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Supraphysiological oxytocin increases the transfer of immunoglobulins and other blood components to milk during lipopolysaccharide- and lipoteichoic acid–induced mastitis in dairy cows

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ABSTRACT

Bacterial mastitis causes pathogen-dependent changes of the blood-milk barrier, and these changes can influence the differential transfer of blood components to milk. It is well known that gram-negative pathogens such as *Escherichia coli* can cause a greater activation of the immune system and thus a more comprehensive transfer of blood components including IgG than gram-positive pathogens such as *Staphylococcus aureus*. Supraphysiological doses of oxytocin (OT) have been shown to increase the permeability of the blood-milk barrier; however, the effect of OT during experimentally induced mastitis has not been investigated. Therefore, the objective of this study was to examine if intravenous administration of OT during lipopolysaccharide (LPS)- or lipoteichoic acid (LTA)-induced mastitis could influence the transfer of blood components to milk. The hypothesis was that OT could induce a greater transfer of blood components during mastitis. Twenty-seven dairy cows were injected via the teat canal with LPS, LTA, or a saline control followed by an intravenous injection of OT 2 h following intramammary challenge. Milk samples were collected every half hour and analyzed for somatic cell count (SCC), IgG, lactate dehydrogenase (LDH), and serum albumin (SA). Due to the chosen dosage of LPS and LTA, there was no difference in SCC between quarters challenged with only LPS or LTA. Quarters challenged with LPS and OT had a higher SCC and a greater transfer of IgG, LDH, and SA compared with quarters challenged with only LPS. Quarters challenged with LTA and OT had a greater transfer of IgG, LDH, and SA, whereas the SCC increase did not differ from quarters only treated with LTA. In quarters treated only with OT, SCC, LDH, and SA increased, but no difference was observed in IgG concentration from untreated control quarters. In conclusion, there are pathogen-specific changes in the blood-milk barrier and OT can induce a greater transfer of blood components to milk in both LPS- and LTA-induced mastitis. Oxytocin could have implications for use as a mastitis therapy, as there was an increased transfer of IgG into the milk.
Key words: mastitis, blood-milk barrier, endotoxin, oxytocin

INTRODUCTION

Mastitis is usually caused by bacterial pathogens invading the mammary gland and these bacteria cause a pathogen-specific activation of the immune system and influence permeability of the blood-milk barrier (Wellnitz and Bruckmaier, 2011). Clinical mastitis cases, such as quarters infected with the gram-negative pathogen *Escherichia coli*, have a greater transfer of various blood proteins including IgG, and a greater stimulation of the immune system. This is compared with subclinical mastitis, which is often associated with gram-positive pathogens such as *Staphylococcus aureus* (Bannerman et al., 2004; Wellnitz et al., 2013). These findings could relate to the mostly chronic infections associated with *S. aureus*, as infections with this bacterium generally do not induce a pronounced immune response that is mirrored by a moderate upregulation of pro-inflammatory cytokines in the mammary gland (Sutra and Poutrel, 1994; Wellnitz and Bruckmaier, 2011).

Bacteria have specific endotoxins embedded in the cell wall: LPS on *E. coli* and lipoteichoic acid (**LTA**) on *S. aureus*. Intramammary injection of these 2 proteins can be used to induce and mimic the inflammation that occurs during mastitis. When dosages of LPS and LTA are chosen to induce equal SCC increases, it has been shown that LPS can induce a greater transfer of blood-

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derived proteins to the milk than LTA (Wellnitz et al., 2013).

Oxytocin (**OT**) is a hormone that causes contraction of myoepithelial cells surrounding the alveoli that leads to milk ejection. Exogenous OT administered in supraphysiological concentrations was previously shown to increase the permeability of the blood-milk barrier (Allen, 1990). The mechanism of action for OT is not fully understood, but it can be speculated that mammary epithelial tight junction integrity is compromised from the mechanical stress of inducing maximum alveolar contraction (Stelwagen and Singh, 2014).

Increased permeability of the blood-milk barrier is characterized by the appearance of blood constituents in milk because of the paracellular diffusion of blood and milk components during mastitis (Nguyen and Neville, 1998). These constituents include the proteins serum albumin (**SA**) and lactate dehydrogenase (**LDH**; Stelwagen et al., 1994; Lehmann et al., 2013; Wall et al., 2015) as markers, as well as immunoglobulins, which have functional properties during an intramammary immune response (Burton and Erskine, 2003).

The aim of the present study was to examine if intravenous OT administration at a supraphysiological dosage would influence the transfer of IgG and other markers of barrier integrity to milk during LPS- or LTA-induced mastitis. Furthermore, this may indicate a basis for the potential use of OT in mastitis therapy. The hypothesis was that OT induces a greater transfer of blood components, including IgG, that may have a functional role in the mammary immune response.

MATERIALS AND METHODS

Animals

All animal trials were approved by the Cantonal Committee of Animal Experiments, Fribourg, Switzerland, and all experimental procedures followed the Swiss law of animal protection. Twenty-seven dairy cows [Holstein (n = 20), Swiss Fleckvieh (n = 7)] in mid-lactation (mean DIM = 199 ± 22) were enrolled. Parities of experimental cows ranged from 1 to 5 (average parity = 2) and cows were producing >15 L of milk/d (mean milk yield = 20.0 ± 0.6). All cows had a SCC $<200 \times 10^3$ cells/mL in all 4 quarters during the 3 d before the experiment and showed no signs of clinical mastitis. Sterile milk samples for bacteriological culture were collected aseptically from all quarters and processed according to Hogan et al. (1999), and all cows were negative for mastitis-causing pathogens. Cows were housed at the Agroscope research station (Posieux, Switzerland) in straw and sawdust bedded tie-stalls for the duration of the experiment. Cows were fed roughage ad libitum and energy concentrate according to their individual production levels. Water was available ad libitum. Cows were machine milked twice daily at 0530 and 1600 h.

Experimental Procedures and Treatments

The day before the experiment, jugular catheters (105 mm long, internal and external diameters of 1.9 and 2.4 mm, 13 gauge, Vygon, Ecouten, France) were inserted and immediately flushed with 0.9% sterile saline and 5,000 IU of heparin (Laboratoire Dr. G. Bichsel SA, Interlaken, Switzerland) to prevent blood clotting overnight.

On the day of the experiment, cows were randomly allocated to 4 treatment groups (LPS, n = 7; LTA, n = 6; LPS+OT, n = 7; LTA+OT, n = 7). Immediately following the morning milking, 2 quarters from each cow (one treatment, one control) were injected with the treatment by sterilizing each teat with gauze soaked in 70% ethanol and inserting a sterilized teat cannula. An upward massage of 15 s was performed immediately after injection to move the injection fluid into the parenchymal tissue. Injected quarters were randomly assigned for each cow to avoid sampling bias. Treatments were prepared as follows: 0.2 μg of LPS (from *E. coli* serotype O26:B6, Sigma-Aldrich, St. Louis, MO) was diluted in 10 mL of 0.9% sterile saline; 20 μg of LTA (from *S. aureus*, Sigma-Aldrich) was diluted in 10 mL of 0.9% sterile saline; control treatment was 10 mL of 0.9% sterile saline. Each cow served as its own control as each cow had one treatment and one control quarter. Quarters were considered independent for this study because previous research showed no differences in control quarters from cows that had an adjacent quarter challenged with LPS, LTA, or saline control (Wall et al., 2016). Time of injection was designated as time 0 h. At 2 h postinjection, either 100 IU of OT (10 IU/mL; Werner Stricker AG, Zollikofen, Switzerland) or 10 mL of 0.9% sterile saline (control) was injected through the jugular catheter. Dosages of endotoxin were chosen to induce similar SCC increases and were based on previous experiments from our group (Wall et al., 2016). Oxytocin dosage was chosen according to previous research by Allen (1990).

Sampling Procedures: Temperature and Milk Samples

The rectal temperature of each cow was measured immediately before injection and every hour until 8 h postchallenge. Milk samples were taken every 30 min