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Serum tryptophan and its metabolites in female dogs undergoing ovariohysterectomy as treatment of pyometra or as elective spay surgery

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ABSTRACT

This study compares serum concentrations of tryptophan (TRP), kynurenine (KYN), kynurenic acid (KYNA), and indoleamine 2,3-dioxygenase (IDO) activity in healthy bitches and bitches with bacterial uterine infection (pyometra). The effects of surgery were also assessed by measuring these variables in both groups of dogs before and after ovariohysterectomy. Presurgery, mean (±standard deviation) TRP, KYN, and KYNA concentrations and IDO activity were 68.44 ± 21.77, 2.00 ± 0.33, 112.11 ± 111.91 μmol/L, and 29.22 ± 10.10, respectively, in the healthy dogs; and 40.16 ± 12.11, 8.27 ± 3.94, 411.11 ± 199.60 μmol/L, and 205.92 ± 154.20, respectively, in the dogs with pyometra. Tryptophan and KYN levels had normalized on suture removal (10 days after surgery) though IDO activity and KYNA concentrations remained elevated during the postoperative period compared with presurgery values in both study groups. Our results suggest that KYNA concentrations and IDO activity could be useful indicators of the inflammation induced by pyometra and could be also used to monitor recovery following ovariohysterectomy in both healthy dogs and dogs with pyometra.

1. Introduction

Blood concentrations of inflammatory mediators vary during the course of an inflammatory process and can be used as a measure of systemic inflammation [1,2]. In clinical practice, inflammatory mediators can be used as biomarkers to detect diseases causing systemic inflammation and assess the severity of the inflammation produced [1–4]. Thus, biomarkers of inflammation are being increasingly used in humans and veterinary clinical practice for diagnostic and prognostic purposes and to follow treatment response and postoperative recovery [5,6]. The inflammatory biomarkers most commonly used in veterinary medicine have been acute-phase proteins [1,7,8]. Among these, C-reactive protein (CRP), a major acute-phase protein in dogs, and many other mediators such as insulin-like growth factor and cytokines have been widely investigated [2,4,9,10]. In human medical research, tryptophan (TRP) and its breakdown products have been assessed as possible biomarkers [11–14]. Tryptophan is an essential amino acid for normal growth in infants and for maintaining nitrogen balance in adults [15,16]. The catabolism of TRP is tightly regulated and controlled by the

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rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) [11]. Importantly, one crucial function of IDO is the production of kynurenine (KYN) *via* the TRP pathway [17,18]. Indoleamine 2,3-dioxygenase catalyzes the first step in TRP degradation, i.e., in the formation of N-formylkynurenine, which is subsequently transformed into KYN [12]. In healthy individuals, blood concentrations of TRP correlate with those of KYN [19]. During an inflammatory process, however, TRP concentrations in serum decrease, whereas concentrations of KYN and other TRP metabolites increase [12]. Kynurenine and its subsequent breakdown products have diverse biological functions, including dilating blood vessels during inflammation and regulating the immune response [20,21]. These many roles make them potential candidates as biomarkers of inflammation severity and the presence of an inflammatory disease. Kynurenic acid (KYNA) is produced from KYN by KYN aminotransferases [22–25]. Kynurenic acid nonspecifically inhibits the receptors of the excitatory amino acids: N-methyl-D-aspartic acid, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainic acid in the central nervous system, thereby having anticonvulsive and neuroprotective properties [14,23,26,27]. Kynurenic acid is also a ligand of G protein–coupled receptor GPR35, which is highly expressed in the immune system [20,28]. Moreover, KYNA has numerous functions such as protecting nerve cells exposed to hypoxia during parturition, bactericidal activity, promoting proper digestion and also acts as a prognostic factor in neoplastic diseases [24–26,28]. To the authors' knowledge, however, no study has examined the role in inflammation played by concentrations of TRP and its downstream metabolites in dogs.

To assess the severity of inflammation and detect inflammatory-associated postoperative complications, accurate monitoring is essential. Before investigating whether an inflammatory mediator will serve to identify and grade systemic inflammation, normal concentrations in healthy animals need to be established. Similarly, before the effects of postoperative complications such as peritonitis or wound infection can be determined, the normal inflammatory response to uneventful surgery has to be assessed. Surgical removal of the uterus and ovaries, i.e., ovariohysterectomy (OHE), is one of the most frequent procedures performed in small animal practices worldwide. This procedure is used both to prevent pet overpopulation (elective spay) and treat diseases such as pyometra. Life-threatening complications associated with inflammation are not uncommon after OHE, and in the early stages, they can be clinically difficult to differentiate from normal postoperative healing. Pyometra is a potentially lethal bacterial infection of the uterus that is associated with systemic inflammation, affecting an average of 19% of all intact bitches [29]. Inflammatory markers that could be helpful to detect complications or for an early disease diagnosis are in high demand. The present study was designed to determine and compare IDO activity and concentrations of TRP, KYN, and KYNA in female dogs scheduled for OHE to treat pyometra or as elective spay surgery. The effects of surgery on these variables were also determined in the two patient groups.

2. Materials and methods

2.1. Ethical approval

All experimental protocols and procedures received local Ethics Committee for Animal Experimentation approval. Informed consent was obtained from each dog owner before its inclusion in the study.

2.2. Animals, sampling, and bacterial isolation

The animals recruited for the experimental group were 12 different breed bitches (Bull Terriers, Golden Retrievers, and Doberman Pinshers) including mixed-breed animals. These female dogs had been admitted to the Department and Clinic of Animal Reproduction, University of Life Sciences, Lublin, Poland, for the surgical treatment of pyometra (OHE). These dogs had participated in an earlier study in which we examined insulin growth factor 1 and CRP concentrations [30]. Briefly, all bitches were examined clinically, and additional tests were performed leading up to a preliminary diagnosis of pyometra and surgical treatment (OHE), as previously described [30]. The clinical examination revealed polydipsia (66.6%), polyuria (41.6%), reduced or complete lack of appetite (anorexia; 83.3%), dehydration (66.6%), apathy (75%), vomiting (33.3%), and abnormal color of the mucous membranes (75%) as common signs of this illness. Additionally, a purulent vaginal discharge was present in 66.6% of the bitches. Vaginoscopy revealed congested serous membranes of the vaginal vestibule; in dogs with open-cervix pyometra, serous membranes were covered with pus. An abdominal ultrasound examination revealed an enlarged uterus of diameter ranging from 3 to 7 cm with hypoechogenic contents. The diagnosis of pyometra was confirmed by a postoperative histopathologic examination of the uterus and ovaries conducted at the Department of Pathological Anatomy, University of Life Sciences, Lublin, Poland.

The control group comprised nine clinically healthy bitches of five different breeds including mixed-breed dogs admitted to the same clinic for elective OHE (spaying). All healthy control bitches were in diestrus as determined by data from medical records, clinical examination findings, and cytologic examination of vaginal smears performed by the first author (Roman Dąbrowski).

Before surgery, blood samples collected from the vena cephalica were submitted for laboratory tests (hematological and biochemical). In addition, blood was collected into silicone tubes containing a clot activator (Vacutest Kima, Piove di Sacco, Padua, Italy) immediately before and 3 and 10 days after OHE and stored frozen. After simultaneously thawing these blood samples, TRP, KYN, and KYNA levels were determined. All blood samples were confirmed free of hemolysis and lipemia.

The surgery was performed under general anesthesia. As premedication, medetomidine (Domitor; Pfizer, Warsaw, Poland) was administered intramuscularly at a dose of 40 μg/kg. Anesthesia was induced with intravenous propofol (2 mg/kg, Plofed; Polfa Warsaw, Poland) and maintained by isoflurane/oxygen inhalation. For pain control immediately after surgery and 2 days postoperatively,