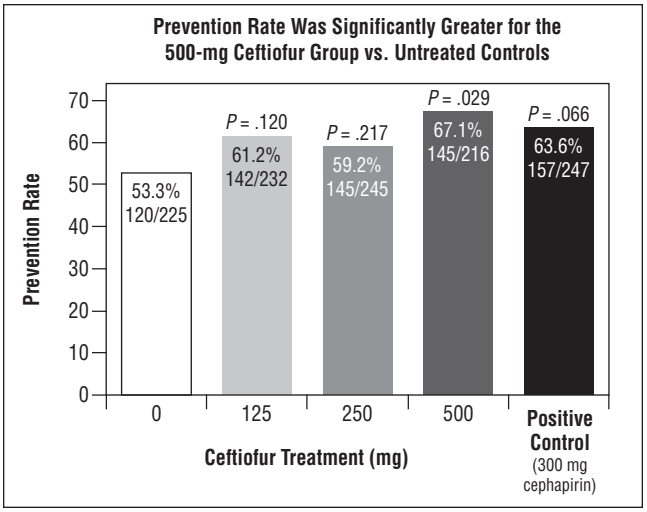

Figure 2. *Prevention of new mammary quarter intramammary infections during the dry period after intramammary infusion of ceftiofur hydrochloride at the last milking of lactation.*

treatment milk sample obtained at the last milking of lactation were absent from both samples obtained at 3 and 5 days after calving. If one of the two postcalving samples was positive, this was considered a treatment failure. Microbiologic cure rates were 46.4%, 65.3%, 54.8%, 61.8%, and 56.3% for 0 mg, 125 mg, 250 mg, and 500 mg ceftiofur and 300 mg cephapirin, respectively (Figure 1). Microbiologic cure rates for 125 mg of ceftiofur (P = .024) and 500 mg of ceftiofur (P = .031) were significantly greater than for untreated negative controls. However, microbiologic cure rates for cephapirin (P = .150) and 250 mg of ceftiofur (P = .138) were not statistically different from untreated negative controls.

Prevention of new IMI during the dry period and comparisons with the untreated control group are presented in Figure 2. A treatment was considered effective for the prevention of new IMI during the dry period if both postcalving milk samples from microbiologically negative pretreatment mammary quarters were still negative after calving. Prevention rates of new IMI during the dry period were 53.3%, 61.2%, 59.2%, 67.1%, and 63.6% for 0 mg, 125 mg, 250 mg, and 500 mg of ceftiofur and 300 mg cephapirin, respectively (Figure 2). The prevention rate for 500 mg of ceftiofur (P = .029) was significantly greater than for untreated negative controls. Prevention rates for cephapirin (P = .066), 125 mg of ceftiofur (P = .120), and 250 mg of ceftiofur (P = .217) were not statistically different from untreated negative controls.

The percentages of mammary quarters uninfected (both postcalving milk samples microbiologically negative) during early lactation regardless of pretreatment infection status were 43.7%, 56.7%, 52.2%, 59.6%, and 55.5% for 0 mg, 125 mg, 250 mg, and 500 mg of ceftiofur and 300 mg cephapirin, respectively (Figure 3). The percentage of mammary quarters uninfected during early lactation for 500 mg of ceftiofur (P = .001), 125 mg of ceftiofur (P = 0.013), and cephapirin (P = .011) were statistically greater than for untreated negative controls. However, the percentage of mammary quarters uninfected during early lactation for 250 mg ceftiofur (P = .075) was not statistically different from untreated negative controls.

DISCUSSION

The objectives of dry cow therapy are twofold: to eliminate infections present during late lactation and to prevent new infections during the early dry period when mammary glands are highly susceptible to new IMI. Intramammary infusion of 500 mg of ceftiofur after the last milking of lactation was effective for

eliminating IMI present during late lactation and for preventing new IMI at a time when mammary glands are highly susceptible to such infections. None of the other doses of ceftiofur or cephapirin met both these criteria.

Experimental evidence suggests that dry cow therapy is very effective in controlling IMI due to *S. agalactiae,* somewhat effective against *S. aureus*, less effective against environmental streptococci, and ineffective against coliform bacteria.[1,3–9] Environmental mastitis pathogens such as *S. uberis, S. dysgalactiae,* and coliform bacteria are ubiquitous in the cows' environment. Consequently, mammary glands are exposed continuously to environmental mastitis pathogens throughout the dry period, especially in herds in total confinement housing.[1,3–9] Many antibiotic preparations approved for use in dry cows are formulated primarily against gram-positive bacteria and have little, if any, activity against gram-negative mastitis pathogens.

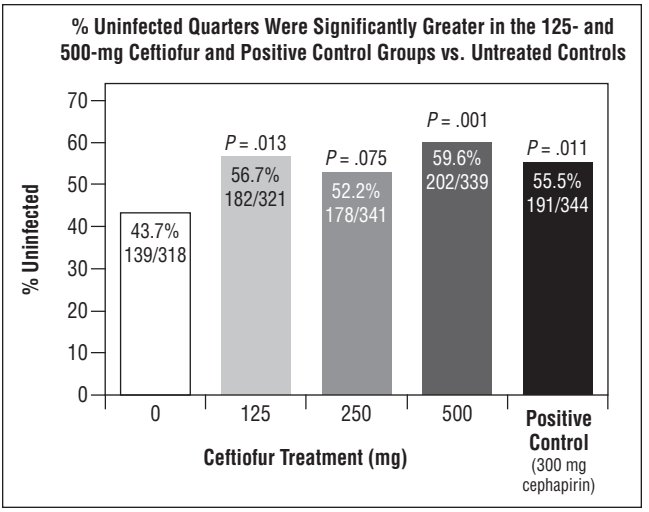

Figure 3. *Percentage of mammary quarters uninfected during early lactation after intramammary infusion of ceftiofur hydrochloride at the last milking of lactation regardless of infection status at drying off.*

In the present study, several bacteria—including CNS, *S. aureus*, environmental *Streptococcus* spp, *Corynebacterium* spp, and such gram-negative mastitis pathogens as *E. coli* and *Klebsiella* spp—were identified from infected mammary quarters. Results of the present study suggest that ceftiofur is effective at eliminating several pathogens capable of causing mastitis. This is consistent with a recent report in which Oliver and associates[12] demonstrated that intramammary infusion of ceftiofur hydrochloride into mammary quarters of lactating cows with subclinical mastitis was effective in eliminating infections caused by several different mastitis pathogens, including *S. aureus*, *S. dysgalactiae,* CNS, and *Corynebacterium bovis*. In another study, ceftiofur therapy was effective in eliminating *S. uberis* experimental IMI in dairy cows that developed clinical mastitis following experimental infection during early lactation.[13] It is noteworthy that ceftiofur has considerable in vitro activity against several environmental mastitis pathogens, including environmental streptococci and gram-negative mastitis pathogens. Ceftiofur may be effective for the treatment and prevention of mastitis caused by gram-negative pathogens during the dry period, which is one important limitation of many dry cow antibiotic preparations approved for use. However, in the present study, there were too few gram-negative IMI to demonstrate efficacy, and, consequently, further studies are needed to substantiate this hypothesis.

CONCLUSION

The results of this large multiple-location field study demonstrated that a single intramammary infusion of 500 mg of ceftiofur was the only treatment in this study that was effec-

tive (statistically) both for treatment of existing IMI at the last milking of lactation and for prevention of new IMI during the dry period. This treatment resulted in a significantly greater percentage of uninfected mammary quarters at calving regardless of pretreatment infection status. Ceftiofur was effective against a broad range of mastitis pathogens. Ceftiofur hydrochloride as an intramammary infusion was well tolerated, and no adverse formulation-related events were noted in any of the cows enrolled in this study. The broad spectrum of antimicrobial activity coupled with the ability of ceftiofur to eliminate IMI present at the time of drying off and to prevent new IMI during the dry period at a time when mammary glands are highly susceptible to new IMI should be of benefit to the dairy industry.

ACKNOWLEDGMENTS

We appreciate the veterinarians, dairy producers, and farm personnel who participated in this study. We also appreciate the laboratory personnel at the Milk Microbiology Laboratory at the Veterinary Teaching and Research Center, University of California, Davis, Tulare, California.

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