

TIME COURSE OF BLOOD C-REACTIVE PROTEIN AND FIBRINOGEN CONCENTRATIONS AFTER EXPERIMENTAL *PSEUDOMONAS* INFECTION IN DOGS

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Summary

Chaprazov, Ts. & I. Borissov, 2009. Time course of blood C-reactive protein and fibrinogen concentrations after experimental *Pseudomonas* infection in dogs. *Bulg. J. Vet. Med.*, 12, No 4, 240–245.

An experimental infection was produced in six mongrel dogs from both genders, at the age of 6–7 years, weighing 16 ± 2 kg, by intravenous injection of 24-hour broth culture of *Pseudomonas aeruginosa* containing 1.2×10^9 CFU/mL. Another six untreated dogs served as controls. Blood C-reactive protein (CRP) was assayed between baseline (hour 0) and post infection hour 24, whereas blood fibrinogen levels were determined between baseline and the 28th day after infection. It was found out that blood CRP concentrations were statistically significantly higher by the 4th hour of infection vs baseline ($P<0.01$), and at hours 6 and 24 vs controls as well ($P<0.001$). Fibrinogen alterations consisted in increased levels by hour 24 (4.25 ± 0.23 g/L) vs baseline concentrations of 2.73 ± 0.27 ($P<0.01$) of treated and 2.17 ± 0.55 g/L in control dogs.

Key words: C-reactive protein, dogs, fibrinogen, *Pseudomonas aeruginosa*

INTRODUCTION

Acute-phase response is a non-specific systemic response in animals to tissue damage (Ebersole & Cappelli, 2000). One of the essential changes related to this response, is the alteration in concentrations of a specific group of plasma proteins, called acute-phase proteins (APPs) (Eckersall, 1995).

C-reactive protein (CRP) is the first described APP. In dogs, its levels increase rapidly under the effect of both aseptic and septic stimuli, and this motivates the utilization of this protein as diagnostic, monitoring and prognostic parameter in this animal species (Otabe *et al.*, 2000).

In dogs, CRP is changes in pneumonias, surgical traumas and inflamma-

tions of various origin (Yamamoto *et al.*, 1993; 1994; Yamashita *et al.*, 1994). Elevated serum CRP concentrations in dogs have been reported in experimental *S. aureus* and *E. coli* infections (Hulton *et al.*, 1985; Hayashi *et al.*, 2001).

A positive correlation was established between serum APPs and the counts of band and segmented neutrophils. APPs are preferred as markers of inflammation and infection, as their concentrations persist for several days, contrary to cytokines and leukocytes, whose elevation is transient (Jain, 1989; Kjelgaard-Hansen *et al.*, 2003).

Fibrinogen is a β -globulin, present in the plasma of all vertebrates (Ceron *et al.*,

2005). It is produced in the liver and thus, the hepatic functional activity and enzyme profile have a major impact on its levels. Systemic inflammation and infection are accompanied by hyperfibrinaemia and change in total white blood cell counts (Dinev, 2002; Dimitrova *et al.*, 2003).

In the early stage of the development of Gram-positive staphylococcal infection, fibrinogen levels were reported to increase (Andonova *et al.*, 2002; Slavov, 2008), but in later stages of infection, its concentrations were reduced to normal in 50% of cases (Aird, 2003).

The present investigation aimed to determine the dynamics of acute phase proteins CRP and fibrinogen after infection with *Pseudomonas aeruginosa* in order to affirm whether the utilization of these APPs is appropriate for early diagnostics of bacteriaemias caused by this Gram-negative agent in dogs.

MATERIALS AND METHODS

Animals

The studies were performed with 2 groups (experimental and control) of 6 mongrel dogs from both genders, aged 6–7 years, weighing 16 ± 2 kg. They were housed in individual cages in the open air with 1.8 m height and area of 1.5 m², with walking yards. Each animals had individual plate for food and a free access to water. Prior to and during the experiment, all dogs received dry food for adult dogs maintenance Canil-21% (Sosil Guyomarc, Sao Paolo, Brazil). All animals were adapted to the new conditions of feeding and housing for 15 days.

One week prior to the beginning, dogs were treated against parasites with praziquantel and abamectin (Prazimec-D, Biovet Co., Peshtera, Bulgaria) applied at a dose of 1 tablet per 10 kg body weight.

Experimental design

Experimental infection was induced by injection of 0.5 mL/kg 24-hour broth culture of *Ps. aeruginosa* with a density of 1.2×10^9 CFU/mL into the jugular vein. The strain was typified with the Sceptor Becton Dickinson Diagnostic System.

Throughout the experiment, blood concentrations of CRP and fibrinogen were assayed in both experimental and control dogs. The CRP levels were determined prior to *Ps. aeruginosa* application (hour 0), and at post infection hours 2, 4, 6, and 24. Fibrinogen was analyzed by these time intervals (except for hour 4) and also by hours 48 and 72, and days 7, 14, 21 and 28 after the infection.

CRP was assayed with a commercial kit Canine C-Reactive Protein Assay (PHASE RANGE, Tridelta Development Ltd, Ireland), and fibrinogen – with HemoStat Fibrinogen test (Human Diagnostica, GmbH, Germany).

Statistical analysis

CRP and fibrinogen concentrations are presented as mean \pm SEM. The differences were estimated by paired or unpaired *t* test using a specialized software (Statmost for Windows, Data Most Corp.) and were considered statistically significant at $P < 0.05$.

RESULTS

Blood CRP concentrations in experimental dogs increased as early as the 4th hour after the intravenous application of *Ps. aeruginosa* – 27.77 ± 4.79 ng/mL vs 16.21 ± 6.40 ng/mL in control dogs (Fig. 1). These values were also statistically significant vs baseline ($P < 0.01$). CRP concentrations in controls by hour 4 were also higher than baseline (16.21 ± 6.40

ng/mL, $P < 0.05$). By the 6th hour, CRP levels in experimental dogs attained 75.65 ± 14.39 ng/mL ($P < 0.01$ vs baseline) and by the 24th hour – 176.91 ± 5.93 ng/mL ($P < 0.001$ vs baseline). These concentrations were also statistically significant vs those of controls at the respective time intervals (2.99 ± 1.01 ng/mL by hour 6 and 9.31 ± 0.37 ng/mL by hour 24; $P < 0.001$).

In the treated group, hyperfibrinaemia occurred by the 24th hour – 4.25 ± 0.23 g/L ($P < 0.01$) and the 72nd hour – 3.80 ± 0.27 g/L ($P < 0.05$) vs baseline (Fig. 2). In controls, fibrinogen levels were 2.17 ± 0.55 g/L and 3.03 ± 0.09 g/L by hours 24 and 72, respectively. In the subsequent course of development of Gram-negative bacteraemia, concentrations remained higher. As compared to baseline (2.73 ± 0.27 g/L) statistically significant differences were observed by hour 24 (4.25 ± 0.23 g/L,

$P < 0.01$); hour 48 (4.38 ± 0.37 g/L, $P < 0.01$); hour 72 (3.80 ± 0.27 g/L, $P < 0.05$); day 7 (3.80 ± 0.35 g/L, $P < 0.05$) and day 14 (4.58 ± 0.28 g/L, $P < 0.001$). In controls, statistically significant differences ($P < 0.05$) vs baseline were established by days 14 and 21 – 4.03 ± 0.35 g/L and 4.05 ± 0.19 g/L respectively.

DISCUSSION

The concentrations of positive acute phase proteins, including CRP and fibrinogen, are reported to increase during the development of inflammation (Szalai *et al.*, 1999).

The results of the present investigation exhibited increased CRP and fibrinogen blood concentrations in the acute phase of infection. The activation of acute phase response by proinflammatory cytokines as IL-1 and IL-6 (Petrov *et al.*, 2003), resul-

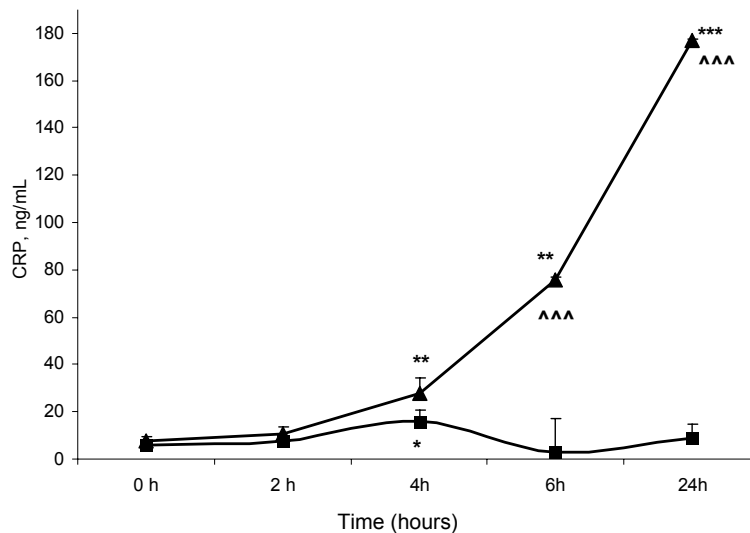


Fig. 1. Time course of blood CRP concentrations (ng/mL) in dogs experimentally infected with *Pseudomonas aeruginosa* at 1.2×10^9 CFU/mL (–▲–) and untreated control dogs (–■–); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ – statistically significant vs baseline; ^^ $P < 0.001$ – statistically significant between groups at a given time interval.

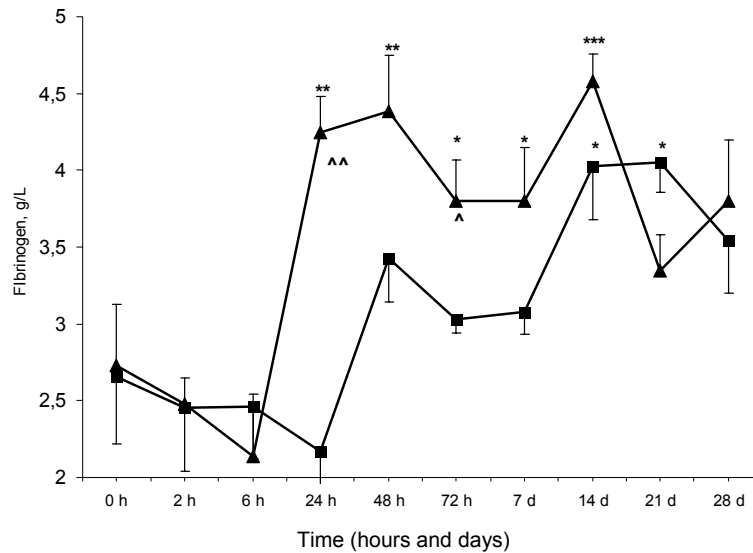


Fig. 2. Time course of blood fibrinogen concentrations (g/L) in dogs experimentally infected with *Pseudomonas aeruginosa* at 1.2×10^9 CFU/mL (—▲—) and untreated control dogs (—■—); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ – statistically significant vs baseline; ^ $P < 0.05$, ^^ $P < 0.01$ – statistically significant between groups at a given time interval.

ted in almost 10-fold higher CRP levels by the 6th hour than baseline (75.65 ± 14.39 ng/mL, $P < 0.001$) and over 20-fold higher concentrations by hour 24 (176.91 ± 5.93 ng/mL, $P < 0.001$).

According to our data as well as results from other investigations, CRP could be used as a marker in the early stages of inflammation development in dogs (Eckersall, 2000; Otabe *et al.*, 2000; Slavov, 2008). Therefore, its inclusion in the group of parameters used for diagnostics of the of systemic inflammatory response syndrome (SIRS) is justified.

The observed increase in fibrinogen concentrations in our study was likely and was anticipated in the course of a bacteriaemia, because plasma levels of a specific group of liver proteins such as CRP and fibrinogen are expected to augment (Vary & Kimball, 1992). What

is more, these APPS modulate the immune response, are involved in tissue reparation and prevent the damage induced by endotoxins and tumour necrosis factor- α (Alcorn *et al.*, 1992).

Because of the later elevation of fibrinogen concentrations (between hour 24 and day 14), the assay of fibrinogen could successfully add to the information obtained by plasma CRP analysis.

In human medicine, the assay of APP concentrations is a routine and essential test for rapid diagnostics of severe infectious diseases (Rikihiya *et al.*, 1994). The investigations with animals showed that the quantitation of these proteins provides useful clinical information about the diagnosis, prognosis and monitoring of therapy of various pathologies in different experimental designs (Martínez-Subiela *et al.*, 2003; Eckersall, 2004). Therefore, the levels of positive acute phase proteins

should be preferably analyzed when determining of health status of animals.

In conclusion, CRP blood concentrations increased in the first 6 hours after the penetration of *Ps. aeruginosa* in the blood circulation in dogs, that could be used in the early diagnostics of this Gram-negative infection. The elevation in fibrinogen concentrations between the 1st and the 14th days could be also utilized in the diagnostics of bacteraemia, induced by *Ps. aeruginosa* together with blood CRP alterations to support the microbiological diagnosis.

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Paper received 27.01.2009; accepted for publication 27.04.2009

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