Efficacy of Two Barrier Teat Dips Containing Chlorous Acid Germicides Against Experimental Challenge with Staphylococcus aureus and Streptococcus agalactiae

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ABSTRACT

Two postmilking teat dips were tested for efficacy against Staphylococcus aureus and Streptococcus agalactiae using experimental challenge procedures recommended by the National Mastitis Council. Both dips contained chlorous acid as the primary germicidal agent and lactic acid or mandelic acid as the chlorous acid activator. The dip activated with mandelic acid significantly reduced new IMI by Staph. aureus and Strep. agalactiae. The IMI rate was reduced 68.7% for Staph. aureus and 56.4% for Strep. agalactiae. The dip activated with lactic acid significantly reduced new Staph. aureus IMI by 69.3% but did not significantly reduce new Strep. agalactiae IMI (35.2% reduction) through the full 11-wk study period. Teat skin condition did not change from pretrial status after using either teat dip during the study.

(Key words: barrier teat dip, chlorous acid, Staphylococcus aureus, Streptococcus agalactiae)

INTRODUCTION

Physical barrier teat dips were originally developed to prevent coliform mastitis (9). An acrylic latex product without a germicide effectively reduced new IMI caused by Staphylococcus aureus, Staphylococcus epidermidis, and coliforms but did not significantly reduce new IMI caused by Streptococcus agalactiae or environmental streptococci (3). The same latex teat dip (3) was subsequently formulated with a germicide of known efficacy. Culture of bacteria from latex dips that did not contain germicides may have prompted the inclusion of germicides in the first latex barrier products (9). The acrylic latex barrier film remaining on teats between milkings must be peeled or washed from the teats with water immediately before the next milking.

A new class of barrier teat dips has recently been developed in which the physical barrier is composed of a polymer gel within which initial germicidal activity is provided by the generation of chlorous acid. Chlorous acid provides continuous broad-spectrum germicidal activity when the teat dip remains moist on the teat skin surface (8, 10). The use of an a-hydroxy organic acid as the activator for generation of chlorous acid provides for the continued maintenance of germicidal activity in the residual film remaining on the teat surface after drying of the product (4). The teat dips tested during the study presented here were of this generic type. Both dips tested were water-soluble and contained a moistening agent to keep the products flexible on teats. Barrier teat dips containing chlorous acid are formulated to kill organisms on teat skin im-
Efficacy of Chlorous Acid Teat Dip

Immediately after milking and during the intermilking period (9).

The objective of this study was to evaluate two postmilking teat dips containing chlorous acid generated from lactic or mandelic acid as the activator using an experimental challenge procedure.

MATERIALS AND METHODS

Sampling Schedule

Bacteriologic status of mammary quarters was determined at the initiation of the trial by collection and culture of duplicate milk samples. A third sample was collected from specific quarters and cultured when results from the first two samples differed. Milk samples were collected and analyzed weekly during the trial. Whenever Staph. aureus or Strep. agalactiae was isolated for the first time in a previously uninfected quarter, a second sample was collected and cultured within 2 d after first isolation. All quarters were eligible for new IMI during the trial except 1) those infected with organisms of the same species as challenge organisms and 2) those with deformed or abnormal teats.

Collection of Milk Samples

Prior to sampling, udders were washed using a hand-held hose and paper towels. After washing, udders were dried thoroughly with additional paper towels, and two or three streams of foremilk were discarded. Each teat apex was scrubbed for several seconds with a cotton pledget moistened with 70% alcohol. Teats on the opposite side of the udder from the technician were sanitized first, and milk samples were collected in reverse order in sterile, snap-cap plastic tubes and refrigerated at 5°C.

Laboratory Cultural Procedures

Samples were mixed by shaking, and a .01-ml aliquot was streaked on trypticase soy agar (TSA) (Becton Dickinson, Cockeysville, MD) containing 5% bovine blood. Plates were incubated at 37°C for 48 h and examined to identify microorganisms present. Staphylococcus aureus were identified presumptively by hemolytic pattern and confirmed by tube coagulase test. Streptococcus agalactiae were identified to serogroup by the Phadebact Streptococcus Test (Boule Diagnostics AB, Huddinge, Sweden). An IMI was confirmed when 1) Staph. aureus or Strep. agalactiae were isolated from a clinical quarter, 2) two consecutive samples yielded ≥500 cfu/ml of the same pathogen, or 3) three consecutive samples contained 100 to 400 cfu/ml of the same pathogen (5).

Description of Experimental Teat Dips

The teat dips were provided as a base solution containing .64% sodium chlorite in a gel formulation and an activator that contained 3% mandelic acid or 2.64% lactic acid (UDDERgold; Alcide Corporation, Norwalk, CT). The base and activator were mixed in equal amounts immediately prior to milking and used fresh daily.

Treatment Method

The milking herd of the Hill Farm Research Station (n = 130) was divided into two groups of cows of equal number. One group was used to determine efficacy of the mandelic acid dip and was milked first; the other group was used to evaluate the lactic acid dip and was milked second. At the afternoon milking, Monday through Friday, all 4 teats of each cow were experimentally exposed by immersion to a depth of approximately 25 mm in a challenge suspension containing Staph. aureus (ATCC 29740) and Strep. agalactiae (ATCC 27956) immediately after milking machines were removed. Immediately thereafter, the distal 25 mm of 2 diagonally opposed teats were dipped with teat dip; the remaining 2 teats served as undipped controls. Teats were exposed to challenge organisms to increase the number of pathogens impinging on the teat apex, resulting in an increased rate of IMI. After 5 wk, sufficient numbers of new Staph. aureus IMI developed in control quarters of both groups of cows to perform a valid statistical analysis; thus, subsequent challenge with Staph. aureus was discontinued, and, for the remaining 6 wk of the study, teats were challenged with Strep. agalactiae only.

Preparation of Challenge Suspensions

Stock suspensions of Staph. aureus were prepared weekly. The contents of one lyophi-
lized vial of *Staph. aureus* were reconstituted in 6 ml of trypticase soy broth (TSB) (Becton Dickinson) and incubated at 37°C for 5 to 7 h. This culture was used to inoculate 500 ml of TSB, which was incubated on a gyratory shaker for 16 h. After incubation, bacterial cells were pelleted by centrifugation, washed twice with 1% proteose-peptone (Difco Laboratories, Detroit, MI), and resuspended to the original volume in proteose-peptone. Serial dilutions were prepared in proteose-peptone, and .1 ml was plated on TSA. Plates were incubated for 24 h at 37°C, and colonies were counted to ascertain the microbial concentration of the stock suspension. This suspension was stored at 5°C and used daily for preparation of the stock suspension. This suspension to obtain a concentration of approximately 10⁷ cfu/ml.

An aliquot of the *Staph. aureus* stock suspension was added to the *Strep. agalactiae* suspension to obtain a concentration of approximately 5 x 10⁷ cfu/ml of *Staph. aureus*. This bacterial suspension was taken immediately to the milking parlor to challenge teats during the afternoon milking. A plate count was conducted daily on challenge suspensions.

**Statistical Methods**

Differences between the percentage of quarters becoming infected in treatment groups were tested as described by Hogan et al. (5) using an approximated t statistic defined as

\[ t = \left( \frac{(x_1/n_1) - (x_2/n_2)}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}(\frac{1}{n_1} + \frac{1}{n_2})} \right)^{\frac{1}{2}} \]

where \( x_1 \) = number of new IMI in control quarters, \( x_2 \) = number of new IMI in treated quarters, \( n_1 \) = (number of control quarters) (time unit), and \( n_2 \) = (number of treated quarters) (time unit). The denominators \( n_1 \) and \( n_2 \) were expressed as the summation of quarter days. A quarter was eligible for only one IMI during the study. The percentage of reduction in rate of new IMI in the treated groups compared with that in the control groups was expressed as

\[ \text{Reduction in rate of new IMI} = 100 \left( 1 - \frac{(x_1/n_1) - (x_2/n_2))}{(x_1/n_1)} \right) \]

Teat germicides are considered to be efficacious when mean percentage of reduction of new IMI is ≥40% and the lower confidence limit of the mean is ≥25% (5).

**Scoring of Teat Skin Condition**

Characteristics of teat skin surface in both teat dip groups were scored immediately before the trial was initiated and at the conclusion of the trial to determine effects of the teat dip products on teat skin. Teat skin was characterized as 1) normal; 2) abnormal: exhibiting a cracked, sloughed, or chapped surface; 3) associated with a nonfunctional quarter; 4) associated with a nonfunctional quarter; or 5) displaying warts.

**RESULTS AND DISCUSSION**

The IMI data collected during the study are summarized in Table 1. The lactic acid dip reduced the number of new *Staph. aureus* IMI by 69.3% (P ≤ .001), and the number of new *Strep. agalactiae* IMI was reduced by 35.2% (P ≤ .001) for the first 11 wk of the study. The mandelic acid dip significantly reduced new IMI by both organisms with efficacies of 68.7% (P ≤ .001) for *Staph. aureus* and 56.4% (P ≤ .01) for *Strep. agalactiae*. Reduction in new *Strep. agalactiae* IMI for wk 1 through 7 for the lactic acid dip was 65% (P ≤ .01), but, during wk 8, 6 new *Strep. agalactiae* IMI in dipped quarters and one new IMI in control quarters were confirmed (data not shown). Thus, during wk 8 through 11, efficacy decreased to 35.2% against *Strep. agalactiae* for this product. This IMI rate with *Strep. agalactiae* was not observed for the first 7 wk of the study or for the last 3 wk of the study, but only during wk 8. The 6 new IMI in dipped quarters during wk 8 occurred in 5 cows with normal teat skin and teat meatus condition. The atypical, increased IMI rate was not related to weather or herd management. The increase in IMI rate during wk 8 was not...
found for *Staph. aureus* in the same dip group or for *Staph. aureus* and *Strep. agalactiae* in the mandelic acid dip group.

Incidence of clinical mastitis (CM) for control and dipped quarters, respectively, was similar for *Staph. aureus* (0 vs. 0%) and *Strep. agalactiae* (1.36 vs. 1.34%) in the lactic acid dip group. In the mandelic acid dip group, the percentage of eligible control quarters that became clinically infected with *Staph. aureus* was higher than that of dipped quarters (4 vs. 2.9%). In contrast, the percentage of eligible quarters with *Strep. agalactiae* CM was greater for quarters dipped in the mandelic acid dip than for control quarters (1.41 vs. .71%). The incidence of CM was too small to test statistically.

Teats in both dip groups were scored for chapping, sloughing, cracks, and other injuries immediately prior to the study and at the end of the study (Table 2). At least 97% of teats were characterized as normal before and after the trial for both groups. In the group treated with lactic acid, 1 undipped control teat of 1 cow (.67%) that was characterized as normal before the trial was characterized as abnormal after the trial. In the group treated with mandelic acid, 2 teats of 2 cows among the dipped quarters that were characterized as abnormal (.69%) or having mechanical injuries (.69%) before the trial improved in condition score after the trial. Thus, teat irritation or abnormalities could not be attributed to use of either product.

Few studies involving experimental exposure to bacteria that cause mastitis tested chlorous acid teat dips using the protocol recommended by the National Mastitis Council (5). Drechsler et al. (1) evaluated a chlorous acid barrier teat dip with lactic acid as the activator during experimental challenge exposure and obtained efficacies of 78.9 and 52.9% against *Staph. aureus* and *Strep. agalactiae*, respectively, after results from two research herds were combined. The same dip was more effective than a positive control teat dip containing 1% iodine against major mastitis pathogens in a natural exposure study (1). In another natural exposure study, a chlorous acid barrier dip with lactic acid reduced new *Staph. aureus* IMI by 67.4% in a research dairy herd (8).

A natural exposure study of five commercial herds compared a chlorous acid barrier teat dip containing lactic acid against a positive control product containing .5% iodophor and found a reduction of 18.8% in new IMI caused by all pathogens and a reduction of 13.6% in major pathogen IMI for the chlorous acid dip group (10). Efficacy of the experimental dip

### TABLE 1. Efficacy data of barrier teat dips containing chlorous acid against experimental challenge with *Staphylococcus aureus* and *Streptococcus agalactiae*.

<table>
<thead>
<tr>
<th>Organism and treatment</th>
<th>Quarters eligible for new IMI</th>
<th>New IMI</th>
<th>Quarter days at risk for new IMI</th>
<th>New IMI per 100 quarter days at risk</th>
<th>Reduction (no.)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staph. aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lactic acid dip</td>
<td>138</td>
<td>10</td>
<td>4939</td>
<td>.202</td>
<td>134</td>
<td>69.3***</td>
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<tr>
<td>Control</td>
<td>134</td>
<td>29</td>
<td>4397</td>
<td>.660</td>
<td></td>
<td></td>
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<tr>
<td><em>Strep. agalactiae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid dip</td>
<td>149</td>
<td>18</td>
<td>9281</td>
<td>.194</td>
<td>147</td>
<td>35.2*</td>
</tr>
<tr>
<td>Control</td>
<td>147</td>
<td>26</td>
<td>8691</td>
<td>.299</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandelic acid dip</td>
<td>136</td>
<td>8</td>
<td>5183</td>
<td>.154</td>
<td>136</td>
<td>68.7***</td>
</tr>
<tr>
<td>Control</td>
<td>125</td>
<td>22</td>
<td>4465</td>
<td>.493</td>
<td></td>
<td></td>
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<tr>
<td><em>Strep. agalactiae</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandelic acid dip</td>
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<td>13</td>
<td>9791</td>
<td>.133</td>
<td>141</td>
<td>56.4**</td>
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<tr>
<td>Control</td>
<td>141</td>
<td>28</td>
<td>9186</td>
<td>.305</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P ≤ .10.

**P ≤ .01.

***P ≤ .001.
TABLE 2. Frequency of teat surface characteristic scores before and after the trial for barrier teat dips containing chlorous acid.

<table>
<thead>
<tr>
<th></th>
<th>Lactic acid dip</th>
<th>Mandelic acid dip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>Dipped Control</td>
<td>Dipped Control</td>
</tr>
<tr>
<td>Normal</td>
<td>93.33 98</td>
<td>99.33 97.33</td>
</tr>
<tr>
<td>Abnormal¹</td>
<td>0 0</td>
<td>.67 .67</td>
</tr>
<tr>
<td>Nonfunctional</td>
<td>.67 2</td>
<td>0 0</td>
</tr>
<tr>
<td>Mechanical injury</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Warts</td>
<td>0 0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Teat skin that was cracked, sloughed, or chapped.

against coagulase-negative staphylococci was 33.3%, and, because these organisms colonize the teat canal, Poutrel et al. (10) theorized that the barrier dip was able to penetrate the teat canal and to kill organisms colonizing this area.

In vitro studies (6) to evaluate germicidal activity of a chlorous acid product showed that addition of growth medium or neutralizing broth did not affect the reduction in number of *Staph. aureus* colonies after 30 s of exposure to the dip. Similarly, effectiveness of the dip against *Staph. aureus* was not affected by time up to 48 h or by the presence of organic matter (6). A chlorous acid disinfectant with a lactic acid activator was 100% effective in killing *Staph. aureus* after exposure of 30 min even when it was diluted 1:1 (vol/vol) with phosphate-buffered saline (11).

The pharmacological activity of chlorous acid that is primarily responsible for the destruction of microorganisms derives from the partial conversion of the chlorite ion (ClO₂⁻) to its corresponding acid form, chlorous acid (HClO₂) (4). This reaction occurs when sodium chlorite is combined with an organic acid under appropriate conditions of pH and chlorite concentration. Chlorous acid is an unstable entity that acts as a source of a series of rapid and highly efficient cidal oxidants as it undergoes a degradation cascade. Chlorous acid can, however, be maintained in a metastable equilibrium under the appropriate conditions of acidity and chlorite concentration, as for the teat dip formulations tested in this study. At the pH achieved in the lactic acid product from ionization of the lactic acid component, approximately 7% of the chlorite ion that is initially present in the formulation has been transformed to the chlorous acid form. The latter undergoes a series of disproportionation reactions on contact with organic matter (e.g., microorganisms) and on evaporation that lead to higher, more unstable concentrations of chlorous acid. The cidal oxidant species that are produced on disproportionation include Cl₂O₂, HClO, ClO₂, and Cl₂O₄ (4).

As chlorous acid is depleted, the system reequilibrates by combining newly ionized organic acid with residual chlorite, thereby replenishing the consumed chlorous acid and continuing the cidal activity. Upon evaporation and eventual drying of the barrier formulation (approximately 10 min), the cidal action from the chlorous acid cascade becomes virtually exhausted. However, the antimicrobial activity during that period has resulted in a minimum reduction of 6 log in surface microorganisms (G. K. Kemp, 1994, Alcide Corp., Norwalk, CT, personal communication).

Once dried, the organic acid that was initially present as the activator of the chlorite species is reduced to about 62% of its original concentration to exist eventually as a lactic acid and lactate buffer. The residual lactic acid, although of less bactericidal ability than the chlorous acid cascade, remains on the teat skin, thereafter to provide continued bacteriostatic and bactericidal activity. The AOAC testing of lactic acid against *Escherichia coli* and *Staph. aureus* has shown population reductions of up to 4 logs and 2 logs, respectively, within 15 s of exposure (G. K. Kemp, 1994, personal communication).
The physical kinetics of destruction of microorganisms following application of a germicidal system of chlorous acid to teat skin is not directly measurable. The mechanism of cidal action is hypothesized to be oxidative, involving the rapid and nonspecific oxidation of labile bonds (e.g., S-S, S-CH₃, phenyl-OH) on amino acid moieties of microbial surface proteins (7). This oxidation disrupts the surface potentials of the organisms, leading to electrolyte leakage and cellular death. The antimicrobial activity of chlorous acid includes an extremely wide range of Gram-positive and Gram-negative bacteria, fungi, mycoplasma, and lipophilic and hydrophilic viruses.

The use of α-hydroxy organic acids other than lactic acid, e.g., mandelic acid, as activators of generation of chlorous acid has no measurable effect on initial germicidal activity. This result is to be expected, because efficacy of the chlorous acid antimicrobial system is based on the generation of comparable amounts of chlorous acid in both formulations. Both lactic acid and mandelic acid at the same pH and in the presence of sodium chlorite initiate the generation of chlorous acid to much the same degree; therefore, no differences in initial efficacy would be anticipated.

The teat dips tested in this study compared favorably with other barrier teat dips containing a chlorous acid germicide against the same mastitis organisms (1, 8, 10, 12). These dips offer antimicrobial activity that is greater than or equal to that of chlorine, with greater residual activity (2), plus the effect of a barrier film, which may seal the teat end.

ACKNOWLEDGMENTS

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