Modulation of the tissue renin-angiotensin-aldosterone system in dogs with chronic mild regurgitation through the mitral valve

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Objective—To investigate whether the tissue and plasma renin-angiotensin-aldosterone system (RAAS) is activated in dogs with mild regurgitation through the mitral valve and determine the contribution of chymase and angiotensin-converting enzyme (ACE) to the activation of the RAAS and potential production of angiotensin II during the chronic stage of mild mitral valve regurgitation.

Animals—5 Beagles with experimentally induced mild mitral valve regurgitation and 6 clinically normal (control) Beagles.

Procedures—Tissue ACE and chymase-like activities and plasma RAAS were measured and the RAAS evaluated approximately 1,000 days after experimental induction of mitral valve regurgitation in the 5 dogs.

Results—Dogs with experimentally induced mitral valve regurgitation did not have clinical signs of the condition, although echocardiography revealed substantial eccentric hypertrophy. On the basis of these findings, dogs with mitral valve regurgitation were classified as International Small Animal Cardiac Health Council class Ib. Plasma activity of renin and plasma concentrations of angiotensin I, angiotensin II, and aldosterone were not significantly different between dogs with mitral valve regurgitation and clinically normal dogs. Tissue ACE activity was significantly increased and chymase-like activity significantly decreased in dogs with mitral valve regurgitation, compared with values in clinically normal dogs.

Conclusions and Clinical Relevance—The tissue RAAS was modulated without changes in the plasma RAAS in dogs with mild mitral valve regurgitation during the chronic stage of the condition. An ACE-dependent pathway may be a major route for production of angiotensin II during this stage of the condition. (*Am J Vet Res* 2007;68:1045–1050)

The goal for the management of dogs with regurgitation through the mitral valve that do not have clinical signs of the condition is to delay the onset of heart failure and prolong survival time. There is abundant experimental evidence[1-3] to indicate the efficacy of ACE inhibitors for use in the treatment of such dogs. However, clinical trials by other investigators[4,a] failed to reveal an obvious benefit of ACE inhibitors in dogs with mitral valve regurgitation and no clinical signs of the condition, despite the fact that activation of the plasma (circulating) RAAS during early stages of the condition implies the efficacy of ACE inhibitors.[5]

Cardiomegaly and intensity of the cardiac murmur have been proposed[4] as factors that affect the time needed to develop CHF in dogs with mitral valve regurgitation that do not have clinical signs of the disease. Therefore, one of the goals for treatment of dogs at that stage of the condition would be to prevent cardiac remodeling as well as delay the onset of CHF.

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Abbreviations

ACE	Angiotensin-converting enzyme
RAAS	Renin-angiotensin-aldosterone system
CHF	Congestive heart failure
AT1	Angiotensin II type 1
AT1RB	Angiotensin II type 1 receptor blocker

It has been suggested[6] that the tissue RAAS may be a critical component in signal transduction that links hemodynamic stress and cardiac hypertrophy. Tissue angiotensin II can activate phospholipase, mitogen-activated protein kinases, and other kinases through G-protein–coupled receptors when there is a hypertrophic stimulus (such as stretch of cardiac myocytes). Activation of these protein kinases results in cardiac hypertrophy and fibrosis.[7]

Increases in the plasma RAAS have been reported[8] in patients with CHF, although the increases did not correlate with the severity of CHF. Early activation of the plasma RAAS has been reported[5] in some dogs with mitral valve regurgitation that do not have clinical signs of the condition, although the plasma RAAS may not always correlate with the tissue RAAS, which would be more important in terms of myocardial remodeling.[9]

The tissue RAAS has been investigated[10-12] in dogs with experimentally induced mitral valve regurgitation. However, in those studies, the experimentally induced mitral valve regurgitation was acute, subacute, or severe, and we are not aware of any studies in which investigators have evaluated the tissue RAAS in dogs with mild mitral valve regurgitation during the chronic stage of the condition. The objectives of the study reported here were to investigate whether the tissue and plasma RAASs are activated in dogs with mild mitral valve regurgitation and determine the contribution of chymase and ACE to the activation of the RAAS and potential production of angiotensin II during the chronic stage in dogs with mild mitral valve regurgitation.

Materials and Methods

Animals—Eleven healthy Beagles between 1 and 3 years of age were used in the study, which was approved by the Animal Research Committee at Azabu University. Each dog was anesthetized by administration of isoflurane,[b] followed by a thoracotomy. Mitral valve regurgitation was created in 5 dogs by direct transection of chordae tendineae, as described elsewhere.[13] The remaining 6 dogs were used as a control group (sham operation). Ketoprofen[c] was administered to provide analgesia immediately after surgery.

General condition, heart rate, and respiratory rate were monitored daily for 1 week after surgery. Medical treatments were used only as necessary to control acute cardiac disease for dogs with induced mitral valve regurgitation after surgery (maximum of 14 consecutive days), and no medication was administered long term during the experimental period after the cardiac condition stabilized. General condition of the dogs with experimentally induced mitral valve regurgitation was monitored daily until they were euthanized at approximately 1,000 days after surgery (mean ± SD, 1,003 ± 34.5 days).

Classification of heart failure—The International Small Animal Cardiac Health Council classification of heart failure[14] was used. Classification of heart failure was determined on the basis of clinical signs and results of physical examination, thoracic radiography, echocardiography, and evaluation by use of cardiac catheterization.

Thoracic radiographs were analyzed by use of a clock analogy method, and vertebral heart score was measured.[15] Left ventricular end-diastolic dimension, left ventricular end-systolic dimension, and fractional shortening were measured on echocardiograms. Values for vertebral heart score, left ventricular end-diastolic dimension, left ventricular end-systolic dimension, and fractional shortening were measured before surgery (baseline) and again on the day the dogs were euthanized, and values were compared within each dog.

Cardiac catheterization was performed in dogs anesthetized by administration of isoflurane.[b] Pulmonary arterial wedge pressure, pulmonary arterial peak systolic pressure, mean pulmonary arterial pressure, left ventricular peak systolic pressure, left ventricular end-diastolic pressure, aortic peak systolic pressure, mean aortic pressure, and diastolic aortic pressure were measured. After the variables were recorded during the final cardiac catheterization, the anesthetized dogs were euthanized by administration of an overdose of anesthetic.

Measurement of the plasma RAAS—Plasma activity of renin and plasma concentrations of angiotensin I, angiotensin II, and aldosterone were measured by use of radioimmunoassays. Blood samples for the analysis of these variables were collected between noon and 4 PM by jugular venipuncture. Samples were immediately centrifuged at 4°C, and plasma was harvested and stored at –20°C until analysis.

Measurement of tissue ACE and chymase-like activity—Immediately after a dog was euthanized, the heart was removed and pieces of the left ventricular free wall were collected and snap-frozen in liquid nitrogen. The small surgically scarred area close to the interventricular septum was avoided during collection of left ventricular samples. The remainder of the heart was immersed in neutral-buffered 20% formalin. The ACE and chymase-like activities were determined for each dog by use of high-performance liquid chromatography, as described elsewhere.[16]

Statistical analysis—Results were reported as mean ± SD. Data from thoracic radiography, echocardiography, and cardiac catheterization were analyzed by use of paired *t* tests. Data for the plasma (circulating) and tissue RAAS were compared between dogs with mitral valve regurgitation and sham-operated dogs by use of the Mann-Whitney *U* test. Differences were considered significant at $P < 0.05$.

Results

Characteristics for dogs with mitral valve regurgitation—None of the dogs with induced mitral valve regurgitation had clinical signs characteristic of the condition. Results of physical examinations were unremarkable except that regurgitant murmurs were loudest at the left apex in dogs with mitral valve regurgitation.

Thoracic radiography did not reveal abnormalities in any dog. There was no significant ($P = 0.133$) change in vertebral heart score obtained before (mean ± SD, 10.0 ± 0.10) and after (10.8 ± 0.33) induction of mitral valve regurgitation.

Echocardiography revealed that the mean ± SD left ventricular end-diastolic and end-systolic dimensions of dogs with mitral valve regurgitation (37.9 ± 1.38 mm and 21.8 ± 0.66 mm, respectively) were significantly ($P = 0.033$ and 0.035, respectively) increased, compared with baseline values obtained for those variables (31.2 ± 2.29 mm and 18.0 ± 0.88 mm, respectively). Fractional shortening did not differ significantly ($P = 0.988$) before (42.4 ± 2.79%) and after (42.4 ± 0.89%) experimental induction of mitral valve regurgitation.

Intracardiac pressures were obtained by use of cardiac catheterization in dogs with mitral valve regurgitation. Mean ± SD values were all within reference ranges (aortic peak systolic pressure, 101.4 ± 11.25 mm Hg; mean aortic pressure, 82.0 ± 11.4 mm Hg; diastolic aortic pressure, 66.6 ± 10.64 mm Hg; pulmonary arterial

wedge pressure, 2.9 ± 2.09 mm Hg; pulmonary arterial peak systolic pressure, 17.6 ± 3.17 mm Hg; mean pulmonary arterial pressure, 9.6 ± 2.71 mm Hg; left ventricular peak systolic pressure, 108.8 ± 11.55 mm Hg; and left ventricular end-diastolic pressure, 7.2 ± 1.20 mm Hg).

Although dogs with mitral valve regurgitation did not have any clinical signs attributable to mitral valve regurgitation, echocardiography revealed substantial eccentric hypertrophy. On the basis of these findings, dogs with mitral valve regurgitation were classified as International Small Animal Cardiac Health Council class Ib.

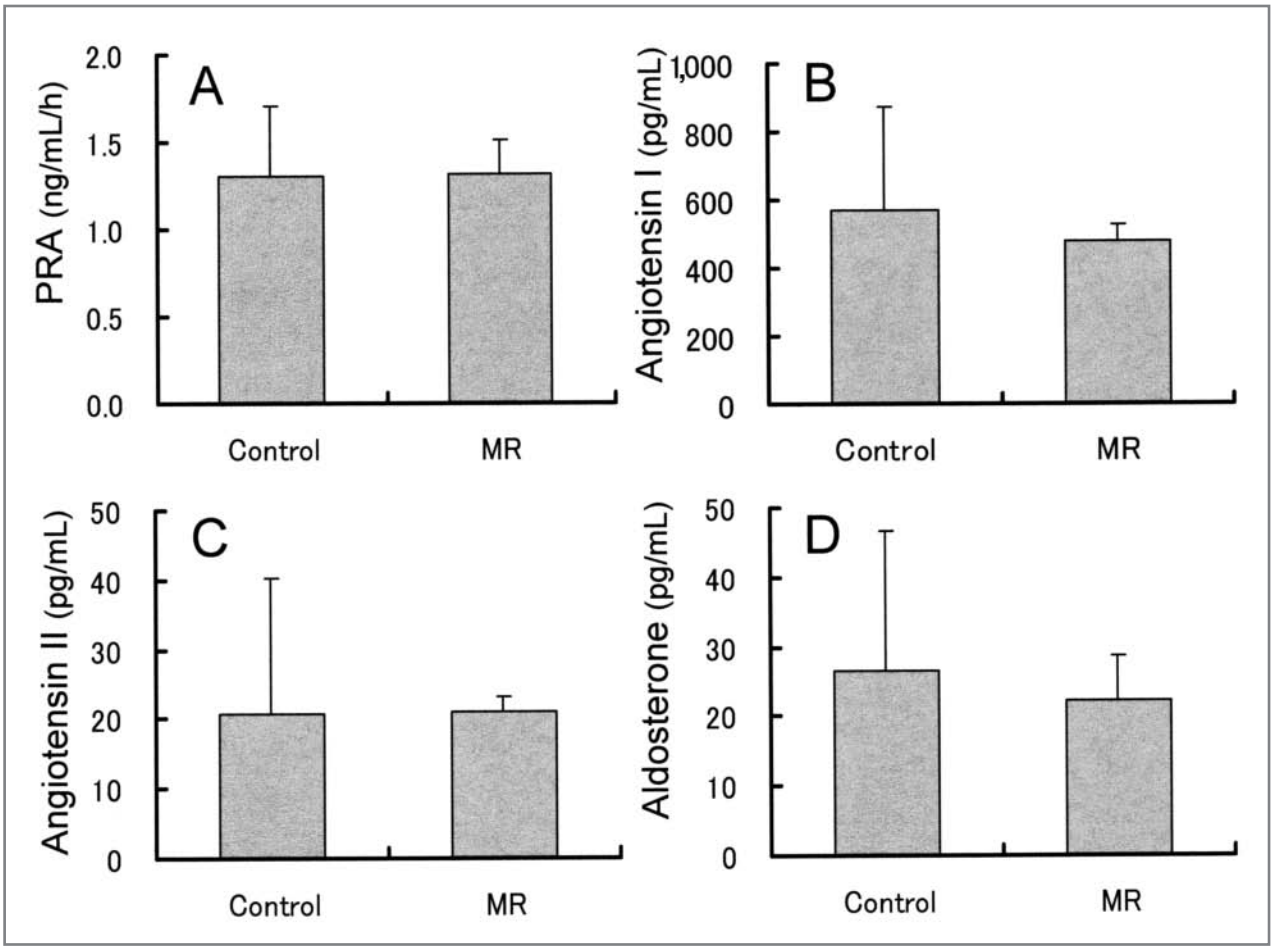

Figure 1—Mean ± SD plasma renin activity (PRA; A) and plasma concentrations of angiotensin I (B), angiotensin II (C), and aldosterone (D) in 5 dogs with experimentally induced mild mitral valve regurgitation (MR) and 6 clinically normal (control) dogs. Within each variable, values did not differ significantly ($P > 0.05$) between groups.

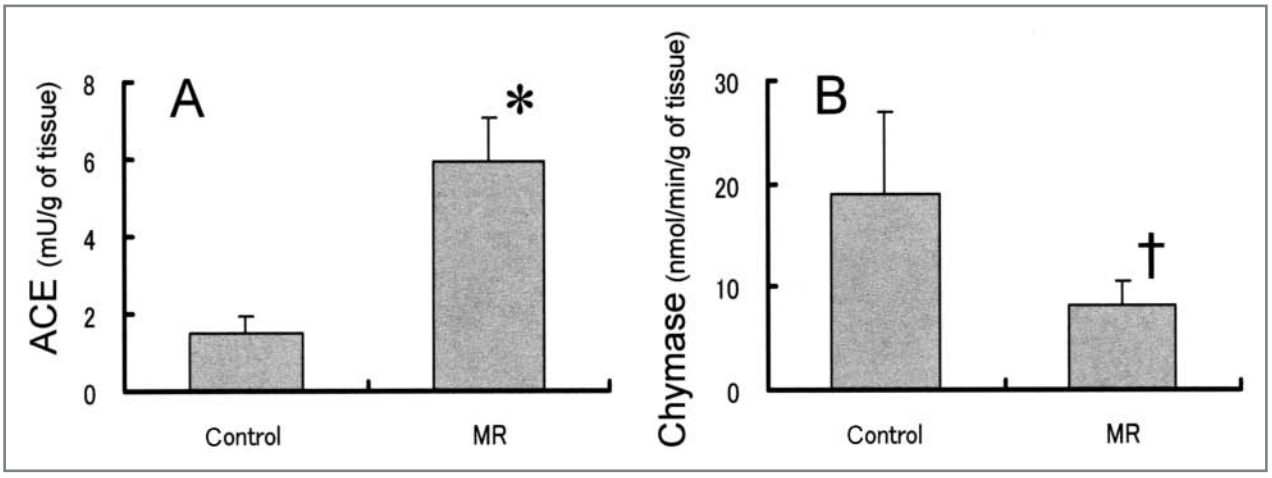

Figure 2—Mean ± SD tissue ACE (A) and chymase-like (B) activities in 5 dogs with experimentally induced mild MR and 6 clinically normal (control) dogs. *,†Within a variable, value differs significantly (*$P = 0.006$; †$P = 0.028$) from the value for the control dogs. *See* Figure 1 for remainder of key.

Plasma RAAS—Mean ± plasma renin activity did not differ significantly ($P = 0.855$) between control dogs (1.32 ± 0.4 ng/mL/h]) and dogs with mitral valve regurgitation (1.32 ± 0.19 ng/mL/h; **Figure 1**). Plasma concentrations of angiotensin I did not differ significantly ($P = 0.806$) between control dogs (570.2 ± 302.26 pg/mL) and dogs with mitral valve regurgitation (477.4 ± 49.29 pg/mL). Plasma concentrations of angiotensin II did not differ significantly ($P = 0.201$) between control dogs (20.7 ± 19.61 pg/mL) and dogs with mitral valve regurgitation (21.0 ± 2.19 pg/mL). Plasma aldosterone concentrations did not differ significantly ($P = 0.715$) between control dogs (26.5 ± 20.10 pg/mL) and dogs with mitral valve regurgitation (22.4 ± 6.39 pg/mL).

Tissue ACE and chymase-like activities—Mean ± SD tissue ACE activity in left ventricular samples obtained from dogs with mitral valve regurgitation (5.92 ± 1.17 mU/g of tissue) was significantly ($P = 0.006$) higher, compared with that of control dogs (1.53 ± 0.17 mU/g of tissue; **Figure 2**). In contrast, chymase-like activity in left ventricular samples obtained from dogs with mitral valve regurgitation (8.22 ± 2.40 nmol/min/g of tissue) was significantly ($P = 0.028$) decreased, compared with that of control dogs (19.03 ± 3.27 nmol/min/g of tissue).

Discussion

The RAAS exists in tissues as well as in the circulation; the tissue RAAS has been recognized as the system that is more responsible for cardiac remodeling. Tissue angiotensin II causes increases in concentrations of transforming growth factor–β1 and activates mitogen-activated protein kinase and phospholipase, which results in increased collagenous tissue and cardiac remodeling.[17,18] Formation of plasma angiotensin II in the vascular space is dependent on endothelium-bound ACE, whereas formation of angiotensin II within tissues relies on both ACE and chymase.[19]

Investigators in 1 study[5] reported that plasma renin activity and plasma aldosterone are activated in Cavalier King Charles Spaniels with naturally developing mitral valve regurgitation, a result that conflicts with findings of the study reported here. However, the range of values for both variables in dogs with mitral valve regurgitation appears to be widely overlapped with that in clinically normal dogs. That study included dogs that had mild clinical signs, which would result in activation of the plasma RAAS. Plasma renin activity and aldosterone concentrations do not correlate with atrial size.[5] Other investigators[8] reported that an increase in the plasma RAAS did not always correlate with the severity of CHF. In another study,[9] plasma angiotensins I and II were activated at the acute stage of volume overload, although values were within the reference ranges during the subacute stage. Because the dogs of the study reported here had only well-compensated, mild mitral valve regurgitation without signs of cardiovascular disease, the hemodynamics should have been minimally affected, which would be 1 reason that the plasma renin activity and plasma concentrations of angiotensin I, angiotensin II, and aldosterone were not changed in our study. The other possible reasons included relatively large variations in data as a result of the small sample number and differences in breeds of dogs (ie, Beagles in our study and Cavalier King Charles Spaniels in the other study[5]).

In the study reported here, tissue ACE and chymase-like activities were changed in dogs with mitral valve regurgitation but without clinical signs of the condition and without significant changes in the plasma RAAS. Changes in the plasma RAAS in rats are not always accompanied by changes in the tissue RAAS,[9] although it is not known whether this can be extrapolated to dogs. In that study, one of the possible reasons for the results was that regulation of the generation of angiotensin II in cardiac tissue is independent of generation of circulatory (plasma) angiotensin II.

Formation of tissue angiotensin II is significantly increased at the time of detection of the disease in New York Heart Association class I human patients.[20] In that report, ACE mRNA concentrations in myocardium of failing hearts were higher than concentrations in clinically normal hearts, although chymase-like activity did not change, compared with activity in clinically normal hearts. Similar results were obtained in the study reported here (ie, ACE activity was significantly increased and chymase-like activity significantly decreased in dogs with mitral valve regurgitation, compared with results for control dogs).

Mechanical stretching is considered a likely candidate to cause ACE upregulation in the tissue RAAS.[11,12,21] On the other hand, chymase released from mast cells increases when there is hemodynamic stress.[22] The experimentally induced mitral valve regurgitation in our study was so mild that hemodynamic stress should have been minimal. Another possible factor that may have influenced the activation of enzymes was the duration of the disease process. It has been reported[16,23] that the degree of enzyme activation changes over time during the course of the disease. Although the exact reason that chymase-like activity decreased in the dogs of our study was unclear, analysis of our results suggested that generation of angiotensin II would be primarily through the ACE-dependent pathway at this stage of the disease.

The tissue ACE activity was increased at the compensated stage of mitral valve regurgitation in our study. Thus, we hypothesized that ACE inhibition may be beneficial in delaying and preventing left ventricular remodeling. However, results of clinical studies[4,a] of long-term treatment with ACE inhibitors in dogs with mitral valve regurgitation did not support our hypothesis. Other investigators[11] reported that treatment with ACE inhibitors prevented the increase in cardiac angiotensin II concentrations in hearts with mitral valve regurgitation but resulted in the upregulation of AT1–receptor mRNA concentrations. In addition in that study,[11] loss of the fine collagen weave was evident in dogs with mitral valve regurgitation treated with ACE inhibitors, which would result in destruction of the structural support of the extracellular matrix that is important in maintaining the normal geometry of the left ventricular chamber. Although that study[11] used dogs with experimentally induced subacute mitral valve regurgitation, treatment with ACE inhibitors alone may not be the

best therapeutic option, even when tissue ACE activity is increased.

Angiotensin II type 1 receptor blocker would be another medication that could possibly prevent left ventricular remodeling. However, in another study,[12] AT1RB did not provide beneficial effects on left ventricular remodeling in dogs with experimentally induced subacute severe mitral valve regurgitation. Thus, results for the study reported here cannot simply be extrapolated to dogs with naturally developing mild chronic mitral valve regurgitation because the clinical stage, duration of the affected period, and cause of the condition are extremely different. Other possible medical interventions for dogs with mild mitral valve regurgitation include β blockers or a combination of an ACE inhibitor and AT1RB. Additional investigations are warranted to determine the best medical treatment for dogs with mitral valve regurgitation without clinical signs of the condition.

The study reported here has some limitations. Although an ACE-dependent pathway has been suggested[20] as a major route for production of tissue angiotensin II, especially during the chronic stage of the condition,[24] it was unclear whether tissue angiotensin II concentrations were increased in the dogs with mitral valve disease in our study. In a preliminary unpublished study conducted by our laboratory group, the percentage area of myocardial fibrosis quantified by computer-based methods was significantly increased in the same dogs with mitral valve regurgitation that were used in the study reported here, compared with values for clinically normal dogs. Because tissue angiotensin II is one of the major factors that induces myocardial fibrosis,[25,26] we believe that it should be increased in dogs with mild mitral valve regurgitation. Another limitation was that experimentally induced mitral valve regurgitation is not the same as the naturally developing condition, although the time course of the disease in our dogs with experimentally induced mitral valve regurgitation appeared to be similar to that for dogs with naturally developing mitral valve regurgitation.

Treatment of dogs with well-compensated, mild mitral valve regurgitation should focus on neurohormonal changes, rather than on hemodynamic changes. It would be clinically useful if the tissue RAAS could be estimated by noninvasive variables, such as stress of the left ventricular wall, because the tissue RAAS cannot be measured without collection of tissue samples.

The study reported here focused on changes in tissue angiotensin II–producing enzymes in dogs with mild chronic mitral valve regurgitation but without clinical signs of the condition, which would be the most common situation encountered in veterinary clinics. Changes in tissue ACE and chymase-like activities have already taken place in these dogs, and it may be that the objective should be to prevent cardiac remodeling for dogs at this stage of the disease. However, a medical regimen as a prophylactic treatment for dogs at this stage remains uncertain. As mentioned previously, evaluation of the influence of β blockers or a combination of AT1RB and ACE or other inhibitors on the tissue RAAS in dogs with mitral valve regurgitation but without clinical signs of the condition would help to reveal the best approach for the management of dogs at this stage of mitral valve regurgitation.

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