

USE OF ENALAPRIL MALEATE, FUROSEMIDE AND SPIRONOLACTONE TO TREAT DOGS WITH DEGENERATIVE MYXOMATOUS MITRAL VALVE

UTILIZAÇÃO DO MALEATO DE ENALAPRIL, FUROSEMIDA, ESPIRONOLACTONA E SUAS ASSOCIAÇÕES EM CÃES COM DOENÇA DEGENERATIVA MIXOMATOSA DA VÁLVULA MITRAL

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SUMMARY

Severe degenerative myxomatous mitral valve disease (DMMVD) is a common condition in dogs. Therefore, a clinical study was conducted to determine the effectiveness of different treatment protocols based on enalapril maleate, furosemide, spironolactone, and their associations, to treat dogs with DMMVD belonging to functional class II congestive heart failure (CHF). For this purpose, 20 dogs were divided into two groups (n = 10), where the first received enalapril maleate (0.5 mg/kg) and furosemide (2 mg/kg) and the second enalapril maleate spironolactone (1 mg/kg) and furosemide, administered once a day for 56 days. The dogs were evaluated four different times regarding clinical signs, radiographic, electrocardiographic, echocardiographic and blood pressure. However, hematological and biochemical-serum, which included mainly serum concentrations of angiotensin converting enzyme (ACE) and aldosterone, were implemented at the beginning and end of the experiment. The results were characterized by animals with mitral murmur grade III to V/VI with reduced clinical signs after administration of therapeutic protocols. Laboratory tests revealed significant reduction (p<0.01) for values of ACE and aldosterone in both groups. The radiographic examination showed significant reductions (p<0.05) in VHS and variable wave Pms ECG in both groups. Moreover, the Doppler echocardiography showed significant decrease (p<0.05) variables LVD d/s IVS d/s, mean LA/Ao, FS% and the speed of mitral valve regurgitation. Therefore, the analysis of the results showed the effectiveness of treatment protocols used, especially when combined with spironolactone.

KEY-WORDS: Endocardiosis. Mitral valve. Canines.

RESUMO

Devido à prevalência de cães portadores da doença degenerativa mixomatosa da válvula mitral (DMMVD) realizou-se uma investigação clínica da eficácia de diferentes protocolos terapêuticos a base de maleato de enalapril, furosemida, espirolactona e suas associações, em cães com DMMVD pertencentes à classe funcional II da insuficiência cardíaca congestiva (ICC). Para tanto, utilizaram-se 20 cães distribuídos em dois grupos (n=10), com o primeiro recebendo maleato de enalapril (0,5 mg/kg) e furosemida (2 mg/kg) e o segundo, maleato de enalapril associado à espirolactona (1 mg/kg) e furosemida, administrados uma vez ao dia durante 56 dias. Os cães foram avaliados em quatro momentos quanto aos sinais clínicos, avaliações radiográficas, eletrocardiográficas, ecodopplercardiográficas e aferição da pressão arterial. Entretanto, em relação aos parâmetros hematológicos e bioquímico-séricos, que incluíram mormente as concentrações séricas da enzima conversora da angiotensina (ECA) e aldosterona, estas foram executadas no início e ao final do experimento. Os resultados obtidos caracterizaram-se animais portadores de sopro foco mitral grau III a V/VI com redução dos sinais clínicos após administração das terapias. Os exames laboratoriais revelaram redução significativa (p<0,01) dos valores de ECA e aldosterona em ambos os grupos. Ao exame radiográfico observou-se reduções significativas (p<0,05) nos valores VHS e na variável onda Pms do eletrocardiograma nos grupos. Outrossim, o ecodopplercardiograma evidenciou diminuição significativa (p<0,05) dos DIVEd/s, ESIVd/s, relação AE/Ao, FEC% e na velocidade da regurgitação válvula mitral. Portanto, os resultados evidenciaram efetividade dos protocolos terapêuticos utilizados, principalmente com associação à espirolactona.

PALAVRAS-CHAVE: Endocardiose. Válvula mitral. Caninos.

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INTRODUCTION

A major challenge in veterinary cardiology today is to adapt and find new therapeutic protocols for the control of congestive heart failure (CHF) due to degenerative myxomatous mitral valve disease (DMMVD). This disease affects older dogs and small breeds (DETWEILER & PATTERSON, 1965; BUCHAMAN, 1997), and clinical signs derive from the activation of compensatory mechanisms of CHF (O'GRADY, 1997; KITTLESON, 1998; MUCHA, 2007). In addition to diagnosis based on clinical history and physical examination, the patient usually presents mitral systolic murmur, with sound intensity graded according to the degree of valvular insufficiency (KITTLESON, 2006), laboratory tests, radiographic, ECG, echocardiogram and blood pressure measurement were performed as well (TILLEY, 1992; BUCHANAN & BÜCHEL, 1995; BOON, 1998; MUZZI et al, 1999).

The patient is classified according to the functional classes of CHF as established by ISACHC (1995), in order to start the most appropriate therapeutic protocol to treat DMMVD. In this case, the use of enalapril maleate is indicated, a vasodilator and inhibitor of angiotensin converting enzyme (ACE) (SEARLE, 1987; SISSON, 1991; ROTH, 1993; KITAGAWA et al, 1997; KEENE & RUSH, 1997; ETTINGER et al, 1998), with or without furosemide, a loop diuretic (JACKSON, 1996; KEENE & RUSH, 1997; PEREIRA, 1996; KOGICA, 1999). Nowadays, spironolactone is added to the association of enalapril maleate furosemide. Spironolactone is a potassium-sparing diuretic and competitive antagonist of aldosterone receptors in heart disease not responsive to conventional therapy (JACKSON, 1996; KOGICA, 1999; DIBARTOLA et al, 2000; RAMIRES & PALANCA, 2001.) The combination of spironolactone and enalapril maleate has shown clinical benefits while used to treat human patients with heart disease and dogs with the studied valvulopathy (MARCY et al, 2006; GOMEZ & ORTEGA, 2007).

Thus, assuming that dogs with DMMVD have increased preload and afterload as well, and that therapeutic protocols based on diuretics and vasodilators may produce clinical improvement of CHF, this study investigates treatment protocols based on enalapril maleate, furosemide and spironolactone and their associations in order to improve dog survival rates. The evaluations will be done through the study of clinical, laboratory, radiographic, electrocardiographic, Doppler echocardiography and blood pressure measurements before and after administration of therapeutic protocols.

MATERIAL AND METHODS

To conduct in depth study, twenty dogs with natural degenerative myxomatous mitral valve (DMMVD) functional class II CHF were used. The disease was characterized by the presence of clinical signs and changes in exams, according to the classification by ISACHC (1995). The diagnosis was based on the

review, clinical history, physical examination and complementary examinations such as hematological, serum chemistry, electrocardiogram, radiography, echocardiography and blood pressure. We excluded dogs with gastrointestinal, liver and kidney disorders, in order to avoid interference on the absorption, metabolism and excretion of the drugs being evaluated.

The inclusion of patients in different treatment protocols was established in advance, according to their order in clinical care. Thus, the dogs were randomly selected into two groups as follows:

- G1: a group of ten dogs with CHF functional class II, treated with 0.5 mg / kg of enalapril maleate and 2 mg / kg of furosemide, both administered orally once a day.
- G2: a group of ten dogs with CHF functional class II, treated with 0.5 mg / kg of enalapril maleate associated with 1mg/kg spironolactone, together with 2 mg / kg furosemide, administered orally once a day.

Each animal was assessed clinically four times during the period of 56 days between the first visit (T0) and after therapy began (T14, T28 and T56 days). In addition, the owners were asked to feed a low sodium content diet. General condition of the animal, occurrence of clinical signs and response to the therapies employed were given by the owners. Moreover, physical examinations were always performed by the same person and if any animal decompensated clinically, it would be treated and excluded from the study. Serum hematological and biochemical parameters were measured before (T0) and 56 days (T56) after administration of therapeutic protocols. Serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total protein (TP) and albumin, were obtained by spectrophotometry using commercially available kits (THRALL et al, 2007). Meanwhile, serum sodium and potassium were measured by photometry (KEENE & RUSH, 1997), aldosterone by radioimmunoassay in duplicate, while the values of ACE were obtained by the enzymatic method simplified (SILVA, 2006).

Definitive diagnosis was achieved by right lateral and dorsal-ventral radiography, while trying to observe the cardiectasia when the sum of Vertebral Heart System (ESR) was above 10.5 vertebral bodies (BUCHANAN & BÜCHELLER, 1995). In turn, an electrocardiogram was performed using a computerized device¹ that allows simultaneous acquisition of six leads (DI, DII, DIII, aVL, aVR and aVF) at a speed of 50 mm/second, calibrated to 1 mV equals one cm, allowing to obtain the electrocardiographic tracing and subsequent interpretation (TILLEY, 1992, WOLF et al, 2000). Systolic, diastolic and mean blood pressures were obtained by the non-invasive oscilometric² method. In the Doppler echocardiography³, the following variables were analyzed from the M mode: right ventricle internal diameter (RVD), left ventricle inner

¹Module for electrocardiogram acquisition for computer model ECG-PC, Teb® - São Paulo - SP.

²DX 2710 - Dixtal® - Manaus - AM.

³300S Pandion Vet - Pie Medical® Equipment - Maastricht - Holanda.

diameter (LVD), interventricular septum thickness (IVS) and free wall thickness of left ventricle (PWLVD), all variables were recorded at the end of diastole (d) and end-systole (s). The fractional thickening of the interventricular septum (IVS%) and left ventricular free wall (PWLV%), ejection fraction (EF%) and shortening (FS%), aortic diameter (Ao) and left atrial (LA), and the ratio LA/Ao were also verified. In the evaluation of pulsed Doppler transmitral flow, we measured the peak velocity of left ventricular filling fast (PEV), slow (PAV) and the relationship between them. After directing the line-cursor through the mitral valve, the time velocity integral (FVI) of mitral regurgitant flow was measured using continuous Doppler, together with the velocity of regurgitant mitral valve (BOON, 1998).

The data were analyzed statistically with the corresponding variables of clinical evolution, using the non-parametric tests and Fisher exact Chi-square test, and considered significant when $p < 0.05$. Moreover, comparing the changes in the four consultations between the G1 and G2, using analysis of variance with repeated measures on factors group and time. Tukey test at $p < 0.05$ was used to evaluate significant variables and the comparison two by two.

RESULTS

Clinical variables such as color of mucous membranes, femoral pulse, heart rate and respiratory rate, rectal temperature and body weight were measured in all clinical evaluations performed, and showed no statistical variation between groups. However, the impact of DMMVD CAE was evident within the age range from nine to 13 years, weighing five to seven kilograms, with racial predisposition: Poodle (52%), Pinscher (20%), Dachshund (12%), Fox terrier (6%) and mixed breed (10%).

During the interview, the owners initially reported on the clinical signs of CHF, such as cough and tiredness. After the implementation of treatment protocols, both groups showed significant reduction ($p < 0.05$) of tiredness and coughing. It should be emphasized, that twelve animals presented tracheal collapse, thus perpetuating clinical signs of cough.

During thoracic physical examination of all evaluated animals, a systolic murmur mitral ranging from grade III to V/VI with absence of pulmonary impairment was observed.

Regarding laboratory tests, erythrocyte and WBC did not show significant changes in both groups. However, G1, did not show significant increase of hematocrit (Ht), erythrocytes (He), hemoglobin (Hb) and plasma total protein (TP) after 56 days of therapy. As for serum biochemistry, both groups showed no significant reduction of the values. However, values of K serum showed lower reduction in G1 when compared to G2. In contrast, serum ACE and aldosterone were not significantly different initially (T0) for the two groups, but significant reductions ($p < 0.01$) were observed at the end of the period (T56 days) (Table 2).

Systolic (SBP), mean (MAP) and diastolic (DBP) blood pressures were measured in all dogs, and showed no significant changes on the baseline. Radiographic evaluation showed reduction of VHS values after standard treatments, but significantly ($p < 0.05$) only in G1. The electrocardiogram showed in T0, an increase of wave duration and complex Pms QRSms in both groups, with the prevalence of sinus rhythm. After 56 days of therapy, a significant reduction ($p < 0.05$) of these variables was observed in both groups.

Finally, Doppler echocardiography results showed no significant changes when comparing both groups. However, at T0, both groups had high values of IVS d/s, LVD d/s LA/Ao and FS%, according to standard values set by Boon (1998). On day 56 of therapy, the values of IVS d/s, LVD d/s, mean LA/Ao and FS% ($p < 0.01$) were significantly lower ($p < 0.05$), while others showed no significant variations. Spectral Doppler mode showed no significant differences for the variables between groups and over time. However, the speed of mitral regurgitation in both groups was significantly lower ($p < 0.05$) after 56 days (Table 2).

DISCUSSION

Mitral valve endocardiosis is a heart condition that affects small dog breeds (O'Grady, 1997) and incidence of valvular disease increases with increasing dog age (DETWEILER & PATTERSON, 1965; BUCHAMAN, 1997). A fact that has been confirmed in this study, since the evaluated animals ranged from 9 to 13 years old, thus correlating the evolution of valvular disease with age. The dogs in this study were characterized as small breeds, with body weight ranging from 5 to 7 kg, confirming the findings reported by Thusfields et al. (1985), Atkins (1995), O'Grady (1997) and Buchanan (1997).

Initially, the dogs of both groups had cough and moderate to severe fatigue, with subsequent significant reduction in grades and/or lack of tiredness after the treatment, as reported by the owners. These data are similar to those described by Calvert (1991) and Hamlin et al. (1996), which reported normalization of the response to exercise after the use of ACE inhibitors in dogs with the same valvular disease. Such improvement was attributed to a reduction in pre and cardiac afterload, and left atrial enlargement as well (ROTH, 1993; SISSON, 1991, PEREIRA et al, 2005). Some animals were suffering from tracheal collapse leading to the appearance of reverse sneezing, characterized by the owners as a mild cough. Therefore, the concomitant development of tracheal collapse in dogs with endocardiosis mitral valve was observed, especially in chondrotyrophic races (KITTLESON, 1998; KITTLESON & SISSON, 1999).

Thoracic auscultation of the dogs showed absence of pulmonary involvement with the presence of holosystolic murmur in the mitral grade III to V/VI, as characterized by Kittleson (2006) when he related the increase in the degree of valvular regurgitation with the evolution of heart disease.

Table 1. Mean values and variance analysis of doppler echocardiographic results obtained in groups of dogs affected by degenerative myxomatous valve mitral with NYHA class II (n = 20) at four different times, according to therapeutic protocols previously stipulated.

Analysis doppler echocardiographic												
VARIABLE	G1 (n=10)				G2 (n=10)				P			
	T0	T14	T28	T56	T0	T14	T28	T56	GROUP	TIME	REFERENCES	
RVDd	0,5 +/- 0,16 ^{Aa}	0,52 +/- 0,17 ^{Aa}	0,48 +/- 0,16 ^{Aa}	0,43 +/- 0,18 ^{Aa}	0,43 +/- 0,17 ^{Aa}	0,40 +/- 0,15 ^{Aa}	0,40 +/- 0,16 ^{Aa}	0,35 +/- 0,21 ^{Aa}	0,359	0,363	0,4 a 0,6	
IVSd	0,93 +/- 0,28 ^{Aa}	0,80 +/- 0,23 ^{Aa}	0,80 +/- 0,13 ^{Aa}	0,79 +/- 0,17 ^{Ba}	0,75 +/- 0,19 ^{Aa}	0,64 +/- 0,20 ^{Aa}	0,62 +/- 0,22 ^{Ba}	0,60 +/- 0,20 ^{Ba}	0,051	0,045*	0,4 a 0,6	
LVDd	3,06 +/- 0,8 ^{Aa}	3,09 +/- 0,77 ^{Aa}	2,98 +/- 0,82 ^{Aa}	2,79 +/- 1,06 ^{Ba}	2,88 +/- 0,79 ^{Aa}	2,67 +/- 1,35 ^{Aa}	2,70 +/- 0,96 ^{Aa}	2,63 +/- 0,92 ^{Aa}	0,596	0,049*	1,6 a 2,8	
PWLVD	0,73 +/- 0,16 ^{Aa}	0,74 +/- 0,22 ^{Aa}	0,86 +/- 0,21 ^{Aa}	0,75 +/- 0,19 ^{Aa}	0,72 +/- 0,24 ^{Aa}	0,70 +/- 0,11 ^{Aa}	0,72 +/- 0,17 ^{Aa}	0,71 +/- 0,19 ^{Aa}	0,771	0,281	0,4 a 0,6	
IVSs	1,32 +/- 0,28 ^{Aa}	1,27 +/- 0,29 ^{Aa}	1,26 +/- 0,42 ^{Aa}	1,18 +/- 0,38 ^{Ba}	1,57 +/- 0,27 ^{Aa}	1,05 +/- 0,31 ^{Aa}	1,05 +/- 0,28 ^{Aa}	1,01 +/- 0,21 ^{Ba}	0,119	0,039*	0,6 a 1,0	
LVDs	1,83 +/- 0,55 ^{Aa}	1,74 +/- 0,47 ^{Aa}	1,71 +/- 0,51 ^{Aa}	1,79 +/- 0,67 ^{Aa}	1,7 +/- 0,58 ^{Aa}	1,72 +/- 0,78 ^{Aa}	1,71 +/- 0,87 ^{Aa}	1,57 +/- 0,54 ^{Ba}	0,906	0,049*	0,8 a 1,6	
PWLVs	1,02 +/- 0,23 ^{Aa}	1,15 +/- 0,26 ^{Aa}	1,15 +/- 0,20 ^{Aa}	1,0 +/- 0,20 ^{Aa}	1,06 +/- 0,34 ^{Aa}	1,11 +/- 0,27 ^{Aa}	1,11 +/- 0,35 ^{Aa}	1,09 +/- 0,18 ^{Aa}	0,642	0,249	0,6-1,0	
IVS%	46,4 +/- 30 ^{Aa}	61,1 +/- 33,08 ^{Aa}	59,4 +/- 64,57 ^{Aa}	68,1 +/- 43,87 ^{Aa}	68,6 +/- 39,89 ^{Aa}	87,4 +/- 90,53 ^{Aa}	91,8 +/- 48,84 ^{Aa}	68,33 +/- 56,58 ^{Aa}	0,282	0,23	34-73,2	
PWLV%	44 +/- 41,13 ^{Aa}	63,8 +/- 50,24 ^{Aa}	30,8 +/- 14,65 ^{Aa}	45 +/- 23,51 ^{Aa}	54,4 +/- 43,37 ^{Aa}	58,1 +/- 17,58 ^{Aa}	55,6 +/- 46,61 ^{Aa}	39,8 +/- 19,61 ^{Aa}	0,439	0,202	45,70-75,90	
EF%	76,5 +/- 10,5 ^{Aa}	78,2 +/- 6,3 ^{Aa}	73,5 +/- 8,27 ^{Aa}	71,6 +/- 13,07 ^{Aa}	79,1 +/- 10,87 ^{Aa}	78 +/- 5,57 ^{Aa}	72,8 +/- 8,53 ^{Aa}	71,3 +/- 3,83 ^{Aa}	0,77	0,913	40-100	
FS%	43 +/- 7,65 ^{Aa}	46 +/- 6,28 ^{Aa}	41,6 +/- 7,50 ^{Aa}	39,9 +/- 7,14 ^{Ba}	48 +/- 6,96 ^{Aa}	40,5 +/- 5,94 ^{Ba}	45 +/- 8,64 ^{Aa}	41,4 +/- 4,47 ^{Ba}	0,788	0,0001**	28-35	
Ao	1,4 +/- 0,50 ^{Aa}	1,4 +/- 0,29 ^{Aa}	1,35 +/- 0,34 ^{Aa}	1,46 +/- 0,42 ^{Aa}	1,23 +/- 0,26 ^{Aa}	1,24 +/- 0,26 ^{Aa}	1,24 +/- 0,31 ^{Aa}	1,24 +/- 0,33 ^{Aa}	0,29	0,416	0,8-1,3	
LA	2,73 +/- 0,59 ^{Aa}	2,62 +/- 0,64 ^{Aa}	2,52 +/- 0,58 ^{Aa}	2,57 +/- 1,45 ^{Aa}	2,42 +/- 1,19 ^{Aa}	2,17 +/- 0,82 ^{Aa}	2,25 +/- 0,90 ^{Aa}	2,29 +/- 0,98 ^{Aa}	0,474	0,245	0,8-1,8	
LA/Ao	2,4 +/- 0,54 ^{Aa}	2,0 +/- 0,52 ^{Aa}	1,8 +/- 0,55 ^{Aa}	1,8 +/- 0,40 ^{Ba}	2,2 +/- 0,58 ^{Aa}	1,85 +/- 0,75 ^{Aa}	1,9 +/- 0,81 ^{Aa}	1,9 +/- 0,89 ^{Ba}	0,127	0,041*	1,3	
PEV	0,9 +/- 0,30 ^{Aa}	0,94 +/- 0,30 ^{Aa}	1,05 +/- 0,31 ^{Aa}	1,01 +/- 0,29 ^{Aa}	0,83 +/- 0,21 ^{Aa}	0,91 +/- 0,28 ^{Aa}	0,89 +/- 0,27 ^{Aa}	0,85 +/- 0,26 ^{Aa}	0,877	0,319	59-118	
PAV	0,69 +/- 0,23 ^{Aa}	0,63 +/- 0,12 ^{Aa}	0,80 +/- 0,23 ^{Aa}	0,71 +/- 0,21 ^{Aa}	0,77 +/- 0,26 ^{Aa}	0,61 +/- 0,33 ^{Aa}	0,72 +/- 0,25 ^{Aa}	0,71 +/- 0,48 ^{Aa}	0,407	0,488	33-93	
E/A	1,39 +/- 0,43 ^{Aa}	1,42 +/- 0,28 ^{Aa}	1,41 +/- 0,31 ^{Aa}	1,39 +/- 0,31 ^{Aa}	1,37 +/- 0,48 ^{Aa}	1,68 +/- 0,54 ^{Aa}	1,33 +/- 0,21 ^{Aa}	1,59 +/- 0,62 ^{Aa}	0,637	0,191	1,04 -2,42	
FVI	0,13 +/- 0,22 ^{Aa}	0,13 +/- 0,03 ^{Aa}	0,12 +/- 0,03 ^{Aa}	0,12 +/- 0,03 ^{Aa}	0,10 +/- 0,03 ^{Aa}	0,10 +/- 0,03 ^{Aa}	0,12 +/- 0,03 ^{Aa}	0,11 +/- 0,03 ^{Aa}	0,877	0,064	0,094	
VREG	5,23 +/- 0,97 ^{Aa}	4,89 +/- 0,56 ^{Aa}	4,65 +/- 0,89 ^{Aa}	4,57 +/- 0,80 ^{Ba}	5,57 +/- 0,50 ^{Aa}	5,15 +/- 0,67 ^{Aa}	4,95 +/- 0,87 ^{Aa}	4,93 +/- 0,44 ^{Ba}	0,05	0,048*	4,7 a 5,9	

* $P < 0.05$ – Significantly different at 5%; ** $P < 0.01$ – Significantly different at 1%;

A= averages followed by same uppercase letter do not differ according to Tukey test over time.

A= averages followed by same lowercase letter do not differ according to Tukey test between groups.

Table 2. Mean values and variance analysis of ACE and aldosterone concentrations obtained in groups of dogs affected by degenerative myxomatous valve mitral with NYHA class II (n = 10) at two different times, according to therapeutic protocols p

VARIABLE	G1 (n=10)		G2 (n=10)		P		
	T0	T56	T0	T56	GROUP	TIME	REFERENCES
ECA (U/L) +	354,7 +/- 56,88 ^{Aa}	153,5 +/- 53,5 ^{Ba}	336,1 +/- 54,62 ^{Aa}	141,5 +/- 48,28 ^{Ba}	0,498	0,001**	239 - 299
ALDOSTERONA (pg/mL) ++	82,87 +/- 68,32 ^{Aa}	26,59 +/- 40,42 ^{Ba}	111,7 +/- 85,33 ^{Aa}	19,62 +/- 37,07 ^{Ba}	0,656	0,001**	5-345

* $P < 0.05$ – Significantly different at 5%; ** $P < 0.01$ – Significantly different at 1%.

A = averages followed by the same uppercase letter do not differ according to Tukey test over time.

a = averages followed by the same lowercase letter do not differ according to Tukey test in the Groups.

Reference: + *B.E.T. Laboratories – Veterinary Endocrinology (Michigan State University, University of California – UC Davis)*; ++ *Silva, R. R.*

Determination of serum angiotensin converting enzyme activity by a simplified enzymatic method – reference values for healthy dogs and evaluation in chronic renal failure. 2006, 49f. Thesis (doctorate) - College of Agrarian and Veterinary Sciences, Universidade Estadual Paulista.

The erythrogram at the end of 56 days of therapy showed no significant increase of Ht, He, Hb and PT, for the dogs that received enalapril maleate and furosemide (G1). A fact that arises from the diuresis promoted by furosemide, a potent loop diuretic, which leads to decreased circulatory volume, and consequently, hemoconcentration serum (PEREIRA, 1996; JACKSON, 1996). However, serum biochemical (ALT, ALP, creatinine, urea and albumin) did not show changes to indicate deleterious effects on kidney function (TEXTOR, 1997) and liver, thus showing the safety of the therapies administered, as previously reported by Haggstron et al. (1996) and Atkins et al. (2002), when evaluating the use of enalapril maleate and furosemide in dogs with the same valvular disease. Serum sodium and potassium ion concentrations did not reduce significantly only in the group treated with enalapril maleate and furosemide (ATKINS et al, 2002; PEREIRA et al, 2005), a fact explained by the increase in diuresis and consequent excretion of sodium, potassium and water, caused by furosemide. However, spironolactone was added to the treatment (G2) to prevent hypokalemia caused by loop diuretics, thus stabilizing blood levels of potassium ions and slightly increasing diuresis by inhibiting the binding of aldosterone to its receptors, as observed in this study and also by Marcy et al. (2006).

ACE serum levels compared between groups showed similarities, thus suggesting that both groups belong to the same functional class of CHF. However, values were above those recommended for dogs (SILVA, 2006) at T0, which is justified by the activation of RAAS in the development of clinical signs of CHF. After the administration of therapeutic protocols, a significant reduction in these values was observed, suggesting a pharmacological action on RAAS with the reduction in preload, which lead to clinical improvement of patients. Therefore, in agreement with Hall & Kalberg (1986) who correlated the use of enalapril maleate in the acute phase of CHF with a reduction of serum angiotensin II and aldosterone. Aldosterone values were similar between groups and reduced significantly after the therapies administered, again justifying the action of drugs against RAAS (WATKINS et al, 1976; HAGGSTRONS et al, 1996; TIDHOLM et al, 2001). In dogs treated with spironolactone associated to therapy (G2), the reduction of aldosterone values was significant and meaningful, due to decreased activation of RAAS and inhibition of its action on receptors, leading to prevention of hypokalemia (MARCY et al, 2006; ORTEGA & GÓMEZ, 2007). These data together suggest the use of ACE serum and aldosterone as independent predictors of CHF, as stated by O'Sullivan et al. (2007) in relation to aldosterone with sudden death.

In this study, blood pressure values did not change significantly between groups and over time as well, which discards the development of hypotension during the therapeutic protocols studied, similarly described by Pereira (1996), Kitagama et al. (1997) and Pereira et al. (2005).

Furthermore, radiographic evaluation showed higher ESR values in both groups at T0, differing from the results reported by Smith et al. (2004), who reported cardiectasia only in some animals with the same valve disease and functional class studied here. However, after therapy, significant reduction of ESR values was observed only for G1, thus suggesting cardiac muscle remodeling with reduction in pre and after load. As for ECG characterization, groups at T0 showed an increase in the duration of P waves and QRS complexes, suggesting left atrial and ventricular overload (WOLF et al, 2000; KITTLESON, 2006; MUCHA, 2007). With further significant reduction and absence of cardiac rhythm disturbances at the end of the evaluation (DEINERT & RIPKEN, 2002; PEREIRA et al, 2005). These data also differed from the ones described by Smith et al. (2005) regarding the electrocardiogram.

Initially, Doppler echocardiography showed an increase of the left ventricular dimensions, FS%, LA and LA/Ao ratio (BOON, 1998; KIENLE & THOMAZ, 2004; BORGARELLO et al, 2007). Subsequent to therapies, a significant decrease of the variables IVS d/s, LVD d/s, LA and mean LA/Ao was observed, thus suggesting a left atrial and ventricular remodeling through reduction of diastolic and end systolic volume, promoted by the reduction in pre and cardiac after load (SISSON, 1991; ROTH, 1993; SEARLE, 1997; BILLER et al, 1998; MORI et al, 2000; DOMENECH et al, 2002; FIRM & PETRIC, 2002). As to FS%, values were higher than 40% (KIENLE & THOMAZ, 2004; KITTLESON, 2006), indicating ventricular hyperkinesis due to Frank Starling mechanism activation, with further reduction at the end of therapies (BORGARELLO et al, 2007). In spectral Doppler, the significant reduction in the rate of mitral regurgitation after treatment suggested a reduction of intraventricular pressure by decreasing pre and afterload as demonstrated by reducing FS% and EF% and left ventricular diameters. However, the reduction in speed cannot be directly correlated with regurgitation volume (KIENLE & THOMAZ, 2004).

CONCLUSIONS

Under the conditions of this study and after analysis, interpretation and discussion of the results, it can be concluded that the therapeutic protocols based on enalapril maleate, furosemide, spironolactone, and their associations, at the end of 56 day trial period, were able to control CHF, and had no deleterious effects on liver and kidney functions while maintaining serum potassium levels when spironolactone was added to the treatment. Furthermore, we suggest the use of serum ACE and aldosterone as indicators of clinical prognosis and therapeutic efficacy, together with echocardiographic variables LVD, IVS, FS%, AE and AE/Ao, in dogs with DMMVD functional class II CHF.

REFERENCES

- ATKINS, C. E. Acquired valvular insufficiency. In: MILLER, M. S., TILLEY, L. P. (Eds) **Manual of Canine and Feline Cardiology**. 2° ed., Philadelphia: W. B. Saunders, 1995, p.129-44.
- ATKINS C.E., BROWN W.A., COATS J.R., CRAWFORD M.A., DE FRANCESCO T.C., EDWARDS J., FOX P.R., KEENE B.W., LEHMKUHL L., LUETHY M., MEURS K., PETRIE J. P., PIPERS F., ROSENTHAL S., SIDLEY J.A., STRAUS J. Effects of long-term administration of enalapril on clinical indicators of renal function in dogs with compensated mitral regurgitation. **Journal of the American Veterinary Medical Association**. v.21, n5, p.654-58, 2002.
- BILLER, B., HORAUF, A., KRAFT, W. Treatment of mitral valve insufficiency in dogs with the ACE inhibitor enalapril. A clinical progress study. **Medizinische Klinik (Praxis-Ausg.)**. v. 26, n. 1, p.21-31, 1998.
- BOON, J. A. Acquired heart disease. In: BOON, J. A. **Manual of Veterinary Echocardiography**. Baltimore: Williams & Wilkins, 1998, cap 4, p.261-382
- BORGARELLI, M., TARDUCCI, A., ZANATTA, R., HAGGSTROM. J. Decreased systolic function and inadequate hypertrophy in large and small breed dogs with chronic mitral valve insufficiency. **Journal of Veterinary Internal Medicine**. v.21, n. 1, p.61-7, 2007
- BUCHAMAN, J. W. Chronic valvular disease (endocardiosis) in dogs. **Advances in Veterinary Science and Comparative**. v.21, p.75-106, 1997
- BUCHAMAN, J. W., BUCHELER, J. Vertebral scale system to measure canine Heart size in radiographs. **Journal of Veterinary Internal Medicine**. v.206, p.194-99, 1995
- CALVERT, C. A. Effect of medical therapy on survival of patients with dilated cardiomyopathy. **The Veterinary Clinics of North America Small Animal Practice**. v21, p. 919-30, 1991.
- CAMACHO, A. A. **Estudo clínico, caracterização morfológica e quantitativa dos neurônios ganglionares atriais da faixa intercalval de cães com miocardiopatia dilatada congestiva idiopática**. Jaboticabal: Universidade Estadual Paulista, 1996. 56f. Tese (livre Docência)- Faculdade de Ciências Agrárias e Veterinárias, 1996.
- DETWEILER, D.; PATTERSON, D. The prevalence and types of cardiovascular disease in dogs. **Annals of the New York Academy of Science**. v.127, p.481, 1965.
- DIBARTOLA, S. P., DE MORAIS, H. S. A. Disorders of potassium: hypokalemia and hyperkalemia. In: DIBARTOLA, S. P. (Ed). **Fluid Therapy in Small Animal Practice**. 2° ed. Philadelphia: W. B Saunders, 2000. p. 83-107
- DOMENECH, O., BUSSADORI, C., PRADELLI, D. Echocardiographic prognostic indicators in mitral insufficiency patients. [2002]. Available on <www.vin.com/proceedings/proceedings.plx/cid=WSA VA 2002>. Access 23/10/ 2007.
- ETTINGER S.J., BENITZ A.M., ERICSSON G.F., CIFELLI S., JERNIGAN A.D., LONGHOFER S.L., TRIMBOLI W., HANSON P.D. Effects of enalapril maleate on survival of dogs with naturally acquired heart failure. The long-term investigation of veterinary enalapril (live) study group. **Journal of the American Veterinary Medical Association**. v. 213, p. 1573-1577, 1998.
- FIRM, I., PETRIC, A. D. Epidemiological study of myxomatous valvular disease – A retrospective study of 195 cases. [2002]. Available on <www.vin.com/proceedings/proceedings.plx/cid=WSA VA 2002>. Access 23/10/ 2007.
- HALL, C., KALLBERG, B. E. Plasma concentrations of angiotensin II and aldosterone during acute left ventricular failure in the dog: Effect of converting enzyme inhibition. **Research in Experimental Medicine**. v. 186, n. 5, p. 387-95, 1986
- HAMLIN, R. L., BENITZ, A. M., ERICSSON, G. F., CIFELLI, S., DAURIO, C. P. Effects of enalapril on exercise tolerance and longevity in dogs with heart failure produced by iatrogenic mitral regurgitation. **Journal Veterinary Internal Medicine**. v. 2, n.10, p.85-7, 1996.
- HAGGSTROMS, J. HARSSON, K., KARLBERG, B. E., KVART, C. MADEJ, A., OLSSON, K. Effects of long-term treatment with enalapril on hidrolazine on the rennin-angiotensin aldosterone system and fluid balance in dogs with naturally acquired mitral valve regurgitation. **American Journal of Veterinary Research**. v. 57, n11, p.1645-52, 1996.
- JACKSON, E. K. Diuréticos. In: HARDMAN, J. G., MOLINOFF, P. B., GOODMAN, G. A. (Eds) **As Bases Farmacológicas das Terapêuticas**. 9° ed. México: McGRAW-Hill. 1996, p.502-22.
- O'GRADY, M R. Cardiomiopatia valvular adquirida. In: ETTINGER, S. J., FELDMAN, E. C. (Eds) **Tratado Medicina Interna Veterinária**. 4° ed. São Paulo: Manole, 1997, p. 1316-37.
- KEENE, B. W., RUSH, J. E. Terapia da insuficiência cardíaca. In: ETTINGER, S. J. FELDMAN, E. C. (Eds) **Tratado Medicina Interna Veterinária**. São Paulo: Manole, 1997, p.1219-53.

- KIENLE, R. D.; THOMAS, W. P. Ecocardiografia. In: NYLAND, T. G.; MATTOON, J. S. **Ultra-som Diagnóstico em Pequenos Animais**. 2° ed, São Paulo: ROCA, 2004, p.368-438.
- KITAGAWA, H., WAKAMIYA, H., KITOH, K., KUWAHARA, Y., OHBA, Y., ISAJI, M.; IWASAKI, T., NAKANO, M.; SASAKI, Y. Efficacy of monotherapy with benazepril, an angiotensin-converting enzyme inhibitor, in dogs with naturally acquired chronic mitral insufficiency. **The Journal of the Veterinary Medical Science**. v. 59, p. 513-20, 1997.
- KITTLESON, M. D., HAMLIN, R. L. Hydralazine therapy for severe mitral regurgitation in a dog. **Journal of the American Veterinary Medical Association**. v.179, n.9, p.903-5, 1981.
- KITTLESON, M. D. Myxomatous atrioventricular valvular degeneration. In: KITTLESON, M. D., KIENLE, R. D. (Eds) **Small Animal Cardiovascular Medicine**. St. Louis: Mosby, 1998, p.297-318
- KITTLESON, M. D. Myxomatous atrioventricular valvular degeneration. In: KITTLESON, M. D. (Ed) **Textbook of Cardiovascular Medicine**, 3° ed. em DVD, St. Louis: Mosby DVD, 2006, 52 pg
- KOGICA, M. M. Diuréticos. In: SPINOSA, M. M. GORNIK, S. L. BERNARDI, M. M. (Eds) **Farmacologia Aplicada a Medicina Veterinária**. 2° ed. Rio de Janeiro: Guanabara, 1999, p.269-76
- MARCY, T. R., RIPLEY, T. C. Aldosterone antagonists in the treatment of heart failure. **American Journal of Health System Pharmacy**. v.63, n1, p.49-58, 2006.
- MORI, Y., NAKAZAWA, M., TOMINATSU, H., MONIMA, K. Long-term effect of angiotensin-converting enzyme inhibitor in volume overloaded heart during growth: controlled pilot study. **Journal of the American College of Cardiology**. v.36, p.270-75, 2000.
- MUCHA, C. J. Insuficiencia valvular mitral. In: BELERIAN, G., MUCHA, C. J., CAMACHO, A. A. (Eds) **Afecciones Cardiovasculares en Pequeños Animales**, 2° ed., Buenos Aires, Inter.-Medica, 2007, p.261-73
- MUZZI, R. A. L., MUZZI, L. A. L., ARAUJO, R. B., PENA, J. L. B., NOGUEIRA, R. B. Diagnostico ecodopplercardiográfico na fibrose crônica da válvula mitral em cão. **Arquivo Brasileiro Medicina Veterinária e Zootecnia**. v. 51, n.6, p.1-6, 1999.
- O'SULLIVAN, M. L., O'GRADY, M. R., MINONS, S. L. Plasma big endothelin-I, atrial natriuretic peptide, aldosterone, and norepinephrine concentrations in normal Dobermans pinchers and Doberman pinchers with dilate cardiomyopathy. **Journal Veterinary Internal Medicine**. v.21, p.92-99, 2007.
- ORTEGA, M.; GÓMEZ, L. Comparación en el manejo de pacientes com degeneración mixomatosa mitral com enalapril e con la combinación enalapril-espironolactona. 2007. Disponível em "www.cardiologiaveterinaria.com". Access on 5/9/2008.
- PEREIRA, J. D. G. **Variações de parâmetros clínicos-laboratoriais de cães tratados com furosemida**. Jaboticabal: Universidade Estadual Paulista, 1996. 71f. Masters Thesis – Faculdade de ciências Agrárias e Veterinárias, 1996.
- PEREIRA, P. M., CAMACHO, A. A., MORAIS, H. A. Tratamento da insuficiência cardíaca com benazepril em cães com cardiomiopatia dilatada e endocardiose. **Arquivo Brasileiro Medicina Veterinária e Zootecnia, Belo Horizonte**, v. 57, p. 1-6, 2005.
- RAMIRES, E. Y., PALANCA, I. M. Manejo de la insuficiencia cardíaca congestiva. In: BELERIAN, G. C., MUCHA, C. J. CAMACHO, A. A. **Afecciones Cardiovasculares en Pequeños Animales**. 1° ed. Buenos-Aires: Inter.-Medica, 2001, p. 83-6
- RIPKEN, A.; DEINERT, M. A clinical trial about efficacy of pimobendan in comparison to enalapril in dogs with mitral valve endocardiosis. Part 1: clinical, laboratory, radiologic and electrocardiographic results. [2002]. Available on <www.vin.com/proceedings/proceedings.plx/cid=WSA VA 2002>. Access on 23/10/2007.
- ROTH, A. L. Use of angiotensin-converting-inhibitor in dog with congestive heart failure. **Comparative Education Review**. v.15, p.1240-44, 1993.
- SEARLE, A. Vasodilator: what they have to offer in management of congestive heart failure. **Australian Veterinary Practitioner**. v.17, n.1, p.11-20, 1987.
- SILVA, R. R. **Determinação da atividade sérica da enzima conversora da angiotensina por método enzimático simplificado – valores de referência para cães sadios e avaliação em insuficientes renais crônicos**. Jaboticabal: Universidade Estadual Paulista, 2006. 49f. Tese (doutorado) - Faculdade de Ciências Agrárias e Veterinárias, 2006
- SISSON, D. Evidence for or against the efficacy of afterload reducers for management of Heart failure in dogs. **The Veterinary Clinics of the North America Small Animal Practice**. v.21, p.945-55, 1991.
- SOARES, E. C., LARSSON, M. H. M. A., PINTO, A. C. B. C. F. Aspectos radiográficos na doença valvular crônica. **Ciência Rural**. v34, n1, p.119-24, 2004
- SOARES, E. C., LARSSON, M. H. M. A., YANATO, R. J. Chronic valvular disease: correlation between

clinical, eletrocardiographic, radiographic and echocardiographic aspects in dogs. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**. v.57, n.4, 2005.
TEXTOR, S. C. Renal failure related to angiotensin-converting enzyme inhibitors. **Seminars in Nephrology**. v.17, n.1, p.67-76, 1997.

THRUSFIELD, M. V., AITKEN, C. G. G., DARKER, P. G. G. Observations on breed and sex in relation to canine heart valve incompetence. **The Journal of Small Animal Practice**. v.26, p.709-17, 1985.

TIDHOLM, A., HAGGSTRONS, J.; HANSSON, K. Effects of dilated cardiomyopathy on the rennin-angiotensin-aldosterone system, atrial peptide natriuretic activity, and thyroid hormone concentrations in dogs. **American Journal of Veterinary Research**. v.62, n.6, p.961-67, 2001.

TILLEY, L. P. **Essentials of canine and feline electrocardiography**. 3º Ed. Philadelphia: Lea & Febger, p. 470, 1992.

TRHALL, M. A. et al. Hematologia e bioquímica clínica veterinária. SãoPaulo: Rocca, 2007.

WATKINS, L. J., BUNTON, J. A., HABER, E., CANT, D. R. SMITH, F. W., BANGER, A. C. The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. **Journal of Clinical Investigation**. v.57, n6, p.1606-17, 1976.

WEBER, K. T. Heart failure: lessons learned over the past 25 years. **Clinical Cardiology**, New York. v.18, p.23-30, 1995.

WOLF, R. CAMACHO, A. A., SOUZA, R. C. A. Eletrocardiografia computadorizada em cães. **Arquivo brasileiro de Medicina Veterinária e Zootecnia**. v.52, n.6, 2000.