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Evaluation of myocardial protective effects of enalapril maleate against experimental supraventricular tachyarrhythmia in dogs by measuring cardiac troponin I levels

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Abstract

The development of ventricular systolic dysfunction in animals with supraventricular tachyarrhythmia (SVTA) has been reported to be largely due to myocardial injury, resulting from activation of the renin–angiotensin–aldosterone system. The objectives of this study were to demonstrate that the development of myocardial injury and the myocardial protective effect of enalapril maleate could be evaluated in a dog model of SVTA by measuring blood cardiac troponin I (cTnI) concentrations. Rapid atrial pacing (RAP) model dogs were divided into a Control group (no medication, n = 7) and an Enalapril group (enalapril maleate at 0.5 mg/kg once per day, n = 7). After 1, 2, and 3 weeks of RAP, significantly lower concentrations of cTnI were observed in the Enalapril group than in the Control group ($P < 0.01$). Upon histopathological examination of heart tissues, the control group showed more severe necrotic changes in myocardiocytes than the Enalapril group. In conclusion, this study demonstrated that both the development of myocardial injury and the myocardial protective effect of enalapril maleate could be assessed by measuring blood cTnI concentrations in SVTA model dogs. The results indicate that such measurements could comprise a useful method to diagnose myocardial injury and determine the therapeutic effect of a drug not only in human medicine but also in the veterinary field.

Key Words: cardiac troponin I, dog, enalapril maleate, supraventricular tachycardia arrhythmia

Introduction

Several biomarkers are considered useful for the assessment of heart disease. Among these, cardiac troponin I (cTnI) has been widely used in clinical settings as a biomarker for the diagnosis of myocardial injury, especially myocardial infarction, in humans[31)]. Damaged cardiomyocytes release cTnI, resulting in an increase in its concentration in the blood[16)]. Increased blood

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concentrations of cTnI have also been reported in some dogs with mitral insufficiency[32], filariasis[1], atrioventricular block[34], and cardiac hemangiosarcoma[27], as well as following administration of cardiomyotoxic anticancer drugs[30]. Thus, cTnI has drawn attention because of its potential use for evaluating the therapeutic effects of drugs and the severity of these diseases[15, 24]. cTnI is considered particularly useful as a convenient examination tool since concentrations of this biomarker can be easily determined in blood samples in commercial laboratories.

In dogs, supraventricular tachyarrhythmia (SVTA) including supraventricular tachycardia, atrial fibrillation, and atrial flutter represents an arrhythmia that is frequently observed independently of or accompanying heart disease and other systemic disorders[14]. SVTA is one of the most frequently encountered arrhythmias in dogs in clinical settings. In SVTA, significantly increased impulses, generated from supraventricular tachycardia, are transmitted into the ventricles, leading to an increased ventricular rate. If this increased ventricular rate is sustained, it can lead to a reduction in ventricular systolic function and ultimately, to heart failure[10, 32, 37]. The development of ventricular systolic dysfunction in animals with SVTA has been reported to be largely due to myocardial injury, caused by fibrosis of myocardial tissue resulting from activation of the renin–angiotensin–aldosterone system (RAAS)[33, 35]. It has been demonstrated that the administration of an angiotensin-converting enzyme inhibitor (ACEI), which regulates RAAS, inhibits the fibrosis of myocardial tissue in a dog model of SVTA[25, 28].

It has also been shown that injury-associated features such as cellular elongation, myofibril misalignment, sarcomere disassembly, and the disappearance of cardiomyocytes occur before the onset of myocardial fibrosis[26, 29]. One of the factors that precedes fibrosis is a tachycardia-induced reduction in coronary blood flow such as myocardial ischemia[21]. Furthermore, direct RAAS involvement in fibrosis formation has been demonstrated[5, 26, 29]. Therefore, it is expected that ACEI, which is a RAAS regulator that also has a coronary dilation effect, could suppress lesion formation in the previous stage of fibrosis. The fibrosis of myocardial tissue develops at the end stage of myocardial injury, and it is important to identify myocardial injury as promptly as possible for early treatment. However, the confirmation and evaluation of myocardial injuries in dogs with SVTA depend on postmortem histopathological examination.

Recently, a meta-analysis evaluated the association between cTnI levels and adverse outcomes in human atrial fibrillation patients[6]. According to this study, elevated cTnI concentration sin atrial fibrillation patients were associated with an increased risk of all-cause mortality and adverse events such as stroke, myocardial infarction, and pulmonary embolism. Therefore, cTnI measurements for canine SVTA patients might also be useful to predict patient prognosis and adverse events.

The objectives of this study were to demonstrate that the development of myocardial injury and the myocardial protective effect of enalapril maleate, an ACEI-inhibiting RAAS, could be evaluated in a dog model of SVTA by measuring blood cTnI concentrations.

Materials and Methods

This study was conducted in accordance with the Ethical Code for Animal Experiments of the Tokyo University of Agriculture and Technology (TUAT) (Approval number: 27–38).

Dogs: Fourteen clinically healthy beagles (seven intact males and seven intact females; body weight: 9–11 kg; age: 2–4 years of age) were used. The dogs were provided food at $1.8 \times \{70 \times [\text{body weight (kg)}]^{0.75}\}$ kcal/day[4] and water ad libitum. They were housed individually from the time of arrival to the end of the study. The dogs were

maintained at a temperature of 21 ± 2 °C and a relative humidity of 50 ± 20 %, with a 12-hr/12-hr light/dark cycle. In addition, the dogs could run freely in a playground for 30 min every day. We used the rapid atrial pacing (RAP) method to produce the SVTA model; this method artificially maintains a state of tachycardia by providing high-frequency electrical stimulation to the atrium[29]. We produced the SVTA model according to the procedures of Ohashi et al.[23] and Gaspo et al.[8], with some modifications. Briefly, the dog's chest was opened at the left-V-intercostals under general anesthesia, and a dipole electrode lead (TY216-033; Unique Medical Co., Ltd., Tokyo, Japan) was sewn onto the left atrium. The electrode lead was introduced through a subcutaneous tunnel and exposed outside of the body from the back before closing the chest using a routine method. The animals were pre-anesthetized with a subcutaneous injection of atropine sulfate (Mitsubishi Tanabe Pharma Co., Osaka, Japan) at 0.05 mg/kg and an intravenous injection of midazolam (Dormicum; Astellas Pharma, Tokyo, Japan) at 0.2 mg/kg. Anesthesia was induced with an intravenous injection of propofol (Fresenius Kabi, Tokyo, Japan) at 5–6 mg/kg and maintained via the inhalation of 1.3–1.7% isoflurane (Isoflur; DS Pharma Animal Health, Osaka, Japan). For perioperative pain management, buprenorphine (Nissin Pharma, Tokyo, Japan) was pre-operatively injected intramuscularly at 0.02 mg/kg, and a total of 2 mg/kg of 0.5% bupivacaine hydrochloride (Marcain; AstraZeneca K. K., Osaka, Japan) and 2% lidocaine (Xylocaine; Aspen Japan, Tokyo, Japan) was administered at the incision area and the surrounding intercostal muscles during operation. Additionally, meloxicam (Metacam 0.5%; Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan) was administered subcutaneously at 0.3 mg/kg on the day of operation and for 3 days post-surgery. After the operation, the dogs were fitted with a jacket containing a pocket with an external cardiac pacemaker (EV4543; Taisho Biomed Instruments Co., Ltd., Osaka, Japan) installed. Fourteen postoperative days were reserved as a recovery period. The 14 dogs were randomly assigned to two groups as follows: a no medication group (Control group, n = 7) and an enalapril maleate-administered group (Enalapril group, n = 7). After the recovery period, the electrode leads were connected to the pacemaker devices in both groups, and RAP (1.0 msec plus width; 2 V stimulation) was initiated at a frequency of 390 bpm. The Enalapril group was orally administered enalapril maleate (Boehringer Ingelheim Animal Health Japan Co., Ltd., Tokyo, Japan) at 0.5 mg/kg once per day during RAP. The duration of RAP was 3 weeks for both groups. Measurement of CTnI concentration and blood pressure as well as echocardiography were performed before starting RAP (baseline) and at 1, 2, and 3 weeks after the initiation of RAP (1 week, 2 weeks, and 3 weeks). All tests were performed without anesthetics. Furthermore, to avoid the influence of diurnal variations on the measured values, all experiments were conducted at a fixed time (4:00–5:00 pm) (Fig. 1).

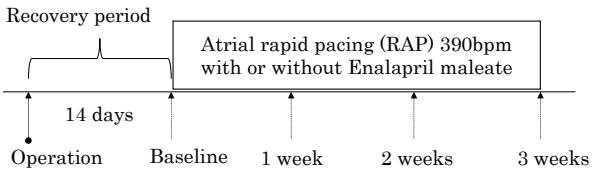

Fig. 1. Schematic representation of the study design. The postoperative recovery period was set to 14 days. Fourteen dogs were randomly assigned to the Control group (n = 7) and the Enalapril group (n = 7). Measurement of cardiac troponin I (cTnI) concentrations and blood pressure as well as echocardiography were carried out at baseline, before the initiation of rapid atrial pacing (RAP), and 1, 2, and 3 weeks after the initiation of RAP. Histopathological examination of the heart was performed at 3 weeks.

cTnI concentration measurements: Blood samples were collected from the jugular vein into a tube with serum-separating medium, followed by centrifugation at 1,500 × g for 10 min. The measurements of cTnI concentrations were entrusted to a commercial laboratory (Fujifilm Monolith Co., Ltd., Tokyo, Japan).

Table. 1 Intra- and Inter assay coefficient of variation for cardiac troponin I measurements

	Intra-assay			Inter-assay		
	Low	Middle	High	Low	Middle	High
Mean (ng/mL)	0.022	0.428	41.770	0.023	0.420	42.670
Standard deviation	0.0016	0.0162	1.4840	0.0027	0.0185	2.6513
CV(%)	7.3	3.8	3.6	11.9	4.5	6.2

Low; Sample of low concentration level dogs cardiac troponin I ,
Middle; Middle concentration level, High; High concentration level,
CV; coefficient of variation

Plasma cTnI concentrations were measured using a highly sensitive chemiluminescence enzyme immunoassay (ADVIA Centaur CP TnI-Ultra; Siemens Healthineers Japan, Tokyo, Japan), which is a three-site, second-generation, sandwich immunoassay employing direct chemiluminometry, with a limit of cTnI detection of 0.006–50 ng/mL. The inter-assay and intra-assay coefficients of variation for this test (Data from Fujifilm Monolith Co., Ltd.) were 11.9% (4.5–11.9%) and 7.3% (3.6–7.3%), respectively (Table.1).

Prior to this study, blood samples were collected (4:00–5:00 pm) from 21 healthy beagles (11 males and 10 females; body weight: 9–11 kg; age: 2–4 years of age), and the cTnI concentrations were measured. The mean ± standard deviation (SD) value was 0.025 ± 0.028 ng/ml cTnI, and thus the reference range for cTnI was set between 0.006 and 0.081 ng/ml at our facility (TUAT), based on the assay's lower limit of detection and the mean experimental value + 2 SD.

Echocardiography: Echocardiography was carried out using an ultrasound unit (Logiq 7; GE Health Care Co., Ltd., Tokyo, Japan). The end-diastolic and end-systolic left-ventricular internal diameter (LVIDd and LVIDs, respectively) and the left-ventricular fractional shortening (FS), an index of the left-ventricular systolic function, were measured using the M-mode on the right parasternal short-axis view at the level of the tendinous cord. Heart rates (HRs) were recorded simultaneously. Using the left parasternal apical 5-chamber view, the ratio between the pre-ejection period and the ejection time (PEP/ET) was determined from the waveform of the left ventricular outflow tract, wherein the sample volume was set on the closed site of the aortic valve. The PEP was defined as the time interval between the Q wave and the beginning of the ventricular ejection[7, 19]. In the same view, cardiac output (CO) and stroke volume (SV) were obtained by measuring the aortic diameter (d) and the velocity time integral (VTI) based on the left-ventricular outflow on pulsed-wave Doppler imaging. The following equations were used: $SV= (d/2)^2 \times \pi \times VTI$ and $CO=SV \times HR$[7]. Echocardiography data were measured over an average of 9 consecutive beats.

Blood pressure measurements: The systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP) were measured at the tail-head using a noninvasive oscillometric sphygmomanometer for animals (BP-100D; Fukuda M-E Kogyo Co., Ltd.) with a cuff width of 40–60% of the circumference of the measured region[3]. Measurements were obtained in triplicate, and when the errors were within 3 mmHg, the mean value of the three measurements was used.

Histopathological examination: After blood samples were obtained at the end of the live study (3 weeks), the animals were euthanized via intravenous administration of sodium pentobarbital (Somnopentyl; Kyoritsuseiyaku, Tokyo, Japan). The heart was removed from each animal and fixed in 4% paraformaldehyde

for 7 days. To obtain myocardial samples for histological examination, a transverse section through the entire heart was made at the level of the mid-ventricular wall. Full-thickness tissue blocks were taken from the anterior wall (AW), lateral wall (LW), and posterior wall (PW) of the left ventricle (LV) and the right ventricle (RV), as well as from the AW, the middle portion (MP), and PW of the interventricular septum (IVS). In addition, two sections were taken from the atria (left atrium and right atrium), transversely across the atrial appendages. Tissue sections were embedded in paraffin wax, sectioned at a thickness of 5 μm, and stained with hematoxylin and eosin (HE) and Masson's trichrome. A semiquantitative evaluation of each specimen was performed using the HE-stained sections by assigning a grade from 0 to 3 for each of 20 consecutive adjacent areas with a 10× objective lens. Each field was graded based on the degree of myocyte necrosis. A grade of 0 was assigned if necrosis was absent, 1 if necrosis was scattered, 2 if necrosis was focal, and 3 if necrosis was patchy or extensive. The range for each grade was arbitrarily determined, and the data were presented as a mean grade for each group. Two independent researchers scored the slides separately in a blinded manner.

Statistical analysis: Measurements were recorded as the mean ± SD. The normality of data distribution was analyzed using the Kolmogorov–Smirnov test. cTnI concentration, echocardiography, and blood pressure data were analyzed using a two-way repeated analysis of variance, and Tukey's post-hoc test was used to further analyze normally distributed data. Histopathological data were analyzed via the Mann–Whitney U-test with a Bonferroni correction. Statistical analyses were performed using statistical software for PC (BellCurve for Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan), and *P*-values < 0.05 indicated statistical significance.

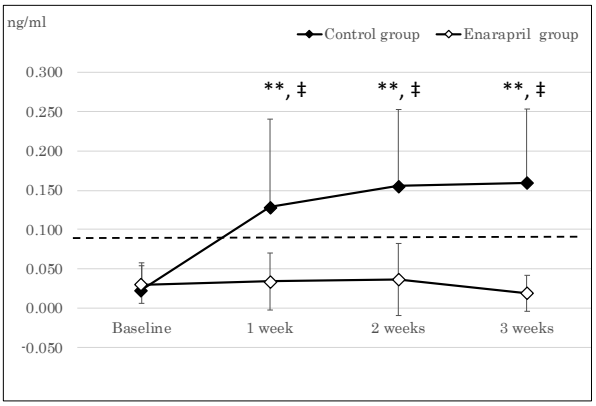

Fig. 2. Concentrations of serum cTnI in the Enalapril and Control groups.
All values are expressed as the mean ± standard deviation. $^{**}P < 0.01$ compared with the baseline; ‡$P < 0.01$ compared with the Control group. The range of reference values for cTnI at our facility was 0.006–0.081 ng/mL.

Results

cTnI concentrations: The ventricular response rates during RAP were 170–190 bpm in both the groups. No significant difference was observed in the baseline cTnI concentrations between the Control and Enalapril groups (0.023 ± 0.034 and 0.030 ± 0.024 ng/mL, respectively). All dogs had cTnI concentrations within the reference value range. In the Control group, the cTnI concentrations were 0.128 ± 0.112, 0.155 ± 0.097, and 0.159 ± 0.094 ng/ml at weeks 1, 2, and 3 after the initiation of RAP, respectively. These values are significantly higher than those recorded at baseline ($P < 0.01$). In contrast, in the Enalapril group, the cTnI concentrations were 0.034 ± 0.036, 0.036 ± 0.046, and 0.019 ± 0.023 ng/ml, respectively. These values are were not significantly different from those recorded at baseline, but were significantly lower than those of the Control group ($P < 0.01$; Fig. 2) at the same time points. In the Control group, 3 weeks after the initiation of RAP, six of seven animals showed higher cTnI concentrations than the upper limit of the reference values. The remaining one cTnI value was within the reference throughout the experiment period. However, cTnI concentrations did not increase in the Enalapril group (0/7

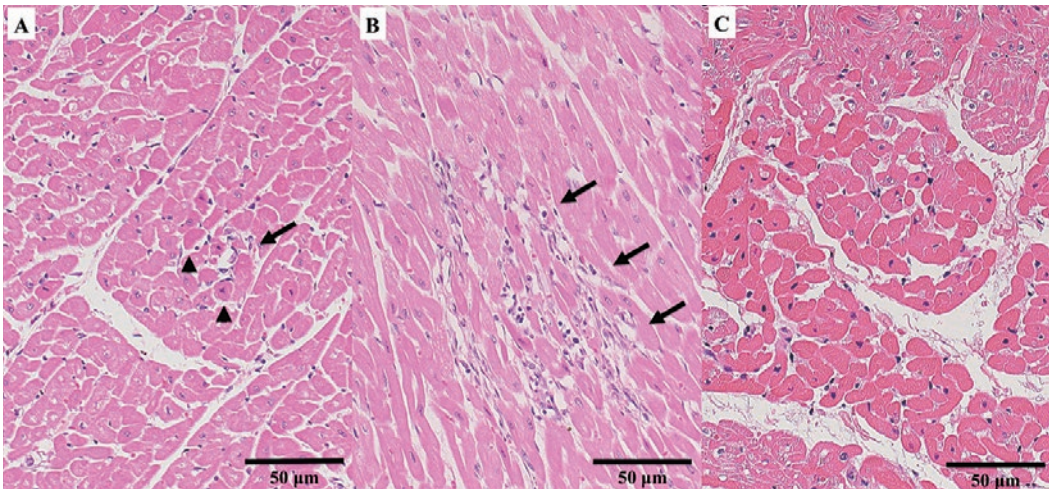

Fig. 3. Grade classification of histopathological findings.
(A) Partial shedding of myocardial cells (arrows), with eosinophilic degeneration of the surrounding cells (arrowheads). Scattered lesions were defined as grade 1.
(B) Shedding of myocardial cells (arrows) was observed, and the focal lesions were defined as grade 2.
(C) An image showing diffuse myocyte necrosis. Patchy or extensive lesions were defined as grade 3.

animals).

Echocardiography: The measured values for each item are shown in Table 2. There were no significant differences between the baseline values of the Control group and the Enalapril group for HR, LVIDd, LVIDs, FS, PEP/ET, SV and CO. Moreover, HR values throughout the measurement period showed no significant differences within or between the Control group and the Enalapril group.

No significant differences in LVIDd were observed at 1, 2, or 3 weeks in comparison with the baseline values, for either the Control group or the Enalapril group. Unlike in the Control group, a significant decrease in LVIDd was detected in the Enalapril group at 1 week ($P < 0.01$).

A significant increase in LVIDs in the Control group was observed at 2 and 3 weeks compared to values at baseline ($P < 0.01$). However, no significant differences were observed in the Enalapril group throughout the entire measurement period.

A significant decrease in FS of the Control group was observed at 1, 2, and 3 weeks ($P < 0.01$) compared to baseline values. Meanwhile, the Enalapril group showed significant reductions at 1 and 2 weeks ($P < 0.01$ and $P < 0.05$, respectively) relative to baseline values. However, the FS of the Enalapril group was significantly higher than that of the Control group at 1, 2, and 3 weeks ($P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively).

PEP/ET of the Control group showed significantly high values at 1, 2, and 3 weeks ($P < 0.01$), compared to baseline values. Furthermore, significantly higher PEP/ET values were observed for the Enalapril group at 1, 2, and 3 weeks compared to those at baseline ($P < 0.01$). However, the PEP/ET of the Enalapril group was significantly lower at 1, 2, and 3 weeks ($P < 0.01$) than that of the Control group.

The Control group showed significantly low SV at 1, 2, and 3 weeks ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively), compared to values at baseline. Meanwhile, SV was significantly lower in the Enalapril group than at baseline at 1 week ($P < 0.01$), but returned to the baseline levels at 2 and 3 weeks. Therefore, the SV of the Enalapril group was significantly higher than that of the Control group at 2 and 3 weeks ($P < 0.01$).

The CO of the Control group was significantly low at 2 and 3 weeks ($P < 0.01$) compared to baseline values. However, no significant

Table 2. Value of echocardiographic parameter in Control group and Enalapril group.

Parameter	Group	Baseline	1 week	2 weeks	3 weeks
HR	Control	128 ± 33	142 ± 32	135 ± 44	122 ± 30
	Enalapril	140 ± 31	156 ± 23	150 ± 29	136 ± 32
LVIDd	Control	31.0 ± 2.2	29.5 ± 3.3	30.4 ± 2.6	29.2 ± 3.5
	Enalapril	30.0 ± 2.4	27.3 ± 1.5 **	30.0 ± 1.8	29.6 ± 2.0
LVIDs	Control	19.2 ± 1.9	21.2 ± 2.7	22.0 ± 3.5 ##	22.5 ± 4.0 ##
	Enalapril	18.8 ± 2.0	18.5 ± 2.0	20.2 ± 2.6	19.7 ± 1.6
FS	Control	38.2 ± 3.8	28.5 ± 4.5 ##	27.1 ± 6.5 ##	23.4 ± 5.6 ##
	Enalapril	37.3 ± 3.5	32.2 ± 5.4 ##, **	32.6 ± 7.5 #, *	33.9 ± 5.9 **
PEP/ET	Control	0.13 ± 0.03	0.27 ± 0.07 ##	0.30 ± 0.07 ##	0.33 ± 0.07 ##
	Enalapril	0.11 ± 0.03	0.21 ± 0.04 ##, **	0.17 ± 0.02 ##, **	0.18 ± 0.06 ##,**
SV	Control	17.0 ± 5.8	13.5 ± 2.5 ##	10.6 ± 2.3 #	10.9 ± 3.4 #
	Enalapril	18.4 ± 2.8	12.9 ± 3.0 ##	16.8 ± 5.9 **	16.8 ± 3.0 **
CO	Control	1.99 ± 0.48	1.90 ± 0.63	1.47 ± 0.49 ##	1.31 ± 0.34 ##
	Enalapril	2.1 ± 0.4	1.80 ± 0.5	2.1 ± 0.8 **	2.2 ± 0.8 **

All values are expressed as Mean ± Stadard deviation.
HR; heart rate, LVIDd; end-diastolic left venricle internal dimension, LVIDs; end-systolic left ventricle internal dimension, FS; fractional shortening, PEP/ET; pre ejection period/ ejection time, SV; stroke volume, CO; caridac output, Baseline; before rapid atrial pacing (RAP), 1 week; 1week after RAP, 2weeks; 2weeks after RAP, 3 weeks; 3weeks after RAP. Control; RAP with no medication, Enalapril; RAP with enarapril malerate administration.
#; P >0.05 vs Baseline, ##; P >0.01 vs Baseline, *; P >0.05 vs Control, **; P >0.01 vs Control.

differences were observed in the CO of the Enalapril group throughout the measurement period. Thus, CO of the Enalapril group was significantly higher than that of Control group at 2 and 3 weeks ($P < 0.01$).

Blood pressure measurements: The measurement values for each item are shown in Table 3. SBP values in the Control group were significantly lower throughout the measurement period at 1, 2, and 3 weeks ($P < 0.01$) than those at baseline. The SBP of the Enalapril group was also significantly low relative to the baseline value throughout the measurement period, at 1, 2, and 3 weeks ($P < 0.05$). At 2 weeks, the values for the Control group were significantly lower than those of the Enalapril group ($P < 0.05$).

The MBP values of the Control group were significantly lower throughout the measurement period, at 1, 2, and 3 weeks ($P < 0.01$, $P < 0.01$, and $P < 0.05$) than those at baseline. The SBP of the Enalapril group was also significantly lower than baseline values throughout the measurement period, at 1, 2, and 3 weeks ($P < 0.01$). However, no significant differences were observed between the two groups throughout the measurement period.

Finally, the DBP values for Enalapril group were significantly low throughout the measurement period, at 1, 2, and 3 weeks ($P < 0.01$) compared to baseline levels. In contrast, no significant changes were observed in the Control group. Further, no significant differences were noted between the two groups throughout the measurement period.

Histopathological examination: The primary pathological condition observed in this study was isolated cardiomyocyte degeneration or necrosis of varying degrees. Myocyte necrosis

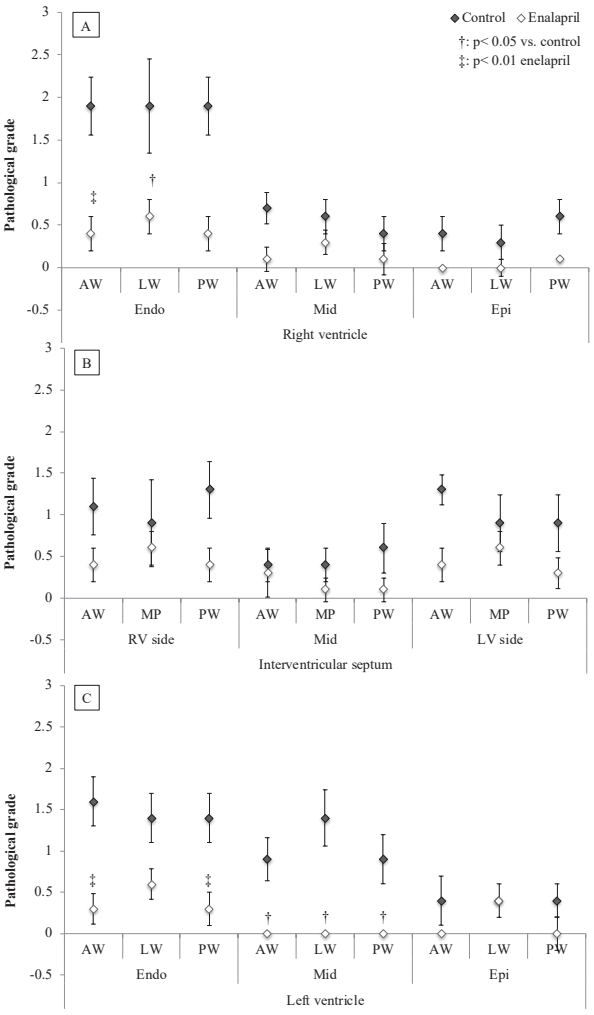

Fig. 4. Pathological grades in each area of the myocardium.
Black diamonds denote the Control group, and white diamonds denote the Enalapril group. RV, right ventricle; IVS, interventricular septum; LV, left ventricle; Endo, endomyocardium; Mid, midmyocardium; Epi, epimyocardium; AW, anterior wall; LW, lateral wall; PW, posterior wall; and MP, middle portion. †$P < 0.05$ and ‡$P < 0.01$ compared with the Control group. All values are shown as the mean ± standard error of the mean.
(A) Pathological grades of RV.
(B) Pathological grades of IVS.
(C) Pathological grades of LV.

was characterized by eosinophilic degeneration of the cytoplasm, a loss of cross-striations, and a pyknotic nucleus. Necrotizing myocytes were frequently observed in association with focal accumulation of mononuclear inflammatory cells. Subsequent evolution to interstitial and focal replacement fibrosis was often observed. These pathological changes were common to both the groups, although a difference in the degree was observed. Myocardial lesions were detected in the left and right ventricular free walls and IVS but were more obvious in the LV and RV. Furthermore, lesions were more severe in the endomyocardium (Endo), whereas the midmyocardium (Mid) and epimyocardium (Epi) were less severely affected.

Semiquantitative analysis showed significant differences in the degree of degenerative or necrotic changes in cardiomyocytes between the Control and Enalapril groups in AW-Endo of the RV (1.86 ± 0.34 and 0.43 ± 0.20, respectively), LW-Endo of the RV (1.86 ± 0.34 and 0.57 ± 0.20, respectively), AW-Endo of the LV (1.57 ± 0.30 and 0.29 ± 0.18, respectively), PW-Endo of the LV (1.43 ± 0.30 and 0.29 ± 0.18, respectively), AW-Mid of the LV (0.86 ± 0.26 and 0, respectively), LW-Mid of the LV (1.40 ± 0.30 and 0, respectively), and PW-Mid of the LV (1.43 ± 0.30 and 0, respectively). There was no significant difference in the myocardium of IVS between the groups (Fig. 4). The pathological changes of one dog in which cTnI was a reference value in the Control group tended to be mild. Further, the Enalapril group tended to exhibit lower pathological scores than the Control group for the right atrial muscle (control: 1.57 ± 0.37 vs. enalapril: 1.57 ± 0.30) and left atrial muscle (control: 1.23 ± 0.79 vs. enalapril: 1.00 ± 1.00) but no statistical significance was observed. Overall, pathological changes of cardiomyocytes were found primarily on the endocardial side of the left and right ventricular walls. All animals in this study were under cage management and did not show clinical signs related to heart failure.

Discussion

In the present study, histopathological observations indicated degeneration or necrosis in cardiac muscles of the canine model of SVTA. Injuries were more pronounced in the endocardial

Table 3. Volue of blood pressure measurement parameter in Control group and Enalapril group.

Parameter	Group	Baseline	1 week	2 weeks	3 weeks
SBP	Control	147 ± 19	130 ± 23 ##	119 ± 15 ##	125 ± 17 ##
(mmHg)	Enalapril	147 ± 11	137 ± 13 #	137 ± 14 #,*	132 ± 17 #
MBP	Control	110 ± 15	106 ± 10 ##	97 ± 13 ##	102 ± 16 #
(mmHg)	Enalapril	121 ± 16	103 ± 11 ##	101 ± 14 ##	100 ± 17 ##
DBP	Control	92 ± 19	94 ± 14 ±	85 ± 19 ±	91 ± 21
(mmHg)	Enalapril	100 ± 11	85 ± 10 ##	83 ± 16 ##	83 ± 18 ##

All values are expressed as Mean ± Standard deviation.
SBP; systolic blood pressure, MBP; mean blood pressure, DAP; diastolic blood pressure,
2weeks after RAP,
3 weeks; 3 weeks after RAP.
Control; RAP with no medication, Enalapril; RAP with enalapril maleate administration.
#; $P > 0.05$ vs. Baseline, ## ; $P > 0.01$ vs Baseline, *; $P > 0.05$ vs. Control

muscle than in the epicardial muscle. However, myocardial necrosis was remarkably attenuated by the administration of enalapril maleate, thereby demonstrating its cardioprotective effect in the canine model of SVTA. Moreover, the cTnI concentration, a biomarker of myocardial injury, was significantly elevated in dogs with SVTA. The administration of enalapril maleate consistently decreased the cTnI concentration, indicating its reliability as a biomarker of cardiac injury in the canine model of SVTA.

The present histopathological observations of cardiomyocyte degeneration or necrosis indicated marked myocardial injury in the dogs with SVTA. Similar observations are commonly reported for the cardiac tissue of human patients after sudden cardiac death, which further suggests cardiac ischemia[12]. In the present study, the myocyte degeneration or necrosis appeared more intensive in the Endo than in the Epi, which might be explained by the course of the coronary artery. In fact, the Epi is less susceptible to ischemic insults than the Endo since the originating coronary artery that supplies nutrients and oxygen to cardiac tissues runs on the surface of the Epi. Therefore, coronary blood is more stably supplied to the Epi than to the Endo[13]. Tachycardia has been shown to increase ventricular pressure, leading to a rise in ventricular wall stress, which, in turn, reduces the coronary flow reserve because of the increased oxygen demand and arterial compression[18]. Moreover, wall stress has been reported to be higher in the Endo than in the Epi[11, 13]. In the present study, cardiomyocyte degeneration or necrosis appeared to be more pronounced in the LV than in the RV. This might be attributed to the physiological mechanism of tachycardia, wherein coronary blood flow has a greater tendency to decrease in the LV than in the RV[13]. Therefore, the multiple factors discussed previously herein might be responsible for the differences in the degree of histopathological changes between the Endo and Epi, as observed in the present study.

In this study, the administration of enalapril maleate significantly reduced histopathological changes, such as eosinophilic degeneration, in the cardiac muscle, compared to those observed in the Control group. Nikolaidis et al.[21] reported that the administration of enalapril maleate reverses the reduction in subendocardial blood flow and vasodilator reserve in dogs with RAP. Although coronary arterial blood flow was not evaluated in this study, it is highly likely, considering the data reported by Nikolaidis et al.[21], that the myocardial protection provided by enalapril maleate can be largely attributed to the reversal of myocardial ischemia. Moreover, reduction in

the afterload and the resultant decrease in the ventricular wall stress could also contribute to the cardioprotective effect of enalapril maleate. In contrast, a variety of mechanisms underlying the myocardial protective action of ACEIs have been reported, including the inhibition of myocardial fibrosis by the reversal of collagen synthesis. The latter is stimulated by transforming growth factor-β1 or endothelin, or through the enhanced reduced activity of a collagen-degrading enzymes, namely matrix metalloproteinases[26], and via the regulation of autonomic nervous system activity[2]. In contrast, in rapid ventricular pacing dogs, hemodynamic improvements have been observed with vasodilators such as hydrazine and isosorbide nitrate, but their effects on myocardial fibrosis have been reported to be inadequate[17]. Therefore, the action of ACEI in controlling the activation of tissue RAAS might also contribute, at least in part, to the cardioprotective effect of enalapril maleate observed in the presented study.

Owing to the sustained RAP throughout the 3-week trial, the Control group in this study showed clearly lower values for FS after the first week, which continued throughout. A decrease in FS means a decrease in cardiac contraction, a reduced preload, or an increased afterload[7]. However, because no significant changes were observed in LVIDd, we concluded that the preload had not been reduced. Furthermore, because the SBP was reduced, we assumed that there was also no increase in the afterload. Therefore, the reduced FS observed in the Control group was considered to be due to a problem that had occurred in the heart muscle function itself owing to LV muscle injury. Furthermore, PEP/ET from Doppler measurements is used as an indicator to assess ventricular myocardial systolic function[7]. PEP/ET has a high value when myocardial contraction function is reduced and a low value when the contraction function accelerates[7]. In addition to the reduced FS in the Control group, the observation of a significant increase in PEP/ET gives stronger support to the theory of myocardial injury due to RAP and reduced LV contraction as a result of that. Furthermore, it is conceivable that this reduction in LV contraction function is also directly related to the reduction in SV and CO.

Meanwhile, the reduction in LV contraction function and CO observed in the Control group was significantly suppressed in the Enalapril group. This leads us to believe that the successful reduction in myocardial injury in the SVTA model dog induced by enalapril maleate was a major contributor. Therefore, we conclude that the administration of enalapril maleate to SVTA dogs not only controls microscopic pathological changes but is also extremely beneficial in controlling cardiac hypoactivity caused by the appearance of those pathological changes. In this study, lower blood pressure values were observed in both the Control and Enalapril groups. The reduction in blood pressure observed in the Control group is believed to perhaps be caused by lower CO values. However, because the Enalapril group had lower blood pressure values despite maintaining the same CO levels, we hypothesized the involvement of peripheral vascular resistance, caused by enalapril maleate. Therefore, no significant differences were observed between the two groups, but when using ACEI in clinical cases of dog SVTA, it is necessary to take complete precaution regarding excessive drops in blood pressure.

The cTnI has been widely used as a potential diagnostic biomarker of cardiac infarction in humans[31]. Furthermore, cTnI concentrations have been reported to be elevated in a variety of cardiovascular diseases including mitral insufficiency in dogs[32]. Therefore, the elevation in the cTnI concentration might reflect histopathological changes associated with a variety of cardiac muscle disorders[9] including myocardial necrosis at the time of myocardial infarction. In this study, an increase in the cTnI concentration was observed in the Control group, indicating myocardial damage such as eosinophilic degeneration after 3 weeks of RAP. Histopathological examination was only

performed at 3 weeks. However, myocardial injury was considered to become more severe over time. Hence, the histopathological change at 1 week would appear mild compared to that at 3 weeks. It seems likely that the measurement of cTnI concentrations can predict cardiac injury at an earlier stage. Meanwhile, no elevation in the cTnI concentration was observed in the Enalapril group, in which myocardial injury was attenuated, as shown based on histopathological analysis. These findings suggest that cTnI is a reliable biomarker of myocardial disorders in living dogs. Additionally, in the Control group, one dog showed no increase in cTnI. The pathological changes in the myocardium of this dog were mild compared to those in the other six dogs. This fact also supports the idea that cTnI measurements can effectively detect myocardial injury in SVTA patients. Moreover, our present findings show that measurements of the cTnI concentration is a simple and non-invasive method that can be useful not only for the prediction of myocardial injury but also for the assessment of the therapeutic effect of administered drugs in dogs with SVTA. Recently, several papers have been published on the relationship between cTnI elevation and prognosis in human atrial fibrillation (AF) patients[6, 20, 36]. According to them, elevated cTnI levels in AF patients were largely responsible for increased mortality and hospitalization, as well as adverse events such as stroke, pulmonary embolism, and major bleeding during treatment. In addition, there is a report on cTnI concentration and survival rate in dilated cardiomyopathy of dogs, demonstrating cardiac morphology and cardiac function similar to those in SVTA dogs, and an increase in cTnI concentration was found to be associated with poor prognosis[22]. Thus, measuring cTnI in SVTA dogs might be useful not only to detect myocardial injury, but also to predict prognosis.

This study has several limitations. First, it was difficult to determine the site of myocardial injury by measuring cTnI concentrations. Although myocardial injury was markedly observed in the LV, the damage was less pronounced in the RV of dogs from the Control group, which showed elevated cTnI concentrations. In addition, although both the right and left atrium showed histopathological changes in the Control and Enalapril groups, no significant difference was found. This suggests that the cTnI value mainly reflects the pathological change in the ventricular muscle with more muscle mass.

Second, the extent of myocardial injury, which is reflected by the cTnI concentration, is unknown. In the present study, definite, albeit less significant, myocardial injury was observed in the Enalapril group, despite the lack of an increase in the cTnI concentration. Third, this study assessed pathological changes in the myocardium only at the third week of RAP initiation. As cTnI results suggest that pathological changes might occur at an early stage of RAP initiation, it would be desirable to confirm pathological changes over time beginning in the early stages of RAP initiation by microscopy. Therefore, further studies are required to determine the extent or the site of myocardial injury. Finally, the reference cTnI values used in the present study were based on the data used at our institution; the precise baseline range of cTnI should be determined by measuring the cTnI concentration in blood samples obtained from a large number of healthy dogs.

In conclusion, cTnI was found to be a reliable biomarker of myocardial injury in dogs with SVTA. Moreover, cTnI was useful to assess the cardioprotective effects of enalapril maleate. Therefore, similar to that used in clinical settings for humans, cTnI measurements could be applicable for the diagnosis of myocardial injury and the evaluation of therapeutic effects of administered drugs in the veterinary field.

Conflict of interest

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