Biochemical Evidence of Myocardial Cell Damage in Heart Failure : A Canine Model of Cardiac Injury

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Abstract : **The objective of the present study was to evaluate the diagnostic efficiency of the new cardiac-specific marker troponin T (cTnT) compared with the conventional markers creatine kinase (CK) and lactate dehydrogenase (LDH) in detecting myocardial injury in dogs with clinical signs and symptoms of various forms of cardiac diseases including congestive heart failure (CHF). The results showed that in animals with clinical valvular heart disease (n = 20), clinical cardiac arrhythmias (n = 11) and heartworm infection (n = 10) without clinical heart failure, as well as in dogs with clinical CHF (n = 18), there was no significant difference either in the activity of CK or LDH. Levels of cTnT in dogs with CHF, on the other hand, were much higher than those in the other 3 groups. The majority of the 18 dogs with CHF had detectable levels of cTnT, with 6 of them (33%) showing values exceeding the upper reference limit of 0.10 ng/ml. The overall mean cTnT level in this animal group was 0.204 ng/ml. These results may imply that CHF in dogs is associated with a small release of cardiac troponin T indicative of minor myocardial cell damage.**

เรื่องย่อ : หลักฐานทางชีวเคมีของการทำลายเซลล์กล้ามเนื้อหัวใจในสุนัขที่มีภาวะหัวใจล้มเหลว

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จุดประสงค์ของการศึกษานี้คือเพื่อประเมินประสิทธิภาพของ cardiac marker ชนิดใหม่ troponin T (cTnT) เปรียบเทียบกับ cardiac enzymes creatine kinase (CK) และ lactate dehydrogenase (LDH) ที่ใช้กันอยู่ในปัจจุบัน ในการบอกถึงภาวะการทำลายของเซลล์กล้ามเนื้อหัวใจ ในสุนัขที่มีโรคหัวใจชนิดต่าง ๆ รวมถึงภาวะหัวใจล้มเหลว ผลการศึกษาแสดงให้เห็นว่าในสุนัขที่มีโรคลิ้นหัวใจ (20 ตัว), โรคหัวใจเต้นผิดจังหวะ (11 ตัว), โรคพยาธิหัวใจ (10 ตัว) และภาวะหัวใจล้มเหลว (18 ตัว) ไม่มีความแตกต่างในระดับของ CK และ LDH ในขณะที่สุนัขที่มีภาวะหัวใจล้มเหลวมีระดับของ cTnT สูงกว่าในสุนัขกลุ่มอื่นอย่างชัดเจน นอกจากนี้ยังตรวจพบว่าสุนัข 6 ใน 18 ตัวที่มีภาวะหัวใจล้มเหลว (33%) มีระดับ cTnT สูงกว่าค่าสูงสุดของค่าปกติที่ 0.10 ng/ml ค่าเฉลี่ยของ cTnT ในสุนัขกลุ่มนี้คือ 0.204 ng/ml ผลการศึกษาแสดงให้เห็นว่าในสุนัขที่มีภาวะหัวใจล้มเหลวมีการปล่อย cTnT ออกมาในกระแสเลือดในปริมาณเล็กน้อย ซึ่งบอกถึงภาวะ minor myocardial cell damage

INTRODUCTION

Congestive heart failure (CHF) is a frequent clinical sequel of several cardiac disorders in human[1,2] as well as in animals.[3,4] Apart from ischemic heart disease, which rarely occurs in small animal species, the common cardiac causes of heart failure in small animals including dogs are dilated cardiomyopathy, valvular heart disease and cardiac arrhythmias.[4] Infection with heartworms represents another frequent form of canine cardiac disease that may lead to right ventricular dysfunction and heart failure.[5]

Over the past decade, the pathophysiological mechanisms underlying impaired ventricular function in CHF have been to a certain extent elucidated. The current concepts of the pathogenesis of heart failure rest on ventricular remodeling and neuroendocrine activation of the renin-angiotensin and sympathetic systems with cytokine production.[1-4] It has been shown in experimental models that release of systemic neurohormone such as norepinephrine results in progressive myocyte damage with loss of cardiac myocytes by apoptosis and necrosis.[6,7] The question thus arises, as to whether myocardial cell injury induced by norepinephrine or cytotoxic cytokines in heart failure is associated with the release of cardiac marker proteins into the circulation.

The aim of the present study was to assess the extent of myocyte damage in heart failure by measuring enzymatic activities of the conventional cardiac markers creatine kinase (CK) and lactate dehydrogenase (LDH) as well as concentrations of the heart-specific contractile protein troponin T (cTnT) in dogs presenting to the hospital with clinical signs and symptoms of CHF. Levels of these marker proteins were also compared with those found in dogs with other cardiac diseases without severe ventricular dysfunction.

MATERIALS AND METHODS

Study Subjects

The study consisted of 59 consecutive cases of dogs brought to the Veterinary Teaching Hospital, Kasetsart University, between January and June 2000 for clinical evaluation and treatment of suspected cardiac diseases. The diagnosis of cardiac disoders was made on the basis of clinical signs and symptoms as well as of results obtained from radiographic and electrocardiographic assessment. Congestive heart failure (CHF) was diagnosed by the presence of cough, dyspnoea, hepatomegaly, peripheral (ventral) edema and ascites as well as by radiographic evidence of pulmonary congestion, pleural effusion and cardiac enlargement. Infection with heartworms was defined as the presence of microfilariae in peripheral blood smear examination and a positive heartworm antigen test kit (Witness).

Final diagnoses were 20 cases of dogs with valvular heart disease (VHD), 10 with heartworm infection (HWI), 11 with cardiac arrhythmias (CAR) and 18 cases of animals with CHF of various etiolo-

gies (3 dogs with VHD, 4 with HWI and 11 with unknown cause, probably of idiopathic dilated cardiomyopathy). The age and weight ranges of the animals studied were between 1-16 years and 2-35 kilograms, respectively.

Laboratory Procedures

Venous blood samples were collected from animals on arrival at the hospital and the serum obtained was immediately frozen at – 20 °C until analysis of the biochemical parameters which was performed within 3 days of the blood sampling procedure. Levels of total cholesterol and triglycerides were measured by standard enzymatic methods. The activity of enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) as well as levels of blood urea nitrogen (BUN) and creatinine were determined by enzymatic colorimetric tests. Total protein and albumin concentrations were measured by using the biuret and bromocresol green methods, respectively. Measurements of all blood chemistry parameters were performed on an automated chemical analyzer (Hitachi 717).

Determination of cardiac troponin T (cTnT) concentration was performed with a third-generation cTnT immunoassay on an immunoanalyzer Elecsys® 2010 (Roche Diagnostic). The test is based on newly developed electrochemiluminescent technology.[8] This assay shows virtually no cross-reactivity (< 0.01%) between the cardiac and skeletal muscle troponin T isoform. The analytical sensitivity and the upper limit of the reference range is 0.01 and 0.10 ng/ml, respectively. In the present study, a cTnT level below the lower detection limit of the assay (< 0.01 ng/ml) was considered as undetectable.

Statistical Analysis

Data are presented as mean ± standard deviation. The significance in the difference in results among the 4 study groups were determined using analysis of variance. A p value of < 0.05 was considered to indicate a significant difference.

RESULTS

The mean levels of selected biochemical parameters in the 4 groups of animals studied are shown in Table 1. Dogs with CHF demonstrated a lower concentration of albumin compared with animals with other cardiac diseases, but the difference achieved statistical significance only with the group of dogs with valvular heart disease. On the other hand, there were no significant differences in the levels of other blood chemistry variables among the 4 animal groups.

Comparisons of the activity of cardiac enzymes CK and LDH in the 4 study groups are displayed in Table 2. Although higher values of CK were observed in dogs with CHF than those in other groups, the differences did not reach statistical significance. Similarly, the relatively high LDH activity found in animals with valvular heart disease did not differ significantly from those in the other 3 groups. In contrast, dogs with CHF exhibited on average a much higher cTnT concentration than those in other groups, reaching a statistical significant difference when compared to the group of animals with valvular heart disease.

Table 3 demonstrates the number and percentage of animals in each study group stratified according to cTnT concentrations. It can be seen that the majority of dogs with valvular heart disease and cardiac arrhythmias, and a certain number of those infected with heartworms displayed undetectable cTnT concentrations of < 0.010 ng/ml. An increased cTnT level (> 0.100 ng/ml), on the other hand, was observed only in the group of dogs with CHF, with concentrations ranging from 0.167 to 1.20 ng/ml.

Follow-up data were available from 12 animals 1 to 4 weeks after treatment. The 4 dogs with an initial cTnT level of < 0.01 ng/ml remained undetectable at follow-up. Of the remaining 8 animals with detectable or increased cTnT concentrations, 6 including 3 dogs with CHF showed a decline in cTnT to levels in the reference range. One dog (with the highest cTnT concentration of 1.20 ng/ml) died suddenly 2 months after treatment.

Table 1. Mean (± SD) levels of selected biochemical parameters in the 4 groups of animals studied.

	VHD (n = 20)	HWI (n = 10)	CAR (n = 11)	CHF (n = 18)
ALT (IU/L)	83 ± 76	120 ± 119	78 ± 72	108 ± 74
AST (IU/L)	56 ± 40	71 ± 76	39 ± 28	84 ± 103
Albumin (g/dl)	3.2 ± 0.6	2.7 ± 0.3	3.1 ± 0.4	2.6 ± 0.6*
Total protein (g/dl)	7.1 ± 0.9	7.6 ± 1.4	7.8 ± 1.2	6.6 ± 1.4
Cholesterol (mg/dl)	201 ± 76	239 ± 60	193 ± 104	184 ± 79
Triglycerides (mg/dl)	76 ± 37	99 ± 50	72 ± 36	95 ± 61
BUN (mg/dl)	23 ± 20	27 ± 9	23 ± 22	39 ± 29
Creatinine (mg/dl)	0.9 ± 0.5	1.2 ± 0.4	0.9 ± 0.3	1.3 ± 0.9

Abbreviation : VHD = valvular heart disease ; HWI = heartworm infection ; CAR = cardiac arrhythmia; CHF = congestive heart failure.
*p = 0.016 compared with VHD group.

Table 2. Comparison of mean (± SD) levels of cardiac marker proteins in the 4 study groups.

	VHD (n = 20)	HWI (n = 10)	CAR (n = 11)	CHF (n = 18)
CK (IU/L)	429 ± 476	206 ± 162	378 ± 524	771 ± 1602
LDH (IU/L)	973 ± 1074	519 ± 438	447 ± 288	898 ± 1004
cTnT (ng/ml)	0.013 ± 0.025	0.012 ± 0.012	0.007 ± 0.012	0.204 ± 0.353*

*p = 0.04 versus VHD group.

Table 3. Number (percentage in parenthesis) of animals in the 4 study groups stratified according to cardiac troponin T levels.

	Cardiac troponin T concentration*		
	Non-detectable	Detectable	Increased
VHD (n = 20)	13 (65%)	7 (35%)	0 (0%)
HWI (n= 10)	4 (40%)	6 (60%)	0 (0%)
CAR (n = 11)	8 (73%)	3 (27%)	0 (0%)
CHF (n = 18)	4 (22%)	8 (44%)	6 (33%)

*Non-detectable = < 0.010, detectable = 0.010 - 0.100, increase = > 0.100 ng/ml.

DISCUSSION

We have demonstrated in this canine model of cardiac injury that a significant number of dogs with clinical signs and symptoms of CHF displayed increased cTnT serum concentration indicative of myocardial cell damage. As indicated by a mean cTnT level of 0.204 ng/ml, however, the extent of myocyte damage is usually minimal and is much less than that usually observed after ischemic myocardial injury.[9] The majority of dogs with CHF, on the other hand, exhibited either non-detectable or detectable cTnT concentration not exceeding the upper limit of normal value.

During the past decade, research into the pathophysiology of heart failure has been focused on the detrimental effect of enhanced sympathetic drive on the myocardium. It has been shown both in animal models[10] and in humans with heart failure[2] that levels of norepinephrine were significantly increased and this enhanced sympathetic activity was even observed in the early and asymptomatic stage of heart failure.[11,12] Since the activation of the sympathetic system has been shown to result in an increase in heart rate and oxygen consumption as well as in myocardial ischemia[1,13], the structural integrity of the myocytes can be lost and necrosis can occur.

The cardiotoxic effect of norepinephrine has previously been experimentally demonstrated in several small animal species including dogs. Mann et al.[14,15] reported that norepinephrine treatment led to an overall significant release of cardiac enzyme CK into cardiac myocyte cultures and there was a concentration-dependent increase in CK released into the medium. However, since CK has been found in a significant amount in skeletal muscle, measurement of this enzyme is not a specific method for detecting damage to the myocardium. Thus, the high CK activity found in present study may possibly be due to ske-letal muscle injury occuring during restraint of the animal.[16] The enzyme LDH, likewise, is not a specific marker of myocardial damage because this enzyme is also abundantly present in several tissues other than myocardium. As in our study, an increase in LDH activity has been reported in patients with valvular heart disease.[17]

Troponin T is a constituent of troponin complex which regulates the interaction of actin and myosin in striated muscle. Tests based on enzyme-linked immunosorbent assay (ELISA) and a novel technology of electrochemiluminescence have been developed which show virtually no cross-reactivity between the cardiac and skeletal muscle troponin T

isoform. Several recent trials conducted in humans have demonstrated that cTnT has a higher diagnostic performance over CK in detecting small damage to myocardial tissue.[18-20] We have performed a study on 72 dogs without and with heartworm infection, but without clinical signs and symptoms of severe cardiac dysfunction using a second-generation TnT assay and found no significant difference in cTnT levels between the 2 groups[21]. The results indicate that the presence of heartworms per se, without causing severe ventricular dysfunction, is not associated with a significant myocardial cell damage.

To our knowledge, there have been two trials investigating the frequency of increased cTnT concentration in patients with CHF. By using a second-generation TnT ELISA and a cut-off value of 0.10 ng/ml, Missov and Mair[22] reported that 5 of 33 patients (15%) with CHF showed an increased cTnT level. Setsuta et al.[23], on the other hand, demonstrated that the majority (52%) of CHF patients displayed detectable ($\geq$ 0.02 ng/ml) cTnT concentrations. The corresponding values from our study using a third-generation TnT assay (electrochemiluminescent assay) were higher, i.e. 33% using a cut-off value of 0.10 ng/ml and 56% with a value of 0.02 ng/ml. We have at present no explanation for these differences but the mean cTnT level in our study of 0.204 ng/ml is only minimally higher than that reported by Missov and Mair (0.140 ng/ml). It is possible that dogs reacted more deleteriously to the cardiotoxic effect of adrenergic overdrive than humans.

Nevertheless, it should be noted that only one-third of our dogs with CHF displayed an increased cTnT concentration, despite the fact that all animals in this group clearly showed clinical signs and symptoms of CHF. One possible explanation is that cTnT may not represent a sensitive indicator of myocardial damage in heart failure, since it has been shown in a study by Voss et al.[9] that the myocardial content of this contractile protein was significantly decreased both in humans and animals with ventricular dysfunction compared with normal controls. Whether the novel marker of CHF brain natriuretic peptide (BNP)[24] is a more sensitive marker of ventricular dysfunction remains to be determined in future investigations.

An apparent limitation of our study, apart from the small sample size, is that the diagnosis of CHF was made on clinical grounds without informations from left ventricular function parameters (i.e. echocardiography). However, echocardiographic assessment of ventricular function is at present not routinely performed in the Veterinary Hospital and thus can not be used as a diagnostic criterion in the present study. Another limitation was the fact that we did not measure CK-MB, at present considered to be the "gold standard" biochemical method for detection of damage to myocardial tissue. Unlike in humans, however, this isoenzyme is present only in a small amount in the myocardium of animals. In addition, unlike the troponins, the immunoassays developed for human application are too selective for epitopes on the human form of CK-MB and will not even cross-react to an appreciable extent with CK-MB from higher primates.[16] Thus, measurement of CK-MB may not represent a sensitive method for detection of myocardial damage in animal species.

CONCLUSION

Results from the present study have shown that the majority of dogs with congestive heart failure displayed a detectable cardiac troponin T serum concentration and a significant number of these animals exhibited a small increase in this cardiac-specific contractile protein indicative of minor myocardial damage. Future clinical trials should focus on investigating the prognostic role of increased cTnT concentration in patients with congestive heart failure.

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