Markers of inflammation in experimentally induced pancreatitis in dogs (Part I): C-reactive protein and white blood cell counts

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SUMMARY

Experimental pancreatitis was induced in 6 dogs by ligation of the pancreatic ducts. The pancreatitis induction was confirmed by conventional histology on the 5th day after surgery. Plasma CRP (C-reactive protein) concentrations and WBC (White Blood Cells) counts were determined 72 hours and immediately before (hour 0) the surgical intervention and 3, 6, 24, 48, 72 and 96 hours after. Both parameters significantly increased compared to basal values since the 3rd hour for CRP concentrations and the 6th hour for WBC counts, reached maximal values at the 24th hours and remained dramatically elevated until the 96th hour and were highly and positively correlated (r = 0.84, P < 0.01). These results showed that the haematological and the biochemical parameters both evidenced the occurrence of an inflammatory response but that plasma CRP was an earlier marker than WBC count.

Keywords: Acute pancreatitis, dog, CRP, WBC, correlation.

Introduction

Acute pancreatitis (AP) is a sudden inflammation of the pancreas with different extent of involvement of adjacent tissues or remote organs and systems [2]. It could range from a moderate self-limiting episode to a severe life-threatening disease. The occurring changes could be both inflammatory and non-inflammatory, due to various aetiological factors and of different pathogenesis, but nevertheless, the clinical picture is fairly similar.

The inflammatory response is an essential element of AP and increased proportionally to its severity. That is the reason why, apart from the routine enzyme determinations, there is an increasing interest towards blood laboratory markers of the intensity of the inflammatory response in acute pancreatitis. This response is generally evaluated by several serum markers: neutrophil-specific elastase, interleukin 6 (IL-6), C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α) and procalcitonin (PCT) [14]. At present, CRP is the most commonly used marker in routine clinical practice for assessment of the inflammation severity. It is discovered in the blood of men during the acute stage of pneumococcal infection and is described in 1930 by TILLUET and FRANCIS [16]. Its name comes form its ability to bind C-polysaccharide from the bacterial membrane by the QUELLUNG reaction [in 16]. Furthermore, it binds phospholipids of destroyed cells and phosphocholine that is part of membrane phospholipid constituents, thus recognizing several foreign bacteria [5]. It is also able to activate the complement system by activation of the complement ligands, suggesting that CRP could initiate the elimination of cells possessing foreign genetic information by interaction with humoral and cellular components of inflammation. In addition, the pro-inflammatory effects of CRP include induction of some inflammatory cytokines and of a number of tissue factors by monocytes [5]. In human medicine, CRP is frequently used for differentiation of viral and bacterial infections, such as viral and bacterial meningitis or pneumonia. Serum concentrations in men and dogs are increased manifold as early as the first hours of the acute phase response (APR) and could increase over 100 times depending on the degree of inflammation. According to
CRP CONCENTRATIONS IN DOGS WITH ACUTE PANCREATITIS

RODOMAN et al. [15], CRP has the disadvantage to increase rather slowly for being utilized as an early predictor for infection severity. Other authors however report a statistically significant increase as early as the 4th hour after surgical intervention with peak values on the 24th hour [9].

It is stated that leukocytosis promote a systemic defence response after challenge with various noxious agents. Different pathologies lead to variable quantitative and functional changes in white blood cells. The increased peripheral white blood cell (WBC) counts in AP reflect the general systemic intoxication in the initial stage of the disease and the starting lytic and destructive inflammation of the pancreas [17, 19]. Leukocytosis is a common finding in severe AP cases, even in the absence of infection. Total leukocyte counts usually increase to 20-25 x 10^9 cells/L.

Regardless of the available information in the literature, the opinions about the role and the diagnostic value of CRP are still very controversial. This point motivated the present study upon the time course of CRP concentrations in experimentally induced acute pancreatitis in dogs and its correlation to the WBC count, a routine and standard parameter of the inflammatory response.

Materials and Methods

EXPERIMENTAL ANIMALS AND PROTOCOL DESIGN

The experimental animals were provided by the municipality of Stara Zagora. Six mongrel dogs from both sexes, 4-5 years old, weighing 13.5-18.0 kg were used. Prior to the experiment the animals were vaccinated with vaccine Nobivac® DHPPiLR (Intervet International B.V) and treated against internal and external parasites by Caniverm ® (Bioveta, A. S. Czech Republic, 1 tablet/10 kg BW, VO) and Bolfo® Powder (Bayer, Germany) respectively. The dogs were fed according to the age and had free access to tap water. The experimental procedure was approved by the Ethical Committee of the Faculty of Veterinary Medicine in Stara Zagora.

Dogs were premedicated with a subcutaneous injection of atropine sulphate (Vetprom Ltd, Bulgaria, 0.02 mg/kg) and 10 minutes later, by an intramuscular injection of acepromazine hydrochloride (Combistress®, Kela – Belgium, 0.2 mg/kg). Twenty minutes after, thiopental sodium (Thiopental VUAB®, Slovakopharma, Slovakia) at 10 mg/kg was intravenously injected for the anaesthesia induction and the anaesthesia was maintained using 1.5-2 V% halothane (Narcotan®, Spofa, The Czech Republic) and oxygen flow 2-2.5 L/min, with a Bain breathing circuit. Fluid maintenance was performed with 0.9% saline (Troyapharm, Bulgaria) at a rate of 5-10 mL/kg/h. The experimental pancreatitis was induced by ligation of both pancreatic ducts. The surgical approach was created by cranial median laparotomy. The initial part of the duodenum was brought outside the abdominal cavity and isolated from inflow of new gastric and duodenal content by intestinal clamps. Duodenotomy was performed by linear incision of the side, opposite to the pancreas. Both ducts were cannulated through the minor and the major duodenal papillae (figure 1). Around the excretory ducts, ligatures were placed around the excretory ducts openings by two-layer (purse-string and cross-stitch) sutures of the absorbable suture material polygalactin (Vicryl®, Ethicomp Inc.) USP 4-0. The duodenal wound was closed by Schmidten’s suture and continuous Lambert polyglyconate sutures (Maxon, Daviscoeck) USP 2-0. The abdominal cavity was closed routinely.

Physical examinations, ultrasonography, ECG, blood and urine analyses were performed 72 hours prior and at the 3, 6, 24, 48, 72 and 96 hours after surgery. Dogs have been fasted for 2 days after surgery but 24 hours after surgery, dogs have had free access to the drinking water.

HAEMATOLOGICAL AND BIOCHEMICAL ANALYSES

Blood samples were collected from the puncture of the v. cephalica antebrachii into 2 sterile tubes, one with EDTA (1 mL) for haematological analysis, and the other with heparinate (10 mL) for blood biochemistry. Complete blood counts were performed within an hour after blood collection on an automated analyzer (Sérono Plus 150 USA). Heparinised blood was centrifuged (1 500g, 10 minutes, at 4°C) within 30 min after collection. Plasma was immediately separated and stored at -20°C until analysis. Plasma C-reactive protein concentrations were assayed on a microplate reader (HP) with species-specific commercial kits produced by Tridelta Development Ltd, Ireland.

HISTOLOGICAL ANALYSIS

On the 5th day after surgery, all dogs were euthanized with thiopental and potassium chloride. Pancreas were sampled, fixed in 10% neutral formalin and embedded in paraffin. Cross sections of 5 µm were stained with haematoxylin/eosin.

STATISTICAL ANALYSIS

Data were statistically evaluated by one-way analysis of variance Anova (StatistiCa for Windows, Stat Soft Inc., USA
LAZAROV (L.) AND COLLABORATORS

Results

Blood C-reactive protein concentrations were significantly influenced by the induced aseptic inflammation. The initial CRP values ranged between 6.3 and 45.5 mg/L, and averaged 15.8 ± 1.04 mg/L 72 hours prior to the beginning of the surgical intervention and 17.4 ± 1.09 mg/L at 0 hour. As early as 3 hours after the surgery, CRP concentrations started to increase and persisted elevated until the end of the experiment (on the 96th hour) compared to baseline values (P < 0.01). The maximal CRP concentrations were recorded on the 24th hour and were nearly 8 times higher than the preoperative values (131.7 ± 6.86 mg/L). Between the 48th and the 96th hours, the CRP concentrations remained statistically significantly elevated and ranged between 112 and 124 mg/L (figure 2 and Table I).

The WBC counts were within 11.20 ± 1.10 x 10^9 cells/L (on -72 hours before surgery) and 12.10 ± 1.25 x 10^9 cells/L immediately before (hour 0). The haematological parameter began to increase and significantly differed from basal values (P < 0.05) 6 hours after ligation of pancreatic ducts, peaked at the 24th hour (24h vs. 6h and 48h: P < 0.01) and then slowly declined and remained significantly elevated compared to initial values until the 96th hour (P < 0.01) (figure 3 and Table I). In addition, the WBC counts were highly and positively correlated with plasma CRP concentrations (r = 0.84, P < 0.01).

Gross examination showed that the pancreas glands were enlarged, with increased density and marbled appearance. On cross sections, this pattern was more marked with some dark-red areas imbibed with blood surrounding islets of intact tissues. Histologically, blood vessels were hyperaemic and oedema was evidenced in the interstitial connective tissue (figure 4).

Discussion

Circulating CRP concentrations in healthy dogs are higher than in healthy humans [13]. According to YAMAMOTO et al. [21], the mean CRP concentrations assayed with ELISA in male dogs of different breeds varied between 2.9 and 30 mg/L, and in bitches from 2.4 and 18.6 mg/L. Usual values for plasma CRP concentrations were also established according to the age classes: 2.7 to 14.1 mg/L in dogs below 2 year old, 2.4 to 18.6 mg/L in 2-5 years old dogs, 3.4 to 30.0 mg/L in 6-9 years old dogs and 3.9 to 14.9 mg/L in dogs older than 10 years [21]. The same authors have reported in 1993 lower CRP concentrations than abovementioned, but this could be attributed to different rearing conditions and eventual stimulation of the immune system [20]. For example, if dogs are housed in private homes or in infection-protected premises, they could exhibit higher CRP concentrations. ULUTAS et al. [18] found similar range values (3.1 - 18.1 mg/L) for plasma CRP concentrations in healthy dogs. In agreement with that,
in the present study, plasma CRP concentrations measured in dogs 72 hours before the surgical intervention were 15.8 ± 1.04 mg/L in average. However, CAPSI et al. [3, 4] reported that plasma CRP concentrations determined using electroimmunoassay was below 5 mg/L in healthy animals and ECKERSALL [7] proposed 10 mg/L as the upper limit for plasma CRP concentrations in healthy dogs. OTABE et al. [13] established that the plasma CRP concentrations were comprised between 0.8 and 22.6 mg/L in dogs and they observed no circadian rhythm and no inter-day fluctuation over a 1 month long period. The lack of influence of blood sampling manipulations on CRP concentrations makes it a reliable and consistent marker of inflammation [9, 13, 20].

In the present study, we have postulated that plasma CRP could be used as an accurate biomarker for an early diagnostic of the inflammation response, in the unapparent stage of disease prior to clinical manifestations and prior to specific immune reactions. A marked and significant increase in the plasma CRP concentrations was evidenced as soon as 3 hours after the induction of acute pancreatic by ligation of the excretory ducts and maximal values (131.7 ± 6.86 mg/L) were obtained on the 24th hour. By contrast, KILANPAA-BACK et al. [11] reported that plasma CRP concentrations have increased later, on the second day of the acute pancreatitis development. It is also stated [1] that CRP values over 200 mg/L 72 hours after the disease induction are strongly associated with pancreas necrosis whereas when they are below the threshold value, tissue necrosis is highly probably absent. In the present investigation, pancreatic necrosis was not observed by histology and plasma CRP concentrations have remained below 200 mg/L. Consequently, necrosis could be anticipated when CRP concentrations were close to and over 200 mg/L.

Different illnesses provoke various quantitative and functional alterations in numeration of the white blood cells. In the most cases, acute pancreatitis is accompanied by a general increase in total WBC counts and specifically by a strong elevation of the neutrophil population whereas lymphocytes and monocytes decreased [17, 19]. Gradual reduction of the leukocyte counts in severe pancreatitis or rapid decrease and normalization of the cell populations in mild forms correlate with the progressive disease remission and indicate a favourable prognosis for the outcome [6]. On the other hand, increase in the neutrophil population is a bad prognostic sign, and indicate complications in the early stage of the acute pancreatitis due to the release of various pro-inflammatory cytokines by activated neutrophils [10, 12]. In the present study, WBC counts dramatically increased after the surgical induction of the pancreatitis confirmed by gross examination and histology since the 6th hour and peaked on the 24th hour. However, a definite prognosis related to the outcome could not be established.

After surgery, significant increases in the WBC counts and in plasma CRP concentrations were observed, with maximal values on the 24th hour and persisting elevated values until the 96th hour for both 2 parameters. The difference in the variation profiles of the 2 parameters was that a significant increase in plasma CRP concentrations has occurred as early as the 3rd hour (P < 0.01) while WBC counts were not yet significantly modified. Consequently, a high positive correlation was obtained between both studied parameters (r = 0.84, P < 0.01). Furthermore, CRP concentrations and WBC counts correlated with the severity of the inflammation and supported the opinion of ECKERSALL [8] that the quantification of CRP is a diagnostic means providing valuable information about the presence of infections, inflammation or trauma.

While the WBC numeration fluctuates according to a circadian rhythm, food intake, blood sampling manipulations, physical exercise etc. [11], CRP concentrations are relatively stable, react earlier and are not influenced by external factors [9, 13, 20]. Another advantage is that the CRP determination is easy and fast to perform and could be used in routine clinical practice for evaluation of the severity and prediction of the course of inflammation.

Table I: Variations of white blood cell (WBC) counts and plasma CRP concentrations (mg/L) in dogs (n = 6) with experimentally induced pancreatitis by ligation of the pancreatic ducts.

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<th>Parameters</th>
<th>Before surgery</th>
<th>After surgery</th>
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<tr>
<td>CRP (mg/L)</td>
<td>15.8 ± 1.04&lt;sup&gt;a&lt;/sup&gt; 17.4 ± 1.09&lt;sup&gt;a&lt;/sup&gt; 40.3 ± 3.62&lt;sup&gt;b&lt;/sup&gt; 75.4 ± 8.09&lt;sup&gt;b&lt;/sup&gt; 131.7 ± 6.86&lt;sup&gt;d&lt;/sup&gt; 123.9 ± 5.51&lt;sup&gt;d&lt;/sup&gt; 112.2 ± 3.68&lt;sup&gt;c&lt;/sup&gt; 111.9 ± 5.16&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>WBC (10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>11.22 ± 1.10&lt;sup&gt;a&lt;/sup&gt; 12.10 ± 1.25&lt;sup&gt;a&lt;/sup&gt; 12.17 ± 1.15&lt;sup&gt;a&lt;/sup&gt; 14.95 ± 1.35&lt;sup&gt;b&lt;/sup&gt; 24.80 ± 2.08&lt;sup&gt;d&lt;/sup&gt; 21.30 ± 1.91&lt;sup&gt;c&lt;/sup&gt; 21.20 ± 1.86&lt;sup&gt;c&lt;/sup&gt; 19.20 ± 1.38&lt;sup&gt;bc&lt;/sup&gt;</td>
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Different superscripts<sup>a,b,c,d</sup> in the same row indicate significant differences (P < 0.05 to P < 0.01).

References