Markers of inflammation in experimentally induced pancreatitis in dogs (Part II): correlation between clinical parameters and haptoglobin

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Keywords: Acute pancreatitis, dog, haptoglobin, respiratory inflammation.

The aim of the study was to investigate changes in plasma haptoglobin (Hp) concentrations during a trauma inflammatory response in dogs and to compare variations with clinical signs. Plasma Hp concentrations measured using a commercial non species specific kit and clinical parameters (body temperature, respiratory and heart rates) were monitored during experimentally induced acute pancreatitis by ligation of the pancreatic ducts in 6 dogs, before and from 3 to 96 hours after the surgery. The body temperature transiently and slightly increased 6-24 hours after, the respiratory rate was significantly accelerated since the 6th hour, remained more rapid than initial values until the 96th hour and was positively correlated with the cardiac frequency whose the increase lasted from the 24th to the 96th hour. However, the clinical parameters have remained in normal ranges or weakly exceeded them. Plasma Hp concentrations have also lately significantly increased (since the 48th hour) and maximal values (percentage of variations compared to initial values: 81.6%) were recorded on the 96th hour, leading to positive correlations with the respiratory and the heart rates. These results showed that Hp can be considered as a moderate acute phase protein in dogs with a trauma inflammation.

SUMMARY

The aim of the study was to investigate changes in plasma haptoglobin (Hp) concentrations during a trauma inflammatory response in dogs and to compare variations with clinical signs. Plasma Hp concentrations measured using a commercial non species specific kit and clinical parameters (body temperature, respiratory and heart rates) were monitored during experimentally induced acute pancreatitis by ligation of the pancreatic ducts in 6 dogs, before and from 3 to 96 hours after the surgery. The body temperature transiently and slightly increased 6-24 hours after, the respiratory rate was significantly accelerated since the 6th hour, remained more rapid than initial values until the 96th hour and was positively correlated with the cardiac frequency whose the increase lasted from the 24th to the 96th hour. However, the clinical parameters have remained in normal ranges or weakly exceeded them. Plasma Hp concentrations have also lately significantly increased (since the 48th hour) and maximal values (percentage of variations compared to initial values: 81.6%) were recorded on the 96th hour, leading to positive correlations with the respiratory and the heart rates. These results showed that Hp can be considered as a moderate acute phase protein in dogs with a trauma inflammation.

Keywords: Acute pancreatitis, dog, haptoglobin, respiratory frequency, cardiac frequency, correlation.

RÉSUMÉ

Cette étude a eu pour objectif de suivre les variations des concentrations plasmatiques d’haptoglobine (Hp) durant une réponse inflammatoire d’origine traumatique et de les comparer avec les manifestations cliniques induites. Les concentrations plasmatiques de Hp mesurées par un kit de dosage non spécifique d’espèce et les paramètres cliniques (température, fréquences respiratoire et cardiaque) ont été déterminées avant et de 3 à 96 heures après induction expérimentale (par ligature des canaux pancréatiques) d’une pancréatite aiguë chez 6 chiens. La température a faiblement et transitoirement augmenté 6 à 24 heures après, le rythme respiratoire a été significativement accéléré dès la 6ème heure, est resté plus rapide qu’initiallement jusqu’à la 96ème heure et a été positivement corrélé avec la fréquence cardiaque dont l’augmentation a duré de la 24ème à la 96ème heure. Néanmoins, les paramètres cliniques sont restés compris (ou ont faiblement dépassé) les intervalles de valeurs usuelles. Les concentrations plasmatiques de Hp ont aussi significativement et tardivement augmenté (à partir de la 48ème heure) et les valeurs maximales (représentant une augmentation de 81,6 %) ont été obtenu à la 96ème heure : des corrélations positives ont été, par conséquent, établies entre le paramètre biochimique et les fréquences respiratoire et cardiaque. Ces résultats montrent que l’haptoglobine peut être considérée comme une protéine modérée de la phase aiguë chez le chien lors d’une réponse inflammatoire d’origine traumatique.

Mots clés : Pancréatite aiguë, chien, haptoglobine, fréquence respiratoire, fréquence cardiaque, corrélation.

Introduction

Acute pancreatitis is defined as suddenly occurring morphological alterations of the pancreas linked only on the basis of the severity of their appearance and development. Occurring changes could be inflammatory or non-inflammatory [1]. In dogs, the causes for acute pancreatitis are difficult to be established [2]. According to the definition accepted by the conference in Atlanta in 1992 [4], acute pancreatitis is a sudden inflammation of the pancreas with different extent of involvement of adjacent tissues or remote organs and systems. Pancreatitis develops when a self-digestion of the pancreatic tissue begins [2]. In this way, trypsin may play a central role since this enzyme is able to activate other proteolytic enzymes of the pancreas (elastase, carboxypeptidase and chymotrypsin), the kallikrein-kinin system and fibrinolysis [3]. As the content of fatty acids in a necrosis locus is 200 times higher than normal according to REGIOLI [14], it is believed that some lipolytic enzymes such as the phospholipase A and the lipase are also involved in the pancreas auto-digestion. Secondary to the enzyme activation, release of biogenic amines, activation of kallikrein-kinin system, plasmin and thrombin systems and to the enzyme attack, toxigens occur in blood and lymph leading to disturbances in central and peripheral hemodynamics and functional failure of parenchymal organs [14].

Acute phase response (APR) is most extensively investigated in men, where acute phase proteins (APP) are divided into 3
groups depending on the extent of increase in their concentrations in various pathological states: i) major APP such as serum amyloid A (SAA), C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), whose 5 to 20-fold increase in circulating concentrations is observed, ii) moderate / minor APP such as haptoglobin (Hp), α1-acid glycoprotein (α1-AGP) and fibrinogen (Fb), whose circulating concentrations exhibit a 2-3 fold increase and iii) APP that are almost unchanged during inflammation like α2-macroglobulin (α2-MG) and serum amyloid P protein [5, 7, 8, 11, 13]. The limited information available on this subject in animals does not still permit an accurate classification of the different APP depending on the rate of their increase in blood in various pathological states. It should be emphasized that the level of expression for the different APP and their role in the acute phase response vary in the different animal species. In men and dogs, Hp is classified as moderate / minor APP as its blood concentrations in healthy subjects is relatively higher and it moderately increased during the acute phase of inflammation.

In the present experiment, blood haptoglobin concentrations were investigated after surgically induced acute pancreatitis in dogs and correlated with clinical parameters in order to verify if this APP can be considered as an early and accurate biomarker of non-infectious inflammation.

Materials and Methods

EXPERIMENTAL ANIMALS AND PROTOCOL DESIGN

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

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 (Combistress®, Kela – Belgium, 0.2 mg/kg). Twenty minutes after, thiopental sodium (Thiopental Spofa, The Czech Republic) and oxygen flow 2-2.5 L/min, to a slight and significant increase in the respiratory frequency and body temperature has weakly but significantly increased at the 6th (p < 0.01) and at the 24th hour (P < 0.05) after surgery compared to the initial values. In the same way, the body temperature, heart and respiratory rates were assessed just before surgery (hour 0) and 3, 6, 24, 48, 72 and 96 hours after. Blood samples were collected in parallel and also 72 hours prior the surgical intervention from the puncture of the v. cephalica antebrachii into sterile heparinised tubes, and after centrifugation (1 500g, 10 minutes, 4°C) within 30 min after blood collection, plasma was immediately separated and stored at -20°C until analysis. Haptoglobin (Hp) concentrations were assayed with a commercial kit manufactured by Tridelta Development Limited, Ireland. The technique is based on the peroxidase activity of free haemoglobin that is inhibited at low pH. When Hp binds to haemoglobin, the peroxidase activity of the formed complex is preserved even at low pH and is proportional to the amount of Hp in the sample.

STATISTICAL ANALYSIS

The effect of experimental pancreatitis on plasma Hp concentrations and on the clinical parameters (body temperature, heart rate and respiratory rate) was evaluated by Anova (StatistIca for Windows, Stat Soft Inc., USA 1993). All results are presented as mean and standard error of the mean (mean ± SEM). The statistical significance of parameters prior to and after the experimental inflammation was determined in the LSD test at P < 0.05. Associations between parameters were calculated with the Pearson test.

Results

Throughout the experiment, the body temperature and the respiratory rate remained within the normal range (38°C-39°C and 20-27 cycles/min, respectively) in all dogs (Table I). However, the body temperature has weakly but significantly increased at the 6th (p < 0.01) and at the 24th hour (P < 0.05) after surgery compared to the initial values. In the same way, the body temperature and the respiratory rate were positively correlated
(r = 0.36, P < 0.05) (Table II). In addition, the heart frequency markedly and gradually increased since the 24th hour after surgery (P < 0.01) for reaching maximal values at the end of the experiment (on the 96th hour). During this time interval, the heart rate was over the normal range (60 - 80 beats/min.) in all dogs. Taking into account the variation profiles of the 3 clinical parameters, a moderate and positive correlation was obtained between the respiratory and the heart frequencies (r = 0.34, P < 0.05) whereas the heart rate and the body temperature were not significantly associated (Table II).

Three and 6 hours after the ligation of the pancreatic ducts, the plasma Hp concentrations remained stable and similar to baseline values recorded 72 hours before and immediately prior the surgery (hour 0). Thereafter, from the 24th hour to the 96th hour, the Hp concentrations gradually increased and reached maximal values at the end of the experiment (Table I). Significant differences with basal values (P < 0.05) were only evidenced since the 48th hour. As shown in Table II, plasma haptoglobin concentrations were significantly and moderately associated with the respiratory and the heart frequencies (r = 0.44 and r = 0.29, respectively, P < 0.05).

Discussion

The quantification of APP is a diagnostic means providing valuable information about the presence of infection, inflammation or trauma [6]. Elevated serum APP concentrations result from the increase in their synthesis rate, evidenced by incorporation of radioactively labelled amino acids in newly synthesized plasma protein during inflammation [15].

ECKERSALL et al. [9] have investigated the amount of APP after surgical intervention for aortic implants and have observed a manifold increase in CRP concentrations (from 0 to 98 mg/L) during the first post operative 24 hours. A statistically significant increase was present as early as the 4th hour (45 mg/L), and peak value was reached on the 24th hour. Thereafter CRP concentrations decreased but were still detected in serum up to the 9th day. Whereas CRP concentrations could increase more than 100 times, haptoglobin is considered to belong to the group of moderate APP in dogs and that it increases 2 to 3 times in response of inflammation, infection or trauma [9]. According to ECKERSALL et al. [9], haptoglobin concentrations in healthy dogs are very low and attain peak values on the 5th day after surgery or trauma. Haptoglobin concentrations range between 0 and 3 mg/L in healthy dogs [10], but they could reach up to 10 mg/L depending on the severity of inflammation. Thus, the monitoring of blood haptoglobin concentrations in dogs could provide valuable information about the extent of the pathological process and the response to the therapy. In agreement with that, plasma haptoglobin concentrations have begun to gradually increase since the 24th hour after the ligation of the pancreatic ducts and reached maximal values on the 96th hour, corresponding to a variation of 81.6% compared to initial values in the present study. Nevertheless, it was also reported that in the same experimental model, plasma CRP concentrations were earlier significantly increased (since the

<table>
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<th>Parameters</th>
<th>Before surgery</th>
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<tr>
<td></td>
<td>- 72h</td>
<td>0 h</td>
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<tr>
<td>Body temperature (°C)</td>
<td>ND</td>
<td>38.4 ± 0.1a</td>
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<tr>
<td>Respiratory rate (cycles / min.)</td>
<td>ND</td>
<td>22.3 ± 2.1a</td>
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<tr>
<td>Heart rate (beats / min.)</td>
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<tr>
<td>Hp (mg/L)</td>
<td>3.37 ± 0.70a</td>
<td>3.80 ± 0.68a</td>
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Table I: Clinical parameters (body temperature, respiratory and heart rates) and plasma haptoglobin (Hp) concentrations observed in dogs before and 3h to 96h after surgical induction of acute pancreatitis by the ligation of the pancreatic ducts in dogs (n = 6). Results are expressed as mean ± SEM.

Table II: Correlation coefficients between clinical parameters (body temperature, respiratory and heart rates) and plasma haptoglobin (Hp) concentrations observed in dogs before and after surgical induction of acute pancreatitis by the ligation of the pancreatic ducts in dogs (n = 6).
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3rd hour after surgery), reached dramatically elevated values on the 24th hour (variation percentage: 733.5%) and thereafter slowly declined but remained high until the 96th hour (multiplied by a factor 6 compared to initial values) [12].

In this experiment, whereas the body temperature was slightly and transiently increased after the surgical intervention (from 6 to 24 hours), the observed increase in the respiratory rate was more persistent (from 6 to 96 hours) but remained moderate. In addition, the cardiac frequency markedly and late increased (from 24 to 96 hours). Consequently, positive correlations were found between the haptoglobin concentrations and the heart and respiratory rates but not with the body temperature. The elevation of the measured clinical parameters however, remained within or weakly above the normal ranges making them irrelevant.

As a conclusion, plasma haptoglobin has increased in dogs with surgically induced acute pancreatitis and may be considered in this way as a marker of inflammation. However, it exhibited a markedly lower sensitivity as compared to the C-reactive protein and slowly increased during the inflammation course. This late moderate APP in dog is easily and relatively quickly measured using a non species specific kit and further studies are required for evaluating its biological interest and its prognostic value during monitoring to anti-inflammatory therapy, similarly to the APP follow-up in human medicine.

References