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Original research article

Identifying diagnostic endocrine markers and changes in endometrial gene expressions during pyometra in cats

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ABSTRACT

Pyometra is a significant reproductive problem in cats. The aims of this study were to evaluate (i) the immunological profile of queens by studying plasma concentrations of metabolites of prostacyclin I_2 (6-keto-$PGF_{1\alpha}$), leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4); and (ii) the gene transcription profiles of Toll-like receptors (TLRs) 2 and 4 (*TLR2/4*), PGE_2-synthase (*PGES*), $PGF_{2\alpha}$-synthase (*PGFS*) and prostaglandin-endoperoxide synthase 2 (*PTGS2*) in the feline endometrium throughout the estrous cycle, after medroxyprogesterone acetate (MPA) treatment and during pyometra.

The concentration of plasma 6-keto-$PGF_{1\alpha}$ in pyometra was increased in comparison to other groups studied ($p < 0.01$). Endometrial mRNA coding for *TLR2* was up-regulated in cats suffering from pyometra compared to other groups ($p < 0.001$). Expression of mRNA for *TLR4* was up-regulated in endometria originating from MPA-treated cats, pyometra and late diestrus cats, compared with tissues from cats during estrus and anestrus ($p < 0.05$). As expected, endometrial mRNA for *PTGS2* was up-regulated only in pyometra, compared with other groups ($p < 0.001$). Similarly, endometrial mRNA for *PGFS* was up-regulated in pyometra, compared with endometria from anestrus, late diestrus and from MPA-treated cats ($p < 0.05$), or from cats during estrus ($p < 0.01$).

Overall, these results indicate that plasma concentrations of LTB_4 and LTC_4 are not useful diagnostic markers since they were not increased in queens with pyometra, in contrast to 6-keto-$PGF_{1\alpha}$. In addition, treatment with MPA evoked neither endocrine nor molecular changes in endometria of cats.

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1. Introduction

Pyometra is a clinically significant reproductive problem in cats, although it is not so frequent as in dogs. Pyometra most often develops in cyclic mature females in the luteal phase of the estrous cycle. The differences in longevity of the luteal phase in these two species, as well as variability in ovulation occurrence in domestic cats, are possible explanations for the much more common incidence of pyometra in dogs. Felids mostly ovulate only after a sufficient luteinizing hormone (LH) peak, evoked after vaginal stimulation caused by mating. Nonetheless, the cat is the only known domesticated species that can exhibit either induced or spontaneous ovulation at the culmination of estrus [1–4]. If ovulation occurs spontaneously, queens develop a non-pregnant luteal phase, which lasts 35–40 days [5]. In contrast to cats, dogs ovulate spontaneously during each estrous cycle and the non-pregnant luteal phase may last longer than 80 days [6], which exceeds the length of the luteal phase in pregnant females.

Besides the occurrence of spontaneous ovulation and development of a non-pregnant luteal phase, progestogen contraceptives are considered major risk factors for the clinical syndrome of pyometra in cats. Endometrial endocrine disturbances are believed to be prerequisite, particularly for development of cystic endometrial hyperplasia, which appears as undesirable morphological and functional changes that mostly affect the endometrial glands. In addition, excessive mucus secretion creates favorable conditions for invading bacteria, supporting their abundant growth in the uterine lumen. Uropathogenic *Escherichia coli* is the most common Gram-negative bacteria isolated from feline [7] and canine [8] uteri with pyometra. Moreover, this microorganism is the most common strain isolated from the lower parts of the reproductive tract of healthy queens [9,10]. The number of *E. coli* binding to canine endometrial epithelium was shown to depend on different stages of the estrous cycle, peaking during spontaneously occurring diestrus [11], or induced diestrus in ovariectomized bitches that were treated with estradiol benzoate and megestrol acetate to induce simulated estrus and diestrus stages [12].

Pyometra is associated with activation and proliferation of immune-specific B and T cells, as well as synthesis and activation of immune and pro-inflammatory molecules. However, this inflammatory response to bacteria is not driven solely by activated immune cells but is also supported by different types of endometrial cells, facilitating the secretion of cytokines or eicosanoids. Recently, we showed that feline endometrial epithelial cells respond to lipopolysaccharide (LPS) with increased secretion of tumor necrosis factor α (TNFα) [13]. Moreover, in contrast to stromal cells, immunostaining for TNFα and its receptor type 1 (TNFR1) [13], as well as Toll-like receptors type 2 and 4 (TLR2/4) [14], were abundantly expressed in superficial and glandular epithelium, confirming our hypothesis of a fundamental role for feline endometrial epithelium in the early response to bacterial infection. Pyometra may begin as a sub-clinical, unrecognized disorder that rapidly evolves into a severe life-threatening disease leading to death, if not promptly treated. This characteristic imposes a need for additional studies to define endocrine markers of this syndrome and to examine involvement of genes that may contribute to pyometra.

The objectives of this study were to evaluate (i) the plasma concentrations of several immunoreactive arachidonate metabolites (metabolite of prostacyclin I_2 [6-keto-$PGF_{1\alpha}$], leukotriene B_4 [LTB_4] and leukotriene C_4 [LTC_4]), and (ii) selected gene expression profiles (*TLR2/4*, *prostaglandin-endoperoxide synthase 2* [*PTGS2*], *PGE_2-synthase* [*PGES*] and *$PGF_{2\alpha}$-synthase* [*PGFS*]) in cats with pyometra compared to cycling, clinically normal queens or with those that were treated with medroxyprogesterone acetate (MPA).

2. Materials and methods

2.1. Animals

All procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (No. 60/2010/DTN). All the cats were ovariohysterectomized with the owners' request and informed consent for venipuncture and tissue collection. The genital tracts were received from private clinics and most of these procedures and history recordings were carried out by private surgeons.

A total of 25 shorthair domestic cats were enrolled in this study, ranging in age from 8 to 180 (mean 31.77 ± 33.7) months. Based on clinical examinations, interviews with the owners, macroscopic observations of the ovaries [15] and circulating concentrations of progesterone in plasma, animals were assigned to the following groups: anestrus ($n = 5$), estrus ($n = 5$), late diestrus ($n = 5$), pyometra ($n = 5$), and a long-term MPA-treated in which animals had previously been orally treated with MPA (Promon Vet®, Pfizer Animal Health, Louvain-la-Neuve, France; 5 mg/animal/week) for at least four to a maximum of twelve months. The last group of cats underwent ovariohysterectomies (OVH) during MPA treatment. In cycling cats, no pharmacological treatment was performed to provoke ovulation.

2.2. Tissue and blood collections

In all queens, 1–1.5 mL of blood for LTB_4, LTC_4 and PGI_2 analyses were collected immediately before surgery into EDTA-containing tubes. Plasma was separated by 10 min centrifugation of whole blood at 1500 × *g*, then frozen at −20 °C and stored.

Immediately after OVH, uteri were rinsed with sterile saline to remove blood contamination and placed into fresh saline at 4 °C and transported on ice to the laboratory. Both horns of each uterus ($n = 25$) were slit longitudinally and the endometrial fragments from the middle part of each uterine horn were separated from myometrium under a binocular microscope (Olympus SZX7, Tokyo, Japan), inserted into cryotubes and placed in 1 mL RNAlater (Ambion Biotechnologie GmbH, Wiesbaden, Germany). The samples were kept overnight at 4 °C, then the RNAlater was removed and the tissues were stored at −80 °C until RNA isolation.

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