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THE EFFECT OF ACUTE INFLAMMATION ON TOTAL ALKALINE PHOSPHATASE ACTIVITY IN DOGS

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ABSTRACT

The main purpose of this study was to investigate the effect of acute inflammation on total alkaline phosphatase (ALP) activity in dogs. In this study total ALP activity was determined in dogs with experimentally induced acute inflammation in order to characterize their potential value in this condition. For that, ALP concentrations were defined in plasmas from 9 mongrel male dogs (in an experimental group) and 6 mongrel male dogs (in a control group) at the age of 2 years and body weight 12-15 kg. The inflammation was reproduced by inoculation of 2 ml turpentine oil subcutaneously in lumbar region and same quantity saline in control dogs. Blood samples were collected into heparinized tubes before inoculation, then at hours 6, 24, 48, 72 and on days 7, 14, 21. The total ALP concentrations were determined with commercial kits (Human-GmbH, Germany) on an automatic biochemical analyzer (BS-3000 P, Sinnowa, LTD Nanjing China). The statistical analysis of the data was performed using one way analysis of variance (ANOVA), Statistica v.6.1 (StatSoft Inc., 2002). Statistically significant difference was not found between the groups, as well as within them. In conclusion, we can say that the total activity of ALP was not significantly affected in dogs with experimentally induced acute inflammation.

Key words: alkaline phosphatase activity, acute inflammation, dogs

INTRODUCTION

Alkaline phosphatase is a non-specific metalloenzyme which hydrolyzes many types of phosphate esters at an alkaline pH in the presence of zinc and magnesium ions. The enzyme is associated with microsomal and cell membranes and is present in many tissues. It is an "ectoenzyme" functioning in the external environment of the cell and is anchored to cell membranes by glycophosphatidylinositol (GPI) proteins (1). Cleavage of these proteins by bile acids, phospholipase D and proteases releases ALP from membranes resulting in increased ALP levels in serum/plasma. There are 2 isoenzymes (products of different genes) and several isoforms (produced from posttranslational modification of isoenyzmes) of ALP. The isoenzymes are produced from intestinal and tissue non-specific ALP genes and differ in amino acid sequence. Isoforms differ in catalytic sites and activity, immunogenecity, and electrophoretic mobility. In domestic animals several variants in ALP are identified: the bones, intestines and liver, but only in dogs there is another isoenzyme - corticosteroid-induced alkaline phosphatase (2). Routine measurement of ALP gives total serum activity (all isoforms) without specificity as to the source. In healthy normal animals, liver-ALP is the predominant isoform in blood. Inflammation accompanied by local and general systematic signs-enhanced fever and increase heart, and respiratory rates, which are indicators for nonspecific response and signs of inflammation. It has been demonstrated in this study that subcutaneous turpentine administration can be used as a simple

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method, which causes a local inflammatory process. The aim of the experiment was to determine the changes in plasma total ALP activity in dogs at different time points in acute inflammation induced by turpentine injection.

MATERIALS AND METHODS

Experimental animals and protocol design

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. 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), and content: extruded cereal products, vegetable proteins, fats, dehydrated poultry meat, amino acids, extracts of sweet chestnut, vitamins, minerals, antioxidants. This food was assigned for dogs of medium physical activity after finishing their growth period (3). The food was given to dogs as a dry in the amount 250 g/day/dog average with permanent access to the clean water. The dog feeding was once a day, always at the same time.

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.

Biochemical analyses

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. Total ALP-activity was determined by a kit from Human Diagnostics (GmbH), Germany. Enzyme analysis of the alkaline phosphatase level was done using a spectrophotometer (SPEKOL 11-Carlzeiss Jena, Germany). The rate of formation of p-nitrophenol is measured as an increase in absorbance of 405 nm wavelength light which is proportional to the alkaline phosphatase activity in the sample.

Statistical analysis

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). 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

The changes in the ALP concentrations during acute inflammation induced by turpentine injection are shown in Table 1. In the experimental and control groups, total alakaline phosphatase activities were followed during a period of 21 days. They were slightly influenced by those local, aseptic inflammatory stimuli. In the experimental group, initial levels (before inoculation) were 81,8±4,85 U/L and 72 hours after this, ALP levels began to rise (87,2±5,22 U/L) and remained high including on day 7 (88,2±5,06 U/L) of the study compared to baselines. After this period, the activities showed consistent downward trend and on the 21st day the mean values were 81,7±4,84 U/L. This study indicated differences in comparison to the control group at the 72th hours. On day 7, ALP levels reached peak elevation compared to the controls, but it was not significantly higher and the concentrations remained in the reference ranges. Inflammation accompanied by local and general systematic signs-enhanced fever (6 hour after inoculation), increase heart and respiratory rates at the 24th h, which are indicators for non-specific response and signs of inflammation (Table 2, 3 and 4).

Table 1. Blood total alkaline phosphatase activities (U/L) in healthy dogs (n = 6) and in dogs (n = 9) with experimentally induced acute inflammation

Time after inoculation	Inoculated dogs (n=9) mean ± SEM	Non-inoculated dogs (n=6) mean ± SEM
0 hour	81,81±4,85	79,53±5,88
6 hour	81,45±4,77	79,40±5,83
24 hours	83,44±4,65	80,10±5,68
48 hours	84,38±5,07	79,53±6,15
72 hours	87,22±5,22	80,03±6,23
Day 7	88,21±5,06	79,45±6,42
Day 14	85,53±4,96	79,31±6,11
Day 21	81,76±4,84	80,30±6,06

Table 2. Dynamics of internal body temperature (IBT) (ºC) in healthy dogs (n = 6) and in dogs (n = 9) with experimentally induced acute inflammation with turpentine. Results are expressed as means ± standard errors of the means (SEM)

Time after treatment	IBT - control dogs	IBT - inoculated dogs
0 hour	38,63±0,16	39,27±0,17
6 hours	38,80±0,18	39,92±0,20^{***c}
24 hours	38,81±0,19	39,46±0,15**
48 hours	38,66±0,17	39,20±0,09*
72 hours	38,68±0,12	39,10±0,17*
Day 7	38,60±0,17	38,65±0,15
Day 14	38,76±0,18	38,46±0,25
Day 21	36,65±0,19	38,85±0,08

For a given biochemical parameter: *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$) indicate significant differences between turpentine inoculated and control dogs. Different superscripts c indicate significant difference ($p < 0.001$) according to time within the experimental group (turpentine inoculated dogs).

Table 3. Dynamics of respiratory rate (RR) in healthy dogs (n = 6) and in dogs (n = 9) with experimentally induced acute inflammation with turpentine. Results are expressed as means ± standard errors of the means (SEM)

Time after treatment	RR - control dogs	RR - inoculated dogs
0 hour	28,66±2,81	35,88±2,44
6 hours	35,00±2,35	41,22±3,10
24 hours	35,00±4,17	48,00±4,21^{*b}
48 hours	38,66±2,90	37,11±4,37
72 hours	34,00±4,47	34,00±2,15
Day 7	34,33±3,87	38,44±2,88
Day 14	35,50±4,42	29,77±3,25
Day 21	28,66±1,33	35,00±3,73

For a given biochemical parameter: *($p < 0.05$) indicate significant differences between turpentine inoculated and control dogs. Different superscripts b indicate significant difference ($p < 0.01$) according to time within the experimental group (turpentine inoculated dogs).

Table 4. Dynamics of pulse rate (PR) in healthy dogs (n = 6) and in dogs (n = 9) with experimentally induced acute inflammation with turpentine. Results are expressed as means ± standard errors of the means (SEM)

Time after treatment	PR - control dogs	PR - inoculated dogs
0 hour	87,33±5,35	101,88±8,88
6 hours	86,33±5,14	103,33±4,28
24 hours	82,33±4,46	98,77±6,92
48 hours	85,33±3,78	100,66±15,15
72 hours	82,00±4,20	82,77±5,25
Day 7	81,16±3,22	104,44±7,43*
Day 14	78,50±5,25	94,55±7,60
Day 21	80,00±4,25	91,66±7,76

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

DISCUSSION

Alkaline phosphatase is a membrane-bound enzyme present in many tissues. Three major isoenzymes in dogs contribute to total serum ALP: bone, liver, and corticosteroid isoenzymes. Bone ALP accounts for about one-third of the total serum ALP and is elevated with conditions associated with increased osteoblastic activity. Liver ALP present on biliary epithelial cells and hepatocytes and has a half-life about 70 hours. Increased ALP activity is one of the most common abnormalities detected on serum chemistry profiles in ill dogs. ALP activity measurement has a high sensitivity (80%) for hepatobiliary disease, but its specificity is low (51%).

Inflammation or trauma can induce local swelling, mild pain, local erythema and variable fevers which are common findings in soft tissue inflammation. It has been demonstrated that subcutaneous turpentine administration can be used as a simple method, which causes a local inflammatory process (4). The results of the study are present in Table 1 and Table 2. In this study, changes in blood alkaline phosphatise concentrations were observed in dogs in response to turpentine injection. In this connection, the activities were slightly affected by aseptic stimuli; the experimental acute inflammation has lead to insignificantly increases in ALP at the 72nd hour and on days 7. In this experiment total ALP activity began to increase at the 72nd h after injection and remained so high up to day 14, although they were within the normal ranges. The referent ranges for total ALP in dogs are 10-150 U/L (4). Turpentine oil which is a very powerful pyrogen, induces significant fever, which peaked 11 h after injection and continues for more than 24 hours (6). In our experimental group, the fever starts to rise 6 hours after treatment (39,92±0,20 ºC) and continued to the 72nd. This period coincides with elevation in ALP activities as compared to the baselines (the levels reached 87,2 U/L at 72nd hour). Some authors (7, 8) reported that the subcutaneous injection of turpentine in mice model induced local tissue damage and the inflammatory reaction to the oil is characterized by local inflammation, abscess formation, fever, loss of body weight, anorexia, lethargy. The same symptoms were observed in our study, whereas loss of body weight and anorexia were insignificant. There are some studies (9) which investigated changes in the ALP concentrations during acute and chronic wounds processes in animal and human models. They suggested that ALP activity is an acute inflammation marker since the enzyme levels are increased in acute wounds, but not in chronic inflammation processes. In our study, the elevation in the ALP levels is not significant because they remained in reference ranges for this species. Although, there are signs of non-specific response and inflammation - enhanced fever 6 hour after inoculation and increased respiratory rates at the 24th h. The fever continued to the 72nd h and at that time slight, insignificant elevation in ALP activities was also observed which continued

until the 7th day (88,21±5,06 U/L), but stayed in the normal ranges (10-150 U/L). 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). The locally increased levels of them, particularly IL-1β, induce IL-6 synthesis and release into the circulation. IL-6 increases dramatically following a systemic inflammatory challenge and correlates significantly with the fever response. No significant changes in total activities of ALP of experimental groups were observed and this lead up to possible slightly increased permeability of liver cells plasma membrane. We could observe slightly elevated enzyme levels, presumably due to cellular damage which is characterized with altered permeability. We can assume that there is no significant disability in the liver function. The magnitude of ALP elevation may be proportional to the number of hepatocytes affected, so the lack of high levels (they were in the reference ranges) leads to the conclusion that in dogs, the liver function is not significantly affected by turpentine injection.

CONCLUSION

In conclusion, these results indicated that the total alkaline phosphatase activity was not significantly affected in dogs with experimentally induced acute inflammation by turpentine injection. Throughout the experiment (21 days), the levels remained in the reference ranges for this species.

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