J. Dairy Sci. 89:3846–3857


# Deferoxamine Reduces Tissue Damage During Endotoxin-Induced Mastitis in Dairy Cows[1]

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## ABSTRACT

The protective effects of 3 antioxidants on polymorphonuclear neutrophil-induced damage to mammary cells were evaluated in vivo using an endotoxin-induced mastitis model. Fifteen healthy, midlactation cows with no history of clinical *Escherichia coli* mastitis were randomly assigned to 1 of the 3 treatment groups corresponding to each modulator to be evaluated, that is, deferoxamine, catechin, and glutathione ethyl ester. Each cow had 1 quarter infused with saline and 1 quarter infused with the selected modulator; a third quarter was infused with lipopolysaccharides (LPS), whereas the fourth quarter received a combination of LPS and the modulator. Infusion of LPS caused acute mastitis as determined by visual observations and by large increases in milk somatic cell count, BSA, and proteolytic activity. These parameters were not affected by antioxidant administration. The extent of cell damage was evaluated by measuring milk levels of lactate dehydrogenase and *N*-acetyl-$\beta$-D-glucosaminidase activity. Levels of these parameters were several times higher after LPS administration. Intramammary infusions of catechin or glutathione ethyl ester did not exert any protective effect, whereas infusion of deferoxamine, a chelator of iron, decreased milk lactate dehydrogenase and NAGase activity, suggesting a protective effect against neutrophil-induced damage. The protective effect of deferoxamine was also evidenced by a lower milk level of haptoglobin. The proteolytic activity of mastitic milk was not influenced by the presence of deferoxamine. Overall, our results suggest that local infusion of deferoxamine may be an effective tool to protect mammary tissue against neutrophil-induced oxidative stress during bovine mastitis.

**Key words:** mammary gland, antioxidant, reactive oxygen species, protease

## INTRODUCTION

Mastitis is an inflammatory reaction that usually occurs following an IMI. The inflammatory response involves the massive transmigration of polymorphonuclear neutrophils (**PMN**) from the blood into the mammary gland (Paape et al., 2000). The presence of functional neutrophils is known to be crucial to host defense against bacterial pathogens (Kehrli et al., 1990). This was also previously demonstrated by Schalm et al. (1976), who showed that treating cows with an antibovine leukocyte serum could turn chronic *Staphylococcus aureus* mastitis into a gangrenous disease. The main functions of PMN are to engulf pathogens and destroy them via a variety of bactericidal mechanisms. Indeed, PMN contain intracellular granules that contain bactericidal peptides, proteins, and enzymes such as elastase and other proteinases and myeloperoxidase that are released into phagocytic vacuoles or the extracellular environment (Borregaard et al., 1993). Additionally, activated PMN have recently been found to release granule proteins and chromatin that together form extracellular fibers. These extracellular traps bind microorganisms and ensure a high local concentration of antimicrobial agents to degrade virulence factors and kill bacteria (Brinkmann et al., 2004). The other mechanism by which PMN eliminates bacteria is oxygen dependent and produces toxic reactive oxygen species (**ROS**). The cornerstone of this process is the generation of superoxide ($O_2^-$) via the enzyme NADPH-oxidase. The superoxide further reacts to yield other toxic ROS such as hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^\cdot$), and hypochlorous acid (HOCl).

In several studies on inflammation, the oxidants and proteases released by PMN have been associated with tissue damage (Weiss, 1989; van Asbeck, 1990; Mehrzad et al., 2005). Oxidative stress can cause damage to all types of biomolecules (DNA, proteins, lipids, and carbohydrates) and therefore induce tissue injury. In cases of acute coliform mastitis, the amount of ROS released by PMN may overwhelm the cow's endogenous antioxidant protection mechanisms and therefore add to the inflammation, causing extensive tissue damage

Received February 14, 2006.
Accepted April 25, 2006.
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that invariably causes losses in milk production and may lead to complete loss of the quarter. For that reason, antioxidants could be used as therapeutic agents to neutralize the effect of an overproduction of ROS. The protective effects of various antioxidants against the cytotoxic effects of ROS have been demonstrated in diverse human diseases both in vitro (Richter-Landsberg and Vollgraf, 1998) and in vivo, as reviewed by Halliwell and Gutteridge (1999).

The involvement of PMN extracellular ROS (Capuco et al., 1986) and pro-ROS cytokines (Shuster et al., 1996) in mammary tissue damage during mastitis has been demonstrated. In vitro, activated blood PMN have been shown to be cytotoxic for mammary epithelial cells (Ledbetter et al., 2001; Lauzon et al., 2005), possibly via the release of extracellular ROS such as hydroxyl radicals (Boulanger et al., 2002). Additionally, we demonstrated that the addition of exogenous deferoxamine (**DFO**), catechin, or glutathione ethyl ester (**GEE**) was able to prevent damage caused by phorbol-12-myristate-13-acetate-activated PMN to mammary cells in culture (Lauzon et al., 2005). Therefore, we hypothesized that the use of these antioxidants in our in vivo study may lower the oxidative stress experienced by the mammary cells and accelerate cellular recovery.

Intramammary infusion of LPS is often used to study events occurring during *Escherichia coli* mastitis because it mimics the symptoms of naturally occurring mastitis without microorganism development and toxin production that could cause direct damaging effects on the mammary epithelial cells (Oliver and Calvinho, 1995). In lesions associated with acute inflammation, bacterial endotoxins damage tissue either directly or by attracting PMN that, in turn, release damaging substances (Birkedal-Hansen, 1993). In this study, an endotoxin-induced model of mastitis was used to evaluate the protective effects of intramammary infusion of catechin, DFO, or GEE on PMN-induced epithelial mammary damages.

## MATERIALS AND METHODS

### Chemicals and Reagents

Unless specified, all reagents used were purchased from Sigma Chemical Co. (St. Louis, MO). Lipopolysaccharide (*E. coli* O55:B5), DFO, and GEE were dissolved in sterile saline. Catechin was dissolved in 100% ethanol before being further diluted in saline. The acrylamide-bis-acrylamide solution, ammonium persulfate, *N*,*N*,*N*′,*N*′- tetramethylethylenediamide, Kaleidoscope prestained standards, and Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories (Hercules, CA).

### Animals and Experimental Procedures

The experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care. Fifteen healthy, high-yielding Holstein cows in midlactation with no history of clinical *E. coli* mastitis were used. Only cows with bacteriologically negative milk samples and a milk SCC of less than $2 \times 10^5$ cells/mL of milk per individual quarter were used in the study. The cows were randomly assigned to 1 of the 3 treatment groups (5 cows per group) corresponding to each modulator evaluated, that is, DFO, catechin, and GEE. Each individual mammary quarter was designated as an experimental unit. For each modulator selected, 5 cows had their left front quarter injected with 20 mL of saline, whereas the right front quarter was injected with 20 mL of the selected modulator. Both front quarters served as controls for the rear quarters. Hence, the left rear quarter of each cow was injected with 500 μg of LPS (*E. coli* O55:B5) in 20 mL of saline, whereas the right rear quarter was injected with 500 μg of LPS in 10 mL of saline plus 10 mL of the modulator. These injections were performed immediately after morning milking. The intramammary doses of DFO, catechin, and GEE were 500, 50, and 50 mg per injection, respectively. All solutions to be injected were prepared aseptically and were fresh. Injection of modulators was carried out immediately after the LPS challenge and was repeated at postchallenge hours (**PCH**) 4, 12, and 24. Milk production data and milk samples for each quarter were collected on d –7, –4, –1, and 0 (immediately before the LPS challenge) and at PCH 12, 24, 36, 48, 60, and 72 using individual quarter milking units. Additional milk samples were collected by hand at PCH 3 and 6. Rectal temperature, visual observations of udder inflammation (redness and swelling), and milk appearance were also recorded, following the same schedule, by 2 observers who were unaware of the treatments that had been given. For a reason unrelated to the experiment, 1 cow assigned to the DFO group had to be removed during the experimental period, leaving only 4 cows in this treatment group.

### Milk Sample Processing

Following milking, aliquots of quarter milk samples were sent to a commercial laboratory (Program d'Analyze des Troupeaux Laitiers du Québec, Sainte-Anne-de-Bellevue, Quebec, Canada) for determination of SCC and infrared evaluation of lactose content and protein content. The remaining quarter milk samples were centrifuged for 15 min at $1,000 \times g$ (4°C) before being defatted and frozen in small aliquots. To obtain whey, a part of the defatted milk was ultracentrifuged ($100,000 \times g$) at 4°C for 20 min, and the aliquots were stored at –20°C.