PROMISING AVENUES IN MASTITIS CONTROL

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Introduction

Despite significant progress in understanding the disease process, bovine mastitis is still the most costly disease in the dairy industry. Reduction of milk yields, reduced milk quality, cost of prevention and therapy including milk discard for drug residue, and testing for drug residue all contribute to the loss of revenue for the industry.

Mastitis results primarily from infection of the mammary gland when bacteria overcome the host's natural defense mechanisms after entering through the teat duct.[1] Reduction of the incidence of mastitis in a herd requires a comprehensive approach to intervene in this process. Good management practices include: ration balancing with vitamin and mineral supplementation; good udder preparation and use of adequate teat sanitization with germicidal teat dips; use of properly functioning milking equipment and maintaining adequate hygiene during milking; identification, isolation and treatment of infected cows during lactation and at dry-off; culling chronically infected cows that are refractory to treatment. Each of these practices have their benefits and limitations in mastitis prevention and control.

Successful antibiotic therapeutic outcome does not always correlate with the *in vitro* sensitivity of a mastitis pathogen. In vitro assays do not adequately reflect conditions prevailing in the mammary gland. Many antibiotics only kill microorganisms as they replicate, and nondividing bacteria in the gland are unaffected by these antibiotics.[2] Some antibiotics fail because of resistance developed by the pathogen, while others are detrimental to phagocytosis and intracellular killing of bacteria by leukocytes.[3,4]

Residues in milk from chemical germicides and antibiotics used for lactating dairy cows are a potential safety problem for the consumer. Control of these residues is coming under increasing scrutiny by regulatory agencies out of concern for public safety,[5] despite the fact that the risk of antibiotic residue in milk is quite low compared to other food safety problems.[6]. Historically, the prevalence of residue violations has declined from 13% prior to 1962[5] to 0.2 to 0.5%[7]. However, headlines in the New York Times and the Wall Street Journal of milk tainted with drugs[8] can destroy the public confidence in the safety of milk. New programs are being instituted to reduce drug residues and promote the safe image of milk.[9]

Because of a greater concern over drug residues, farmers using antibiotics to treat lactating animals are requesting better methods for testing milk before it is offered for sale. There is an increasing use of cowside drug residue testing, but data on the accuracy and reliability of these tests is incomplete[6]. False positive results when antibiotic is not present damage producers' confidence in these tests.[10] Reliable, specific user friendly tests are desirable. In the future, antimicrobials used for mastitis control should preferably be less hazardous than those used today in order to allay concern over safety of the milk supply.

Alternatives to Antibiotics

Recent advances in biotechnology may provide improved methods for treating and preventing mastitis as well as enhancing the resistance to disease. These products include molecules directed towards the specific pathogens (bacteriocins),[11] or products used to enhance the host's immune system (cytokines).[12,13] Transgenic animals that express cloned foreign genes have been produced.[14,15] These include the staphylocidal bacteriocin, lysostaphin, which has been expressed in transgenic mice and produced in the mammary gland during lactation.[16] Expression of antimicrobial proteins in the mammary gland to increase the host's resistance to new intramammary infections(IMI) is more likely to result in development of resistant bacteria as has resulted from the excessive exposure to antimicrobial.

Antimicrobial Proteins

An alternative to chemical germicides and antibiotics is to use of antimicrobial proteins effective toward mastitis pathogens.[11] A wide range of naturally occurring proteinaceous antimicrobials are known. Some classes of these have been listed in Table 1.

Defensins are antimicrobial peptide naturally occurring inside granules of bovine leukocytes.[18] They are part of the host's natural defense mechanisms to combat bacterial infections. They do not usually work outside of the leukocyte and can be toxic to mammalian cells. Nevertheless, augmentation of their activity within the neutrophil or providing a more acceptable form through biotechnology could add to the repertoire of agents for use in mastitis control.

Lysostaphin and nisin are bacteriocins particularly active against staphylococci and streptococci. Both bacteriocins are rapidly bactericidal, equally active against dividing and nondividing bacteria, and are active against antibiotic-resistant isolates. They are digested by intestinal protease. Nisin has been approved for use by the Food and Drug Administration in dairy products as a food preservative.[17]

Table 1. Example of naturally occurring antimicrobial proteins

Type	Bacteriocins[1,2]	Cecropins[1,4]	Marginins[3,4]	Defensins[2,3,4]
Source	bacteria	insects	amphibians	mammals
Target	bacteria	bacteria yeast fungi	bacteria yeast	bacteria

1 Activity generally restricted to related bacteria
2 Activity restricted by environment (pH, salt, serum)
3 Toxicity to mammalian cells
4 Complicated expression by recombinant methods

Lysostaphin: Lysostaphin was isolated from *Staphylococcus simulans* and first described in 1963.[19] The gene for lysostaphin has been cloned[20] and the recombinant product is available as AMBICIN L® [Applied Microbiology, Inc., New York], produced as a secretory product by a nonpathogenic strain of *Bacillus sphaericus*. AMBICIN L is essentially identical to the natural protein. Lysostaphin is highly active toward staphylococci and has shown efficacy toward various types of staphylococcal infections. No significant adverse responses have been reported for lysostaphin administered by

different routes at a wide range of doses in various animals including lactating dairy cows.[11,21]

Nisin: This antimicrobial peptide is a natural product of certain strains of *Lactococcus lactis*. Nisin's activity is usually limited toward certain Gram-positive bacteria, principally species of streptococci, lactobacilli, and certain spore forming bacilli including *Clostridium botulinum*.[22] Recently, nisin's activity has been demonstrated toward a broad range of Gram-positive and Gram-negative bacteria.[23](Table 2). In its highly purified form, AMBICIN N® [Applied Microbiology, Inc. New York], has been developed for a range of applications including the prevention and treatment of mastitis.

Table 2: Nisin enhanced bactericidal activity against pathogens

Gram-positive organisms	Gram-negative organisms
Streptococci	*Escherichia coli*
Staphylococci	*Klebsiella pneumoniae*
Corynebacteria	*Pseudomonas aeruginosa*
Bacilli	Salmonellae
Clostridia	
Listeria	

Mastitis Treatment

Lysostaphin and nisin have both been used as the active agents in formulations for intramammary infusions for treatment of mastitis. Both nisin and lysostaphin are rapidly bactericidal in milk and serum toward pathogens responsible for mastitis. Nisin is principally active toward streptococci while lysostaphin is 1000 times more active than nisin toward staphylococci. The combination of lysostaphin with nisin is synergistic toward *Staphylococcus aureus*.[11]

We have shown that aqueous infusions of lysostaphin were able to eliminate *S. aureus* from 30% of experimentally infected glands[24] confirmed with collaborative studies by researchers at American Cyanamid.[25] However, after suitable formulation, lysostaphin efficacy can be improved to 50%. However, simple aqueous infusions of combinations of lysostaphin with nisin have proven most effective against established infections of *S. aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis*.[26,27] No adverse reactions were observed at any dose following intramammary infusion of these antimicrobial peptide. These agents are now in development as products for treatment of mastitis in lactating cows. (Applied Microbiology, Inc./Ciba Geigy)

Mastitis Prevention

Nisin has been used to formulate a germicidal teat sanitizer to be used both before and after milking to achieve better hygiene and control of mastitis. This formulation was designed to have rapid (< 1 minute) broad spectrum germicidal activity toward mastitis pathogens, and to be nonirriting on teat skin and leave behind no residues that could be hazardous to the milk supply.

This nisin-based formulation was evaluated for germicidal activity on teat skin of cows and its clinical efficacy has been demonstrated in experimental challenge trials and under natural field exposure. The product has demonstrated rapid germicidal activity, achieving greater than 99.9% kill after 1-minute exposure on live teat skin (>3 log reductions) with a range of mastitis pathogens including *S. aureus, Str. agalactiae, Str. uberis, Klebsiella pneumoniae,* and *Escherichia coli*[28] (Table 3).

Efficacy trials using an experimental challenge demonstrated mastitis control equivalent to that of 0.5% iodine. Applied before and after milking, a nisin-based formulation was as effective as 0.5% iodophor teat dip in preventing new intramammary infections with *S. aureus* and *Str. agalactiae* (81% reduction) and was superior to 0.5% iodophor teat dip applied only postmilking (70% reduction). The nisin-based formulation was also effective in reducing new environmental streptococcal intramammary infections (75% reduction) for premilking and postmilking teat dipping was comparable to the 0.5% iodophor teat dip (Galton, Cornell University, unpublished).

The performance of premilking and postmilking dipping with the nisin-based formulation has been confirmed under natural exposure field studies (Pankey, University of Vermont, personal communications). This product is now available. (Concept®, Babson Bros.)

Table 3: Germicidal activity of a nisin-based teat dip formulation

Pathogen	Log reduction (LR[1])	Percent Reduction (LR[2])
Staphylococcus aureus	3.90	61.77
Streptococcus agalactiae	4.43	98.60
Streptococcus uberis	3.68	67.10
Klebsiella pneumoniae	4.00	76.49
Escherichia coli	4.22	85.51

[1] Log reduction = Log colony forming units/milliter negative control minus log colony forming units/milliter treated teats.

[2] Percent LR = 100 (LR) ÷ log negative control.

Conclusions

Lysostaphin and nisin are examples of nontoxic antimicrobial proteins that have efficacy toward mastitis pathogens. Other examples of antimicrobial proteins exist in nature. The application of modern technology may make certain of them available in the future. Immunomodulators, transgenic animals, and breeding practices may help improve the host resistance to mastitis infections. Any advances should be focused on improving dairy producers'ability to maintain the quality of the product and reduce the cost of mastitis.

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SUMMARY

The control of bovine mastitis commonly relies on the use of antibiotics and chemical germicides to treat and prevent intramammary infections. The failure of antibiotics to eliminate mastitis pathogens in lactating cows can be attributed to microorganisms sequestered in microabcesses or within leukocytes where they lie dormant and inaccessible to antibiotics. There is an increasing consumer concern over the potential risk of residues in the milk supply from mastitis treatment. Advances in biotechnology have made available alternative compounds that can be used for the treatment and control of mastitis. Examples are the antimicrobial proteins, nisin and lysostaphin, that are bactericidal against dividing and dormant organisms, and against antibiotic resistant mastitis pathogens. These proteins are nontoxic and may be used in the prevention and therapy of mastitis infections while reducing the risk of hazardous milk residues.

RESUMEN

El control de mastitis se basa comunmente en el uso de antibióticos y germicidas químicos para tratar y prevenir nuevas infecciones intramamarias. El fracaso de los antibióticos en la eliminación de los patógenos causantes de mastitis en vacas lactantes puede ser atribuído a que el microorganismo puede hacerse inaccesible para la droga ya sea en microabcesos ó dentro de leucocitos. Además, existe en los consumidores una preocupación en aumento debido al riesgo potencial de la presencia de residuos en la provisión de leche como consecuencia del uso de antibióticos en el tratamiento de la mastitis. La biotecnología ha hecho posible la disponibilidad de compuestos alternativos que pueden ser usados en el tratamiento y en el control de esta enfermedad. Las proteínas antimicrobianas son un ejemplo. De éstas, la nisina y la lisostafina son bactericidas contra los patógenos causantes de mastitis ya sea en multiplicación ó en latencia. Estas proteínas son atóxicas y pueden ser inactivadas y digeridas por enzimas intestinales. Estos agentes antimicrobianos tienen un uso potencial en la prevención y la terapia de infecciones mastíticas, y al mismo tiempo en la disminución del riesgo de residuos peligrosos en la leche.

Resume

Le contrôle des mammites bovines repose habituellement sur l'utilisation d'antibiotiques et d'antiseptiques traitant et prévenant les infections intramammaires. L'impuissance des antibiotiques à éliminer les pathogènes chez les vaches en lactation peut être attribuée aux microorganismes séquestrés au sein de microabcès ou dans les leucocytes ou ils reponsent dormants et in accessibles aux antibiotiques. Par ailleurs il y a un soucis croissant du consommateur à l'égard du risque potentiel lié aux résidus dans le lait suite au traitement des mammites. Les biotechnologies ont mis à disposition d'autres composés pouvant être utilisés dans le traitement et la prévention des mammites. Les protéines antimicrobiennes en sont un éxemple. Entre autres, la nisine et la lysostatine sont des bacteridides actifs contre les agents pathogènes des mammites qu'ils soient en multiplication, dormant ou résistants aux antibiotiques. Ces protéines ne sont pas toxiques et peuvent être inactivées et digérées par les enzymes intestinales. Une formulation appropriée de ces agents antimicrobiens a un potentiel pour un usage dans la prévention et le traitement des mammites infectieuses tout en réduisant les risques liés aux résidues dans le lait.