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Creatine kinase activity in dogs with experimentally induced acute inflammation

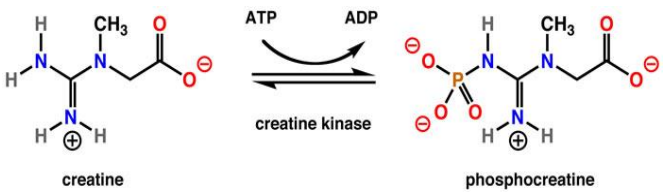
ABSTRACT

The main purpose of this study was to investigate the effect of acute inflammation on total creatine kinase (CK) activity in dogs. In these animals, CK is an enzyme found predominantly in skeletal muscle and significantly elevated serum activity is largely associated with muscle damage. Plasma increases in dogs are associated with cell membrane leakage and will therefore be seen in any condition associated with muscular inflammation. The study was induced in 15 mongrel male dogs (n=9 in experimental group and n=6 in control group) at the age of two years and body weight 12-15 kg. The inflammation was reproduced by inoculation of 2 ml turpentine oil subcutaneously in lumbar region. The plasma activity of creatine kinase was evaluated at 0, 6, 24, 48, 72 hours after inoculation and on days 7, 14 and 21 by a kit from Hospitex Diagnostics. In the experimental group, the plasma concentrations of the CK-activity were increased at the 48th hour (97.48±6.92 U/L) and remained significantly higher ($p<0.05$) at the 72 hour (97.43±2.93 U/L) compared to the control group (77.08±5.27 U/L). The results of this study suggest that the evaluation of creatine kinase in dogs with experimentally induced acute inflammation has a limited diagnostic value. It was observed that the creatine kinase activity is slightly affected by the experimentally induced acute inflammation in dogs.

Key words: creatine kinase activity, acute inflammation, turpentine, dogs

Introduction

Creatine kinase (CK) (EC 2.7.3.2) catalyses the reversible exchange of high-energy phosphate bonds between phosphocreatine (PCr) and adenosine diphosphate (ADP), regenerating adenosine triphosphate (ATP) from ADP produced during muscle contractions (Shelton, 2010).



The main function of the cytosolic CK is replenishment of ATP at sites of high-energy demand (Genet et al., 2000). The reaction catalyzed by CK allows energy storage as phosphocreatine when demand is low, and when energy demand increases CK enables rapid restoration of the intracellular pool of ATP necessary for muscle contraction. Three cytosolic isoenzymes have been describes: MM-muscle type; MB-heart and other tissues, and BB-brain type (Genet et al., 2000). Creatine kinase is an enzyme found predominantly in skeletal muscle and significantly elevated serum activity is largely associated with muscle damage. It is a sensitive indicator of muscle damage, but is not specific as to cause. Plasma increases in dogs are associated with cell membrane leakage and will therefore be seen in any condition associated with muscular inflammation, necrosis or degeneration (Nevill et al., 2010). Inflammation, infection or trauma in dogs can induce secondary muscle involvement, which may be indicated by increased activities of the CK. Stoikovski (2001) reported that normal blood creatine kinase activity in dogs varied between 25-467 UI/L. The aim of this study was to determine plasma CK activity at different time points after turpentine injection.

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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°C). They were fed a commercially available diet of dog pellet twice daily and had free access to water. Prior to the experiment the animals were vaccinated with vaccine Nobivac® DHPPiLR, 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 collected and from the control animals. 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 CK-activity was determined by a kit from Hospitex Diagnostics.

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 CK concentration after turpentine injection are shown in Table 1. In both experimental and control groups, total creatine kinase activities were followed during a period of 21 days.

Table 1. *Blood creatine kinase concentrations (U/L) in dogs with acute inflammation experimentally induced by turpentine oil.*

Time after inoculation	CK (U/L) in infected dogs (n=9) means±SEMs	CK (U/L) in non-infected dogs (n=6) means±SEMs
0 h	92.10 ± 7.04	97.26 ± 10.8
6 h	89.66 ± 4.54	86.36 ± 9.00
24 h	84.54 ± 6.92	81.48 ± 11.2
48 h	97.48 ± 6.92	91.46 ± 7.05
72 h	97.43 ± 2.93*****	77.08 ± 5.27
Day 7	86.52 ± 3.44	71.98 ± 4.99
Day 14	77.63 ± 2.91	77.11 ± 5.1
Day 21	70.06 ± 3.54**b**	81.45 ± 4.74

***** – compared with the control group $p<0.05$
b – from baseline (0 hour)

Blood creatine kinase activity was slightly influenced by the local, aseptic inflammatory stimuli. In the experimental group, the initial levels (before inoculation) were 92.10±7.04 U/L and 48 hours after this, CK levels began to rise (97.48±6.92 U/L) and remained high at the 7th day (97.43±2.93 U/L) of the study compared to the controls. From the 72th hour, the CK concentration showed consistent downward trend and on 7th day the mean values were 86.52±3.44 U/L. This study indicated significant differences (*$p<0.05$) in dogs with experimentally induced acute inflammation in comparison to the control group at 72th hours. At the 72th hour, CK levels reached significant peak elevation – 97.43±2.93 U/L compared to the controls – 77.08±5.27 U/L. Infection accompanied by local and general systematic signs-enhanced fever (6 hour after inoculation), increased heart and respiratory rates at 24th h, which are indicators for non-specific response and signs of inflammation.

Discussion

In the dog, CK release from the cells and reaches the plasma mostly via the lymphatic route and then remains in the plasma compartment. It is rapidly cleared with a half-life of about 2 hours. Muscle disorders are the main source of plasma CK elevations, moreover sex has no influence on the plasma CK activity, which is higher in young dogs than in adults (Aktas et al., 1993).

Inflammation or trauma can induce secondary muscle involvement, which may be indicated by increased activities

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of the CK. The mechanisms of this injury are not fully understood, but raised protein catabolism in the muscle cell is suspected (Neumann, 2005). This author demonstrates that dogs and cats with metabolic diseases have increased CK-activity. Myositis may result from contiguous sites of infection, penetrating trauma, vascular insufficiency, or by hematogenous dissemination. In most cases this involves only a single muscle group. The first stage occurs with 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 (Muthny et al., 2008).

The results of our study are present in Table 1. In this experiment CK activity began to increase at 48th h after injection and remained so high up to 72 hours, although they were within the normal ranges. Turpentine oil is very powerful pyrogen, induced significant fever, which peaked 11 h after injection and continue more than 24 hours (Aguilar-Valles et al., 2007). Renckens et al. (2005) and Leon et al. (1996) 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 observed in our study in dogs. Stoikovski (2001) showed that in dogs normal blood creatine kinase activity varied between 25-467 UI/L.

The increase of CK-activity after s.c. injection in this study indicated that this enzyme activity in dogs is influenced by the local inflammatory process caused by turpentine oil (Table 1). Tóthová et al. (2009) measured 5 fold enhance of CK-activity at the 24th h after s.c. injection of a combined mineral preparation in calves, with consistent decrease of activity. A similar, but not significant trend of increasing we observed in our experiment. We matched peak 48th h after s.c. injection, with consistently decline of activity. The reduction of the CK-activity after peak values could be explained by the direct elimination and by intravascular inactivation (Tóthová et al., 2009). Most notably CK is moderately to markedly elevated in necrotizing, inflammatory and dystrophic myopathies and is usually normal or only mildly elevated in non-inflammatory muscle diseases. Intramuscular injections of drugs can cause a transient increase in serum CK but should return to baseline values in about 3 days Transient elevation of serum CK activity may follow external muscle damage from many causes (Shelton, 2010). The results from this study showed that the plasma CK of experimental dogs has little changed shortly in the course of acute inflammation induced by turpentine oil. This may be due to destruction of fascia and musculature, as well as the proteolytic enzymes of accumulated leukocytes, which led to muscle tissue lysis at the site of injection.

Conclusion

In conclusion, we think that our results show that local, acute inflammation caused by turpentine injection may induce secondary elevation of creatine kinase activity in dogs and is an indicator for muscle damage at the site of injection. In the experimental group the plasma CK concentration was significantly ($p<0.05$) higher than in the control group at the 72th hour. These changes may be due to progression of inflammation caused by injection and this elevation of CK-activity could not be used as a parameter for detection of acute inflammation in dogs.

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