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Short Term Echocardiographic and Clinical Effects of Ramipril on Dogs with Asymptomatic Degenerative Mitral Valve Disease

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Original Article

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Abstract

Angiotensin converting enzyme (ACE) inhibitors have beneficial effects on degenerative mitral valve disease (DMVD) dogs with stages C and D (ACVIM classification) and on dogs with congestive heart failure. However, ACE inhibitors' effects on stage B2 or asymptomatic DMVD dogs have still been uncertain. Ramipril is an ACE inhibitor that has lipophilic effects and can suppress ACE in cardiac tissue effectively. We hypothesized that ramipril had beneficial effects on dogs with naturally occurring DMVD in stage B2. Twenty dogs with stage B2 DMVD, weighing between 3-12 kg and being older than 6 years, were recruited into the study. The dogs were single blinded randomized and divided into 2 groups. Owners made decisions whether or not to supplement their dogs with ramipril. Dogs in the ramipril group (n = 10) received ramipril once a day at dose of 0.22 mg/kg PO. The control group (n = 10) did not receive any drugs for 91 days. Complete physical examination, electrocardiography and echocardiography were performed on days 0, 28, 56 and 91. Echocardiographic examination was used to determine cardiac sizes and structural changes. Independent *t*-test was performed to compare differences between dogs in ramipril and control groups. Repeated ANOVA was used to compare differences within groups between days 0, 28, 56 and 91. $P < 0.05$ was considered statistically significant. Cardiac chamber size, systolic function and severity of mitral regurgitation were not significantly different between the 2 groups throughout the study period. In conclusion, ramipril did not affect cardiac size, severity of mitral regurgitation and systolic function in 91-day study period.

Keywords: angiotensin converting enzyme inhibitors, congestive heart failure, dog, mitral valve disease, ramipril

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บทคัดย่อ

ผลระยะสั้นของยารามิพริลต่อค่าคลื่นเสียงสะท้อนความถี่สูงและอาการทางคลินิกในสุนัขที่มีภาวะลิ้นหัวใจเสื่อมในกลุ่มที่ยังไม่แสดงอาการทางคลินิก

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ยากลุ่ม angiotensin converting enzyme (ACE) inhibitors ให้ผลดีในการรักษาสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อมระยะ C และ D (ACVIM classification) และสุนัขที่อยู่ในภาวะหัวใจล้มเหลว อย่างไรก็ตามการศึกษา ผลของ ACE inhibitors ในระยะ B2 หรือสุนัขที่ยังไม่แสดงอาการ ยังไม่มีข้อสรุปที่แน่ชัด รามิพริลเป็นยาในกลุ่ม ACE inhibitors ที่มีคุณสมบัติในการละลายในไขมันได้ดีทำให้สามารถยับยั้ง ACE ในเนื้อเยื่อของหัวใจได้อย่างมีประสิทธิภาพ คุณสมบัติการยับยั้ง ACE ในเนื้อเยื่อหัวใจนี้น่าจะส่งผลให้รามิพริลมีประสิทธิภาพในการรักษาสุนัขที่มีภาวะลิ้นหัวใจเสื่อมได้ดีกว่ายาตัวอื่นๆในกลุ่มเดียวกัน สมมติฐานการทดลอง คือ รามิพริล สามารถรักษาสุนัขที่ป่วยด้วยโรคลิ้นหัวใจไมทรัลเสื่อมแต่ยังไม่แสดงอาการ หรือในระยะ B2 ศึกษาในสุนัข 20 ตัวที่มีน้ำหนักระหว่าง 3-12 กก. มีอายุมากกว่า 6 ปี และป่วยเป็นโรคลิ้นหัวใจไมทรัลเสื่อมในระยะ B2 แบ่งสุนัขเป็น 2 กลุ่มโดย เจ้าของเป็นผู้เลือกว่าจะให้ยาสุนัขหรือไม่ กลุ่มที่ได้รับยาจำนวน 10 ตัว ได้รับยารามิพริลปริมาณ 0.22 มก.ต่อกก. วันละครั้ง กลุ่มที่ไม่ได้รับยาจำนวน 10 ตัวเป็นกลุ่มควบคุมระยะเวลาการศึกษา 91 วัน สุนัขทุกตัวได้รับการตรวจร่างกายทางคลินิก ตรวจคลื่นไฟฟ้าหัวใจ และตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงในวันที่ 0 28 56 และ 91 การศึกษาขนาดและโครงสร้างของหัวใจใช้ค่าของการตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเป็นตัวประเมิน การวิเคราะห์ทางสถิติใช้ independent T-test ในการเปรียบเทียบระหว่างกลุ่มที่ให้ยารามิพริลกับกลุ่มควบคุม และใช้ Repeated ANOVA ในการเปรียบเทียบในกลุ่มเดียวกันระหว่างวันที่ 0 28 56 และ 91 ค่า $p < 0.05$ บ่งชี้ว่ามีนัยสำคัญทางสถิติ ผลการศึกษาพบว่า ขนาดของหัวใจ ความสามารถในการบีบตัวของหัวใจและความรุนแรงของภาวะลิ้นหัวใจไมทรัลเสื่อมของสุนัขทั้ง 2 กลุ่มไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติตลอดระยะเวลา 91 วันที่ศึกษา

คำสำคัญ: กลุ่มยายับยั้งแองจิโอเทนซินคอนเวอร์ตติงเอนไซม์ ภาวะหัวใจล้มเหลว สุนัข โรคลิ้นหัวใจไมทรัล รามิพริล

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Introduction

Degenerative mitral valve disease (DMVD) is a contribution of cardiovascular morbidity and mortality in dogs (Egenvall et al., 2006). This chronic progressive disease increases incidence in small breeds of old dogs and is found more frequently in males than females. (Häggström et al., 2009). DMVD is characterized by a long preclinical period. It has been found in about 3-7% of all canine population (Borgarelli and Buchanan, 2012). Some dogs may develop heart failure in a short period of time whereas some dogs may stay healthy and have no clinical signs or progress to heart failure for several years (Kvart et al., 2002). The incidence is particularly high and shows strong component to a polygenic mode of inheritance in some breeds, for example, Dachshund and Cavalier King Charles spaniel (CKCS) (Swift, 1996). The prevalence of DMVD is strongly associated with age and breed. In early stage, affected valves may function adequately without hemodynamic effects (Borgarelli et al., 2011). The clinical signs of DMVD vary from dogs to dogs including cough, anorexia, exercise intolerance, dyspnea to sudden death. DMVD involves complex connective tissue degeneration. The histological changes by excessive destruction and derangement of valve layer structure with accumulation of glycosaminoglycans in the leaflet and chordae tandinae (Fox, 2012). In later stage, mitral valves and chordae tendinae severely thicken and become redundant, causing improper leaflet coaptation and regurgitation of blood across the closed mitral valve during ventricular systole. The valve regurgitation leads to drop in forward stroke volume, cardiac output and increased intraatrial pressure resulting in renin angiotensin aldosterone system (RAAS) activation to maintain cardiac output. The compensatory process is able to maintain cardiac output for a long period of time. Once the heart fails to compensate, the decompensatory heart failure develops and death can occur.

Several reports have proven that angiotensin converting enzyme (ACE) inhibitors benefit management of congestive heart failure in dogs by increasing survival rate, improving quality of life in dogs with classes II and III International Small Animal Cardiac Health Council (ISACHC) classification heart failure (The BENCH Study group, 1999; Häggström et al., 2008). The beneficial effects of ACE inhibitors in other stages of heart failure particularly in asymptomatic dogs have been debated. Previous multicenter double blinded studies found that ACE inhibitors did not have significant effects in treatment asymptomatic dogs with DMVD (Kvart et al., 2002; Atkins et al., 2007). A retrospective study of Pouchelon et al. (2008) reported a possible benefit of early treatment with benazepril in asymptomatic dogs with DMVD. Thus, the beneficial effects of ACE inhibitors in asymptomatic DMVD dogs are uncertain and remain in the area of debate.

ACE is present in both plasma and tissues. In addition, there are differences in the relative tissue or circulating affinity of ACE inhibitors (Apurva et al., 2005). Ramipril is a long acting ACE inhibitor, which has a lipophilic property. Therefore, it can penetrate to local tissues and suppress ACE releasing there effectively. Erman et al. (1991) demonstrated that ramipril had high tissue affinity property and could reduce both tissue and plasma ACE activity. Ramipril is one of ACE inhibitors in dicarboxylate containing agent group. ACE inhibitors in this group are prodrugs or precursors that need to be converted to active metabolites by liver esterase enzyme before they can works. Because of a more lipophilic effect and a long acting duration, ramipril may be superior to other ACE inhibitors in treatment of DMVD dogs. Several reports show that use of ramipril is safer than the other ACE inhibitors in renal impairment patients (Hope study investigators, 2000; Lefebvre et al., 2006). The study of ramipril in naturally occurring DMVD is still lacking, particularly in asymptomatic dogs. We hypothesized that ramipril had beneficial effects on dogs with naturally occurring canine DMVD in stage B2 (ACVIM classification) or dogs with DMVD that have no clinical signs but have cardiac structural changes. The aim of this study was to evaluate short term echocardiographic and clinical effects of ramipril in naturally occurring asymptomatic stage B2 DMVD dogs (ACVIM classification).

Materials and Methods

This study was a single blinded randomized prospective study. All dogs enrolled into the study were patients of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The protocol used in the present study was approved by Chulalongkorn University Animal Care and Use Committee. Dogs affected with stage B2 DMVD (ACVIM classification) (Atkins et al., 2009) presented at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University were included in the study. Due to possible differences in the nature of DMVD between small and large breeds of dogs (Borgarelli et al., 2004), only small breeds that weighed between 3-12 kg and were older than 6 years were selected for study. All dogs had systolic murmur heard best over the left cardiac apex or the mitral area. The dogs underwent complete physical examination, cardiac auscultation, blood collection, radiography, echocardiography and electrocardiography. Results were collected as baseline data. Thoracic radiography was performed to assess hemodynamic significance of DMVD and rule out primary respiratory diseases as well as signs of pulmonary edema secondary to congestive heart failure. Echocardiography was used to confirm the diagnosis of mitral valve degeneration. Cardiac remodeling was determined by M-mode echocardiography including enlarged left atrium (increased left atrium to aorta dimension ratio more than 1.3 (Boon et al., 1983) and/or increased left ventricular chamber size with decreased left ventricular wall thickness. Blood samples were submitted to evaluate renal and liver status before starting the study and to assess clinical tolerance as well as undesirable side effects from ramipril after enrollment in the study. All dogs with acute and chronic renal insufficiency (defined as creatinine(Cr) above 1.8 mg/dl and BUN above 27mg/dl) (Douglass et al., 2005) were excluded from the study. Due to teratogenic effects of ramipril, the study was not performed in pregnant and lactating bitches. Dogs with clinical signs of heart failure including ascites, dyspnea, exercise intolerance, cough or dogs receiving any drugs that might have effects on cardiovascular system and dogs with other kinds of heart diseases or abnormalities rather than DMVD were excluded from the study.

Because there have not been any standard protocols for treatment of dogs with stage B2 DMVD recently (Atkins et al., 2009), the owners themselves made decisions whether or not to supplement their dogs with the drug. In the ramipril group, dogs were supplemented with ramipril 0.125 mg/kg per OS once a day for 91 days. Dogs in the control group did not receive any drugs. Complete physical examination, electrocardiography and echocardiography were performed on days 0, 28, 56, and 91. Radiography and blood collection for hematology and biochemistry analysis were performed on days 0 and 91. Quality of life and clinical signs were evaluated by clinical score in Table 1. Cardiac structure remodeling was evaluated from two dimensional and M-mode echocardiography assessed by ultrasound machine (LogicTM5 Pro) with multi-frequency 6-10 MHz microconvex and 5-6 MHz phrase array transducers. Left ventricular internal diastolic diameter (LVIDd), left ventricular internal systolic diameter (LVIDs), left ventricular free wall thickness during diastole (LVWd) and systole (LVWs), ventricular septal thickness during diastole (VSd) and systole (VSs), and the ratio of left atrium to aorta dimension (LA/Ao) were measured from right parasternal short and long axis views. To evaluate the chamber size and wall thickness, the echocardiographic indices including LVIDdi (LVIDd/Body surface area (BSA)), LVIDsi (LVIDs/BSA), VSdi (VSd/BSA), VSsi (VSs/BSA), LVWdi (LVWd/BSA), and LVWsi (LVWs/BSA) were used to reduce body weight variation between dogs. Fractional shortening was calculated by {(LVEDd- LVEDs)/LVEDdx100}.

Table 1 Scoring protocols for clinical signs

Clinical signs	Score
Appetite	I : decreased appetite, II : normal appetite, III : increased appetite
Cough	I : normal, II : few cough, III : cough all day
Exercise intolerance	I : dog is able to fully exercise, II : Dog is less active than normal, avoided long walk, III : Dog is inactive and will only get up to eat and drink
Attitude	I : alert and responsive, II : moderately lethargic, III : minimal responsive
Respiratory effort	I : normal, II : increase rate or effort, III : severe respiratory distress

Modified from: Häggström et al., 2008.

Regurgitant flow velocity (MR), area of regurgitant jet as a proportion to the area of left atrium (ARJ/LA) and Proximal Isovelocity Surface Area (PISA) were measured for evaluating severity of mitral valve regurgitation. All echocardiographic examinations were performed in conscious un-sedated dogs.

The clinical score was evaluated as descriptive analysis. Independent *t*-test was used to compare echocardiographic values between ramipril and control groups on days 0, 28, 56 and 91. Repeated ANOVA was used to compare differences within control and ramipril groups. Paired *t*-test was used to compare differences within groups between days 0, 28, 56 and 91. *p*-value less than 0.05 was considered statistically significant.

Results

A total number of 23 dogs enrolled in the study. Twelve dogs (9 males and 3 females) were assigned to the ramipril group while the other 11 dogs (5 males and 6 females) were in the control group. In the ramipril group, two dogs were excluded from the study due to an increase in creatinine concentration in one dog (Cr > 1.8 mg/dl) and death from car accident in the other. In the control group, one dog was excluded due to the car accident. Therefore, only 20 dogs were included for final analysis. The intensity of heart murmur of all dogs in the control group was grade IV. Two dogs had grade III and 8 dogs had grade IV murmur in the ramipril group. The control group consisted of 4 males and 6 females. Breeds included 7 Poodles, 2 mixed breeds and 1 Miniature Pinscher. Ten dogs in the ramipril group consisted of 7 males and 3 females, including 2 mixed breeds, 2 Chihuahuas, 2 Poodles and one each of Splitz, Shih tzu, Yorkshire Terrier and Dachshund. At baseline, age, weight, heart rate, respiratory rate were similar and not statistically different between the control and ramipril groups (Table 2). The average dose of ramipril in the ramipril group was 0.18 ± 0.03 mg/kg (ranging from 0.14-0.22 mg/kg).

Effects of ramipril on echocardiographic values: LVIDdi and LA/Ao parameters were used to evaluate cardiac chamber size. LVIDdi and LA/Ao were not statistically different between the control and ramipril groups on days 0, 28, 56 and 91 (Fig 1). To assess the degree of mitral regurgitation between the control and ramipril groups, the MR, ARJ/LA and PISA were determined. The results of PISA, MR and ARJ/LA showed insignificant difference between the control and ramipril groups on days 0, 28, 56 and 91 (Fig 2). %FS and LVIDsi were used to assess systolic function. Both values were not statistically different between the two groups throughout 91 days of the study (Fig 3). The echocardiographic values are presented in Table 3.

Progressive of disease in control and ramipril groups: The progressive of disease in the control and ramipril groups was studied on days 0, 28, 56 and 91. On the control group, PISA on days 28 and 56 was the same as day 0. In day 91, PISA was significantly different compared to day 0 (p = 0.01). ARJ/LA changed significantly on day 56 compared to day 0 and day 28 (p = 0.03) but not different from day 91 (Table 4). In the ramipril group, all echocardiographic values were not statistically different between days 0, 28, 56 and 91 (Table 5).

Effects of ramipril on clinical signs, potential adverse reactions and causes of withdrawal: Three dogs in the ramipril group showed an improvement of clinical status. Two dogs increased appetite and one dog reduced frequency of cough. One dog was withdrawn because of increase in Cr and BUN after two months of treatment with ramipril.

Table 2 Baseline characteristics of 20 dogs with degenerative mitral valve disease

Variable	Ramipril (n = 10)	Control group (n = 10)	*p*-value
Age (years)	11.70 ± 2.41	12.10 ± 2.51	0.72
Weight (kg)	7.27 ± 3.17	6.59 ± 3.30	0.65
Heart rate (bpm)	145.60 ± 15.85	137.00 ± 21.10	0.39
Respiratory rate (rpm)	39.00 ± 7.95	36.80 ± 7.89	0.25

Data presented as mean ± SD (bpm: beat per minute, rpm: respiratory rate per minute)

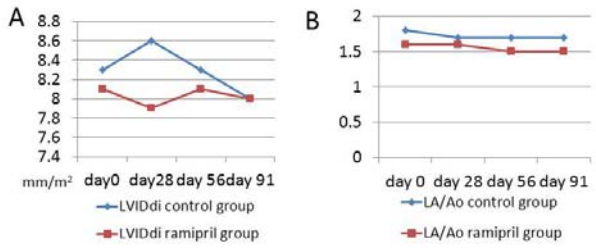

Figure 1 Effect of ramipril on LVIDdi and LA/Ao. These graphs show echocardiographic value on days 0, 28, 56 and 91 of ramipril and control groups. (A) LVIDdi (Left ventricular internal diameter diastolic index, (B) LA/Ao (Left atrial/Aorta).

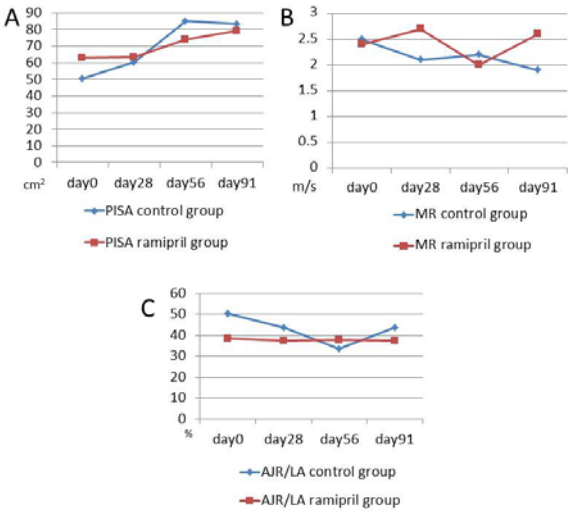

Figure 2 Effect of ramipril on PISA, MR and AJR/LA. These graphs show PISA, MR and AJR/LA on days 0,28, 56 and 91 of control and ramipril groups. (A) PISA (Proximal Isoveloity Surface Area), (B) MR (Mitral regurgitant flow velocity), (C) AJR/LA(area of regurgitant jet as a proportion to the area of left atrium).

Effect of ramipril on heart rate, respiratory rate, heart murmur grade and electrocardiography: Heart rate and respiratory rate were not significantly different between the ramipril and control groups throughout the study period (Table 6). Grade of heart murmur in the control group was grade IV in all dogs throughout 91 days. In the ramipril group, heart murmur was grade III in 2 dogs and grade IV in 8 dogs. On days 28, one dog in the ramipril group had decreased

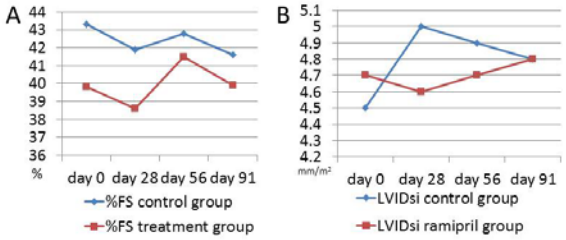

Figure 3 Effects of ramipril on %FS and LVIDsi. These graphs show %FS and LVIDsi on days 0, 28, 56 and 91 of ramipril and control groups. (A) %FS (Fraction shortening), (B) LVIDsi (Left ventricular internal diameters systolic index).

intensity of heart murmur from grade IV to grade III. On day 91, heart murmur in 2 dogs was increased intensity from grade III to grade IV. Electrocardiography was normal and no evidence of arrhythmia was seen in both control and ramipril groups.

Effects of ramipril on complete blood count and blood chemistry: SGPT in the control group was higher than in the ramipril group on day 0 (p = 0.04). However, the SGPT was still in the normal limit in both groups (Table 7). Values of other blood parameters between the control and ramipril groups were not significantly different on days 0 and 91. All blood hematology and blood chemistry values in the control group between day 0 and day 91 were not significantly different. In the ramipril group, platelet number on day 91 decreased compared to day 0 (p = 0.03) and white blood cell count on day 91 increased significantly compared to day 0 (p = 0.01) (Table 8).

Table 3 Baseline characteristics of 20 dogs with degenerative mitral valve disease

Variables	Day0		Day 28		Day 56		Day 91	
	mean ± SD	*p*-value	mean ± SD	*p*-value	mean ± SD	*p*-value	mean ± SD	*p*-value
LVIDdi (mm/m²)								
Control	8.30 ± 1.60	0.74	8.60 ± 2.00	0.41	8.30 ± 1.60	0.74	8.00 ± 1.50	0.94
Ramipril	8.10 ± 1.20		7.90 ± 1.40		8.10 ± 1.20		8.00 ± 1.60	
LA/Ao								
Control	1.80 ± 0.30	0.07	1.70 ± 0.30	0.30	1.70 ± 0.50	0.66	1.70 ± 0.50	0.21
Ramipril	1.60 ± 0.10		1.60 ± 0.20		1.50 ± 0.20		1.50 ± 0.20	
PISA(cm²)								
Control	0.98 ± 0.45	0.18	1.26 ± 0.37	0.78	1.71 ± 0.79	0.64	1.75 ± 0.78	0.96
Ramipril	1.56 ± 1.21		1.19 ± 0.59		1.54 ± 0.79		1.73 ± 1.05	
MR (m/s)								
Control	4.50 ± 0.80	0.85	4.60 ± 0.90	0.55	4.90 ± 1.10	0.62	4.80 ± 1.10	0.94
Ramipril	4.70 ± 1.30		5.00 ± 1.10		4.70 ± 1.00		4.80 ± 1.20	
AJR/LA								
Control	50.50 ± 20.10	0.05	43.70 ± 10.40	0.25	33.60 ± 10.80	0.43	43.80 ± 15.10	0.19
Ramipril	38.30 ± 18.00		37.30 ± 17.60		37.90 ± 16.80		37.40 ± 13.70	
%FS								
Control	43.30 ± 7.50	0.27	41.90 ± 6.40	0.11	42.84 ± 6.00	0.83	41.60 ± 6.20	0.54
Ramipril	39.80 ± 6.30		38.60 ± 4.80		41.50 ± 7.70		39.90 ± 6.00	
LVIDsi (mm/m²)								
Control	4.50 ± 0.80	0.85	5.00 ± 1.10	0.55	4.90 ± 1.10	0.62	4.80 ± 1.10	0.48
Ramipril	4.70 ± 1.30		4.60 ± 0.90		4.70 ± 1.00		4.80 ± 1.20	

LVIDdi : left ventricular diameter diastolic index; LA/Ao : left atrial /aorta ratio; PISA : Proximal Isovelocity Surface Area; MR : mitral regurgitant flow velocity; ARJ/LA : atrial regurgitant jet/left atrial area; % FS : % fraction shortening; LVIDsi : Left ventricular internal diameter systolic index

Table 4 Comparison of echocardiographic values obtained from dogs in control group on days 0, 28, 56 and 91

Variables	Day 0	Day 28	Day 56	Day 91	*p*-value
IVSsi	3.30 ± 0.70	3.30 ± 0.70	3.20 ± 0.80	3.40 ± 0.80	0.67
LVIDsi	4.50 ± 0.80	4.60 ± 0.90	4.90 ± 1.10	4.80 ± 1.10	0.42
LVFWsi	3.00 ± 0.50	3.10 ± 0.40	3.10 ± 0.50	2.90 ± 0.40	0.22
IVSdi	2.10 ± 0.40	2.40 ± 0.20	2.30 ± 0.30	2.20 ± 0.20	0.24
LVIDdi	8.30 ± 1.60	8.60 ± 2.00	8.30 ± 1.60	8.00 ± 1.50	0.44
LVFWdi	2.00 ± 0.30	2.00 ± 0.30	1.90 ± 0.30	1.80 ± 0.30	0.66
Lai	6.50 ± 1.70	6.40 ± 1.70	6.40 ± 1.70	6.80 ± 1.90	0.39
Aoi	3.50 ± 0.50	3.80 ± 0.70	3.90 ± 0.80	3.80 ± 0.60	0.30
LA/Ao	1.80 ± 0.30	1.70 ± 0.30	1.70 ± 0.50	1.70 ± 0.50	0.57
PISA	0.98 ± 0.45	1.26 ± 0.37	1.71 ± 0.79	1.75 ± 0.78[a]	0.01
MR	2.50 ± 1.40	2.10 ± 1.20	2.20 ± 1.80	1.90 ± 1.00	0.52
ARJ/LA	50.50 ± 20.10	43.70 ± 10.40	33.60 ± 10.80 [a,b]	43.80 ± 15.10	0.03
%FS	43.30 ± 7.50	41.90 ± 6.40	42.80 ± 6.00	41.60 ± 6.20	0.84

Data are illustrated in mean ± SD

[a] $p < 0.05$ indicates significant difference from day 0

[b] $p < 0.05$ indicates significant difference from day 28

Table 5 Comparison of echocardiographic values obtained from dogs receiving ramipril on days 0, 28, 56 and 91

Variables	Day 0	Day 28	Day 56	Day 91	*p*-value
IVSsi	2.90 ± 0.80	3.00 ± 0.60	3.10 ± 0.70	3.20 ± 1.00	0.14
LVIDsi	4.70 ± 1.30	5.00 ± 1.10	4.70 ± 1.00	4.80 ± 1.20	0.66
LVFWsi	2.80 ± 0.70	2.90 ± 0.60	2.90 ± 0.60	2.80 ± 0.50	0.74
IVSdi	2.20 ± 0.60	2.10 ± 0.50	2.10 ± 0.60	2.20 ± 0.50	0.87
LVIDdi	8.10 ± 1.20	7.90 ± 1.40	8.10 ± 1.20	8.00 ± 1.60	0.87
LVFWdi	2.00 ± 0.50	2.00 ± 0.60	2.10 ± 0.50	1.90 ± 0.30	0.63
Lai	6.20 ± 1.50	6.10 ± 1.20	5.90 ± 1.00	6.00 ± 1.30	0.76
Aoi	3.70 ± 0.80	3.80 ± 0.60	3.80 ± 0.60	3.90 ± 0.70	0.46
LA/Ao	1.60 ± 0.10	1.60 ± 0.20	1.50 ± 0.20	1.50 ± 0.20	0.90
PISA	1.55 ± 1.21	1.19 ± 0.59	1.54 ± 0.79	1.73 ± 1.05	0.39
MR	2.40 ± 1.40	2.70 ± 1.60	2.00 ± 1.40	2.60 ± 1.30	0.53
ARJ/LA	38.30 ± 18.00	37.30 ± 17.60	37.90 ± 16.80	37.40 ± 13.70	0.99
%FS	39.80 ± 6.30	38.60 ± 4.80	41.50 ± 7.70	39.90 ± 6.00	0.99

Data are illustrated in mean ± SD

Table 6 Heart rate assessed by electrocardiography and respiratory rate in control and ramipril groups

Variables	Day0		Day 28		Day 56		Day 91	
	mean ± SD	*p*-value	mean ± SD	*p*-value	mean ± SD	*p*-value	mean ± SD	*p*-value
Heart rate								
Control	137.0 ± 24.6	0.34	140.0 ± 22.1	0.69	140.0 ± 13.3	1.00	139.0 ± 21.2	0.70
Ramipril	145.0 ± 15.1		142.0 ± 9.9		142.0 ± 22.9		143.0 ± 24.9	
Respiratory rate								
Control	37.0 ± 8.0	0.58	40.2 ± 8.9	0.47	41.2 ± 7.7	0.37	41.4 ± 9.2	0.86
Ramipril	39.0 ± 7.9		42.6 ± 10.8		43.2 ± 9.0		39.6 ± 7.8	

Discussion

This study aimed to investigate the short-term effect of ramipril in dogs with stage B2 DMVD. The study demonstrated that ramipril did not affect cardiac chamber size, mitral regurgitation severity and systolic function assessed by echocardiography in 91-day period of treatment.

Renin-angiotensin-aldosterone system (RAAS) is a complex neurohormonal compensatory system functioning to maintain blood pressure and tissue perfusion. In DMVD, the valvular structure deformation can cause ineffective valve coaptation resulting in regurgitation of blood back into the left atrium as well as decrease in forward stroke volume and blood pressure. This phenomenon activates RAAS by releasing renin from juxtaglomerular apparatus. Renin, then, breakdowns angiotensinogen to angiotensin I (ATI). ATI is converted into angiotensin II (ATII) by angiotensin-converting enzyme in the lungs. ATII, a potent vasoconstrictor, activates sympathetic nervous system (SNS) and increases the release of aldosterone and anti-diuretic hormone (ADH) to maintain cardiac output, blood pressure and tissue perfusion in normal state (Sisson, 2004). This mechanism is useful in acute phrase of hypotension. However, in chronic phrase, ATII releases growth factors that promote remodeling of vessels and myocardium resulting in decreased vascular compliance and increased afterload. In long term, ATII causes pathological ventricular hypertrophy, myocardial necrosis from the cytotoxic effect and loss of myocardium contractility causing cardiac systolic dysfunction. Moreover, chronic activation of SNS and RAAS will increase the cardiac preload by increasing blood volume and venous return to the heart leading to excessive volume retention and eccentric hypertrophy or cardiac dilatation. The chronic volume overload leads to ventricular remodeling, ventricular dysfunction and heart failure (Dávila et al., 2005). ACE inhibitors have been used to reduce effect of AII, RAAS stimulation and over compensatory mechanism which can cause heart failure.

Table 7 Comparison of blood hematology and chemistry values obtained from dogs in control and ramipril groups at day 0 and 91

Variables		Day0		Day 91	
		mean ± SD	*p*- value	mean ± SD	*p*- value
RBC (x 10^6)	Control	6.39 ± 1.19	0.96	6.25 ± 1.37	0.15
	Ramipril	6.41 ± 1.03		7.01 ± 0.87	
Hb (g/dl)	Control	14.57 ± 2.52	0.51	14.60 ± 3.27	0.87
	Ramipril	15.30 ± 2.36		14.80 ± 3.39	
Hct (%)	Control	43.70 ± 8.01	0.50	43.80 ± 8.00	1.00
	Ramipril	46.00 ± 6.85		43.80 ± 8.70	
WBC (x 10^3)	Control	16.60 ± 8.33	0.06	15.37 ± 6.18	0.62
	Ramipril	10.37 ± 4.78		14.16 ± 4.70	
Neutrophil (x 10^3)	Control	12.21 ± 7.11	0.07	10.91 ± 4.17	0.13
	Ramipril	7.28 ± 3.66		8.06 ± 3.99	
Band cell	Control	21.90 ± 57.8	0.47	13.00 ± 41.10	0.34
	Ramipril	46.30 ± 80.2		0.00	
Monocyte (x 10^2)	Control	11.80 ± 12.3	0.33	12.81 ± 11.17	0.92
	Ramipril	7.28 ± 7.44		12.35 ± 11.10	
Eosinophil (x 10^2)	Control	11.91 ± 15.51	0.33	15.44 ± 25.01	0.26
	Ramipirl	6.66 ± 5.51		6.05 ± 2.80	
Lymphocyte (x 10^3)	Control	2.34 ± 1.47	0.20	2.07 ± 11.43	0.86
	Ramipril	1.61 ± 9.65		2.15 ± 11.10	
Platelet (x 10^4)	Control	29.51 ± 7.60	0.16	30.34 ± 74.08	0.72
	Ramipril	32.99 ± 16.35		32.34 ± 10.43	
SGPT (Unit/litre)	Control	65.20 ± 28.60	0.04*	66.00 ± 36.80	0.51
	Ramipril	40.10 ± 22.10		55.00 ± 37.40	
ALP (Unit /litre)	Control	157.00 ± 133.00	0.77	120.30 ± 61.90	0.56
	Ramipril	140.00 ± 136.00		154.00 ±163.00	
BUN (mg/dl)	Control	15.50 ± 4.93	0.50	24.60 ± 11.50	0.70
	Ramipril	17.67 ± 8.84		27.70 ± 22.60	
CREA (mg/dl)	Control	0.89 ± 0.32	0.98	0.93 ± 0.23	0.69
	Ramipril	0.89 ± 0.19		0.97 ± 0.21	

P < 0.05 indicates significant difference between control and ramipril groups (RBC : red blood cell, Hb : hemoglobin, Hct : hematocrit, WBC : white blood cell, SGPT : serum glutamic pyruvic transaminase, ALP : alkaline phosphatase, BUN : blood urea nitrogen, CREA : creatinine)

Table 8 Comparison of blood hematology and chemistry values obtained from dogs in control and ramipril groups

Blood parameter		Control	*p*-value	Ramipril	*p*-value
RBC (x 10^6)	Day 0	6.39 ± 1.18	0.59	6.41 ± 1.03	0.14
	Day 91	6.25 ± 1.36		7.01± 0.87	
Hb (g / dl)	Day 0	14.57 ± 2.52	0.96	15.30 ± 2.35	0.59
	Day 91	14.60 ± 3.27		14.83 ± 3.38	
Hct	Day 0	43.70 ± 8.01	0.96	46.00 ± 6.84	0.35
	Day 91	43.80 ± 8.09		43.80 ± 8.76	
WBC (x 10^3)	Day 0	16.60 ± 83.32	0.66	10.37 ± 4.78	0.01*
	Day 91	15.37 ± 61.03		14.18 ± 4.25	
Neutrophil (x 10^3)	Day 0	12.21 ± 71.13	0.65	7.28 ± 3.66	0.49
	Day 91	10.91 ± 41.77		8.06 ± 3.99	
Band cell	Day 0	21.85 ± 51.82	0.35	46.30 ± 80.24	0.30
	Day 91	0		13.00 ± 41.10	
Monocyte (x 10^2)	Day 0	11.83 ± 12.39	0.54	7.27 ± 7.44	0.08
	Day 91	12.81 ± 11.17		12.35 ± 11.10	
Eosinophil (x 10^2)	Day 0	11.91 ± 15.51	0.35	6.65 ± 5.50	0.73
	Day 91	15.43 ± 25.01		6.04 ± 2.80	
Lymphocyte (x 10^3)	Day 0	23.44 ± 14.77	0.12	1.61 ± 0.96	0.25
	Day 91	20.70 ± 11.43		2.15 ± 1.10	
Platelet (x 10^4)	Day 0	29.51 ± 7.60	0.72	37.99 ± 16.35	0.03*
	Day 91	30.34 ± 7.40		32.34 ± 10.43	
SGPT (Unit /litre)	Day 0	65.20 ± 28.56	0.94	40.10 ± 22.05	0.08
	Day 91	66.00 ± 36.82		55.00 ± 37.40	
ALP (Unit /litre)	Day 0	157.00 ± 132.52	0.34	139.60 ± 135.76	0.62
	Day 91	120.30 ± 61.90		153.50 ± 163.26	
BUN (mg/dl)	Day 0	15.50 ± 4.92	0.06	17.67 ± 8.84	0.17
	Day 91	24.60 ± 11.47		27.70 ± 22.63	
CREA (mg/dl)	Day 0	0.89 ± 0.32	0.71	0.89 ± 0.19	0.73
	Day 91	0.93 ± 0.23		0.97 ± 0.21	

**p* < 0.05 indicates significant difference between day 0 and 91 (RBC : red blood cell, Hb : hemoglobin, Hct : hematocrit, WBC : white blood cell, SGPT : serum glutamic pyruvic transaminase, ALP : alkaline phosphatase, BUN : blood urea nitrogen, CREA : creatinine)

Despite the common mechanism of action, ACE inhibitors differ with regard to properties such as half-life, duration of action, active metabolites, affinity to ACE, lipo- or hydrophilicity. These different properties may account for different response to various ACE inhibitors in the same patients. Ramipril is an ACE inhibitor that is widely used to treat heart failure in human medicine. However, clinical study of ramipril in veterinary medicine is still lacking. Ramipril is a precursor that has to metabolite into an active product, ramiprilat in the liver before it works. Ramipril has lipophilic and high affinity to ACE. Thus, it can penetrate tissues and suppress systemic and local RAAS more effectively than other ACE inhibitors that have low tissue ACE affinity such as enalapril and lisinopril (Wolfgang, 1992). Pilote et al. (2008) found that ramipril could decrease mortality rate in human with congestive heart failure more effectively compared to enalapril and captopril. A previous study in humans showed the beneficial effects of ramipril in lowering the risk of mortality in patients that had cardiovascular problem (Hope study, 2000). A study of Hartman et al. (1993) found that local angiotensin II might play a role as a growth factor that could promote cardiac hypertrophy in heart failure condition. Due to a high density of angiotensin II receptors and angiotensin converting enzyme (ACE) in canine myocardium (Dell'italia et al., 1997), use of high lipophilic ACE inhibitors such as ramipril may suppress local renin angiotensin aldosterone system (RAAS) in the myocardium more effectively than other ACE inhibitors such as enalapril or captopril (Kvart et al., 2002). However, the present study did not show any beneficial effects of ramipril compared with the untreated group. Firstly, this lack of effect may occur from other angiotensin II forming pathways in cardiac tissues (McDonald et al., 2001). Chymase is another pathway that can trigger angiotensin II from angiotensin I (Balcells et al., 1996). Ramipril can suppress ACE pathway but not chymase pathway. In other words, ramipril cannot completely inhibit production of angiotensin II. Thus, hemodynamic changes secondary to RAAS stimulation can still occur. Secondly, RAAS may not be fully activated in asymptomatic DMVD dogs. A human study by Francis et al. (1990) showed that plasma renin in patients affected by left ventricular dysfunction without heart failure was not higher than normal patients, suggesting that RAAS had not been activated. Same as in people, the RAAS was suggested not to be stimulated in the heart diseased dogs without congestive heart failure (Häggström et al., 1997). However, the RAAS stimulation has not been studied in stage B2 DMVD dogs yet. Thirdly, the short duration of the study may not be enough to show changes of disease that have long progressive period like DMVD. Lastly, the small sample size may affect the power of the study.

For the clinical effects of ramipril, one dog in the ramipril group had decreased cough frequency and two dogs increased in appetite. These findings suggest that ramipril might improve clinical status of diseased dogs. The beneficial effects of ACE inhibitors on improvement in clinical signs of dogs with DMVD have been previously reported (The COVE study, 1995; The improve study, 1999). The effect of ramipril on blood chemistry values and complete blood count was determined before and after treatment. The blood chemistry values of dogs in the ramipril group were not significantly different on days 0 and 91. Most of the dogs were well-tolerated to ramipril except one dog that had azotemia which was transient and did not need specific treatment. This result indicates that ramipril is similar to other ACE inhibitors which may have side effects on renal function and can cause azotemia (Weinberg, 1993). Therefore, dogs that receive ramipril or other ACE inhibitors should be monitored renal function continually.

Echocardiography is a non-invasive technique that can investigate cardiac chamber size, structural abnormalities and function. Since echocardiography is a non-invasive technique, it is practical for a repeated, follow-up measurement. In this study, several echocardiographic parameters were used to assess cardiac enlargement, severity of mitral valve regurgitation and systolic function of the heart. The mitral regurgitant flow is dependent on left atrial pressure, systolic left ventricular function, and preload and systemic arterial pressure (Chetboul and Tissier, 2012). The regurgitant flow may decrease in case of systolic ventricular impairment and high left atrial pressure. Therefore, the mitral regurgitant flow velocity may not be a good indicating for evaluation the regurgitant severity. A previous study found that atrial regurgitant jet/left atrial area (ARJ/LA) and LA/Ao were correlated with the severity of mitral regurgitation (Gouni et al., 2007). Although ARJ/LA can subjectively differentiate mild and moderate degree of DMVD, difference between moderate and severe degrees is difficult to evaluate by this technique. PISA or flow convergence method is more reliable for the discrimination of mitral regurgitant severity compared to the color mapping technique or ARJ/LA (Chetboul and Tissier, 2012). However, PISA method has some limitations. First, PISA is accurate for regurgitant flow with circular orifice only. Second, some dogs with multiple regurgitant jet can cause PISA inaccuracy. Third, PISA is inaccurate if precise location of the orifice and the flow convergence shapes could not be determined. If misalignment or eccentric jet occurs, it will underestimate flow velocity and overestimate orifice area (Zoghbi et al., 2003). The regurgitant severity assessed by PISA, ARJ/LA and MR in the ramipril group was unchanged throughout the study period and similar to the control group suggesting a lack of short-term effects of ramipril in decreasing mitral regurgitant severity. The regurgitant severity in the control group was more varied during the period of the study. The severity changed at some points of time. ARJ/LA and PISA were significantly different on days 56 and 91, respectively, when compared to day 0 in the control group. These findings suggest that without any drug the severity of regurgitation may change within a short period of time. With ramipril, the progression of regurgitation severity may be controlled or delayed.

In conclusion, based on echocardiographic values, this study showed that ramipril did not affect cardiac size, mitral regurgitation severity and systolic function in short term treatment duration. However, due to the slow progression with long preclinical period of this disease, further studies with long-term treatment duration should be performed. Because the current available data from this clinical trial and other studies do not confirm or support that early treatment with ACE inhibitors is beneficial, dogs with stage B2 DMVD should be individually evaluated and treated on a case by case basis.

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