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Evaluation of antioxidant and proinflammatory gene expression in bovine mammary tissue during the periparturient period

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ABSTRACT

The incidence and severity of mastitis can be high during the period of transition from pregnancy to lactation when dairy cattle are susceptible to oxidative stress. Oxidative stress may contribute to the pathogenesis of mastitis by modifying the expression of proinflammatory genes. The overall goal of this study was to determine the relationship between critical antioxidant defense mechanisms and proinflammatory markers in normal bovine mammary tissue during the periparturient period. Mammary tissue samples were obtained from 12 cows at 35, 20, and 7 d before expected calving and during early lactation (EL, 15 to 28 d in milk). Enzyme activities for cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase were relatively low during the dry period, but increased during EL, whereas activity of thioredoxin reductase 1 did not change significantly as a function of time. In contrast, gene expression for these antioxidant selenoproteins and for heme oxygenase-1 gradually decreased as parturition approached and then increased during EL. The expression of intercellular vascular adhesion molecule-1 and vascular cell adhesion molecule-1 followed a similar trend where mRNA abundance gradually declined as parturition approached with a slight rebound in EL. Gene expression of the pro-oxidant, 15-lipoxygenase 1, which is known to increase during times of oxidative stress, also increased dramatically in mammary tissue from EL cows. Expression of the proinflammatory cytokines, IL-1β, IL-6, and IL-8 did not change significantly during the periparturient period. Strong positive correlations were found between several antioxidant enzymes (cytosolic glutathione peroxidase, thioredoxin reductase 1, and heme oxygenase-1) and vascular adhesion molecules (intercellular vascular adhesion molecule-1, vascular cell adhesion molecule-1) suggesting a protective response of these antioxidants to an enhanced proinflammatory state. Ability to control oxidative stress through manipulation of key antioxidant enzymes in the future may modify the proinflammatory state of periparturient cows and reduce incidence and severity of some diseases such as mastitis.
Key words: mammary gland, periparturient period, oxidative stress, inflammation

INTRODUCTION

Dairy cattle experience increased incidence of disease, such as mastitis, during the periparturient period when host defense mechanisms are compromised. Several physiological changes occur in periparturient dairy cows that may contribute to altered immune and inflammatory responses including overall nutritional status, energy metabolism, and changes in hormone profiles (Sordillo, 2005). Another factor contributing to compromised immunity and increased incidence of disease may be the progressive development of oxidative stress (Miller et al., 1993; Sordillo, 2005; Sordillo and Aitken, 2009). Oxidative stress occurs when there is an imbalance between production of reactive oxygen species (**ROS**) and reduced host antioxidant capabilities (Valko et al., 2007). During the periparturient period, dairy cows experience extreme shifts in cellular metabolism as the mammary gland prepares for the ensuing lactation (Sordillo and Aitken, 2009). The onset of copious milk synthesis and secretion requires large amounts of molecular oxygen for aerobic metabolism. Free radicals are formed as a normal end product of cellular metabolism arising from either the mitochondrial electron transport chain or from stimulation of $NADPH_2$ (Valko et al., 2007). Therefore, the considerable increase in oxygen requirements during heightened metabolic demands results in augmented rates of ROS production. Indeed, several recent studies showed that production of excess ROS in the peripheral blood of dairy cattle during the periparturient period can overwhelm certain

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antioxidant defenses, resulting in increased oxidative stress (Bernabucci et al., 2005; Castillo et al., 2005; Sordillo et al., 2007).

Dairy cattle have several known endogenous antioxidant defense mechanisms that can counteract the harmful effects of ROS accumulation, but it is the selenium-dependent selenoproteins that have been studied extensively with respect to mammary gland health (Sordillo and Aitken, 2009). Selenium supplementation reduces the incidence and severity of mastitis (Smith et al., 1984). The beneficial effects of selenium supplementation are thought to be due to the actions of certain antioxidant selenium-dependent enzymes, which have a selenocysteine residue incorporated into their active site. These selenoenzymes function in part by reducing harmful ROS and other fatty acid hydroperoxides to less-reactive waters and alcohols, respectively. Approximately 25 selenoproteins have been identified in humans (Papp et al., 2007), but the selenoprotein most often associated with antioxidant functions in cattle is cytosolic glutathione peroxidase (**GPX1**; Smith et al., 1997). Indeed, GPX1 activity is often used as a diagnostic tool when assessing the selenium status of dairy cows or as an indicator of increased ROS accumulation. Several recent studies, however, now document the presence of other selenoprotein enzymes in the blood and tissues of dairy cattle that may play an important role in controlling oxidative stress, including thioredoxin reductase 1 (**TrxR1**) and phospholipid hydroperoxide glutathione peroxidase (**GPX4**; Bruzelius et al., 2007; Sordillo et al., 2007). In addition to their ROS scavenging functions, many selenoproteins are essential for regulating cellular redox status and influencing the expression of redox-regulated genes. For example, TrxR1 regulates expression of other important antioxidant defenses such as heme oxygenase 1 (**HO-1**), which is upregulated in response to ROS and converts the pro-oxidant heme into bilirubin, carbon monoxide, and iron (Balla et al., 2007). Although HO-1 has not been studied in dairy cattle in vivo, HO-1 is upregulated in cultured bovine aortic endothelial cells under oxidative stress conditions following reduced TrxR1 activity (Trigona et al., 2006). Despite significant evidence supporting a role for antioxidants in enhancing resistance to mastitis (Sordillo and Aitken, 2009), there is no information as to how the expression of specific antioxidant defenses change in mammary tissues during the periparturient period when dairy cattle experience increased oxidative stress.

There are several human inflammatory-based diseases that occur as a consequence of oxidative stress, including cardiovascular disorders, diabetes, and cancer (Valko et al., 2007; Bonomini et al., 2008). The pathologies of these diseases may result from the enhanced expression of redox regulated proinflammatory factors such as eicosanoids and cytokines. For example, the metabolism of arachidonic acid through the 15-lipoxygenase 1 (**15-LOX1**) pathway causes the formation of 15-hydroperoxyeicosatetraenoic acid (**15-HPETE**), which is enhanced during oxidative stress (Cao et al., 2000). Increased 15-HPETE concentration in tissue and cells is associated with enhanced expression of certain proinflammatory genes such as intercellular adhesion molecule-1 (**ICAM-1**) and vascular cell adhesion molecule-1 (**VCAM-1**) (Bonomini et al., 2008). Vascular adhesion molecules are essential for transendothelial leukocyte migration to the site of infection. Enhanced expression of either ICAM-1 or VCAM-1, however, can lead to pathologic proinflammatory conditions (Radi et al., 2001; Sordillo et al., 2008). Relative to dairy cattle health, oxidative stress enhanced 15-LOX1 activity and accumulation of 15-HPETE in bovine endothelial cells and caused a significant increase in ICAM-1 expression (Sordillo et al., 2008). The expression of VCAM-1 protein in bovine mammary tissues also was reported to increase significantly during colostrogenesis when dairy cattle are known to experience oxidative stress (Hodgkinson et al., 2007).

Oxidative stress increases the expression of acute phase cytokines that also can exacerbate tissue damage during severe inflammatory responses (Cuschieri and Maier, 2007). Proinflammatory cytokines are thought to play an important role in the mammary gland's response to a variety of mastitis-causing organisms including *Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli* (Oviedo-Boyso et al., 2007). Indeed, numerous studies showed that tumor necrosis factor-α (**TNF-α**), IL-1β, IL-6, and IL-8 were linked with the severity of coliform mastitis during the periparturient period when dairy cattle experience oxidative stress (Oviedo-Boyso et al., 2007). Expression of TNF-α from isolated mononuclear cells in either peripheral blood or supramammary lymph nodes was greater in the periparturient period compared with mid to late lactation (Sordillo et al., 1995). An inverse relationship between TrxR1 activity and TNF-α production by peripheral blood mononuclear cells obtained from cows experiencing oxidative stress also was recently reported (O'Boyle et al., 2006).

Collectively, these previous data support the contention that reduced antioxidant capacity and enhanced proinflammatory status may be related and that this relationship may play a role in dairy cattle disease susceptibility during the periparturient period. However, there is no information linking the antioxidant and inflammatory status in normal bovine mammary tissue during the periparturient period when dairy cattle are susceptible to increased incidence of disease. Such