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Lipopolysaccharides, cytokines, and nitric oxide affect secretion of prostaglandins and leukotrienes by bovine mammary gland during experimentally induced mastitis in vivo and in vitro

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ABSTRACT

The aim of the study was to determine the effects of lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF), interleukin-1-alpha (IL-1α), and nitric oxide donor (NONOate) on both in vivo and in vitro secretion of prostaglandin (PG)E_2, $PGF_{2\alpha}$, leukotriene (LT)B_4, and LTC_4 by the bovine mammary gland. In the first experiment, tissues isolated from the teat cavity and lactiferous sinus were treated in vitro with LPS (10 ng/mL), TNF (10 ng/mL), IL-1α (10 ng/mL), NONOate (10^{-4} M), and the combination of TNF + IL-1α + NONOate for 4 or 8 h. PGE_2 or $PGF_{2\alpha}$ secretion was stimulated by all treatments ($P < 0.05$) excepting NONOate alone, which did not stimulate $PGF_{2\alpha}$ secretion. Moreover, all factors increased LTB_4 and LTC_4 secretion ($P < 0.05$). In the second experiment, mastitis was experimentally mimicked in vivo by repeated (12 h apart) intramammary infusions (5 mL) of (1) sterile saline; (2) 250-μg LPS; (3) 1-μg/mL TNF; (4) 1-μg/mL IL-1α; (5) 12.8-μg/mL NONOate; and (6) TNF + IL-1α + NONOate into 2 udder quarters. All infused factors changed PGE_2, 13,14-dihydro,15-keto-$PGF_{2\alpha}$, and LT concentrations in blood plasma collected from the caudal vena cava, the caudal superficial epigastric (milk) vein, the jugular vein, and the abdominal aorta ($P < 0.05$). In summary, LPS and other inflammatory mastitis mediators modulate PG and LT secretion by bovine mammary gland in both in vivo and in vitro studies.

1. Introduction

The negative economic impact of acute coliform mastitis on the dairy industry demands new insights on the pathophysiology of the process [1]. Inflammation of the mammary gland may be associated with several bacteria, which invade the udder through the teat canal. Mastitis caused by coliform bacteria *Escherichia coli* is typically more severe and more frequently observed in high producing dairy cows around parturition and during early lactation [2].

Primary cells involved in the initial defense line are the resident mammary macrophages and the bovine mammary epithelial cells [3]. Pathogen-associated molecular patterns such as lipopolysaccharide (LPS) may be sufficient to elicit mastitis by *E coli* [2] and trigger an innate immune response with subsequent release of proinflammatory cytokines [4,5]. Several studies have suggested that levels

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of tumor necrosis factor alpha (TNF), interleukin-1-alpha (IL-1α), and free oxygen radicals are significantly increased in milk and serum of cows during LPS-induced mastitis [6,7]. Differential regulation of local secretion of cytokines and other inflammatory mediators may result in acute mastitis, which causes different clinical symptoms [5,6]. As a result, reproductive performance in high production cows may be affected [1,8].

Arachidonic acid (AA) metabolites, such as prostaglandins (PGs) and leukotrienes (LTs), are enzymatically generated by prostaglandin endoperoxide synthase 2 and arachidonate 5 lipoxygenase during the inflammatory process. They act as major proinflammatory mediators and might play a critical role in the severity of mastitis in cows and their susceptibility to *E coli* [9,10]. Certainly, eicosanoids locally released during mastitis in the bovine mammary gland are important mediators of vascular permeability, chemotaxis, or hyperalgesia [11].

Our previous studies have shown that basic mediators of inflammation during *E coli* mastitis (TNF, IL-1α, and NO) may locally modulate the secretion of PG and LT by isolated bovine mammary epithelial cells; this response may be an important first-line defense mechanism for the mammary gland [12]. Consequently, we hypothesize that during inflammation of bovine mammary gland, locally produced inflammatory mediators TNF, IL-1α, NO, and AA metabolites act systemically and impact reproductive function. To address this question, we investigated: (1) the in vitro effect of inflammatory mediators on PG and LT secretion by bovine mammary gland tissues and (2) the in vivo effect of LPS and mastitis inflammatory mediators on PG and LT secretion in bovine mammary gland.

2. Materials and methods

2.1. Animals and surgical procedures

All animal procedures were approved by the Local Animal Care and Use Committee, University of Warmia and Mazury in Olsztyn, Poland (Agreement No. 83⁄2009). Normally cycling Polish Holstein–Friesian cows (4–6 lactations) were used for this study (n = 36). There was no history of mastitis in any of the cows used. The animals were culled by the owners from 2 dairy herds because of their low level of milk production. The cows were examined and scored generally for rectal temperature, general attitude, respiratory and heart rates, and specifically, for udder quarter swelling and pain (data not shown). The cows used in this study were free of major mastitis pathogens, diagnosed negative by bacteriologic examinations with a quarter foremilk somatic cell count below 100,000 cells/mL (data not shown). To carry out the in vivo experiments, the estrus was synchronized using an analog of $PGF_{2\alpha}$ (dinoprost, Dinolytic; Pharmacia and Upjohn, Belgium) injected twice with an interval of 11 d, as previously described [13]. The onset of estrus was taken as day 0 of the estrous cycle.

During the experiment, animals were premedicated with xylazine (25–30 mg/animal intramuscular; Xylavet 2%, ScanVet, Poland). Local epidural anesthesia was induced by injecting 4 mL of 2% procaine hydrochloride (Polocainum Hydrochloricum; Biowet Drwalew, Poland) between the first and second coccygeal vertebrae, and 4 catheters (Tomel Sp, Poland) were inserted for frequent collection of blood samples as follows:

(1) (outer diameter (o.d.) = 1.6 mm and inner diameter (i.d.) = 1.2 mm) into the caudal vena cava via the coccygeal vein as previously described [13];

(2) (o.d. = 2.1 mm and i.d. = 1.6 mm) into the milk vein;

(3) (o.d. = 2.1 mm and i.d. = 1.6 mm) into the jugular vein;

(4) (o.d. = 1.5 mm and i.d. = 1.1 mm) into the posterior abdominal aorta through the coccygeal artery as previously described [13,14].

2.2. Mammary gland tissue collection

Mammary glands (n = 30) were collected postmortem at a local abattoir, from cyclic cows in the luteal phase of the estrous cycle (day 8–12). Milk samples were collected from selected animals and taken to the laboratory for bacteriologic tests (data not shown). Health of the udders (cows free from clinical mastitis), as well as the phase of the estrous cycle, was confirmed by veterinary inspection. The whole mammary gland was collected within 5 min after death, washed, and kept on ice during transport to the laboratory.

2.3. Collection of tissues from the teat cavity and lactiferous sinus of the bovine mammary gland

Tissues representing mammary gland mucosa were collected from the teat cavity and lactiferous sinus of bovine mammary gland and used in this study. Udders were separated into quarters, which were cut along the teat canal. Briefly, strips from the teat cavity and lactiferous sinus were washed 3 times in sterile phosphate-buffered saline containing 20-μg/mL gentamicin (G1397; Sigma–Aldrich, USA). The tissue was then cut into small pieces (30–50 mg), washed again in phosphate-buffered saline with 20-μg/mL gentamicin, and finally placed in a glass culture tube (12 × 75 mm) containing 2 mL of Dulbecco's Modified Eagle's medium (DMEM) and Ham's F-12 phenol red free culture medium (DMEM/F12, D-2906; Sigma–Aldrich), supplemented with 0.1% bovine serum albumin (A9056; Sigma–Aldrich), 10,000 U/mL penicillin G, 10-mg/mL streptomycin, and 25-μg/mL amphotericin B (antibiotic antimycotic solution, A5955; Sigma–Aldrich). Tissues were preincubated in a water bath with shaking at 38.5°C under 5% CO_2 for 1 h and then incubated in the same conditions for 4 or 8 h.

Time and doses of LPS, TNF, IL-1α, and NONOate in vitro and in vivo treatments were previously determined (data not shown).