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Effects of the angiotensin-converting enzyme inhibitor alacepril in dogs with mitral valve disease

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ABSTRACT. Alacepril is a relatively novel angiotensin-converting enzyme inhibitor; however, the safety, tolerance, and efficacy of alacepril in terms of cough suppression in dogs with mitral valve disease (MVD) remain unknown. The aim of this study was to investigate the safety, tolerance, and cough suppression efficacy of alacepril in dogs with MVD. This was a multi-center, prospective study. Forty-two dogs with echocardiographic or radiographic evidence of cardiac enlargement in addition to cough were enrolled. Dogs were treated with alacepril (1.0–3.0 mg/kg/day) for at least 4 weeks. One dog (2.4%) developed complications, including appetite loss, lethargy, and vomiting. Thirty-six dogs were re-evaluated after 4 weeks of treatment. Cough resolved or improved in 20 dogs (55.6%) after treatment. Based on the efficacy of alacepril, the dogs were divided into an effective group (n=20) and an ineffective group (n=16). After treatment, the left ventricular end-diastolic internal diameter corrected for body weight was significantly increased from baseline in the ineffective group but was significantly decreased in the effective group. Univariate binomial logistic regression analyses showed that high atrial natriuretic peptide level, N-terminal pro-B-type natriuretic peptide level, and E wave velocity at baseline were significantly correlated with alacepril inefficacy. Alacepril as treatment for MVD is well tolerated in most dogs, and different conditions of cardiac loading may influence the effect of the drug. Alacepril is expected to improve the quality of life of dogs with early stage MVD.

KEY WORDS: alacepril, canine, cough, heart failure, vasodilator

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Mitral valve disease (MVD), secondary to degeneration of the mitral valve, is the most common acquired cardiac disease and cause of heart failure in dogs. Heart failure is a complex clinical syndrome characterized by symptoms such as exercise intolerance, dyspnea, respiratory effort, and fatigue [5, 6, 14]. Affected dogs may ultimately develop left-sided congestive heart failure (CHF), especially in association with pulmonary edema, and the prognosis for these dogs is very poor [6]. In addition, cough is the most common complication of MVD even in dogs without CHF [12, 16, 36]. Both mainstem bronchial compression and cardiac enlargement are common sequelae of MVD and appear to interact with cough in a complex manner [36, 37].

Several angiotensin-converting enzyme (ACE) inhibitors are available clinically, and they are divided into two groups according to the presence of sulfhydryl; furthermore, captopril, zofenopril, and alacepril contain sulfhydryl groups. The presence of a sulfhydryl group may confer useful properties such as an antioxidant effect and enhancement of vascular endothelial nitric oxide production [24, 27, 28, 30]. Such effects may improve vascular endothelial function and encourage vascular remodeling

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Table 1. Scoring for cough

Variable	Score	Clinical correlation
Presence or absence	1	None
	2	Yes
Time of day	1	Dog coughs during the night/early morning
	2	Dog coughs consistently during the day
Situation	1	Dog coughs only during excitement
	2	Dog coughs at rest during the daytime
Change in cough after alacepril	1	Cough disappeared
	2	Cough decreased but remained
	3	Unchanged or increased

[28, 30, 31]. In addition, the effects of alacepril are mediated by its metabolites, captopril and deacetyl-alacepril [25, 40]. Alacepril exerts potent and prolonged ACE inhibition compared with captopril [38–40]. In addition to vasodilation [26, 40, 43], laboratory studies have demonstrated that alacepril restores myocardial beta-adrenoceptor density and norepinephrine content in the failing myocardium [43], while deacetyl-alacepril exerts long-lasting antihypertensive effects [25, 40] and inhibits norepinephrine secretion from the isolated artery [26]. Alacepril attenuated sympathetic activation and enhanced the arterial baroreflex in human patients with heart failure [22].

As with other cardiac diseases, the renin-angiotensin-aldosterone system is activated in dogs with MVD [32]. Previous studies demonstrated that ACE inhibitors attenuated myocardial fibrosis in animal models of heart failure [11, 42]. Pharmacological blockade of ACE plays a significant role in the management of heart failure in humans [20]. Although a recent retrospective study showed that ACE inhibitors prolonged the survival time in dogs with asymptomatic MVD [33], no prospective studies have shown that ACE inhibitors delay the onset of CHF and prolong the survival time in dogs with MVD [4, 10, 14, 15]. Furthermore, the use of ACE inhibitors to treat dogs with asymptomatic MVD, i.e., American College of Veterinary Internal Medicine (ACVIM) stage B2, remains controversial [3, 12].

Although a few studies have investigated the effects of alacepril in laboratory dogs [21, 34], clinical information of alacepril in dogs with spontaneous MVD is lacking. The objectives of the current study were to investigate the safety and tolerance of alacepril in dogs with MVD and to determine whether alacepril improves clinical symptoms, i.e., cough, in dogs with MVD in ACVIM stage B2.

MATERIALS AND METHODS

This was a prospective multi-center study. All dogs were examined between August 2015 and July 2016. The study was conducted in accordance with the experimental animal guidelines of the Japanese Ministry of Education, Culture, Sports, Science and Technology. Owners provided informed consent before their dogs participated in the study.

Dogs

A total of 42 dogs (20 males and 22 females) aged 4.5–16.0 years and weighing 2.4–12.0 kg were enrolled in the study. All dogs underwent physical examination, echocardiography, thoracic radiography, and blood sampling, all of which were performed without sedation in a quiet examination room. All owners were asked detailed questions on whether their dogs had received previous treatment for cardiovascular disease.

Inclusion criteria for dogs with MVD were at least a grade 3/6 heart murmur and confirmation of mitral valve regurgitation on echocardiography. Dogs had echocardiographic or radiographic evidence of cardiac enlargement, i.e., ACVIM stage B2 [3]. In detail, dogs that met two or more of the following criteria were regarded as stage B2: vertebral heart scale (VHS) score of ≥10.5, left atrial-to-aortic diameter ratio (LA/Ao ratio) of ≥1.6, and left ventricular end-diastolic internal diameter corrected for body weight (LVIDDN) of ≥1.7 [3, 7]. Furthermore, to investigate the suppression of cough by alacepril, only dogs with a cough were included. Following the initial examination, dogs were treated with alacepril (1.0–3.0 mg/kg/day, once-daily oral administration; Apinac, DS Pharma Animal Health, Osaka, Japan) for at least 4 weeks. The alacepril dose was not changed during the study. Each dog was examined at the time of enrollment and after treatment. Cough was scored based on the owner's assessment (Table 1). After data collection, subgroup analyses were conducted based on the improvement in total cough score. The dogs were divided into an effective group if the total cough score improved by ≥1 after treatment and into an ineffective group if the total cough score did not improve. The clinician was not blinded to the dog's history or the echocardiography results.

Exclusion criteria for the study were asymptomatic dogs without cardiac enlargement (ACVIM stage B1), and dogs with current or past cardiogenic pulmonary edema (ACVIM stage C). Other exclusion criteria were concurrent systemic illness in addition to cardiovascular disease such as pulmonary, endocrine, renal, hepatic, or inflammatory diseases or malignancies. In addition, dogs that exhibited an obvious respiratory disease, such as tracheal collapse, bronchomalacia, pneumonia, laryngeal, or thoracic cavity disease, and/or a lung tumor were excluded after thoracic radiography.

Thoracic radiography and echocardiography

The cardiothoracic ratio and VHS score were evaluated using thoracic radiography according to a previously described method [8, 17]. Transthoracic echocardiography was performed by experienced echocardiographers using an ultrasonographic unit with a 7.5- to 12-MHz probe. MVD was diagnosed based on characteristic valvular lesions of the mitral valve apparatus (thickened and/or prolapsing mitral valve leaflets), and demonstrated mitral valve regurgitation evident on color flow Doppler echocardiography. The LA/Ao ratio was measured on the right parasternal short-axis view, and M-mode echocardiography was performed. Fractional shortening was calculated as (left ventricular end-diastolic internal diameter [LVIDd]–left ventricular end-systolic internal diameter [LVIDs])/LVIDd × 100. LVIDDN was calculated as LVIDd (cm)/(body weight [kg]$^{0.294}$) [7]. In the left parasternal apical four-chamber view, pulsed-wave Doppler echocardiography was used to measure the transmitral flow velocity with the sample volume positioned at the tip of the mitral valve leaflets. The mitral early diastolic flow (E wave) and late diastolic flow (A wave) velocities were measured, and the ratio of the E to A wave (E/A ratio) was calculated. Three dogs could not undergo detailed echocardiographic measurements due to excitement or panting but were diagnosed based on the results of B-mode using color Doppler flow echocardiography.

Cardiac biomarker measurements

Blood samples were collected from the jugular vein at the initial examination in all dogs. The blood samples were collected in tubes containing aprotinin (for measurement of atrial natriuretic peptide [ANP]) and ethylene diamine tetraacetic acid (for measurement of N-terminal pro-B-type natriuretic peptide [NT-proBNP]), then centrifuged at 1,500 *g* for 10 min at 4°C. The plasma C-terminal ANP concentration was determined using a chemiluminescence enzyme immunoassay for human α-ANP in a commercial laboratory (Fujifilm Monolis, Co., Ltd., Tokyo, Japan). The precision of the ANP assay has been reported previously [18]. Plasma NT-proBNP concentrations were determined using an enzyme immunoassay for canine NT-proBNP in a commercial laboratory (Cardiopet proBNP test; IDEXX Laboratories, Ludwigsburg, Germany).

Statistical analysis

Statistical analyses were performed using a statistical software (Statmate III, Ver 3.16, Avice, Inc., Tokyo, Japan). Normality of data was assessed with the Kolmogorov–Smirnov test. All data were expressed as medians (min–max or interquartile range [IQR]). The Mann–Whitney *U* test was used to determine statistical significance between the effective and ineffective groups, and the paired *t*-test was used to determine statistical significance between before and after treatment. Statistical significance was determined using a Bonferroni-corrected alpha of 0.0166 to adjust for multiple comparisons. The relationship between the inefficacy and each variable was further investigated using univariate binomial logistic regression analysis. Odds ratios are given as 2-tailed with 95% confidence intervals. A P value of <0.05 was considered statistically significant.

RESULTS

A total of 42 dogs were enrolled in the study and started treatment with alacepril. One of the 42 dogs (2.4%) developed complications, including appetite loss, lethargy, and vomiting after alacepril administration, but serious adverse effects were not observed. This dog recovered following discontinuation of alacepril for 3 days and reduction of the dose by approximately 50% but was excluded from the study due to owner noncompliance. In addition, 5 dogs were excluded from the final analyses because they were lost to follow-up. As a result, data from 36 dogs (17 males and 19 females; age 4.5–14.1 years; weight 2.4–12.0 kg) were included in the final analyses. The study population consisted of 16 Chihuahuas, 4 Cavalier King Charles Spaniels, 2 Shih Tzus, 4 mixed breed dogs, 2 Shiba Inus, 2 Pomeranians, and 2 Yorkshire Terriers, with the remaining dogs representing 4 other breeds. Twenty-five dogs were prescribed alacepril alone, and the remaining 11 dogs were prescribed one or more other medications concomitant with alacepril: pimobendan (n=7), loop diuretics (n=7), spironolactone (n=2), carvedilol (n=2), nitric oxide (n=2), or sildenafil (n=1). One dog had been prescribed benazepril before study enrollment, at which time the drug was changed to alacepril. The median daily oral dosage of alacepril was 1.8 (IQR: 1.3–2.2) mg/kg/day, and the median duration of alacepril treatment was 35 (IQR: 30–56) days. There were no significant changes in biochemical parameters before and after alacepril treatment (data not shown).

Regarding the cough score, the prevalence of cough observed consistently throughout the day was reduced from 69.4 to 36.1%, while that at rest during the daytime was reduced from 30.6 to 16.7% (Table 2). Furthermore, cough disappeared in 5 (13.9%) dogs and decreased in 15 (41.7%) dogs after alacepril treatment, but the remaining 16 (44.4%) dogs showed no change. Next, the dogs were divided into two groups based on cough scores: an effective group (20 dogs) and an ineffective group (16 dogs). The results of each examination in both groups before and after alacepril treatment are shown in Table 3. The dose and the treatment duration of alacepril was equivalent between the groups. Compared to baseline, the adjusted *P*-values of pairwise comparisons indicated a significant increase in LVIDDN after alacepril in the ineffective group, but LVIDDN in the effective group showed a significant decrease after alacepril. Finally, univariate binomial logistic regression analyses showed that a higher ANP level, NT-proBNP level, and E wave velocity were useful to predict the risk of failure of alacepril for dogs with MVD (Table 4).

DISCUSSION

ACVIM stage B2 is defined as dogs with MVD that have evidence of cardiac enlargement but have never had CHF [3]. Some

Table 2. Cough scores before and after alacepril treatment

Number (%)	Baseline	After treatment
Presence of cough	36 (100)	31 (86.1)
Time range		
Dog coughs during the night/early morning	6 (16.7)	8 (22.2)
Dog coughs during the day	25 (69.4)	13 (36.1)
Situation		
Dog coughs only during excitement	19 (52.8)	17 (47.2)
Dog coughs also at rest during the daytime	11 (30.6)	6 (16.7)
Change in cough after alacepril		
Cough disappeared	–	5 (13.9)
Cough decreased but remained	–	15 (41.7)
Unchanged or increased	–	16 (44.4)

Table 3. Comparison of results before and after alacepril treatment

	Ineffective group		Effective group	
	Baseline	After treatment	Baseline	After treatment
Treatment duration (days)	–	39 (30–61)	–	34 (30–55)
Dose (mg/kg/day)	2.0 (1.5–2.0)	–	1.5 (1.2–2.3)	–
Body weight (kg)	4.5 (3.1–7.0)	4.4 (2.8–7.0)	4.5 (3.3–7.5)	4.3 (3.4–7.2)
Respiratory (/min)	38 (30–50)	36 (28–48)	40 (29–42)	35 (30–48)
Heart rate (/min)	138 (132–158)	142 (120–156)	129 (112–150)	136 (122–156)
CTR (%)	61.3 (57.3–68.7)	60.1 (54.8–71.4)	59.3 (53.9–66.5)	60.0 (58.0–66.0)
VHS score	11.8 (10.4–12.6)	11.7 (10.9–12.5)	11.1 (10.5–11.7)	11.0 (10.2–11.5)
ANP (*p*g/m*l*)	117.2 (50.5–187.0)	161.2 (98.3–193.1)	53.2 (31.3–83.0)	72.8 (42.1–87.2)
NT-proBNP (pmol/*l*)	3,336 (1,769–5,031)	3,777 (1,654–4,736)	1,942 (942–3,000)	1,702 (1,370–2,334)
LVIDDN	2.02 (1.80–2.21)	2.24 (1.82–2.35)[a)]	1.93 (1.78–2.06)	1.77 (1.62–1.98)[b)]
Fractional shortening (%)	48.7 (42.8–52.1)	49.0 (44.8–52.2)	49.6 (43.7–53.8)	46.9 (42.7–54.7)
LA/Ao ratio	2.1 (1.8–2.4)	2.4 (2.1–2.8)	1.9 (1.7–2.4)	2.0 (1.8–2.4)
MR flow (m/sec)	6.0 (5.4–6.5)	5.8 (5.2–6.3)	6.0 (5.7–6.2)	5.8 (5.4–6.2)
E wave (cm/sec)	120.2 (103.6–138.5)	123.0 (110.8–150.0)	99.0 (89.4–125.9)	89.0 (84.3–113.4)
A wave (cm/sec)	84.0 (74.2–100.2)	85.7 (66.4–106.6)	86.0 (67.7–109.8)	85.0 (68.7–98.3)
E/A ratio	1.3 (1.0–1.6)	1.5 (1.2–2.0)	1.2 (1.0–1.6)	1.1 (0.9–1.4)

Data are expressed as medians (interquartile range [IQR]). ANP, atrial natriuretic peptide; A wave, mitral late diastolic flow; CTR, cardiothoracic ratio; E/A ratio, the ratio of the E to A wave; E wave, mitral early diastolic flow; LA/Ao ratio, left atrial-to-aortic diameter ratio; LVIDDN, left ventricular end-diastolic internal diameter corrected for body weight; NT-proBNP, N-terminal pro-B-type natriuretic peptide; MR, mitral valve regurgitation; VHS, vertebral heart scale. a; $P<0.0166$ vs. baseline, b; $P<0.0033$ vs. baseline.

Table 4. Results of univariate binomial logistic regression analyses before treatment for the effective and ineffective groups

	Coefficients	Odds ratio	df	*P* value	95% confidence interval
E wave	0.881	1.029	1	0.049	1.001–1.059
ANP	1.078	1.018	1	0.021	1.003–1.034
NT-proBNP	0.915	1.0005	1	0.031	1.000–1.001

df, degrees of freedom; E wave, mitral early diastolic flow; ANP, atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

dogs with stage B2 can show clinical signs before the onset of cardiogenic pulmonary edema. For example, cough is the most common complication of dogs with MVD [12, 16, 36]. Indeed, 40% of dogs with asymptomatic MVD of ACVIM stage B2 have cough [7]. In the present study, MVD dogs with ACVIM stage B2 concomitant with cough were selected. We confirmed that the included dogs had no primary respiratory disease, but it is possible that cardiac enlargement, e.g., left atrial dilation, contributed to coughing via mainstem bronchial compression [37].

Previous studies reported that dogs with MVD treated with ACE inhibitors showed complications including vomiting, diarrhea, loose feces, hematochezia, anorexia, lethargy, and azotemia [5, 10, 14]. The prevalence of adverse effects in these dogs treated with ACE inhibitors varied from 6 to 50% [5, 10, 14, 15]. This large range may reflect study differences such as enrollment criteria,

dog breeds used, specific ACE inhibitor used, and severity of CHF. One study found a high prevalence of adverse effects, but most of these effects were reportedly caused by aggravation of heart failure [5]. In the present study, complications (appetite loss, lethargy, and vomiting) were observed in one dog (2.4%) after alacepril treatment. A possible explanation for these complications is that alacepril may induce systemic hypotension due to vasodilation effects [38, 41]. Similar to other ACE inhibitors [10, 14], discontinuation of alacepril for 2 or 3 days and reduction of the dose by approximately 50% resolved these complications. These results indicate that alacepril treatment may be a safe and well-tolerated treatment for dogs with MVD.

Previous studies have shown that the ACE inhibitors imidapril and enalapril improve clinical symptoms, especially cough, in dogs with MVD and dilated cardiomyopathy [5, 14, 15]. Our results also showed that cough completely or partially resolved in 55.6% of dogs treated with alacepril. Possible mechanisms by which alacepril improves cough in dogs with MVD are multifactorial. Alacepril has been reported to induce vasodilation, thus decreasing systemic arterial pressure, in a canine model of MVD [21]. Deacetyl-alacepril inhibits catecholamine-induced vasoconstriction [40]. In addition, alacepril reduced LVIDDN in the effective group in the present study. LVIDDN is known as an indicator of cardiac hypertrophy in dogs, which reportedly improved after surgical treatment for patent ductus arteriosus [35]. Similarly, in humans, alacepril was reported to improve the functional class of heart failure and decrease left ventricular hypertrophy [23]. Therefore, these mechanisms may be relevant to cough resolution, but further studies are needed to clarify the mechanism of alacepril-attenuated cough.

Several studies have reported that ANP and NT-proBNP levels can be used to predict severity in dogs with MVD [2, 9], and higher ANP and NT-proBNP levels in dogs with heart failure are reported to be significantly associated with poor prognosis [9, 13, 29]. In the present study, univariate logistic regression analyses showed that higher ANP and NT-proBNP levels before treatment were significantly correlated with the inefficacy of alacepril. Circulating natriuretic peptide levels increase in response to atrial and ventricular loading related to a hemodynamic abnormality in dogs [2, 9, 19]. Elevated left atrial pressure and left ventricular end-diastolic pressure contribute to increases in intracardiac dimensions and natriuretic peptide levels in dogs with MVD [2, 9]. Therefore, different conditions of cardiac loading between the effective and ineffective groups may have potentially influenced our results, despite the same functional stage and medication dose.

Previous studies have indicated that the sulfhydryl moiety is associated with antioxidant activity, and that sulfhydryl-containing ACE inhibitors (such as captopril) moderate atherosclerosis [24, 27, 30, 31]. In addition, captopril enhances left ventricular relaxation in isolated guinea pig hearts by activating the bradykinin–nitric oxide pathway, whereas the non-sulfhydryl–containing ACE inhibitors lisinopril and quinaprilat do not [1]. As alacepril is converted into captopril *in vivo* [25, 40], we hypothesize that the sulfhydryl group may confer properties additional to ACE inhibition. One study found that alacepril prevented atherosclerosis development by reducing low-density lipoprotein levels and vascular ACE activity in monkeys [28]. Furthermore, alacepril, but not temocapril or enalapril, reduces heart rate in dogs [21]. Few studies have compared the effects of alacepril with those of other ACE inhibitors in dogs; further work is needed.

The present study had several limitations. First, a placebo control group and a blinded design were lacking. Second, although 11 of 36 dogs had been prescribed alacepril in conjunction with other cardiovascular drugs, the latter drugs were not withheld during the study. Thus, the possibility that use of these other cardiovascular drugs may have affected our results cannot be dismissed. A large-scale, placebo-controlled, prospective, double-blinded, randomized, multi-center trial is still required to determine the efficacy of long-term alacepril treatment in dogs with MVD. Finally, because the present study commenced before publication of the results of the EPIC study [7], we did not prescribe pimobendan for all dogs. Notably, pimobendan is currently recommended as the first-line therapy for dogs with ACVIM stage B2 [12].

This is the first study to investigate the efficacy of alacepril in terms of cough suppression in dogs with spontaneous MVD. The drug was safe and well tolerated. Alacepril treatment resolved or improved cough in 20 dogs (55.6%). Alacepril may be ineffective in dogs with MVD who have high pre-treatment levels of ANP and NT-proBNP. Different conditions of cardiac loading may influence the effectiveness of alacepril. Long-term clinical trials should be performed to determine the benefits of alacepril based on the onset of CHF, duration until treatment failure, and survival time.

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