การประเมินผลทางคลินิกของยารามิพริลในสุนัขที่มีภาวะลิ้นหัวใจเสื่อมในกลุ่มที่ยังไม่แสดงอาการทางคลินิก

นายประกิจ เกาะกายสิทธิ์

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CLINICAL EVALUATION OF RAMIPRIL IN DOGS WITH ASYMPTOMATIC DEGENERATIVE MITRAL VALVE DISEASE

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ประกิจ เกาะกายสิทธิ์ : การประเมินผลทางคลินิกของยารามิพริลในสุนัขที่มีภาวะลิ้นหัวใจเสื่อมในกลุ่มที่ยังไม่แสดงอาการทางคลินิก (CLINICAL EVALUATION OF RAMIPRIL IN DOGS WITH ASYMPTOMATIC DEGENERATIVE MITRAL VALVE DISEASE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: สพ.ญ. ดร. สิริลักษณ์ สุรเชษฐพงษ์, 42 หน้า.

โรคลิ้นหัวใจไมทรัลเสื่อมเป็นโรคหัวใจที่พบได้บ่อยในสุนัขจากการศึกษาพบว่าการให้ยากลุ่ม Angiotensin converting enzyme (ACE) inhibitors ให้ผลดีในสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อมระยะ C และ D (ACVIM classification) อย่างไรก็ตามการศึกษาถึงผลของ ACE inhibitors ในระยะ B2 ยังไม่มีข้อสรุปที่แน่ชัด

รามิพริลเป็นยาในกลุ่ม ACE inhibitors ที่มีคุณสมบัติในการละลายในไขมันได้ดีกว่ายาตัวอื่นๆในกลุ่มนี้ทำให้สามารถยับยั้ง ACE ในเนื้อเยื่อของหัวใจได้อย่างมีประสิทธิภาพ คุณสมบัติการยับยั้ง ACE ในเนื้อเยื่อหัวใจนี้น่าจะส่งผลให้รามิพริลมีประสิทธิภาพในการรักษาสุนัขที่มีภาวะลิ้นหัวใจเสื่อมได้ดีกว่ายาตัวอื่นๆในกลุ่มเดียวกัน

ในการศึกษานี้ทำการศึกษาในสุนัข 20 ตัวที่น้ำหนักระหว่าง 3-12 กิโลกรัม และมีอายุมากกว่า 6 ปีที่มีภาวะโรคลิ้นหัวใจไมทรัลเสื่อมในระยะ B2 ทำการแบ่งสุนัขเป็น 2 กลุ่มโดยที่เจ้าของจะเป็นผู้เลือกว่าจะให้ยาในสุนัขหรือไม่ กลุ่มที่ได้รับยาจำนวน 10 ตัวจะได้รับยารามิพริลปริมาณ 0.22 มิลลิกรัมต่อกิโลกรัมวันละครั้ง กลุ่มที่ไม่ได้ทำการให้ยา 10 ตัวเป็นกลุ่มควบคุม ซึ่งจะไม่ได้รับยาอะไรเลยเป็นระยะเวลา 91 วัน สุนัขทุกตัวจะได้รับการตรวจร่างกายทางคลินิก ตรวจคลื่นไฟฟ้าหัวใจและตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงในวันที่ 0 28 56 91 วันตามลำดับ การศึกษาขนาดและโครงสร้างของหัวใจจะใช้ค่าของการตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเป็นตัวประเมิน การวิเคราะห์ทางสถิติใช้ independent T-test ในการเปรียบเทียบระหว่างกลุ่มที่ให้ยารามิพริลกับกลุ่มควบคุม และใช้ Repeated ANOVA ในการเปรียบเทียบในกลุ่มเดียวกัน ระหว่างวันที่ 0 28 56 91 ค่า $p < 0.05$ บ่งชี้ว่ามีนัยสำคัญทางสถิติ

ผลการศึกษาในครั้งนี้พบว่า ขนาดของหัวใจ ความสามารถในการบีบตัวของหัวใจและความรุนแรงของภาวะลิ้นหัวใจไมทรัลเสื่อมทั้ง 2 กลุ่มไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติตลอดระยะเวลาที่ทำการศึกษา อย่างไรก็ตามในกลุ่มที่ได้รับยาพบว่าสุนัข 2 ตัวมีความอยากอาหารเพิ่มมากขึ้น สุนัข 1 ตัวมีอาการไอลดน้อยลง และสุนัข 1 ตัวมีค่าของ BUN และ CREA สูงมากกว่าปกติภายหลังจากได้รับยา

สรุปผลจากการศึกษานี้พบว่ายารามิพริลไม่มีผลต่อการเปลี่ยนขนาดของหัวใจ ความสามารถการบีบตัวของหัวใจและความรุนแรงของภาวะลิ้นหัวใจรั่วในสุนัขเทียบกับกลุ่มที่ไม่ได้รับยาภายในระยะการศึกษา 91 วัน

ภาควิชา....อายุรศาสตร์.....ลายมือชื่อนิสิต.....

สาขาวิชา..อายุรศาสตร์สัตวแพทย์...ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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KEYWORDS : ANGIOTENSIN CONVERTING ENZYMES INHIBITORS, CONGESTIVE HEART FAILURE, DOG, MITRAL VALVE DISEASE, RAMIPRIL

PRAKIT KOHKAYASIT: CLINICAL EVALUATION OF RAMIPRIL IN DOGS WITH ASYMPTOMATIC DEGENERATIVE MITRAL VALVE DISEASE.

ADVISOR: SIRILAK SURACHETPONG, D.V.M., Ph.D., 42pp.

Degenerative mitral valve disease (DMVD) is the most common cardiac disease in dogs. Angiotensin converting enzyme (ACE) inhibitors have beneficial effects on DMVD dogs with (ACVIM classification) stage C and D. However, the study determining effects of ACE inhibitors in DMVD stage B2 is still controversy.

Ramipril is an angiotensin converting enzyme (ACE) inhibitors that has more lipophilic effects than other ACE inhibitors. It can suppress ACE in cardiac tissues effectively, so this drug may have more beneficial effects in treatment dogs with naturally occurring canine DMVD stage B2 (ACVIM classification) than other ACE inhibitors.

In this study, we used 20 dogs with asymptomatic DMVD stage B2 with body weight between 3-12 Kg and older than 6 years. Dogs were single blinded randomized dividing into 2 groups. The owners themselves selected to either supplement or not supplement the drugs. Dogs in ramipril group (n= 10) received ramipril once a day at dose 0.22 mg/kg PO and control group (n= 10) did not receive any drug for 91 days. Complete physical examination, electrocardiography and echocardiography were performed in day 0, 28, 56 and 91. Echocardiographic examination was used to compare cardiac sizes and structural changes. For statistical analysis, independent t-test was performed to compare between dogs in ramipril and control groups. Repeated ANOVA was used to compare within groups between days 0, 28, 56, 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. Two dogs in ramipril group increased in appetite, one dog had reduced frequency of cough and one had increased blood urea nitrogen and creatinine.

In conclusion, the ramipril could not change cardiac size, severity of mitral regurgitation and systolic function compared with the control group in 91 days study period

Department……Veterinary Medicine….. Student's Signature……....

Field of study….Veterinary Medicine..... Advisor's Signature……

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CHAPTER I
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 breed old dogs, more frequent in males. (Häggström et al., 2009). DMVD is characterized by a long preclinical period. It has been found 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. DMVD, which is a cause of mitral valve regurgitation, can be detected by thickening of mitral valve and chordae tendineae, left heart chamber enlargement and systolic murmur heard best at left apex. In some dogs, affected valves may function adequately without hemodynamic effects (Borgarelli et al., 2011). The quality of murmur can be different with various stages of disease due to the degree of mitral valve regurgitation. 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 with little inflammatory reaction. The histological changes occur in the atrialis and spongiosa layers by excessive destruction and derangement of stroma with accumulation of proteoglycan and glycosaminoglycan in the leaflet and chordae tandineae (Fox, 2012). Later stage, mitral valve and chordate tendineae are severe thickening and distortion, causing improper leaflet coaptation and regurgitation of blood across the closed mitral valve during ventricular systole. The regurgitant valve allows blood to flow back from left ventricle to left atrium during ventricular systole leading to drop in stroke volume, cardiac output and increase intraatrial pressure. Renin-angiotensin-aldosterone system (RAAS) is activated 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 have benefits in management congestive heart failure in dogs by increasing survival rate, improving quality of life in dogs with class 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 therapy with ACE inhibitors in asymptomatic dogs with mitral valve disease had not shown significant effects (Kvart et al., 2002; Atkins et al., 2007). The retrospective study of Pouchelon et al., (2008) reported a possible benefit of the early treatment with benazepril in asymptomatic dogs with mitral valve disease. However, because of the limitations of the retrospective study, the clinical prospective study should be performed to confirm the result. Ramipril is a long acting ACE inhibitor, which has more lipophilic than enalapril, thus it can suppress ACE releasing in local tissues more effectively. Several reports also show that use of ramipril is safer than the other ACE inhibitors in renal impairment patients (Hope study, 2000; Lefebvre et al., 2006). Because of a more lipophilic effect and a long acting duration, ramipril may be superior to other ACE inhibitors in treatment of DMVD dogs. In addition, the study of ramipril in naturally occurring DMVD is still lacking, particularly in asymptomatic dogs. Therefore, ramipril was chosen in this study.

Objectives of the Study

The aim of this study was to clinically evaluate the short term effects of ramipril in naturally occurring degenerative mitral valve disease in asymptomatic dogs with heart failure stage B2 (ACVIM classification).

Hypothesis of the Study

Ramipril has beneficial effects in treatment dogs with naturally occurring canine DMVD stage B2 (ACVIM classification).

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

Keyword (Thai): กลุ่มยายับยั้งแองจิโอเทนซิน-คอนเวอร์ติงเอ็นไซม์, ภาวะหัวใจล้มเหลวแบบมีเลือดคั่ง, สุนัข, โรคลิ้นหัวใจไมทรัล, รามิพริล

CHAPTER II
LITERATURE REVIEW

Anatomy and function of mitral valve

The mitral valve is a structure that lies between left ventricle and left atrium. The posterior left atrial wall, mitral valve annulus, mitral valve leaflets, chordate tendineae, left ventricular papillary muscles and associated left ventricular wall are components of mitral valve apparatus (Perloff and Robert 1972). Mitral valve apparatus operates through complex interplay and synergistically maintain valve integrity. The mitral leaflets are composed of anterior (septal) and posterior (mural) leaflets separated at their commissures. The mitral valve leaflet surface has a rough zone near free margins and a smooth zone at annular junction. Normal mitral leaflets are thin, smooth and translucent glistening attached to the papillary muscle by chordae tendineae. The leaflets consist of four layers from atrial to ventricular aspects. The atrialis is a thin layer on inflow side of mitral leaflet being covered by endothelial cells overlying elastic fibers. The spongiosa layer is extended from annulus to free edge of leaflet. This layer is comprised of glycosaminoglycans, proteoglycans and collagen

fibers. The fibrosa layer is composed of a dense collagen fiber. The ventricularis layer is a thin layer that is similar to the atrialis layer and being covered by endothelium (Ahmed et al., 2009). In normal dogs, mitral leaflets completely coapt with a little or without regurgitation through the valve orifice. When mitral valve leaflets close, the left ventricle ejects blood through the aorta (Tilley et al., 2008).

Natural history

Degenerative mitral valve disease (DMVD) is a disease causing mitral valve degeneration. Its appearance varies from a small focal thickening to an irregular surface with large nodules. This disease transforms normal thin, translucent leaflets into opaque structures that become thickened and progress to diffuse valve thickening, nodularity and deformity. In severe cases, myxomatous transformation causes the distal portion of leaflets to become markedly thickened and may protrude into the left atrium. The chordae tendineae might also become thickened causing rupture of chordae tendineae (Fox, 2012). This disease is the most common acquired cardiovascular disease accounting for approximately 75% of all cardiovascular diseases in dogs which mostly progresses to congestive heart failure (Häggström et al., 2009; Borgarelli and Buchanan, 2012). Valve degeneration is most commonly affected the left atrioventricular valve or mitral valve. DMVD has been found 1.5 times more frequently in male than in female (Atkins et al., 2009). This disease is related to age and breeds of dogs. In small breed dogs (body weight < 20) the incidence of this disease is almost 100 percent (Borgarelli and Buchanan, 2012). Large breed dogs are occasionally affected but with less frequency than small breed dogs (Borgarelli et al., 2004). DMVD can be categorized into large 4 groups using an A through D paradigm of American College of Veterinary Internal Medicine (ACVIM) classification. *Category A* for dogs without evidence of heart disease but are at risks (e.g. Cavalier King Charles spaniels and Dachshund). In *category B*, dogs have heart disease without an evidence of heart failure and cardiomegaly (B1) or cardiomegaly (B2). *Category C* includes dogs with clinical signs of heart failure. In *category D,* dogs have end-stage heart failure or in refractory stage.

Pathophysiology of degenerative mitral valve disease

. Due to DMVD, the structure of mitral valves changes in cellular constituents, collagen contents and alignment of collagen fibrils. Valve leaflets and chordae tendineae are affected by deposition of mucopolysaccharides within the spongiosa layer (Perloff and Roberts, 1972). DMVD can also be classified into 4 types according to Whitney's classification. Mitral valve leaflet is thickened and have some small discrete nodules at the tip of leaflets in *type1*. *Type*2, DMVD has some larger nodules than type 1 which tend to fuse at the edge of valve leaflet. In *type3*, mitral valve has been found severe thickening and nodular distortion without involvement of chordae tendineae, while chordae tendineae are lengthened and may rupture in *type 4* (Whitney, 1967). In severe stage, the leaflets are redundant. The free edge of the leaflets is rolling toward the ventricular endocardium causing improper coaptation and resulting in valve regurgitation (Tilley et al., 2008).

The cause of DMVD is unknown. In some dog breeds, this disease appears to have an inherited component (Olsen et al., 1999). Dogs with mild to moderate disease show no clinical signs of cardiac diseases whereas severe diseased dogs are at risk of congestive heart failure (Häggström et al., 2009). Mild DMVD regards as benign condition and low risk for sudden death (Atkins et al., 2007).

Neurohormonal system of heart failure

Renin-angiotensin-aldosterone system (RAAS) is a complex neurohormonal compensatory system functioning to maintain blood pressure and tissue perfusion within normal limit. While valvular structure deformation progresses, an ineffective coaptation of the valves can occur. The forward stroke volume and systemic blood pressure decrease due to regurgitation of blood back into the left atrium. This phenomenon activates RAAS by releasing renin from juxtaglomerlular apparatus. Renin, then breakdowns angiotensinogen to angiotensin I which will be converted into angiotensin II (ATII) by angiotensin-converting enzyme in the lungs. ATII, which is 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 sympathetic nervous system (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 (Davila et al., 2005).

Diagnosis

The diagnosis of DMVD can be performed by auscultation holosystolic murmur heard best at the left apex. Thoracic radiography should be performed in all dogs with DMVD to assess the hemodynamic significance of the murmur and to obtain baseline information when the patients are in an asymptomatic stage (Atkins et al., 2009). Although history taking, physical examination and radiography are strongly suggested of DMVD, echocardiography is required to confirm the diagnosis and to exclude other cardiovascular diseases that can cause mitral valve regurgitation. The characteristic of DMVD includes prolapse or thickening of one or both mitral valve leaflets. In cases under suspicion of DMVD, at least 2 echocardiographic views should be performed (Atkins et al., 2009).

Treatment

Therapy of DMVD can be done by surgical or medical treatment. The surgical treatment of DMVD in dogs can be achieved by valve replacement or mitral valve repair technique. This treatment is effective for severe mitral valve regurgitation, which can improve survival rate and prognosis in dogs (Griffiths et al., 2004). However, due to the complex procedures such as cardiopulmonary bypass, mitral chordal replacement and mitral annuloplasty (Uechi, 2012), the operation has to be performed by an experienced veterinary surgeon. Moreover, because of the very expensive operation cost, the management of DMVD in some country is not a suitable choice. For medical treatment, it normally starts when dogs have cardiac structural changes and clinical signs of heart failure (stage C or D) (Atkins et al., 2009). Drugs used for DMVD treatment include diuretics,

pimobendan, ACE inhibitors. The COVE study and LIVE study provide the evidence that ACE inhibitors added to the standard therapy can improve quality of life and survival times in dogs with chronic heart failure caused by DMVD (The COVE study group, 1995; Ettinger et al., 1998). However, the use of ACE inhibitors in asymptomatic DMVD dogs is still the subject of controversy. The HOPE investigators, the study in people found that ACE inhibitors can reduce mortality, morbidity and prolong life in people who are at risk of heart disease but without organic heart disease (HOPE study investigators, 2000). Therefore, it is assumed that canine DMVD stage B may have some benefits from ACE inhibitors treatment (Atkins and Häggström, 2012). However, the study of Kvart et al., (2002) had shown no significant effect of enalapril in delaying or preventing the onset of congestive heart failure in asymptomatic DMVD dogs. Another retrospective study reported a possible benefit of early treatment with benazepril in DMVD asymptomatic dogs (Pouchelon et al., 2008). Thus, the beneficial effects of ACE inhibitors in DMVD asymptomatic dogs are uncertain and remain in the area of debate.

Pharmacodynamic and pharmacokinetic of ramipril

Ramipril is an ACE inhibitor. An inactive product ramipril metabolites into an active product, ramiprilat in the liver. Ramipril has more lipophilic and higher affinity to ACE than enalapril (Xiang et al., 1985; Wolfgang, 1992). Due to the long duration of action, ramipril can prescribe once daily. Ramipril is excreted via bile (60%) and urine (40%). Thus, it can be administered in dogs with impaired renal function without dose adjustment (Lefebrve et al., 2006). The presence of angiotensinI and angiotensinII has found in numerous tissues. Conversion of inactive angiotensin I into active angiotensin II can occur in tissue locally through intrinsic RAAS or in systemic RAAS. Due to a more lipophilic effect, ramipril can penetrate tissues and suppress systemic and locally RAAS more effectively than other ACE inhibitors (Xiang et al., 1985). Pilote et al. (2008) found that ramipril can decrease mortality rate in human with congestive heart failure more effectively comparing to enalapril and captopril. There is no prospective study to investigate the clinical effects of ramipril in asymptomatic DMVD dogs. Therefore, this study was created to determine the short term clinical

effects of ramipril in naturally occurring DMVD dogs with cardiac structural remodeling but have no clinical sign or DMVD dogs in heart failure stage B2 (ACVIM classification).

CHAPTER III
MATERIALS AND METHODS

This study was a single blinded randomized prospective study. All dogs enrolled into this 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.

Animals

All 20 degenerative mitral valve disease dogs with stage B2 (ACVIM classification) heart failure (Table 3.1) presented at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University were used in this study.

Table3.1 American College of Veterinary Internal Medicine (ACVIM) classification of heart failure (Atkins et al., 2009)

ACVIM stage	Clinical signs
Stage A	Dogs at risk developing DMVD that have no identifiable cardiac structure disorder
Stage B1	Dogs with DMVD that have never developed clinical sign and have no radiographic or echocardiographic evidence of cardiac remodeling
Stage B2	Dogs with DMVD that have never developed clinical sign but have radiographic or echocardiographic evidence of cardiac remodeling
Stage C	Dogs with DMVD and past or current clinical signs of heart failure associated with structural heart remodeling
Stage D	Dogs with end stage DMVD and heart failure that is refractory to standard therapy

Inclusion Criteria

Due to possible difference in the nature of DMVD between small and large breed dogs (Borgarelli et al., 2004). Dogs in this study were older than 6 years and they were small breeds with body weight between 3-12 kilograms. All dogs had systolic murmur heard best over the left cardiac apex. Dogs had no clinical signs of heart failure including depression, ascites, dyspnea, exercise intolerance or persistent cough. 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 murmur and rule out primary respiratory diseases. Echocardiography was performed to confirm the diagnosis of mitral valve degeneration. Cardiac remodeling was determined by M-mode echocardiography including enlarged left atrium (increase 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. Dog recruited into this study were in class B2 heart failure. Blood sample analysis including complete blood count, blood urea nitrogen (BUN), creatinine (CREA), alanine aminotransferase (ALT), alkaline phosphatase (ALP) 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.

Exclusion Criteria

All dogs with acute and chronic renal insufficiency (defined as creatinine 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 did not perform in pregnancy and lactating bitches. Dogs with clinical signs of heart failure including ascites, dyspnea, exercise intolerance, cough or dogs received any drugs that might have effects on cardiovascular system were excluded. Dogs with other kinds of heart diseases or abnormalities rather than DMVD were also excluded from the study.

Experiment Protocol

Dogs were single blinded randomized dividing into 2 groups. Because it has no standard protocol for treatment dogs with DMVD stage B2 recently (Atkins et al., 2009), the owners themselves

selected to either supplement or not supplement the drugs to their own dogs. In the treatment group (n = 10), dogs were supplemented with ramipril 0.125 mg/kg per os q24 for 91 day. In control group (n = 10), dogs did not receive any drugs. Complete physical examination, electrocardiography and echocardiography were performed in day 0, 28, 56, and 91. Radiography, blood collection for hematology and biochemistry were performed at day 0 and 91. Echocardiographic examination was used to compare cardiac sizes and structural changes. The quality of life and clinical signs were evaluated by the clinical score in Table 3.2

Table 3.2Scoring 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.

Physical examination

Physical examination included cardiac and lung auscultation. Cardiac auscultation was performed in the quiet place. The intensity of heart murmur and location were recorded at day 0, 28, 56, 91.

Electrocardiography

The dogs were performed electrocardiography (KENZ ECG-110 ®, Nagoya Japan) in the right lateral recumbent position. The heart rate and electrocardiographic abnormalities were evaluated at day 0, 28, 56 and 91.

Radiography

All radiographs were taken at right lateral and ventro-dorsal projections at day 0 and 91. The size of the heart was evaluated by measuring vertebral heart score (VHS). Cough from cardiac disease will be defined if VHS is more than 11.4 (Guglielmini, et al, 2009). The dogs with pulmonary edema were excluded from this study.

Echocardiography

The cardiac structure remodeling was evaluated from two dimensional and M-mode echocardiography assessed by ultrasound machine (Logic^{TM}5 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, LVIDdi (LVIDd / Body surface area (BSA)), LVIDsi (LVIDs / BSA), VSdi (VSd / BSA), VSsi (VSs / BSA), LVWdi (LVWd / BSA), LVWsi (LVWs / BSA) were used to reduce body weight variation between dogs. The fractional shortening was calculated by {(LVEDd-LVEDs) / LVEDdx100}. The regurgitant flow velocity, area of regurgitant jet as a proportion to the area of left atrium and PISA technique (Proximal Isovelocity Surface Area) were measured for evaluating the severity of mitral valve regurgitation. All examinations were performed in conscious un-sedated dogs at day 0, 28, 56 and 91. The research plan and experimental protocol is presented in figure 3.1

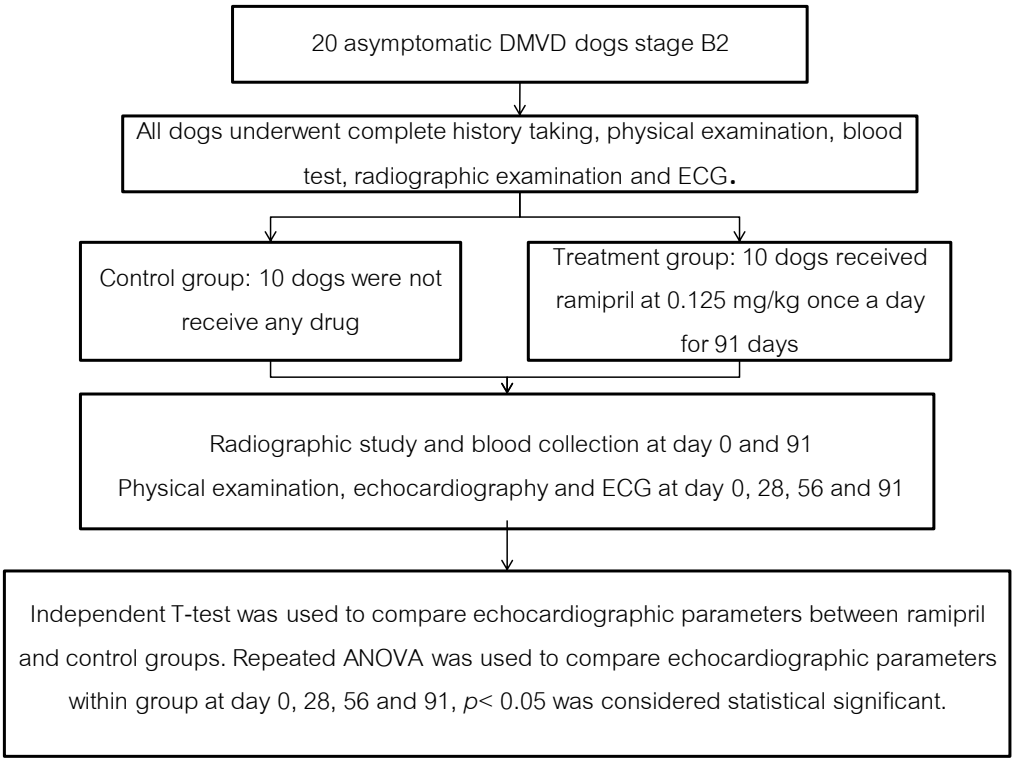

Figure 3.1 Research plan and experimental protocol

Data acquisition and statistical analysis

In this study, a group of dogs that did not receive any drug were served as a control group. The clinical score was evaluated as descriptive analysis. Independent t-test was used to compare echocardiographic parameters between ramipril and control groups at day 0, 28, 56 and 91. Repeated ANOVA was used to compare differences within control and ramipril groups. Compare difference within group between day 0, 28, 56, 91 with Paired T-test. p-value less than 0.05 was considered statistically significant.

CHAPTER IV
RESULTS

Baseline characteristics

A total number of 23 dogs were enrolled in this study. Twelve dogs (9 males, 3 females) were assigned to the ramipril group while the other 11 dogs (male 5, female 6) 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 (CREA > 1.8mg/dl) and dead from car accident in the other. In the control group, one dog was excluded due to car accident. Therefore, only 20 dogs were included. The age, body weight, heart rate and respiratory rate were shown in Table 4.1. The intensity of heart murmur in the control group was grade IV in 10 dogs. In the ramipril group, 2 dogs was grade III and eight dogs in grade IV. The control group consisted of 4 males (40%) and 6 females (60%). Breed included 7 Poodles, 2 mixed breed and 1 Miniature Pinscher. Ten dogs in the ramipril group consisted of 7males (70%) and 3 females (30%). Two mixed breed, 2 Chihuahuas, 2 Poodles and one each of Splitz, Shitzu, Yorkshire Terrier and Dachshund were included in the ramipril group.

Table4.1Baseline 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)

At baseline, age, weight, heart rate, respiratory rate were similar and not statistically different between control and ramipril groups (Table4.1). The average dose of ramipril in the ramipril group was 0.18 ± 0.03 mg/kg (range from 0.14-0.22mg/kg).

Effect of ramipril on echocardiographicvalue

Cardiac chamber size

In this study, we used LVIDdi and LA/Ao parameters to evaluate cardiac chamber size. LVIDdi and LA/Ao were not statistically different at day 0, 28, 56 and 91 between control and ramipril groups (Figure 4.1) (Table 4.2).

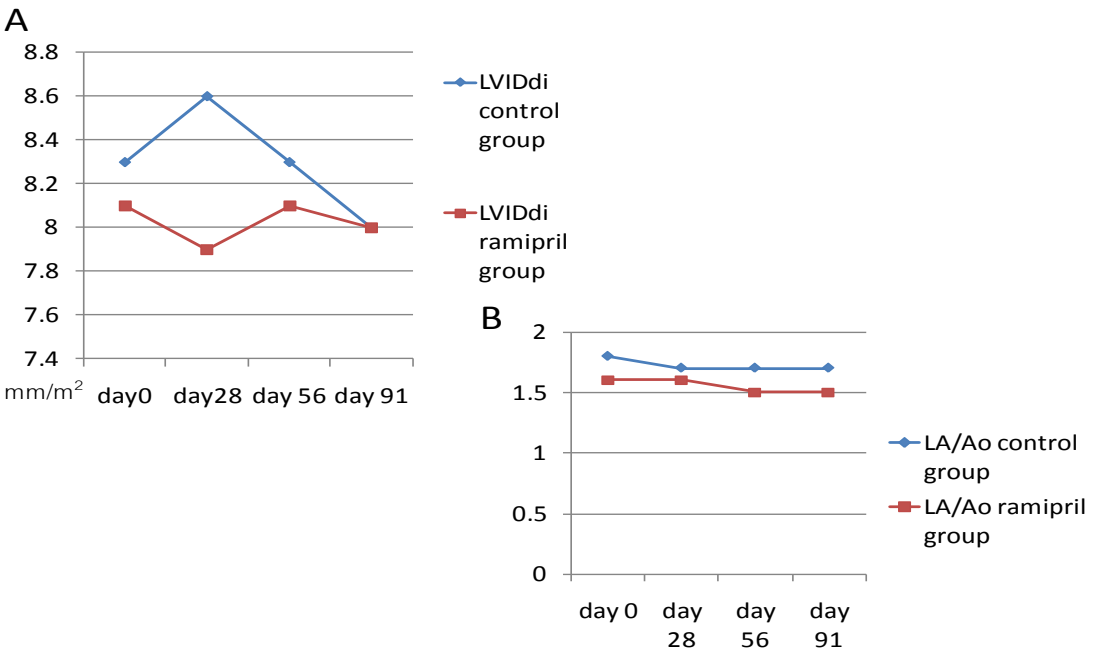

Figure 4.1 Effect of ramipril on LVIDdi and LA/Ao. These graphs show echocardiographic value at day 0, 28, 56 and 91 of ramipril and control groups. (A) LVIDdi (Left ventricular internal diameter diastolic index, (B) LA/Ao (Left atrial / Aorta)

Variables	Day0		Day 28		Day 56		Day 91	
LVIDdi (mm/m^2)	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Control group	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 group	8.10 ± 1.20		7.90 ± 1.40		8.10 ± 1.20		8.00 ± 1.60	
LA/Ao								
Control group	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 group	1.60 ± 0.10		1.60 ± 0.20		1.50 ± 0.20		1.50 ± 0.20	

Table 4.2 LVIDdi and LA/Ao parameters of dogs between ramipril and control group at day 0, 28, 56, 91

(LVIDdi = left ventricular diameter diastolic index, LA/Ao= Left atrial / Aorta)

Severity of mitral regurgitation

To assess the degree of mitral regurgitation between control and ramipril groups, the mitral regurgitant flow velocity (MR), regurgitant fraction area (RF) and PISA were determined. The result of PISA, regurgitant fraction and mitral regurgitant flow showed the non-significant difference between control and ramipril groups at day 0, 28, 56, 91 (Figure 4.2) (Table 4.3).

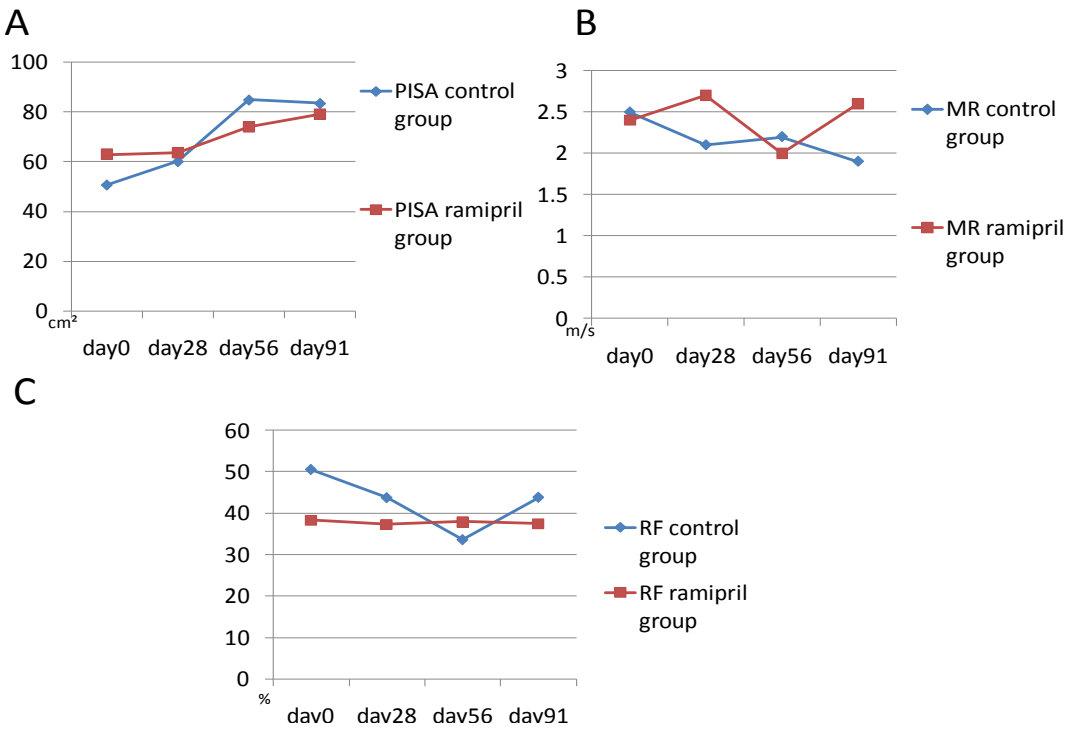

Figure 4.2 Effect of ramipril on PISA, MR and RF. These graphs show PISA, MR and RF at day 0,28, 56, 91 of control and ramipril groups, (A) PISA (Proximal Isoveloity Surface Area), (B) MR (Mitral regurgitatant flowvelocity), (C) RF (Regurgitant fraction)

Table 4.3 PISA, MR and RF parameters of dogs between control and ramipril group at day 0, 28, 56 and 91

Variables	Day0		Day 28		Day 56		Day 91	
PISA(cm^2)	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Controlgroup	0.98 ± 0.45	0.18	1.26 ± 0.37	0.78	1.71 ± 0.79	0.64	1.75 ± 0.78	0.96
Ramiprilgroup	1.56 ± 1.21		1.19 ± 0.59		1.54 ± 0.79		1.73 ± 1.05	
MR (m/s)								
Control group	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 group	4.70 ± 1.30		5.00 ± 1.10		4.70 ± 1.00		4.80 ± 1.20	
RF (%)								
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
groupRamipril	38.30 ± 18.00		37.30 ± 17.60		37.90 ± 16.80		37.40 ± 13.70	
group								

(PISA =Proximal Isovelocity Surface Area; MR= mitral regurgitant flow velocity; RF = regurgitant fraction).

Systolic function

In this study, percent fraction shortening (%FS) and Left ventricular end systolic diameter index (LVIDsi) were used to assess systolic function. %FS and LVIDsi were not statistically different between two groups throughout 91days of the study (Table 4.4). %FS and LVIDsi remained the same in both groups throughout 91 days of the study period (figure 4.3).

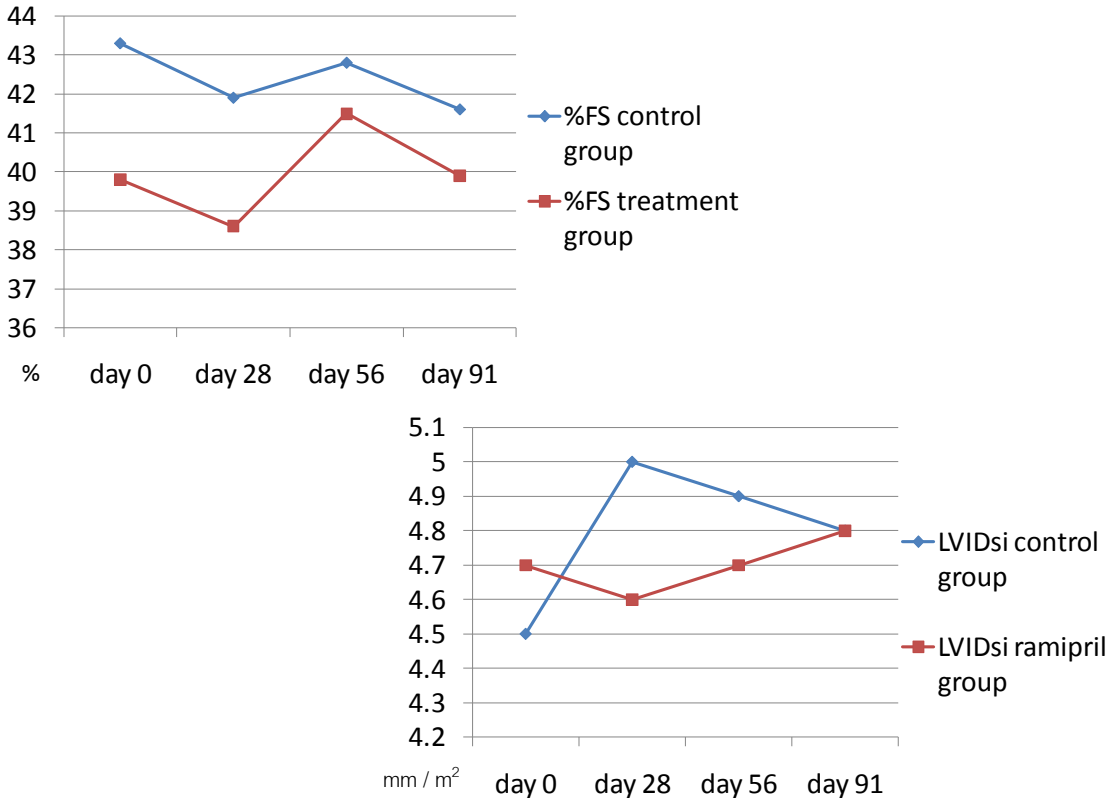

Figure 4.3 Effects of ramipril on %FS and LVIDsi. These graphs show %FS and LVIDsi at day 0, 28,56 and 91 of ramipril and control groups. (A) %FS (Fraction shortening), (B) LVIDsi (Left ventricular internal diameters systolic index).

Table 4.4. %FS and LVIDsi parameters of dogs between control and ramipril groups at day 0, 28, 56, 91

Variables	Day 0		Day 28		Day 56		Day 91	
%FS	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Control group	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 group	39.80 ± 6.30		38.60 ± 4.80		41.50 ± 7.70		39.90 ± 6.00	
LVIDsi (mm/m^2)								
Control group	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 group	4.70 ± 1.30		4.60 ± 0.90		4.70 ± 1.00		4.80 ± 1.20	

(%FS = % fraction shortening; LVIDsi = Left ventricular internal diameter systolic index)

Progressive of degenerative mitral valve disease in the control group

The progressive of degenerative mitral valve disease in the control group was studied at day 0, 28, 56 and 91. In the control group, PISA parameter at day 28 and 56 was the same as day 0. In day 91 PISA was increase ($p = 0.01$) significantly compare to day 0. RF was increase significantly at day 56 compare to day 0 and day 28 ($p = 0.03$) but not different from that at day 91 (Table 4.5).

Table 4.5 Comparison of echocardiographic parameters obtained from dogs in control group on day 0, 28, 56, 91 Results are illustrated in Mean ± SD

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
RF	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

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

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

Progressive of degenerative mitral valve disease in the ramipril group

The progressive of degenerative mitral valve disease in the ramipril group was determined at day 0, 28, 56 and 91. All echocardiographic parameters were not statistically different throughout 91 day period of this study (Table 4.6).

Table 4.6 Comparison of echocardiographic parameters obtained from dogs receive ramipril on day 0, 28, 56, 91 Results are illustrated in Mean ± SD

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
RF	38.30 ± 18.00	37.30 ± 17.60	37.90 ± 16.80	37.40 ± 13.70	0.99

Effect of ramipril on clinical signs

Three dogs in the ramipril group showed an improvement of clinical status. Two dogs increased appetite and one dog reduced frequency of cough compare to the control group (Table 4.7).

Potential adverse reaction and cause of withdrawal

Adverse reaction was recorded for dogs in ramipril groups. One dog was withdrawn because of increase in CREA and BUN after two months of treatment.

Table 4.7 Clinical status of ramipril and control groups at day 0 and 91

Clinical status	Ramipril group (n = 10)		Control group (n = 10)	
	Day 0	day91	Day 0	Day 91
Appetite score				
Score I	0	0	0	0
Score II	10	8	10	10
Score III	0	2	0	0
Cough score				
Score I	7	8	8	8
Score II	3	2	2	2
Score III	0	0	0	0
Exercise intolerance score				
Score I	10	10	10	10

Score II	0	0	0	0
Score III	0	0	0	0
Attitude score				
Score I	10	10	10	10
Score II	0	0	0	0
Score III	0	0	0	0

Clinical status	Ramipril group (n = 10)		Control group (n = 10)	
	Day 0	day91	Day 0	Day 91
Respiratory effort score				
Score I	7	7	6	5
Score II	3	3	4	4
Score III	0	0	0	0

Effect of ramipril on heart rate, respiratory rate, heart murmur grade and electrocardiography

Heart rate and respiratory rate were not significantly different between ramipril and control groups throughout the study period (Table 4.8). Grade of heart murmur in the control group was grade IV in all dogs throughout the study period. In the ramipril group, heart murmur was grade III in 2 dogs and in grade IV in 8 dogs. At day 28, one dog in the ramipril group had decreased intensity of heart murmur from grade IV to grade III (Table 4.9). At day 91, heart murmur in two 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.

Table 4.8 Mean ± SD of heart rate assessed by electrocardiography and respiratory rate in ramipril and control groups

Variables	Day0		Day 28		Day 56		Day 91	
Heart rate	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Control group	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 group	145.0 ± 15.1		142.0 ± 9.9		142.0 ± 22.9		143.0 ± 24.9	
Respiratory rate								
Control group	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 group	39.0 ± 7.9		42.6 ± 10.8		43.2 ± 9.0		39.6 ± 7.8	

Table 4.9 Heart murmur grade in ramipril and control groups

	Day 0	Day 28	Day 56	Day 91
Murmur intensity				
Control group(III)	0 (0%)	0(0%)	0(0%)	0(0%)
Control group (IV)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Ramipril group (III)	2 (20%)	3 (30%)	3 (30%)	1 (10%)
Ramipril group(IV)	8 (80%)	7 (70%)	7 (70%)	9 (90%)

Effect of ramipril on complete blood count and blood chemistry between ramipril and control groups at day 0 and 91

SGPT in the control group was higher than the ramipril group in day 0 ($p < 0.04$). However, the SGPT was still in normal limit in both groups. The value of other blood parameters of dogs between control and ramipril groups were not significantly different at day 0 and 91 (Table 4.10)

Table 4.10 Comparison of blood value 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 group	6.39 ± 1.19	0.96	6.25 ± 1.37	0.15
	Ramipril group	6.41 ± 1.03		7.01 ± 0.87	
Hb (g/dl)	Control group	14.57 ± 2.52	0.51	14.60 ± 3.27	0.87
	Ramipril group	15.30 ± 2.36		14.80 ± 3.39	
Hct (%)	Control group	43.70 ± 8.01	0.50	43.80 ± 8.00	1.00
	Ramipril group	46.00 ± 6.85		43.80 ± 8.70	

Variables		Day 0		Day 91	
		Mean ± SD	p- value	Mean ± SD	p- value
WBC	Control group	16.60 ± 8.33	0.06	15.37 ± 6.18	0.62
(x 10^3)	Ramipril group	10.37 ± 4.78		14.16 ± 4.70	
Neutrophil	Control group	12.21 ± 7.11	0.07	10.91 ± 4.17	0.13
(x 10^3)	Ramipril group	7.28 ± 3.66		8.06 ± 3.99	
Band cell	Control group	21.90 ± 57.8	0.47	13.00 ± 41.10	0.34
	Ramipril group	46.30 ± 80.2		0.00	
Monocyte	Control group	11.80 ± 12.3	0.33	12.81 ± 11.17	0.92
(x 10^2)	Ramipril group	7.28 ± 7.44		12.35 ± 11.10	
Eosinophil	Control group	11.91 ± 15.51	0.33	15.44 ± 25.01	0.26
(x 10^2)	Ramipirl group	6.66 ± 5.51		6.05 ± 2.80	
Lymphocyte	Control group	2.34 ± 1.47	0.20	2.07 ± 11.43	0.86
(x 10^3)	Ramipril group	1.61 ± 9.65		2.15 ± 11.10	
Platelet	Control group	29.51 ± 7.60	0.16	30.34 ± 74.08	0.72
(x 10^4)	Ramipril group	32.99 ± 16.35		32.34 ± 10.43	
SGPT	Control group	65.20 ± 28.60	0.04*	66.00 ± 36.80	0.51
(Unit / litre)	Ramipril group	40.10 ± 22.10		55.00 ± 37.40	
ALP	Control group	157.00 ± 133.00	0.77	120.30 ± 61.90	0.56
(Unit / Litre)	Ramipril group	140.00 ± 136.00		154.00 ± 163.00	
BUN	Control group	15.50 ± 4.93	0.50	24.60 ± 11.50	0.70
(mg / dl)	Ramipril group	17.67 ± 8.84		27.70 ± 22.60	
CREA	Control group	0.89 ± 0.32	0.98	0.93 ± 0.23	0.69
(mg / dl)	Ramipril group	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

Comparison of blood hematology and chemistry value within control and ramipril groups

All blood hematology and blood chemistry value in the control group between day 0 and day 91 were not significantly different (Table 4.11). In the ramipril group, platelet number at day 91 was decrease compare to day 0 ($p = 0.03$) and WBC in day 91 was increased significantly compared to day 0 ($p = 0.01$) (Table 4.12).

Table 4.11 Comparison of blood hematology and chemistry value obtained from dogs in the control group

Blood parameter		Mean ± SD	p-value
RBC (x 10^6)	Day 0	6.39 ± 1.18	0.59
	Day 91	6.25 ± 1.36	
Hb (g / dl)	Day 0	14.57 ± 2.52	0.96
	Day 91	14.60 ± 3.27	
Hct	Day 0	43.70 ± 8.01	0.96
	Day 91	43.80 ± 8.09	
WBC (x 10^3)	Day 0	16.60 ± 83.32	0.66
	Day 91	15.37 ± 61.03	
Neutrophil (x 10^3)	Day 0	12.21 ± 71.13	0.65
	Day 91	10.91 ± 41.77	
Band cell	Day 0	21.85 ± 51.82	0.35
	Day 91	0	
Monocyte (x 10^2)	Day 0	11.83 ± 12.39	0.54
	Day 91	12.81 ± 11.17	
Eosinophil (x 10^2)	Day 0	11.91 ± 15.51	0.35
	Day 91	15.43 ± 25.01	
Lymphocyte (x 10^3)	Day 0	23.44 ± 14.77	0.12
	Day 91	20.70 ± 11.43	
Blood parameter		**Mean ± SD**	**p-value**
Platelet (x 10^4)	Day 0	29.51 ± 7.60	0.72
	Day 91	30.34 ± 7.40	
SGPT (Unit / Litre)	Day 0	65.20 ± 28.56	0.94
	Day 91	66.00 ± 36.82	
ALP (Unit / Litre)	Day 0	157.00 ± 132.52	0.34
	Day 91	120.30 ± 61.90	

BUN (mg / dl)	Day 0	15.50 ± 4.92	0.06
	Day 91	24.60 ± 11.47	
CREA (mg / dl)	Day 0	0.89 ± 0.32	0.71
	Day 91	0.93 ± 0.23	

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 4.12 Comparison of blood hematology and chemistry value obtained from dogs in the ramipril group

Blood parameter		Mean ± SD	p-value
RBC (x 10^6)	Day 0	6.41 ± 1.03	0.14
	Day 91	7.01± 0.87	
Hb (g / dl)	Day 0	15.30 ± 2.35	0.59
	Day 91	14.83 ± 3.38	
Hct (%)	Day 0	46.00 ± 6.84	0.35
	Day 91	43.80 ± 8.76	
WBC (x 10^3)	Day 0	10.37 ± 4.78	0.01*
	Day 91	14.18 ± 4.25	
Neutrophil (x 10^3)	Day 0	7.28 ± 3.66	0.49
	Day 91	8.06 ± 3.99	
Blood parameter		**Mean ± SD**	**p-value**
Band cell	Day 0	46.30 ± 80.24	0.30
	Day 91	13.00 ± 41.10	
Monocyte (x 10^2)	Day 0	7.27 ± 7.44	0.08
	Day 91	12.35 ± 11.10	
Eosinophil (x 10^2)	Day 0	6.65 ± 5.50	0.73
	Day 91	6.04 ± 2.80	
Lymphocyte (x 103)	Day 0	1.61 ± 0.96	0.25
	Day 91	2.15 ± 1.10	

Platelet (x 104)	Day 0	37.99 ± 16.35	0.03*
	Day 91	32.34 ± 10.43	
SGPT (Unit / Litre)	Day 0	40.10 ± 22.05	0.08
	Day 91	55.00 ± 37.40	
ALP (Unit / Litre)	Day 0	139.60 ± 135.76	0.62
	Day 91	153.50 ± 163.26	
BUN (mg / dl)	Day 0	17.67 ± 8.84	0.17
	Day 91	27.70 ± 22.63	
CREA (mg / dl)	Day 0	0.89 ± 0.32	0.71
	Day 91	0.93 ± 0.23	

***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)**

CHAPTER V
DISCUSSION

This study aimed to investigate the short-term effect of ramipril in canine DMVD stage B2. The study demonstrated that ramipril did not affect cardiac chamber size, mitral regurgitation severity and systolic function assessed by echocardiography. The disease was still progressive in both control and ramipril groups in the same manner.

A previous study in humans showed the beneficial effects of ramipril in lower the risk of mortality in cardiovascular system (Hope study, 2000). In the study of Hartman et al.(1993) found that local angiotensin II may play a role as a growth factor that promotes cardiac hypertrophy in heart failure condition. Due to high angiotensin II receptor and angiotensin converting enzyme (ACE) in

canine myocardium (Dell'italia et al., 1997), use of high lipophilic ACE inhibitor, such as ramipril may suppress local RAAS in myocardium more effectively than other ACE inhibitors, such as enalapril or captopril (Kvart et al., 2002). However, this study did not show any beneficial effect of ramipril compared with untreated group. This lack effect may occur from another angiotensin II forming pathways in cardiac tissues (Dzau, 1989). Chymase is the other pathway that can trigger angiotensin II from angiotensin I (Balcells et al., 1996). Ramipril can suppress ACE pathway but cannot inhibit chymase pathway. Thus, the beneficial effect of ramipril could not be seen in this study. Second, renin-angiotensin system may be not fully activated in asymptomatic DMVD dogs (ACVIM stage B2). In human study by Gary 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 renin angiotensin aldosterone system (RAAS) has not been activated yet. 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). The RAAS stimulation has not been studied in stage B2 DMVD dogs. Third, short duration of the study may not enough to show the changes of disease that have long progressive period like DMVD. In addition, 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. The beneficial effects of ACE inhibitors in improve clinical signs of dogs with degenerative mitral valve disease has been reported previously in The COVE study (1995). The result of the present study suggests that ramipril might improve clinical signs of diseased dogs. The effects of ACE inhibitors in improve clinical signs were apparently seen after 1month of treatment (The IMPROVE study, 1999). In ramipril group, the effect of ramipril to blood chemistry and complete blood count was checked before and after treatment with ramipril. In this study CREA, SGPT and BUN of both groups at day 0 and 91 were not different significantly. Moreover, most of the dogs in this study were well tolerated to ramipril with only a few side effects. Only one dog had azotemia which was transient and did not need specific treatment. This result indicates that ramipril similar to other ACE inhibitors that may have some side effects on renal function (Weinberg, 1993). Therefore, dogs those received 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, preload and systemic arterial pressure (Chetboul and Tissier, 2012). The

mitral regurgitation may decrease in case of systemic ventricular impairment and high left atrial pressure. Therefore, the mitral regurgitant flow velocity may not be a good indicator 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 degree was difficult to evaluate by this technique. PISA or flow convergence method is more reliable for the discrimination of mitral regurgitant severity compare to the color mapping technique or ARJ/LA ratio (Chetboul and Tissier, 2012). However, PISA method has some limitations. First, PISA will be accurate for regurgitant with circular orifice only, when the regurgitation is a non-circular orifice, the PISA method will not be accurate. Second, some dogs with multiple regurgitant jet will make PISA method inaccurate. Third, PISA method will be inaccurate if the 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 mitral regurgitance flow velocity in the ramipril group were unchanged throughout the study and not different from the control group suggesting a lack of short term effects of ramipril in decreasing disease severity.

Limitations of this study are the small number of sample groups and the short period of the study. In general, the main difficult of this study was sample lost during following protocol.

In conclusion, based on echocardiographic value, this study showed that ramipril did not change cardiac size, mitral regurgitation severity and systolic function compared with the control group in short term treatment duration. However, due to the slow progression with long preclinical period of this disease, another study with long-term treatment duration should be performed. Although, the current available data from this clinical trial and another study do not confirm or support that early treatment with ACE inhibitors will be beneficial. Therefore, canine DMVD stage B2 should be individually evaluated and treated on a case by case basis.

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