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Effect of lipopolysaccharide and cytokines on synthesis and secretion of leukotrienes from endometrial epithelial cells of pigs

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ABSTRACT

In the present study, effects were studied of lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4 and IL-10 on the mRNA and protein expression of 5-lipooxygenase (5-LO), leukotriene (LT)A_4 hydrolase (LTAH) and LTC_4 synthase (LTCS), and secretion of LTB_4 and LTC_4 from endometrial epithelial cells of pigs, as well as on viability of these cells. Cells were incubated for 24 h with LPS (10 or 100 ng/ml of medium), TNF-α, IL-1β, IL-4 or IL-10 (each cytokine: 1 or 10 ng/ml of medium). Larger doses of TNF-α and IL-10 and both doses of IL-1β increased the relative abundance of mRNA/protein of 5-LO in the cells. A similar effect was exerted by the smaller dose of LPS on 5-LO mRNA content. Smaller doses of LPS and IL-4, and the larger dose of IL-10 increased the relative abundance of mRNA/protein LTAH, while both doses of TNF-α and the larger dose of IL-1β increased the protein content of this enzyme. Relative abundance of the mRNA/protein of LTCS was greater with the smaller dose of LPS, both doses of TNF-α and greater doses of IL-1β and IL-10, while relative abundance of LTCS mRNA was greater in response to the larger dose of LPS and both doses of IL-4. The LTB_4 and LTC_4 release was increased by the smaller dose of LPS, both doses of TNF-α and larger doses of IL-1β and IL-10. The IL-4 at the smaller dose exerted a stimulatory effect on LTB_4 release. Larger doses of TNF-α and IL-4 enhanced cell viability. Interactions with LPS and cytokines revealed in this study may represent mechanisms important for the regulation of endometrium functions of pigs under physiological or pathological conditions.

1. Introduction

Leukotriene (LT), prostaglandin (PG) and thromboxane synthesis is initiated by the release of arachidonic acid (AA) from cell membranes under the influence of phospholipase A_2. In relation to the LT production, AA is converted in a two-step process into an unstable intermediate, LTA_4, by a catalytic complex: 5-lipooxygenase (5-LO) and the 5-LO activating protein. This substance is subject to further transformation by the cytosolic LTA_4 hydrolase (LTAH) to LTB_4 or converted to the tripeptide glutathione under the influence of LTC_4 synthase (LTCS) to generate LTC_4. LTC_4 and its derivatives, LTD_4 and LTE_4, are known as cysteinyl-LTs (Rinaldo-Matthis and Haeggström, 2010). The LTs are produced mainly in immune system cells and are of great importance for regulating innate immune and inflammatory responses. The role of LTs in the pathogenesis of many inflammatory diseases was previously documented (Liu

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and Yokomizo, 2015). The physiological LT effects refer to the modulation of many processes in the reproductive tract (Abu and Konje, 2000).

Tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-4 and IL-10 are important for the many processes under physiological and pathological conditions. These factors are produced and released by immunological cells and other cell types. There is a presence of TNF-α in the endometrium and fetal membranes of pigs (Tayade et al., 2007; Linton et al., 2010; Khalaj et al., 2015). The TNF-α and its receptors are also present in the cattle (Okuda et al., 2010) and horse (Galvão et al., 2013) endometrium indicating a role of these factors in endometrial function. In pigs, IL-1β produced by peri-implantation embryos and the endometrium could affect conceptus-uterine communications during early pregnancy (Ross et al., 2003). This cytokine also activates uterine enzymes involved in the synthesis of PGE_2 (Franczak et al., 2010). Moreover, Seo et al. (2012) described functional receptors for IL-1β in the endometrium of pigs. IL-4 and its receptor have been identified in utero-placental tissues indicating an immunobiological role of this cytokine during pregnancy (De Moraes-Pinto et al., 1997). Similarly, IL-10 was detected in the endometrium and trophoblast of pigs (Linton et al., 2010). Furthermore, the presence of IL-10 was described in the inflamed endometrium of mares (Woodward et al., 2013) while receptors for IL-10 are present in human adenomiotic tissues (Qin et al., 2012).

Uterine inflammation is the most frequent reproductive disorder in livestock, leading to both economic and animal welfare problems; and is caused mainly by bacteria. One of the factors initiating uterine inflammation is lipopolysaccharide (LPS, a cell wall component of gram-negative bacteria), recognized by Toll-like receptor 4 and the CD14 complex present in immune and endometrial cells. As a consequence, mediators of inflammatory processes are synthesized and released (Cronin et al., 2012). Uterine inflammation in rats induced by LPS or Escherichia coli (E.coli) resulted in increased TNF-α and IL-1β concentrations in peripheral blood (Jana et al., 2005). Similarly, large amounts of these cytokines and IL-6 and IL-8 are synthesized and secreted during uterine inflammation in ruminants and mares; in the latter species, IL-10 gene expression was also increased (Gabler et al., 2009; Shao et al., 2012).

Little information is available about the synthesis and release of LTs from the uterus, nor about the mechanisms involved in controlling these processes. Studies on this subject have demonstrated that LTB_4 and LTC_4 synthesis occurs in the pig (Jana et al., 2014) and horse (Guzeloglu et al., 2013) uterus. Moreover, uterine inflammation in pigs (Jana et al., 2014) and cattle (Belluzzi et al., 2004; Barański et al., 2013) led to the increased synthesis and release of both LTs. LPS, TNF-α, IL-1β, IL-4 and IL-10 all stimulate increased relative abundance of LTAH and LTCS and secretion of LTB_4 and LTC_4 from endometrial strips of pigs following saline and E.coli intrauterine infusions (Czarzasta et al., 2014). Moreover, strong 5-LO, LTAH and LTCS immune responses were discovered in epithelial cells of these tissues (Jana et al., 2014). Therefore, it was hypothesized that LPS, TNF-α, IL-1β, IL-4 and IL-10 function locally to modulate LT synthesis and secretion from endometrial epithelial cells and influence cell viability. The aim of the present study was to examine the influence of LPS, TNF-α, IL-1β, IL-4 and IL-10 on the relative abundance of mRNA and protein of 5-LO, LTAH and LTCS in pig endometrial epithelial cells and on release of LTB_4 and LTC_4 from these cells. The viability of endometrial epithelial cells in response to these factors was also assessed.

2. Materials and methods

2.1. Animals and uterine tissue collection

Uteri from six gilts were obtained from a local abattoir. Entire uteri were removed within 5 min after the gilts were slaughtered. A macroscopic examination of the gonads was conducted to identify the phase of estrous cycle (Akins and Morrissette, 1968). Uteri obtained on day 8 of the estrous cycle were used in the study. This day of the estrous cycle was chosen because the isolated cells easily become confluent in culture conditions when collected on this day of the estrous cycle. The uteri were kept on ice, and transported immediately to the laboratory. All procedures for tissue collection were approved by the Local Ethics Committee in Olsztyn (Agreement No 31/2010). The authors of the study conformed to the principles of animal care (NIH publication No 86-23, revised in 1985).

2.2. Isolation of endometrial epithelial cells

Epithelial cell isolation from the endometrium was performed following the methodology described earlier (Blitek et al., 2011). Using sterile phosphate-buffered saline (PBS, 137 mM NaCl, 27 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4, pH 7.4; POCH, Gliwice, Poland, # 794121116, 739740114, 799250111 and 742020112, respectively) supplemented with antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin; Life Technologies, Bleiswijk, The Netherlands, # 15140–122), horns of uteri were washed twice. The mid-portions of uterine horns were opened longitudinally on the mesometrial surface and the endometrium was separated from the myometrium by careful scraping using a scalpel blade. The separation of these layers was confirmed under the dissecting microscope and then the endometrium was minced into small slices. The endometrium was digested with 0.48% (w/v) dispase (Life Technologies, Grand Island, NY, USA, # 17105041) in Hank's balanced salt solution (HBSS, Ca^{2+}, Mg^{2+} and phenol red-free, pH 7.4; Sigma-Aldrich, St Louis, MO, USA, # H8991) at room temperature for 60 min with gentle shaking. The released epithelial cells were pelleted by centrifugation at 200 x *g* for 10 min and washed once with Medium 199, containing 4% (w/v) bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA, # M5017 and # A2153, respectively) and antibiotics. Red blood cell lysing buffer (Sigma-Aldrich, St. Louis, MO, USA, # R7757) was applied to remove red blood cells from cell suspensions. The epithelial cells were subsequently rinsed three times with fresh Medium 199 containing 4% BSA, re-suspended in 2 ml of culture medium (Medium 199 supplemented with 2% BSA, 10% newborn calf serum/NCS; Sigma-Aldrich, St. Louis, MO, USA, # N4637/and antibiotics), counted in a