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Research Article

Changes in the Concentrations of Some Plasma Proteins During Acute Inflammation in Dogs

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Abstract
The purpose of the present study was to analyse the changes in concentrations of total proteins, albumin, globulins and albumin/globulin ratio in dogs with experimentally induced acute inflammation. The study was performed on 9 mongrel dogs (experimental group) and 6 mongrel dogs (control group) at the age of 2 years and body weight 12-15 kg. The acute inflammation was reproduced by inoculation of 2 ml turpentine oil in the lumbar region subcutaneously and in same quantity saline in control dogs. Blood samples were collected into heparinized tubes before inoculation (hour 0) then at hours 6, 24, 48, 72 and on days 7, 14, 21. The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The level of albumin statistically decreased in the experimental dogs from at 72nd h to day 14 while the concentration of globulins increased from the 72nd h to day 21. On days 7 and 14 the albumin/globulin ratio slightly decreased. During the whole post inoculation period the values of total protein have not changed. The dates of the present study confirm that albumin, albumin/globulin ratio and globulins are sensitive factors in inflammatory conditions in dogs.

Key words: plasma proteins, acute inflammation, dogs.

Introduction

Inflammation accompanied by local and general systematic signs-enhanced fever and increase heart, and respiratory rates, which are indicators for non-specific response and signs of inflammation. In this study has been demonstrated that subcutaneous turpentine administration can use as a simple method, which causes local inflammatory process. The present study was conducted to evaluate the changes in the concentrations of total protein, albumin, globulins and albumin/globulin ratio at different time points in dogs with acute inflammation induced by turpentine injection.

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The changes in blood protein concentrations and in the respective proportions are resulting from different clinical signs and are related to a number of pathologies as well as to synthesis of acute phase proteins (APP) [1]. APPs are synthesized during an acute phase response (APR). Plasma concentration of some proteins increases and these proteins are called positive acute-phase proteins whereas plasma concentration of others decreases and these proteins are called negative acute-phase proteins (albumin).

The biochemistry laboratory routinely measures total protein and albumin concentrations, usually in a serum specimen, and reports the globulin fraction as the difference between the first results. Albumin is synthesized in the liver as all other plasma proteins except immunoglobulins, and is catabolised by all metabolically active tissues. Its molecular weight is 69 kDa and its half-life is 8.2 days in dogs. It is a negative acute phase protein, which concentrations decrease during infection or inflammation processes. Globulin concentrations in dogs vary from 15 to 35 g/L according to Kaneko [2] and from 18 to 39 g/L according to Hines [3]. The ratio albumin to globulins is often considered as more informative than the respective concentrations in various disorders that could not be identified only by total protein determination.

The present study aimed to establish the alterations occurring in blood circulating proteins, i.e. total proteins, albumin (as a negative APP), globulins and the albumin/globulin ratio during acute inflammation, using dogs as model.

Material and Methods

The experiment was approved by the Ethic Committee at the Trakia University, Faculty of Veterinary Medicine. The experimental animals were provided by the municipality of Stara Zagora. The study was performed on 9 mongrel dogs (experimental group) and 6 mongrel dogs (control group) at the age of 2 years and body weight 12-15 kg. The dogs were housed in metal cages. They were exposed to a 12h light-dark cycle at room temperature (20-22^{0}C). The dogs were fed of commercial extruded dry food (Jambo dog, Gallisman S.A., Bulgaria).

Prior to the experiment, the animals were in the adaptation period of one month. During this period they were vaccinated with vaccine Nobivac, Intervet International B.V and treated per oral against internal parasites with Caniverm, Bioveta, A. S. Czech Republic, 1 tablet/10 kg b.w., and external parasites with Bolfo Puder, Bayer, Germany. The acute inflammation was reproduced by inoculation of 2 ml turpentine oil in the lumbar region subcutaneously (s. c.) in experimental animals whereas the control dogs were injected with the same volume of saline solution.

Blood samples were collected from the puncture of the *v. cephalica antebrachii* into heparinized tubes before inoculation (hour 0) then at hours 6, 24, 48, 72 and on days 7, 14, 21 after turpentine injections. At the same time blood was taken from controls. Heparinised blood was centrifuged (1500g, 10 minutes, room temperature) within 30 min after collection. Plasma was immediately separated and stored at -20°C until analysis. The total protein and albumin concentrations were determined with commercial kits (Human-GmbH, Germany) where determination of albumin was based on the bromocresol green assay, after mixing blood plasma (10 μL) with the reactive buffer. After 3 minutes, the absorption was measured at 546 nm. Globulins were determined by subtracting albumin concentration from total protein concentration.

The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The results were processed with software Statistic v.6.1 (StatSoft Inc., 2002). All results are presented as mean and standard error of the mean (Mean ± Err). The statistical significance of parameters was determined in the LSD test at $p < 0.05$.

Results and Discussions

The changes in the protein concentrations – total protein (TP), albumin (Alb), globulins (Glb) and albumin/globulin ratio (A/G ratio) during acute inflammation induced by turpentine injection are shown in Table 1. In the experimental and control groups, the blood protein levels were followed during a period of 21 days. They were influenced by local, aseptic inflammatory stimuli. The total protein, albumin and globulin concentrations as well as the albumin/globulins ratios remained stable according to time in the control dogs. In experimental animals, the total protein concentrations were in reference ranges-from 68 to 70 g/L. By contrast, the globulin concentrations gradually increased in inoculated dogs and peaked on day 14 (36.98±0.86 g/L). Highly significant differences in globulin concentrations between the experimental and control groups ($p < 0.05$) were found from the 72nd h (35.47±1.52 and 29.42±1.8 g/L, respectively) to day 21 (34.38±1.21 and 29.92±1.4 g/L, respectively). In parallel, acute inflammation caused marked fall in serum albumin concentrations: they were significantly decreased compared to the mean control values at 72nd h (34.14±1.48 and 37.8±0.8 g/L, respectively) and on days 7 and 14 ($p < 0.05$). The lowest values were noted on the 14th day after inoculation (32.14±1.13 g/L). Additionally, the A/G ratio progressively declined in the experimental group, reaching significantly low values on day 14 (0.8) ($p < 0.01$) compared to the control ratio (1.2).

As reported in Table 2, strong positive associations were observed between proteinemia and albumin concentrations ($r = 0.85$, $p < 0.05$) or A/G ratios ($r = 0.83$, $p < 0.05$). The A/G ratios were also negatively coupled to globulin concentrations ($r = -0.97$, $p < 0.05$) as well as we observed negatively association between Alb and Glb ($r = -0.97$, $p < 0.05$). In addition, albuminaemia was significantly and strong positively coupled to the A/G ratios ($r = 0.99$, $p < 0.05$).

Table 1

Plasma concentrations (g/L) of total protein (TP), albumin (Alb) and globulins (Glb) and albumin/globulin (A/G) ratio in healthy dogs (n = 6) and in dogs with experimentally induced acute inflammation (n = 9) according to time after subcutaneous inoculation. Results are expressed as means ± standard errors of the means (SEM)

Time	TP		Alb		Glb		A/G	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
0 hour	67.8±0.90	70.97±1.42	36.2±0.4	37.27±1.73	31.65±1.1	33.7±1.07	1.1±0.05	1.1±0.08
24 hours	66.0±0.94	69.93±1.37	37.4±0.8	37.34±0.72	28.67±3.2	32.59±0.84	1.4±0.17	1.1±0.04
48 hours	65.9±0.90	69.84±0.97	36.8±0.6	36.83±1.22	29.13±2.2	33.01±1.26	1.3±0.08	1.1±0.07
72 hours	67.3±0.73	69.61±0.96	37.8±0.8	34.14±1.48*	29.42±1.8	35.47±1.52*	1.2±0.12	0.9±0.08
Day 7	66.2±0.83	68.58±1.08	36.2±0.5	32.21±1.66*a	29.95±1.2	36.37±1.52*a	1.3±0.08	0.8±0.07**
Day 14	65.4±0.81	69.12±1.26	36.8±0.9	32.14±1.13*a	28.62±1.7	36.98±0.86*a	1.2±0.11	0.8±0.04**
Day 21	67.6±0.84	70.02±1.52	37.6±0.8	35.64±1.12	29.92±1.4	34.38±1.21a	1.3±0.11	1.0±0.06

For a given biochemical parameter: * ($p < 0.05$) and ** ($p < 0.01$) indicate significant differences between turpentine inoculated and control dogs.

Different superscript "a" indicate significant difference ($p < 0.05$) according to time within the experimental group (turpentine inoculated dogs).

Table 2

Correlations between total protein (TP), albumin (Alb), globulins (Glb) concentrations and albumin/globulin (A/G) ratio in dogs with turpentine-induced acute inflammation. Correlations were calculated from all time points (from 0 h to the 21st day) and significant associations (*P* < 0.05) were in bold.

Parameters	TP	Alb	Glb	A/G ratio
TP	r=1.0	**r=0.85 p<0.05**	r= -0.70 NS	**r=0.83 p<0.05**
Alb	**r=0.85 p<0.05**	r=1.0	**r= -0.97 p<0.05**	**r=0.99 p<0.05**
Glb	r= -0.70 NS	**r= -0.97 p<0.05**	r=1.0	**r= -0.97 p<0.05**
A/G	**r=0.83 p<0.05**	**r=0.99 p<0.05**	**r= -0.97 p<0.05**	r=1.0

NS: not significant

Inflammation accompanied by local and general systematic signs-enhanced fever (6 hour after inoculation), increase heart and respiratory rates at 24th h, which are indicators for non-specific response and signs of inflammation.

Inflammation or trauma can induce local swelling, mild pain, local erythema and variable fevers are common findings in soft tissue inflammation. It has been demonstrated that subcutaneous turpentine administration can use as a simple method, which causes local inflammatory process [3]. The acute phase proteins (APPs) are reactants synthesized during an acute phase response (APR). This response is part of the innate defense system of an animal and can be due to infection, inflammation, stress, trauma or tissue damage [4, 5].

The mode of action of this potent pyrogen (turpentine injection) involves the production of localized necrotic damage which results in the sequential induction of some interleukins (TNFα and IL-1β) at the site of injury [6].

In this study, changes in blood protein concentrations were observed in dogs in response to turpentine injection. The experimental inflammation has lead to significant increases in globulins at the 72nd h and on days 7, 14 and 21, as well as to significant decreases in albumin concentrations at the 72nd h and on days 7 and 14. The reference range of plasma Alb concentrations in animals is between 29 and 36 g/L [1]. It belongs to the negative APP and constitutes between 35 and 50% of the total serum proteins by contrast with humans and nonhumans primates in which albumin accounts for 60-67% of the total [7]. In the present study, in experimental dogs, levels of albumin started significantly decrease since at 72nd h (34 g/L) compared to the controls and remained lowered until the 14th day after inoculation (32 g/L). In contrast to that, Tous et al. [8] have not observed a reduction of albumin in mice after turpentine injection (25.8 g/L compared to controls-26.2 g/L). Plasma proteins (albumin and globulin) in dogs have half lives varying from 8 to 10 days, and therefore a decrease in albuminaemia is usually not apparent early in the infection. Hypoalbuminemia is very common in many illnesses and results in most instances from one or more of the following factors: impaired synthesis in liver diseases, increased catabolism, reduced absorption of amino acids or turn off amino acids for the synthesis of other proteins (like acute phase proteins) in liver [9]. Nowadays, albumin is classified as negative APPs. The observed decrease in serum albumin concentration supports the view that albumin is a negative APPs in all mammalian species by other researchers [7, 10, 11, 12]. The decreasing of albumin in our study confirm relating of it as a negative APPs. The reduction in the plasma concentration of negative APP is probably caused by a preferential synthesis in liver of positive APPs which are important components of systemic defence mechanisms [13], the partial deficiency of α-amino acids induced by increased production of APP being compensating by the strong reduction of the synthesis of the negative APPs, including albumin.

Globulins are divided into main groups, the α-, β-, and γ-globulins. A tendency for slightly elevation of immunoglobulins as part of γ-globulins in our experiment is in an agreement with the data of Burtis, [14], who stated that increased levels of immunoglobulins are seen in both acute and chronic infections. The experimental inflammation in the present study has lead to significant increases in globulins at the 72nd h and on days 7, 14 and 21 whereat reached maximal values on day 14.

The normal albumin/globulins (A/G) ratios in dogs are from 0.7 to 2.00 according to Kaneko [2] or from 0.8 to 2.2 according to Hines [3]. In healthy dogs and in experimental dogs before inoculation the A/G ratios determined in the present study were included in these usual ranges. On days 7 and 14 after inoculation this parameter was markedly lowered compared to controls, indicating globulin overproduction or albumin reduction or both. Similarly, Zapryanova *et al*. [16] observed declined in the A/G ratios 3 days after induction of an acute pancreatitis in dogs. The sensitivity and specificity of the A/G ratio for detecting clinical or subclinical disease are not as high as those of positive APPs [4].

In healthy dogs for the whole experimental period and in dogs before inoculation, the plasma total protein concentrations were closely related to the upper limits of the usual ranges defined in adult dogs, as indicated by some authors [10] (48-66 g/L) and by Valladares et al. [17] (54-73 g/L). During the whole post inoculation period the TP levels in our study remain unchangeable. Similarly, according to Lampreave et al., [15] in pigs, the concentrations of TP did not change after experimentally turpentine-induced inflammation.

Conclusions

Blood proteins altered visible their levels in dogs affected by turpentine injection and it was observed elevation in the concentration of globulins at 72nd h and the same time reduction in concentration of albumin which support relating of an albumin as a negative acute phase protein.

A tendency for slightly reduction of A/G ratio noted 7 day after acute inflammation.

The dates of the present study confirm that albumin, albumin/globulin ratio and globulins are sensitive factors in inflammatory conditions in dogs.

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