Theriogenology 79 (2013) 470–485

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Synthesis of prostacyclin and its effect on the contractile activity of the inflamed porcine uterus

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ARTICLE INFO

Article history:
Received 25 April 2012
Received in revised form 3 October 2012
Accepted 24 October 2012

Keywords:
Pig
Inflamed uterus
Prostacyclin synthesis
Contractile activity

ABSTRACT

The goal of the study was to estimate the content of prostacyclin (PGI_2), the levels of PGI synthase (PTGIS) and receptor (PTGIR) protein expression, and the cellular localization of these factors in the inflammatory-changed porcine uterus. The effect of PGI_2 on the contractility of the inflamed uteri was also determined. On Day 3 of the estrous cycle (Day 0 of the study), 50 mL of either saline or *Escherichia coli* suspension (10^9 colony-forming units/mL) were injected into each uterine horn. Acute endometritis developed in all bacteria-inoculated gilts, however on Day 8 of the study a severe form of acute endometritis was noted more often than on Day 16. Bacteria injections increased the contents of 6-keto-prostaglandin $F_{1\alpha}$ in endometrium, myometrium, washings, and the level of PTGIS in endometrium on Days 8 and 16, and the content of PTGIR in endometrium on Day 16. In the inflamed uteri on both study days, stronger immunoreactivity for PTGIS was observed in part of the luminal and glandular epithelial cells and in a portion of the endometrial arteries, and for PTGIR in part of the luminal epithelium and endothelial cells in a portion of the endometrial arteries. On Day 8, PGI_2 decreased contraction intensity in endometrium/myometrium and myometrium of the saline-treated uteri and increased the contraction intensity in both types of strips from the inflamed organs. Our study reveals that inflammation of the porcine uterus upregulates PGI_2 synthesis and that PGI_2 increases contractility, which suggests that PGI_2 might be essential for the course of uterine inflammation.

1. Introduction

Prostacyclin (PGI_2) is a member of the prostaglandin family of bioactive lipids and is a derivative of the 20-carbon omega-6 fatty acid, arachidonic acid. Both cyclooxygenase enzymes (COX-1 and COX-2) convert arachidonic acid into the prostaglandin precursor PGH_2, from which PGI_2 is rapidly synthesized by a specific PGI synthase (PTGIS). Prostacyclin is unstable at a physiological pH and thus has a very short half-life *in vivo*, rapidly forming the inactive hydration product, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). Prostacyclin elicits its effects on target cells by interacting with its G protein-coupled receptor (PTGIR) which has a typical seven-trans-membrane structure [1,2]. Moreover, PGI_2 might also signal through nuclear receptor-mediated pathways such as the peroxisome proliferator-activated receptor δ pathway [3]. The physiological effects of PGI_2 are vast, but many still remain to be elucidated. It has been reported that PGI_2 is a potent vasodilator, inhibits platelet aggregation [4] and vascular smooth muscle cell proliferation [5], reduces pulmonary blood pressure and bronchial hyperresponsiveness [6], and regulates renal blood flow and the release of renin [7]. Prostacyclin is also a very important inflammatory mediator. Together with prostaglandin (PG)E_2, it induces changes in vascular

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http://dx.doi.org/10.1016/j.theriogenology.2012.10.020

permeability associated with hyperemia and edema during acute inflammation [8,9]. Prostacyclin is also well known to elicit nociceptive pain response [9] and is involved in pain transmission at the spinal cord level during peripheral inflammation [10]. Its contribution to inflammatory diseases such as rheumatoid arthritis, pulmonary vascular disease, atherosclerosis [11,12], and allergy [13] has been extensively documented.

Endometritis is a common reproductive disorder in female domestic animals with consequences ranging from no effect on reproductive performance to permanent sterility. This pathological state can occur after parturition and in animals that have not yet given birth after artificial insemination or natural mating. It has been reported that a wide range of bacteria, mainly *Escherichia coli*, *Staphylococcus spp*., *Streptococcus spp*., and in some cases *Actinomyces pyogenes, Pasteurella multocida*, and *Klebsiella pneumonae*, were isolated from the uteri of sows with and without endometritis [14]. Endometritis causes considerable alterations in the rate of synthesis of inflammatory mediators, i.e., metabolites of arachidonic acid such as PGs [15–18] and tromboxane A_2 [19,20] and nitric oxide [21] and proinflammatory cytokines—tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8 [22,23]. Intrauterine infusions of lipopolysaccharide (LPS), the immunoactive component in the walls of Gram-negative bacteria, in postpartum cows and spontaneous or experimentally induced bacterial infections of the uterus in mares [24,25], cows, [19,26] and pigs [16,17] were associated with the increased concentration of the peripheral plasma $PGF_{2\alpha}$ metabolite, PGFM. Inoculation of porcine uteri with *E. coli* suspension resulted in an increase in the content of $PGF_{2\alpha}$ and PGE_2 in the endometrium (ENDO) and myometrium (MYO) [17]. Moreover, the rate of synthesis of these PGs in inflamed bovine [15] and porcine [17] uteri depends on the intensity of the inflammatory process, its duration, and the type of uterine tissue affected. It was also reported that elevated amounts of both PGE_2 and $PGF_{2\alpha}$ in the inflamed porcine uterus coincide with an increase in the expression of COX-2, microsomal PGE synthase-1 (enzyme down streaming COX-2, terminating PGE_2 synthesis) and 9-ketoreductase (enzyme converting PGE_2 to $PGF_{2\alpha}$) [17,18,27]. Moreover, our studies have shown that $PGF_{2\alpha}$ enhances [28], and PGE_2 decreases [29] the contractile activity of inflamed porcine uteri.

Although many studies have examined the production and importance of PGI_2 in several physiologic and pathologic processes, little information is available concerning the synthesis of PGI_2 in inflamed uteri. So far the only studies on this subject reported that the levels of the PGI_2 metabolite were higher in the peripheral blood of postpartum dairy cows with spontaneous uterine infections [19] and after intrauterine infusions of LPS [20]. Based on the above-mentioned findings, we hypothesize that PGI_2 synthesized in an inflamed uterus, can be important for the course and/or outcomes of this pathological condition. Therefore, to study the synthesis of PGI_2 in the process of uterine inflammation, we examined the effects of the intrauterine injection of *E. coli* suspension in gilts on the: (1) concentrations of PGI_2 in uterine tissues and washings, and in peripheral blood, (2) the levels of PTGIS and PTGIR protein expression, and (3) cellular localization of PTGIS and PTGIR. Moreover, the effect of PGI_2 on the contractile activity of the inflamed uteri was determined.

2. Materials and methods

2.1. Animals

Thirty crossbred gilts (Large White × Landrace) of similar age (7 to 8 months) and body weight (BW; 107 to 122 kg) were used in the present study. Estrous behavior was detected using the boar-tester. The animals originated from a herd with no abnormal discharge or fertility disorders. The gilts were individually housed in stalls under natural light and temperature conditions. They were fed a commercial grain mixture with water available *ad libitum*. We followed the principles of animal care (NIH publication No. 86-23, revised in 1985) and the specific national law on animal protection. The experimental procedures were approved by the Local Ethics Committee, University of Warmia and Mazury in Olsztyn (Agreement No. 31/2010).

2.2. Experimental procedure

On Day 3 of the estrous cycle (Day 0 of the study), twenty-four gilts were randomly assigned to one of two groups: group I, gilts receiving saline (N = 12), and group II, gilts treated with *E. coli* (N = 12). In all the gilts median laparotomy was performed under general anesthesia induced by azaperone (2 mg/kg BW Stresnil; Janssen Pharmaceutica, Beerse, Belgium) and sodium pentobarbital (30 mg/kg BW Vetbutal; Biowet, Pulawy, Poland). After the abdominal incision, both uterine horns were gently removed and either 50 mL of saline or 50 mL of *E. coli* (strain O25:K23/α/:H1; National Veterinary Research Institute, Department of Microbiology, Puławy, Poland) suspension containing 10^9 colony-forming units per mL, were applied into each uterine horn. Each uterine horn was injected 5 times (10 mL of solution per each injection) using a syringe equipped with a 0.8 mm ga needle, keeping a similar distance between the places of the injections. To avoid the outflow of the solution from the uterus, soft pressure was applied with a gauze to the areas of injection which were next gently wiped with the gauze with saline. To evenly distribute either saline or bacterial suspension within the uterine horn, both horns were carefully massaged. Additionally, in the saline- (N = 6) and *E. coli* (N = 6)-treated gilts whose slaughter was planned on Day 16 after treatment, the polyvinyl cannula (outer diameter 2.2 mm, inner diameter 1.8; Tomel, Tomaszów Mazowiecki, Poland) was inserted into the jugular vein to collect blood samples for determining PGI_2 metabolite–6-keto-$PGF_{1\alpha}$ concentration. The samples were collected on Day 0 (before saline or bacteria infusion) and from Days 1–16 after treatment, twice a day (8:00 AM and 8:00 PM). Immediately after collection, the samples were put into the ice bath, where they were kept until centrifugation (1500× *g*, at 4 °C). The plasma was decanted and stored at −20 °C until further processing. The gilts were not treated with antibiotics during the whole period of the study. The first subset of the saline- (N = 6) and *E. coli* (N = 6)-treated gilts