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EVALUATION OF CERULOPLASMIN AS AN ACUTE PHASE PROTEIN IN INFECTED DOGS

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Abstract

Ceruloplasmin (Cp) is a sensitive indicator of inflammation and infection. It is a liver-derived a2-glycoprotein and is synthesized primarily as a positive acute-phase reactant. Staphylococcus aureus (S. aureus) is oft associated with suppurative infections and is recognized as an inherent member of the microflora of the skin of humans and dogs and is responsible for a diverse spectrum of diseases. This infection was chosen because Staphylococcus aureus presented in a lot of animals –dogs, horses, cats, pigs, cattle, pigeons. Our aim in this experiment was to examine Cp concentrations in dogs that were exposed to Staphylococcus aureus infection and then evaluate how Cp could be used as an indicator of this bacterial infection in dogs. For that, Cp concentrations were determined in plasmas from 9 mongrel male dogs (in experimental group) and 6 mongrel male dogs (in control group) at the age of 2 years and body weight 12-15 kg. The infection was reproduced by inoculation of 5 ml 24 h broth culture of S. aureus strain with density of 3.1x109 c.f.u./ml in the lumbar region of experimental animals and same quantity saline in control (non-infected) 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 at the same time was taking blood and from controls. One day after inoculation, we observed that Cp showed consistent upward trend and between 24 to 72 hours, the values were from 66.55 to 66.88 mg/L, after that we noticed a peak of the concentration on the 7th day (109.5±7.82 mg/L). At the end of the experimental period (21 days) this acute phase protein remained still higher (81.88±6.59 mg/L) compared to baseline. In conclusion, dogs experimentally infected with Staphylococcus aureus showed an acute phase response characterized by statistically significant ($p<0.001$) twofold increase in Cp concentration on the seventh day after inoculation. Therefore, the results of this study suggest that ceruloplasmin can be a useful marker for the presence of staphylococcal infection in dogs.

Key words: *dogs, ceruloplasmin, infection, Staphylococcus aureus.*

INTRODUCTION

The acute phase proteins (APPs) are reactants synthesized during an acute phase response (APR). This response can be due to infection, inflammation, stress, trauma or tissue damage (Petersen et al., 2004; Ceron et al., 2005).. The synthesis and role of APPs may differ depending on the animal species. Ceruloplasmin (Cp) is an α2-glycoprotein which is considered as one of the major positive acute phase proteins in dogs (Ceron et al., 2005). Cp is a 132 kD protein of blood plasma regarded as an acute phase reactant Additionally it is involved in copper transport and antioxidant defence. CP displays a large variety of enzymatic activities and behaves as a universal antioxidant due to the presence in its molecule of six tightly bound copper ions (Sokolov et al., 2007). It plays an important role in protecting host tissues from toxic oxygen metabolites released from phagocytic cells during inflammatory states (Ceron and Martinez-Subiela, 2004).

Cp, a ferroxidase (Fe^{+2}, oxygen oxidoreductase EC.1.16.3.1) binds about 95% of plasma copper each molecule attaching to 6 or 7 cupric ions. Measurement of this protein provides valuable information on the inflammatory status to clinicians in canine practice (Solter et al., 1991).

The frequency of *Staphylococcus aureus* (*S. aureus*) infection is permanently increasing (Thiemermann, 2002). The bacterial components and secreted products that affect the pathogenesis of *S. aureus* infections are numerous and include surface-associated adhesins, exoenzymes, exotoxins, and capsular polysaccharide (O'Riordan and Lee, 2004; Tzianabos et. al., 2001). According to some

authors De Kimpe (1995) and Thiemermann (2002) the key elements in *S. aureus* are peptidoglycan (PepG) and lipoteichoic acid (LTA), which are a component of cell wall, synergize to cause shock and organ dysfunction. In this study ceruloplasmin levels were determined in dogs with *Staphylococcus aureus* infection in order to determine their potential value in the diagnosis.

MATERIALS AND METHODS

The experiment was approved by the Ethic Committee at the Faculty of Veterinary Medicine. The experimental animals were provided by the municipality of Stara Zagora (Bulgaria). The study was performed on 9 mongrel male dogs (in experimental group) and 6 mongrel male dogs (in control group) at the age of 2 years and body weight 12-15 kg. Prior to the experiment the animals 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 dogs were housed in metal cages. They were exposed to a 12h light-dark cycle at room temperature (20-22^{0}C). They were fed a commercially available diet of dog pellet twice daily and had free access to water. The infection was reproduced by inoculation of 5 ml 24h broth culture of *S. aureus* strain with density of $3.1x10^9$ c.f.u./ml in the lumbar region of experimental animals and same quantity saline in control (non-infected) 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 at the same time was taking blood and from controls. Manual method for ceruloplasmin determination based on the oxidation of different compounds has become widely adopted for routine use in clinical chemistry laboratories. The method is based on the *in vitro* oxidase activity that ceruloplasmin shows with substances such as p-phenylenediamine (PPD). A colored oxidation product is formed from the oxidation of p-phenylendiamine by Cp using Ravin's method described by Bestujeva and Kolb, (1982). These measurements were made on a spectrophotometer (λ=530 nm).

The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The results were processed with software Statistica v.6.1 (StatSoft Inc., 2002).

RESULTS AND DISCUSSIONS

The changes in this acute phase protein concentrations during the staphylococcal infection in our study are shown in Table 1. In the experimental and control groups, ceruloplasmin was followed during a period of 21 days. Cp concentrations were strongly influenced by staphylococcal infection. In the experimental group, initial levels (before inoculation) were 56.58±3.83 mg/L and 24 hours after this, Cp levels began to slightly rise (60.55±2.69 mg/L). From the 24th hour, the Cp concentration showed consistent upward trend and at 48th h the mean values were 66.88±2.63 mg/L. At the 72nd hour, Cp levels reached significant elevation –79.88±5.67 mg/L in compared to the controls –57.95±2.32 mg/L.

Table 1. Plasma ceruloplasmin concentrations (mg/L) in healthy dogs (n = 6) and in dogs with experimental Staphylococcus aureus infection (n = 9) according to time after subcutaneous inoculation. Results are expressed as means ± standard errors of the means (SEM)

Time after inoculation	Inoculated group *Staphylococcusaureus*	Control group
	mean ± SEM	mean ± SEM
0h	56.58±3.83	56.41±1.75
24h	60.55±2.69	55.80±1.72
48h	66.88±2.63 ^{c}	58.72±2.39
72h	79.88±5.67^{***c}	57.95±2.32
d7	109.55±7.82^{***c}	58.66±1.97
d14	98.55±5.92^{***c}	57.92±2.23
d21	81.88±6.59^{***c}	56.19±1.95

For a given biochemical parameter: ***($p<0.001$) indicate significant differences between *S. aureus* inoculated and control dogs.
Different superscripts c indicates significant difference ($p<0.001$) according to time within the experimental group (*S. aureus* inoculated dogs).

This study indicated significant differences ($p<0.001$) in dogs with staphylococcal infection in comparison to the control group on day 7-109.55±7.82 mg/L and on day 14-98.55±5.92 mg/L. On the 21st day, Cp remained still higher –81.88±6.59 mg/L. As shown in table 1, the plasma ceruloplasmin concentrations were significantly increased since the 72nd h after *S. aureus* inoculation compared to the control values and remained significantly enhanced

until the 21st day ($p<0.001$).Infection accompanied by local and general systematic signs-enhanced fever, increase heart and respiratory rates at 24th h, which are indicators for non-specific response and signs of inflammation. At the 6th h after *S. aureus* implication observed painfulness and oedema of the soft tissue. We watched at the 24th h enlargement of inguinal lymphatic nodes in the limp which was injected and reduced appetite and impaired motor activity in dogs. These clinical symptoms are observed by other authors at the 24th h after inoculation of bacteria (Georgieva et al., 2010). One of the experimental dogs had oedema on the scrotum. After 48th h, some of the dogs were purulent conjunctivitis eye infection and at this time the Cp concentrations were higher (66.88±2.63 mg/L) than initial levels (56.58±3.83 mg/L). In area of the inoculation of bacteria, hair loss and appeared erosions on tissues.

The acute phase response is part of the innate defense system of an animal against trauma, inflammation, and infection (Ulutas et al., 2007). Ceruloplasmin belongs to the acute phase proteins which level can increase during infection or other tissue damaging factor. The main source of Cp is hepatocytes and it can also be synthesised in the mammary gland and places of tissue damage (Szczubiał et al., 2012). Cp activity increases during inflammation, infection, and injury, suggesting that serum Cp acts possibly as an antioxidant and as an acute phase protein Furthermore, since the generation of oxidation products, including O_2 and H_2O_2, is associated with conditions that increase plasma Cp, which can serve as a scavenger of superoxide radicals, the functional properties of Cp in vitro have led to suggestions that it serves as a serum antioxidant in vivo. (Eum et al., 2005). It takes participation in iron homeostasis, copper transport, coagulation, defence against oxidant stress as an antioxidant and low density lipoprotein oxidation. Has the ability to inhibit the oxidation of lipids and has the ability to scavenge superoxide and sequester free copper ions (Tapryal et al., 2009). It has ability to oxidize Fe^{2+} to Fe^{3+} and thus it may reduce the oxidant capacity of iron by inhibition of the Fenton reaction (Tapryal et al., 2009).

The increases in circulating Cp likely caused by increased rate of synthesis probably stimulated by cytokines, growth factors, hormones, and other cellular effectors. Ulutas et al., (2007) establish rising concentrations in dogs with skin problems, fractures, trauma, enteritis, gastritis, pneumonia, hepatosoonozi, malignant neoplasia, otitis. They estimated that statistical significance increase in Cp in diseased dogs was observed in groups of trauma, leishmaniasis, babesiosis and dermatitis. Canine serum ceruloplasmin levels increase during infection, inflammation or trauma. In the available literature there is not information about the concentrations of this protein in dogs after staphylococcal infection. Some authors (Georgieva et al., 2012) reported that Cp concentrations in rabbits elevated after infection induced by *Staphylococcus aureus.* They reported that Cp increased from 24th h to on day 7, and the highest level reached at 48th h. This results are closely to our, but we showed peak concentrations on 7th day. In additions, Ceron et al. (2005) showed that elevation of ceruloplasmin concentrations can observe in dogs with different infectious diseases. The studies of Solter et al. (1991) and Ceron and Martinez-Subiela, (2004) in dogs showed than inflammatory conditions, measurement of Cp and haptoglobin which increased from 2 to 6 time, are more important than determining fibrinogen and leukocyte count. At the time of same study this authors confirmed the elevation of ceruloplasmin, which is contrary to other authors (Bildik et al., 2004) who claim that this APP decreased in infected dogs. According to them, lower concentrations of antioxidants during infection are typical for Cp, which belong to this group (as an extracellular antioxidant). Reducing is result of a defence mechanism against increased oxidation caused by infectious agents.

CONCLUSIONS

As a conclusion, these results indicate that plasma Cp may be considered as an early positive acute phase protein and may be used as a helpful indicator for an early diagnostic of the staphylococcal infection in dogs. The comparing with other clinical parameters

(temperature, heart and respiratory rates) Cp concentrations showed that this parameter could serve as a marker for inflammation for a long period (till day 21).

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