ABSTRACT

Efficacy of three different treatment regimens in the elimination and prevention of Staphylococcus aureus intramammary infection was studied in 106 dry cow periods. At drying off, norfloxacin nicotinate was given subcutaneously to 44 cows at 10 mg/kg, oxytetracycline-HCl was administered intramuscularly to 18 cows at 20 mg/kg, 500 mg cephapirin benzathine were infused into each udder quarter of 21 cows, and a group of 23 cows served as an untreated control.

Number of existing Staphylococcus aureus intramammary infections was reduced only in the norfloxacin nicotinate treatment group. New infection rate appeared lower in the two systemic treatment groups. The percentage of infected quarters remained the same throughout the dry period in the norfloxacin treatment group but number of infected quarters increased by 33 to 85% (significant in the cephapirin group) in the other groups. Minimal inhibitory concentration of the drugs for 57 S. aureus isolates was determined. Isolates were sensitive to norfloxacin and cephapirin and moderately sensitive to oxytetracycline.

Results suggest that systemic dry cow therapy using norfloxacin nicotinate, which possesses large distribution volume, long half-life, and is highly active against the pathogen involved, was more effective than the other treatments. (Key words: mastitis, therapy, pharmacokinetics)

INTRODUCTION

Mastitis is probably the most important disease of the lactating cow, causing economical losses estimated to be more than 2 billion dollars in the US (13). Effective treatment regimens could substantially reduce these losses. Acute mastitis is treated during lactation, but subclinical intramammary infections (IMI) are usually subject to dry cow therapy. Antibiotic treatment at drying off aims at both eliminating the existing IMI and preventing new infections (6). Dry cow therapy at present exclusively implies intramammary infusion of antimicrobial agents. The udder is most susceptible to new infections during the first and last weeks of the dry period (10, 18), and optimally, the therapy should be extended over the whole dry period. This is claimed to be achieved by the use of special slow release ointment bases in the intramammary preparations. Although large amounts of antibiotics are used annually on mastitis therapy, the resulting bacteriological cure has been low, especially in the case of Staphylococcus aureus intramammary infections (2, 4).

The pharmacokinetic basis and the theoretical soundness of intramammary antibiotic therapy was discussed recently (19). Antibiotic concentrations in the milk or in the dry udder secretions exceeding the minimal inhibitory concentration (MIC) have been generally accepted to be equated with potentially effective therapeutic concentrations (23). Mastitis is, however, a disease of the mammary tissue. This is especially pronounced when S. aureus, considered to be a deep tissue invader, is involved. Antibiotic concentrations attained in the milk will have only a low, if any, correlation to the concentrations reached at the foci of infection, i.e., the tissue. Pharmacokinetic calculations showed that the absorption of the drug from the mammary tissue to blood circulation is faster than the absorption of the drug from milk to the tissue (19). When absorption is further slowed by the slow release base, drug concentrations in tissue are very low. Because the drug clearance...
mechanisms in the body are able to eliminate the drug faster than it enters the system, no systemic antimicrobial effect can be anticipated. In order to reach adequate antibiotic tissue concentrations, systemic administration of antimicrobial agents at drying off was suggested (19).

The third generation antibacterial fluoroquinolones are well distributed into the body fluids, including the intracellular fluid (22). They are also able to kill phagocytosed but intact staphylococci residing inside the macrophages (14, 15). The water-soluble norfloxacin nicotinate, due to its pharmacokinetic and antimicrobial properties in the dairy cow (25), appeared potentially promising for the systemic therapy.

The purpose of this study was to evaluate a new approach to dry cow therapy in the form of systemic treatment for subclinical, staphylococcal mastitis. The norfloxacin nicotinate treatment was compared with systemic oxytetracycline therapy, intramammary cephapirin therapy, and untreated control groups.

**MATERIALS AND METHODS**

**Animals and Housing**

Israeli Holstein cows were used from a commercial herd totaling 225 milking cows at a kibbutz type farm located in the central part of Israel. The herd’s average annual milk production was 9333 L per cow, and the mean SCC was 619,000 cells/ml. Mastitis control procedures such as teat dipping or dry cow treatment were not employed during the last 5 yr. Cows were milked three times daily with computer-controlled milking equipment (Westfalia Separator AG., Oelde, West-Germany) in a double-sided, 14-stall herringbone parlor. The cows were housed in free stalls. Cows were dried off approximately 60 d before the calculated calving date or when the daily yield declined to less than 12 L. One hundred and six dry periods were studied. The dry cows were housed in separate stalls and moved to a maternity unit 1 wk before the estimated calving date. After calving, cows were placed among the milking cows. No management changes were made during the study.

**Sampling and Microbiological Procedures**

All cows were sampled (quarter milk samples) at drying off and between 7 and 21 d postpartum. Prior to collection of the milk samples, the teat ends were cleaned and disinfected with cotton swabs soaked with 70% alcohol. The first three or four streams of milk were discarded, the teat ends were disinfected again with alcohol, and 5 to 10 ml of milk were collected in a sterile test tube. The uncooled samples were taken to the laboratory for culture within 1 to 2 h after collection. All samples were examined microbiologically by streaking .05 ml of milk onto the surface of one-fourth plate of trypticase soy agar (Difco, Detroit, MI, US) containing 5% whole bovine blood and .05% esculin with .01% of ferric citrate (Difco). After 14 to 16 h and an additional 24 h of incubation at 37°C, the colonies on the plates were tentatively identified according to morphology, CAMP test, and type of hemolysis produced. Those initially characterized as staphylococci were tested in tubes for coagulase production to confirm the diagnosis of *S. aureus*. For the purpose of the present report all other major and minor udder pathogens isolated were disregarded.

**Treatments**

All treatments were given at random immediately after obtaining the quarter milk samples at the cessation of milking. Forty-four cows were given subcutaneous injection of a freshly prepared aqueous solution of norfloxacin nicotinate (250 mg/ml) (“Quinabic”, Abic Ltd., Netanya, Israel) at 10 mg/kg and 18 cows were administered intramuscularly oxytetracycline-HCl (“Engemycin 10%/LA”, Gist-Brochades, Delft, Holland) at 20 mg/kg. Twenty-one cows were infused intramammarily (full insertion of the cannula) with benzathine cephapirin (“Cephapirin D. C.”, Vitamed Ltd., Bat-Yam, Israel) at 500 mg to each quarter. Twenty-three cows served as untreated controls.

**Minimal Inhibitory Concentrations**

The MIC of the three antimicrobial agents used in the study for 57 *S. aureus* isolates were determined by procedures using the twofold agar dilution method (21). Bacteria were iso-
lated from milk samples taken from cows with subclinical mastitis at the cooperator farm. Stock solutions (1.0 mg/ml) of the antimicrobials were prepared in .9% saline, and plates of Mueller-Hinton agar (Difco, Detroit, MI) at pH 7.2 were prepared containing twofold antibiotic dilutions in concentrations ranging from .01 to 100 μg/ml. Overnight, nutrient broth (Difco) cultures of each isolate were used as inocula diluted 1:100 in sterile saline solution. Using a modified Steers' inoculation device delivering .01 ml of bacterial culture, the plates were inoculated, incubated aerobically at 37°C for 16 h and examined for MIC. The inoculation size was approximately 1 x 10⁵ organisms. The lowest concentration that totally inhibited bacterial growth was the MIC.

Statistics

Chi-square test was used for statistical analysis of the data. A value of P<.05 was considered significant.

RESULTS

Table 1 illustrates the in vitro antimicrobial activity of the three drugs used. The range of MIC values gives an overall indication of the susceptibility of the bacterial isolates examined, whereas the median MIC (MIC₅₀) and 90% MIC (MIC₉₀) provide information on the distribution of MIC values within the population. The tested staphylococcal isolates appeared to be sensitive to cephapirin and to norfloxacin with MIC₅₀ at .39 and .2 μg/ml. The isolates were only moderately sensitive to tetracycline: MIC₅₀ was 6.25 μg/ml and MIC₉₀ was >100 μg/ml.

The cure and new infection rates are shown in Table 2. The norfloxacin-treated group had a higher cure rate (66.7%) than the other groups. The cure rate of the other two treatment groups (25.0% for the oxytetracycline-treated and 30.8% for the cephapirin-treated groups) did not differ from that of the untreated control group (33.3%). Significantly fewer new infections were diagnosed in the norfloxacin and oxytetracycline treatment groups (17.1% and 9.7%) than in the untreated control group (29.2%). The new infection rate in the intramammary treated group (51.7%) was significantly higher than in the control group.

DISCUSSION

To the best of our knowledge, systemic dry cow therapy has never been reported or implemented, although parenteral treatment was recommended as an adjunct to intramammary dry cow therapy (16). The theoretical pharmacokinetic and pharmacodynamic basis for this type of therapy was reported (19). Results of this study (Table 2) indicate that the cure rate seen after parenteral norfloxacin nicotinate therapy was significantly better than those of the other antimicrobial agents used and the spontaneous cure in the untreated control group. Pharmacokinetically (Table 3), both norfloxacin and oxytetracycline are well distributed into the body fluids and should be able to reach the site of infection, but only norfloxacin has been shown to kill intracellular, phagocytosed staphylococci (14, 15). The terminal half-lives of the two drugs in serum following intravenous administration are 6.1 and 9.5 h (Table 3), which is relatively long and a desired feature for parenteral dry cow therapy. The MIC values of tetracycline against Staphylococcus aureus isolates were high (Table 1), and it seems possible that the serum and tissue drug concentrations were insufficient for successful treatment.
thermore, tetracyclines lost their antibacterial activity in the presence of milk (11). There were only eight infected quarters in the oxytetracycline treatment group, which limited conclusions. The third generation fluoroquinolones are excreted well (peak concentration of milk: plasma is >5) into the milk (5, 25), which may indicate high mammary tissue concentrations.

The MIC values for the isolates of S. aureus were lowest with cephapirin. However, the cure rate recorded for the cephapirin treatment group was similar to that of the oxytetracycline-treated and control groups and was significantly lower than for the norfloxacin treatment group. Therefore, route of administration, the dose, or the stability of the cephapirin formulation used seems to have been inappropriate. The β-lactam antibiotics do not enter the intracellular space and, therefore, the phagocytosed staphylococci are not affected by these antibiotics. Systemic β-lactam administration was superior to intramammary infusion of this group of antibiotics in the treatment of staphylococcal mastitis in lactating cows that previously were unsuccessfully treated with another intramammary preparation (12). This result indicates that the route of administration may be the determining factor. The basic therapeutic principles for lactating and dry cow treatment should actually be equivalent, but different spontaneous cure and new infection rates would apparently influence the outcome.

The new infection rate in each group studied was directly proportional to the number of quarters infected at drying off, which emphasizes the need to reduce the number of infective agents at the beginning of the dry period. The new infection rate was highest in the intramammary treatment group. Full insertion of the syringe cannula was used, and a likely explanation for the higher new infection rate could be

### TABLE 3. Selected pharmacokinetic variables of norfloxacin nicotinate and oxytetracycline in lactating cows after intravenous injection.

<table>
<thead>
<tr>
<th>Pharmacokinetic variable</th>
<th>Norfloxacin nicotinate, 10 mg/kg</th>
<th>Oxytetracycline-HCl, 5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{1/2} h(^2)</td>
<td>6.1</td>
<td>9.5</td>
</tr>
<tr>
<td>MRT, h(^3)</td>
<td>4.5</td>
<td>NC(^4)</td>
</tr>
<tr>
<td>V_{ml/kg}</td>
<td>2.50</td>
<td>NC</td>
</tr>
<tr>
<td>V_{area} L/kg(^6)</td>
<td>NC</td>
<td>.92</td>
</tr>
<tr>
<td>CL(_T), ml/min/kg(^7)</td>
<td>10.18</td>
<td>1.24</td>
</tr>
</tbody>
</table>

\(^1\)Number of reference.  
\(^2\)T_{1/2} = \text{Terminal half-life.}  
\(^3\)MRT = \text{Mean residence time.}  
\(^4\)NC = \text{Not calculated.}  
\(^5\)V_{ml} = \text{Volume of distribution at steady state.}  
\(^6\)V_{area} = \text{Volume of distribution according to area method.}  
\(^7\)CL\(_T\) = \text{Total body clearance.}
introduction of bacteria residing in the teat canal to the milk cistern with the penetrating syringe (1). Accordingly, the higher the prevalence of IMI at the time of drying off, the higher is the likelihood of this event to occur. In a herd free of S. aureus IMI, this effect would probably not be seen. The poor performance of intramammary cephapirin treatment in prevention of new IMI contradicts the results reported from our laboratory and by other researchers on intramammary dry cow therapy (8, 9, 17, 24), which are very similar to the results obtained by norfloxacin nicotinate therapy in this study. Further investigation using other intramammary dry cow products in comparison with systemic treatment, preferably in several herds, is required to substantiate the reasons we suggest for the discrepancy. However, new infection rate of S. aureus ranging from 2 to 40% between herds was reported (20) in a study comparing the efficacy of two intramammary preparations indicating very large herd variation regardless of the antibiotics used. Higher new infection rate of coagulase-negative staphylococci as a function of number of intramammary infusions during the dry period was reported (3).

After parenteral norfloxacin nicotinate therapy, the number of the IMI remained the same over the entire dry period. The number of IMI increased in the oxytetracycline treatment group during the dry period, which can be attributed primarily to the failure of the drug to cure infections existing at time of drying off. The oxytetracycline tissue concentrations were apparently insufficient for a cure but could have been satisfactory for the prevention of new infections. The increase in the number of IMI during the dry period was significant only in the cephapirin treatment group.

The cooperator herd was heavily infected with S. aureus (34.8% of the quarters and 68.4% of the cows in the herd). No mastitis control procedures had been implemented for several years, and the majority of subclinical IMI were chronic. The clinical mastitis cases during lactation were routinely treated with parenteral administration of penicillin and streptomycin. The annual bacteriological screening of this herd revealed that more than 50% of the staphylococcal isolates were resistant to penicillin by the agar disc sensitivity test (Difco). The MIC determinations showed, however, that each tested isolate was sensitive to cephapirin, i.e., there seemed to be no crossresistance between the two β-lactam antibiotics. The dynamics of IMI during the dry period may be different in a herd with few staphylococcal IMI (2). It is also possible that each drug, depending on formulation and route of administration, may give different results. However, the only mastitis control measure implemented in this study was the antimicrobial treatment at drying off, and the results obtained can apparently be attributed principally to this factor.

The results generated in this study suggest that effective antimicrobial dry cow treatment is the outcome of several determinants fulfilled simultaneously. Adequate pharmacokinetic data must support the assumption that the duration of therapy is sufficient and that appropriate tissue concentrations can be reached. The latter prerequisite is of special significance in the treatment of S. aureus IMI. The MIC determinations rather than sensitivity testing of the antibiotic for the pathogens involved should be used to select the most appropriate antibiotic and dose determination. The results emphasize also that susceptibility testing alone does not predict the clinical outcome of the treatment. The dose and dosing interval must always be confirmed in clinical trials.

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