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Internal Medicine

Long-term effect of sildenafil on echocardiographic parameters in dogs with asymptomatic myxomatous mitral valve degeneration

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ABSTRACT. Sildenafil is a selective phosphodiesterase-5 inhibitor that has been demonstrated to delay ventricular remodeling in humans and experimental animals. The aim of this prospective study was to assess the chronic effects of sildenafil administration on echocardiographic indices and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in dogs with naturally occurring, asymptomatic myxomatous mitral valve degeneration. Thirty client-owned dogs with ACVIM class B1 or B2 were enrolled. Dogs were randomly assigned to treatment (sildenafil 1–3 mg/kg, PO, BID for 180 days) or control groups. A total of 12 dogs completed the 180 days trial in the sildenafil group, whereas 10 dogs remained in control group. When comparing the difference from baseline values obtained over time between groups, the stroke volume (SV) at day 30 was significantly higher in the sildenafil group (P=0.038). The LA/Ao and the MR jet area were significantly lower beginning at day 30 (only MR jet area; P=0.006), day 90 (P=0.006 and P=0.027, respectively) and day 180 (P=0.029 and P=0.032, respectively). The 2D-LA was significantly lower at day 90 when compared with control group (P=0.028). The differences of NTproBNP from baseline were significantly lower when compared with control group at the same timepoint (D90, P=0.017 and D180, P=0.013). In conclusion, this study suggested that long-term treatment with sildenafil prevented aggravation of disease progression as suggested by several echocardiographic indices (i.e. SV, LA/Ao, MR jet area, 2D-LA) and reduced NTproBNP level at the indicated timepoints in dogs with asymptomatic mitral valve degeneration.

KEY WORDS: asymptomatic, dog, echocardiography, mitral valve degeneration, sildenafil

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Myxomatous mitral valve degeneration (MMVD) is the most common acquired heart disease in older dogs [13]. This disease affects small breed dogs more than larger breeds, and some breeds are predisposed to the heart disease, especially the Cavalier King Charles Spaniel [14]. The process begins with valve thickening which prevents complete valve closure resulting in backward flow of blood into the left atrium known as mitral regurgitation [18, 22]. Several compensatory mechanisms are triggered including the stimulation of sympathetic nervous system (SNS) and the renin-angiotensin aldosterone system (RAAS) [28]. Unfortunately, in small animal veterinary medicine, there are no medications that are proven to prevent left ventricular remodeling or delay the MMVD progression. Once the clinical signs develop, angiotensin converting enzyme inhibitor (ACEi), diuretic and pimobendan are mostly prescribed [2].

Sildenafil is a selective phosphodiesterase-5 inhibitor causing nitric oxide-mediated vasodilation [27]. In veterinary medicine, sildenafil is widely used for the treatment of pulmonary arterial hypertension (PAH) [4]. Recently, sildenafil has been demonstrated to delay ventricular remodeling in humans and experimental animals induced by volume or pressure overload as well as heart failure [9]. In a rat model of chronic mitral regurgitation (MR), sildenafil prevents LV remodeling and cardiac dysfunction by inhibition of inflammation and apoptosis [17]. In patients with stable systolic heart failure, sildenafil improves LV diastolic dysfunction, cardiac geometry and clinical status [12].

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Although the inhibition of PDE5 by sildenafil is a promising approach for the treatment of ventricular remodeling in several animal models and human patients, there are no data to support the use of sildenafil in dogs with naturally occurring, asymptomatic mitral valve degeneration. The aim of this prospective study was to assess the chronic effects of sildenafil administration on echocardiographic indices and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in dogs with naturally occurring, asymptomatic class B1 or B2 MMVD.

MATERIALS AND METHODS

Animals and criteria

Thirty client-owned dogs were enrolled at the Cardiac Unit, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. Consent was obtained from all owners involved with the study, and the study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University (protocol no.1431008). Dogs affected with MR attributable to MMVD in a stage of B1 or B2 according to the Consensus Statements of the American College of Veterinary Internal Medicine (ACVIM) were enrolled in the present study [2]. All enrolled dogs had a typically heart murmur (i.e. systolic murmur with point of maximal intensity at left apex). Some dogs had enlargement of the left atrium (LA) and ventricle, but none had clinical or radiographic signs of congestive heart failure (CHF). All dogs also had the ratio between the diameter of LA and aortic root (AO) (LA/AO) greater than 1.13. The exclusion criteria were: 1) dogs receiving other treatments for heart failure within 2 months before entering the study, 2) dogs with signs of other systemic diseases and 3) dogs presented with clinical or radiographical signs of CHF.

Treatment

Before the beginning of the treatment, all dogs were subjected to physical examination, radiographic examination, blood pressure measurement, body surface electrocardiography, echocardiography and venous blood drawn for complete blood count, blood chemistry profiles and NT-proBNP. Dogs were randomly assigned to treatment or control groups. Sildenafil (Sidegra® 50 mg/tab, Government Pharmaceutical Organization, Bangkok, Thailand) was given to all dogs in treatment group at a dose of 1–3 mg/kg, orally, twice a day for 6 months, whereas dogs in control group were not given any medicine. Owners were instructed to administer the drug in the morning and evening approximately 12 hr apart, and about 1 hr before feeding. All parameters, except for the thoracic radiograph, were re-evaluated at 1, 3 and 6 months after treatment. Dogs were considered to have reached the endpoint of the study when one of the following occurred: increased the stage of heart failure, sudden cardiac death, drug intolerance or end of study period.

Clinical evaluation

Blood sample collection and analytical procedures: Blood was collected in both EDTA and heparinized tubes. Complete blood count (CBC) was measured using an automated hematology analyzer (The CELL-DYN 3700, Abbott Laboratory, Abbott Park, IL, U.S.A.). Blood chemistry profiles including blood urea nitrogen (BUN), creatinine (Cr), alanine aminotransferase enzyme (ALT) and alkaline phosphatase (ALP) were measured using a chemistry analyzer (The IL ILab 650 Chemistry Analyzer, Diamond Diagnostic, Holliston, MA, U.S.A.). Additional blood was collected into EDTA tube for measurement of NT-proBNP. Plasma was separated by centrifugation within 30 min of collection and stored at −80°C until batched analysis according to guidelines established by the manufacturer and was analyzed using a commercially available assay for measurement of canine NT-proBNP (MyBioSource, San Diego, CA, U.S.A.).

Radiography

In order to confirm the presence of cardiomegaly, pulmonary edema and measurement of cardiac dimension at baseline and the end of study period, thoracic radiography in right lateral and dorso-ventral projections were used. Cardiomegaly was assessed with the vertebral heart score (VHS) method [5].

Electrocardiography

Electrocardiograms (ECG) were obtained from conscious dogs positioned on right lateral recumbency using a Ponemah12-lead ECG amplifier (Data Sciences International, Valley View, OH, U.S.A.). All limbs were perpendicular to the long axis of the body, and electrodes were attached to the skin of all limbs using alligator clips. ECG tracings (lead II) were evaluated for any changes in heart rate, rhythm or QRS morphology. Measurements were made of at least 12 consecutive cardiac cycles, and the average was used [15]. The QT interval was corrected for heart rate using van de Water formula: QTc (V)=QT−0.087*(RR-1000) [25].

Blood pressure measurement

Systolic, diastolic and mean blood pressure were obtained using oscillometric device (Datascope Passport 2, Datascope Corp., Mahwah, NJ, U.S.A.). A pressure cuff with the width of approximately 40% of the leg's circumference was placed on the median artery between the elbow and carpal pad. Three consecutive measurements of blood pressure were obtained and averaged.

Echocardiography

Standard M-mode, 2-dimensional (2D), color-flow and spectral Doppler were performed by a single blinded examiner on

Table 1. Baseline characteristics of the study population dogs with naturally occurring, asymptomatic myxomatous mitral valve degeneration

Variables	MMVD dogs (n=22)	
	Control group (n=10)	Sildenafil group (n=12)
Age (years)	12.5 ± 1.0	13.5 ± 0.6
Weight (kg)	6.5 ± 1.2	7.4 ± 1.1
Breeds		
Poodle	3	3
Pomeranian	2	2
Shih Tzu	2	3
Small mixed breed	2	1
Beagle	1	1
Cocker	-	1
Schnauzer	-	1
Gender		
M/Mc/F/Fs	3/2/0/5	3/2/2/5
Classify the stage of cardiac diseases (ACVIM)		
B1	7	9
B2	3	3

Data are presented as mean ± standard error mean (SEM). MMVD: Myxomatous mitral valve degeneration, n: Number, kg: Kilogram, M: Male, Mc: Castrated male, F: Female, Fs: Spayed female, ACVIM: American College of Veterinary International Medicine.

echocardiographic machine (EKO 7, Samsung Medison Co., Ltd., Seoul, Korea) equipped with 2–4 and 4–10 MHz phased array cardiac probes (Samsung Medison Co., Ltd.) and continuous ECG recording. All examinations were performed in unsedated dogs positioned in right and left lateral recumbency. Guidelines for the American Society of Echocardiography were followed during all examinations. Two-dimentional, M-mode, color flow and pulse wave Doppler were used to evaluate cardiac function and structure, chamber dimensions, valvular competence and flow patterns of aortic, pulmonary and atrio-ventricular. Standard M-mode dimensions were obtained in right parasternal short axis views using 2D guidance projection at the level beneath mitral valve and measured using the leading edge-to-leading edge method [23]. The 2-dimentional left atrium size (2D-LA) was obtained in the right parasternal short axis views in the first frame after aortic valve closure [8]. The quantification of MR jet (%) was determined by using Doppler echocardiography from left apical 4 chamber view. The area of mosaic color observed during systole inside the left atrium was measured and compared with the total area of the left atrium as described previously [7].

Statistical analysis

Values are presented as mean ± standard error of mean. Percent changes from baseline (D0) were calculated, and the differences between groups at the same timepoint were compared using Student *t*-test. Statistical analysis was performed by using commercial software. Level of significance was set at $P<0.05$.

RESULTS

General characteristics

The initial population of this study included 13 male and 17 female dogs (age range, 6–18 years; and weight range, 1.94–16 kg). There are several breeds enrolled in the study including Poodle (10), Shih Tzu (6), Pomeranian (4), Beagle (2), Yorkshire terrier (2), Schnauzer miniature (1), American cocker (1) and mixed breeds (4). Four dogs in the control group did not complete the study period owing to progression of their disease to congestive heart failure. Two dogs in sildenafil group were lost due to follow-up, and two dogs were excluded due to non-compliance. Therefore, these dogs were excluded from the present study. A total of 12 dogs completed the 180 days trial in the sildenafil group, whereas 10 dogs remained in control group. At baseline (D0), both groups were similar in terms of age (sildenafil=13.5 ± 0.6 versus control=12.5 ± 1.0, $P=0.380$), sex, weight (sildenafil=7.4 ± 1.1 versus control=6.5 ± 1.2, $P=0.582$) and severity of the disease based on the ACVIM classification (Table 1).

Echocardiographic data

The echocardiographic data of sildenafil and control groups are summarized in Table 2. When comparing the difference from baseline values obtained over time between groups, there were some parameters that changed significantly. The stroke volume (SV) at day 30 was significantly higher in the sildenafil group ($P=0.038$). The LA/Ao and the MR jet areas of sildenafil group were significantly smaller than those of the control group beginning at day 30 (only MR jet area; $P=0.006$), day 90 ($P=0.006$ and $P=0.027$, respectively) and day 180 ($P=0.029$ and $P=0.032$, respectively). The 2D-LA was significantly lower at day 90 when compared with control group ($P=0.028$). Other parameters did not achieve statistical significance when compared between groups at any timepoint in the study (i.e. LVIDd, LVIDs, FAC, EF, EDV, ESV and MV E/A).

Table 2. Effects of sildenafil on echocardiographic parameters in dogs with naturally occurring, asymptomatic myxomatous mitral valve degeneration

Parameters	Time (Day)	Sildenafil	Control	*P* value
LVIDd (cm)	0	2.61 ± 0.20	2.61 ± 0.12	0.498
LVIDd (% change from BL)	30	3.05 ± 3.17	−2.50 ± 1.29	0.085
	90	3.01 ± 4.52	−1.39 ± 2.60	0.232
	180	−2.09 ± 2.10	−3.37 ± 3.78	0.354
LVIDs (cm)	0	1.51 ± 0.14	1.49 ± 0.09	0.467
LVIDs (% change from BL)	30	1.63 ± 4.27	2.00 ± 3.18	0.372
	90	−0.51 ± 6.44	3.25 ± 0.11	0.356
	180	−2.52 ± 3.33	−1.68 ± 5.32	0.327
FAC (%)	0	73.10 ± 2.72	75.83 ± 2.99	0.254
FAC (% change from BL)	30	6.20 ± 5.17	−4.89 ± 2.36	0.093
	90	5.75 ± 4.55	−3.16 ± 2.99	0.138
	180	5.77 ± 5.67	−2.27 ± 2.91	0.263
EF (%)	0	75.22 ± 2.14	75.33 ± 2.51	0.488
EF (% change from BL)	30	0.90 ± 2.55	−4.33 ± 2.85	0.103
	90	2.36 ± 3.02	−5.83 ± 3.55	0.108
	180	0.49 ± 2.09	−0.32 ± 2.69	0.388
EDV (m*l*)	0	27.54 ± 4.60	25.7 ± 2.99	0.376
EDV (% change from BL)	30	10.16 ± 9.35	−5.57 ± 3.20	0.088
	90	12.12 ± 12.87	−2.23 ± 5.84	0.190
	180	−4.11 ± 5.22	−5.53 ± 8.13	0.406
ESV (m*l*)	0	7.31 ± 1.66	6.35 ± 0.87	0.318
ESV (% change from BL)	30	8.58 ± 13.55	7.37 ± 9.44	0.432
	90	8.39 ± 19.51	10.48 ± 8.01	0.488
	180	−3.94 ± 8.71	2.58 ± 13.45	0.388
SV (m*l*)	0	20.23 ± 3.17	19.35 ± 2.40	0.417
SV (% change from BL)	30	10.87 ± 9.38	−9.56 ± 4.30	0.038
	90	14.82 ± 13.13	−6.60 ± 7.30	0.122
	180	−3.81 ± 5.48	−6.42 ± 8.01	0.422
HR (bpm)	0	106 ± 6.53	9 ± 7.94	0.234
HR (% change from BL)	30	3.41 ± 7.26	29.35 ± 13.62	0.115
	90	−1.78 ± 5.56	22.00 ± 14.40	0.112
	180	6.00 ± 5.78	16.95 ± 12.14	0.376
LA/Ao	0	1.55 ± 0.09	1.57 ± 0.09	0.422
LA/Ao (% change from BL)	30	−4.06 ± 4.15	9.54 ± 7.05	0.083
	90	−8.10 ± 3.46	7.26 ± 6.30	0.026
	180	−7.98 ± 3.59	4.44 ± 4.35	0.009
MV E/A	0	1.07 ± 0.16	0.95 ± 0.09	0.258
MV E/A (% change from BL)	30	1.04 ± 0.09	6.55 ± 5.41	0.268
	90	0.37 ± 7.94	0.82 ± 7.20	0.080
	180	−2.13 ± 10.81	1.00 ± 0.18	0.428
Jet area (%)	0	32.49 ± 4.23	28.04 ± 12.27	0.199
Jet area (% change from BL)	30	−24.16 ± 8.20	30.91 ± 3.28	0.006
	90	−0.05 ± 7.60	28.02 ± 9.16	0.027
	180	−4.51 ± 8.68	50.77 ± 22.65	0.032
2D-LA (cm)	0	5.04 ± 0.69	3.76 ± 0.26	0.062
2D-LA (% change from BL)	30	−2.82 ± 5.79	4.80 ± 4.16	0.096
	90	−6.93 ± 2.59	9.67 ± 5.51	0.028
	180	4.52 ± 3.56	15.94 ± 8.64	0.119

Values at day 0 are presented as actual number, whereas values at days 30, 90 and 180 are presented as percent change from baseline. Data are presented as mean ± standard error mean (SEM). The differences between groups at the same timepoint were compared using Student *t*-test, and values of $P<0.05$ were considered significance. BL: Baseline, LVIDd: Left ventricular internal diastole diameter, LVIDs: Left ventricular internal systole diameter, FAC: Fractional area of change, EF: Ejection fraction, EDV: End-diastolic volume, ESV: End-systolic volume, SV: Stroke volume, HR: Heart rate, LA/Ao: Left atrial-to-aortic root diameter ratio, MV E/A: The mitral valve early filling/atrial filling velocities, 2D-LA: The 2-dimentional left atrium size.

Table 3. Effects of sildenafil on N-terminal pro-B-type natriuretic peptide (NT-proBNP), electrocardiographic parameters and blood pressures in dogs with naturally occurring, asymptomatic myxomatous mitral valve degeneration

Parameters	Time (Day)	Sildenafil	Control	*P* value
NTproBNP (*pg*/m*l*)	0	92.8 ± 9.0	56.1 ± 7.9	0.007
NTproBNP (% change from BL)	30	14.3 ± 9.8	45.2 ± 22.8	0.200
	90	−7.4 ± 10.9	63.4 ± 26.9	0.017
	180	−2.2 ± 12.4	73.3 ± 26.6	0.013
RR (ms)	0	614.0 ± 26.4	590.9 ± 52.4	0.683
RR (% change from BL)	30	5.08 ± 5.75	7.10 ± 10.50	0.879
	90	6.19 ± 3.66	10.76 ± 10.09	0.693
	180	−0.37 ± 4.08	13.17 ± 11.45	0.307
PQ (ms)	0	92.3 ± 4.7	98.1 ± 6.3	0.466
PQ (% change from BL)	30	3.02 ± 1.90	−1.43 ± 3.09	0.280
	90	−0.62 ± 2.42	−1.02 ± 4.05	0.940
	180	0.75 ± 2.06	2.98 ± 3.31	0.610
QRS (ms)	0	46.7 ± 2.3	47.5 ± 2.3	0.799
QRS (% change from BL)	30	14.35 ± 3.82	11.92 ± 6.41	0.771
	90	12.34 ± 3.07	4.94 ± 2.33	0.122
	180	19.24 ± 3.34	8.04 ± 5.72	0.140
QT (ms)	0	193.4 ± 4.0	195.6 ± 3.9	0.701
QT (% change from BL)	30	0.60 ± 2.65	−1.51 ± 1.78	0.588
	90	-0.06 ± 1.85	−1.42 ± 1.59	0.640
	180	−2.00 ± 1.84	2.03 ± 1.04	0.134
QTc (V) (ms)	0	193.4 ± 4.0	195.6 ± 3.9	0.701
QTc (V) (% change from BL)	30	0.60 ± 2.64	−1.50 ± 1.78	0.588
	90	-0.06 ± 1.85	−1.42 ± 1.59	0.640
	180	−1.58 ± 1.82	2.03 ± 1.04	0.171
SBP (mmHg)	0	122.77 ± 4.57	127.73 ± 5.26	0.428
SBP (% change from BL)	30	16.26 ± 4.33	1.72 ± 6.80	0.175
	90	9.11 ± 4.57	2.11 ± 4.77	0.500
	180	5.96 ± 4.05	8.54 ± 6.32	0.905
MBP (mmHg)	0	98.58 ± 4.47	101.58 ± 4.53	0.606
MBP (% change from BL)	30	13.27 ± 4.26	3.66 ± 8.31	0.384
	90	4.38 ± 4.70	1.52 ± 4.71	0.874
	180	3.62 ± 4.43	8.93 ± 7.37	0.765
DBP (mmHg)	0	81.55 ± 3.93	82.55 ± 4.28	0.445
DBP (% change from BL)	30	10.87 ± 4.76	0.94 ± 7.61	0.143
	90	0.10 ± 5.97	−1.34 ± 4.41	0.707
	180	2.00 ± 5.66	10.85 ± 8.60	0.520

Values at day 0 are presented as actual number, whereas values at days 30, 90 and 180 are presented as percent change from baseline. Data are presented as mean ± standard error mean (SEM). The differences between groups at the same timepoint were compared using Student *t*-test, and values of $P<0.05$ were considered significance. BL: Baseline, SBP: Systolic blood pressure, MBP: Mean blood pressure, DBP: Diastolic blood pressure.

Other parameters

During the study period, several parameters including NTproBNP, VHS, ECG and blood pressure were obtained. There was no significant change for those parameters when compared between groups at any timepoint in the study, except for the NTproBNP (Table 3). In sildenafil group, the differences of NTproBNP from baseline were significantly reduced when compared with control group at the same timepoint (D90, $P=0.017$; and D180, $P=0.013$). All ECG tracings demonstrated no significant arrhythmia during the study period in both groups. CBC and blood chemistry profiles obtained during the 180-day period of time did not demonstrate any clinically abnormal change.

DISCUSSION

In this study, the control MMVD dogs were recruited to evaluate the natural progression of disease over the same study period, while the sildenafil group was enrolled to assess the effects of drug. The majority of dogs enrolled in this study were Poodle (33.3%) with an age ranging from 7 to 18 years. This is consistent with previous canine studies that demonstrated small breed dogs are suffered from MMVD more than large breeds [1, 7].

This study demonstrated that sildenafil prevented aggravation of disease progression as indicated by several echocardiographic

indices (i.e. SV, LA/Ao, MR jet area and, 2D-LA) at the indicated timepoint when compared with the control group. However, the significant changes of some parameters seen at day 30 (SV) or day 90 (2D-LA) were not detected at subsequent evaluations. This may be due to the small number of enrolled dogs in each group and increased variability of those indices. The increased SV at day 30 may result from an increased EDV, since the ESV was unaltered. It has been known that PDE5 gene is minimally expressed in normal myocardium, but its expression is increased in a number of myocardial diseases (i.e. cardiomyopathies due to pressure overload or ischemia) [23, 26]. It was demonstrated previously that the process of cardiac remodeling in heart failure was blunted by protein kinase G (PKG) which was activated by cyclic guanosine monophosphate (cGMP) [24]. It has been known that sildenafil inhibits PDE5; therefore, the effects of sildenafil on LA/Ao, MR jet area and 2D-LA of MMVD dogs in the present study may relate to delay adverse remodeling process. This is consistent with previous studies that showed to attenuate progressive remodeling in a rat model of chronic mitral regurgitation, a murine model of pressure overload and patients with congestive heart failure [12, 17, 20]. In those studies, the authors suggested that sildenafil possesses antiapoptotic and anti-inflammatory effects which are underlying the mechanism of cardiac remodeling.

It has been known previously that inactive precursor molecule of BNP is released in response to increase stretch of the heart in order to promote naturesis. A previous study in dogs demonstrated that NTproBNP, an inactive N-terminal portion of the molecule, is correlated with progress of heart disease in which the plasma NTproBNP elevated with progressively increasing severity of mitral valve disease [19]. However, the use of NTproBNP to assess response to therapy is still skeptical. In the present study, NTproBNP level at baseline in sildenafil group was higher than that of control group, and it was decreased significantly after receiving sildenafil when compared with those values in the control group. Although it is statistical significance, the clinical importance is ambiguous. The reduction of NTproBNP in the present study may not be enough to ensure that sildenafil is helpful with the stress or stretch of the myocardium in MMVD dogs. Further large clinical trials need to elucidate the use of NTproBNP for assessment of patient's response to therapy, especially in the MMVD dogs [21].

Based on results of a previous study in dogs with pulmonary arterial hypertension, the lack of statistical significance of ECG parameters was expected [16]. In human embryonic kidney (HEK) cell line, the therapeutic level of sildenafil was not potent blocker of the human ether-a-go-go related gene (HERG) [10]. However, one study reported that the pharmacological properties of sildenafil at higher concentration may affect the cardiac repolarization, since it was demonstrated blocking effect on the rapid component of the delayed rectifier potassium current in isolated guinea pig heart, HEK293 cells and Chinese hamster ovary cells [11].

It is noteworthy to mention that sildenafil is not currently used for treatment in MMVD dogs. The so-called "triple therapy" (i.e. furosemide, pimobendan and ACEi) was recommended for ACVIM class C2 or more [2]. PAH secondary to MMVD has been recognized for several years in veterinary medicine, and its prevalence has increasing due to advance in diagnostic techniques [6]. Previous studies have shown that sildenafil improved clinical signs and increased quality of life without effect on blood pressure in dogs with PAH [3, 16]. Hence, some practitioners may prescribe sildenafil for MMVD dogs with stage D heart failure complicated by PAH [1]. Consistently, sildenafil used in the present study did not show any alteration in blood pressure or hematology.

Potential limitations

There are several limitations in the present study; therefore, the result must be interpreted with cautions. Firstly, the study was performed in a small sample size due to the time restriction. The lack of significant difference in some parameters between sildenafil and control group or times of assessment may be influenced by a small sample size. Although this study demonstrated beneficial effects of clinically relevant dosages of sildenafil in MMVD dogs, a larger sample size would have been more suitable to confirm the finding of this study. Secondly, the authors do not know when the disease began, since all dogs used in this study were naturally occurring heart disease. The use of medication at different stages of heart disease may affect the outcome. However, all dogs were with ACVIM class B1 or B2. Finally, the concentrations of sildenafil in dogs were not performed.

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