Effectiveness of therapy with low-dosage masitinib on pulmonary hypertension in dogs: a pilot study

Yu Sahashi[1,2], Yoshiaki Hikasa[1]

[1]Tottori University, Joint Graduate School of Veterinary Sciences, Department of Clinical Veterinary Science, Tottori, Japan
[2]Sahashi Veterinary Hospital, Inagawa, Kawabe-gun, Hyogo, Japan

Received May 23, 2022
Accepted October 10, 2022

Abstract

The purpose of this pilot study was to assess the efficacy of long-term masitinib therapy at low doses on echocardiographic, cardiovascular, haematological, and blood biochemical indicators, as well as clinical symptoms in dogs with pulmonary hypertension (PH) caused by advanced chronic degenerative mitral valve disease or heartworm disease. Seven client-owned dogs with severe PH were recruited prospectively and given low-dose masitinib orally, 3 mg/kg body weight (approximately one-fourth of the recommended antineoplastic dosage), q24h, for 123–928 days. Examinations were performed prior to masitinib administration, as well as 1, 2, 3, 6, and 12 months later. At 1–12 months, low-dose masitinib significantly reduced systolic pulmonary arterial pressure ($P < 0.05$ or 0.01) and dramatically improved clinical symptoms. Low-dose masitinib treatment improved right ventricular function indicators such as right atrium/aorta ratio, maximum tricuspid regurgitation velocity, right ventricular Tei index, and tricuspid annular plane systolic excursion, without worsening left ventricular function indicators. These findings suggest that low-dose masitinib may be effective as an adjunctive therapeutic for chronic heart failure in dogs with PH and may increase the survival of PH dogs.

Chronic heart failure, tyrosine kinase inhibitor, pulmonary arterial pressure, right ventricular function

Pulmonary hypertension (PH) is defined as systolic pulmonary arterial pressure (sPA) of > 30 mmHg or mean pulmonary arterial pressure (mPAP) of > 20 mmHg (Stepien 2009; Kellihan and Stepien 2010). In humans, PH is one of the predictors of mortality and refractory cases, and it frequently results in a poor prognosis (Humbert et al. 2004). Dogs with PH have a poor prognosis, with a median survival duration of 3–91 days after diagnosis (Johnson et al. 1999; Bach et al. 2006). Increased pulmonary vascular resistance caused by pulmonary artery vasoconstriction and vascular remodeling causes PH (Mandegar et al. 2004). Several extracellular and intracellular signaling abnormalities have been implicated in this remodeling, including platelet-derived growth factor (PDGF) receptors and c-Kit receptors (Mandegar et al. 2004; Barst 2005; Schermuly et al. 2005; Rabinovitch 2008; Montani et al. 2011). Moreover, overexpressed PDGF and its receptors may play a pathogenic role in the development of human PH, and novel therapeutic strategies targeting the PDGF pathway should be tested in clinical trials (Perros et al. 2008).

Imatinib and sorafenib, tyrosine kinase inhibitors targeting PDGF and c-Kit receptors, reverse pulmonary vascular remodeling in PH model rats by inhibiting the mitogen-activated protein kinase (MAPK) pathway (Schermuly et al. 2005; Leong and Hikasa 2018; Leong et al. 2018), and imatinib also have pulmonary vasodilatory effects in guinea pigs (Maihöfer et al. 2017). Using high doses of imatinib in humans with PH has yielded mixed results (Ghofrani et al. 2010), with side effects including nausea, thrombocytopaenia, and anaemia (Frost et al. 2015). Similar adverse effects have been reported in dogs given antineoplastic doses (Bonkobara 2015). On the other hand, imatinib or sorafenib reversed pulmonary arterial remodeling and right ventricular systolic pressure in PH model rats

Address for correspondence:
Yoshiaki Hikasa
Joint Department of Veterinary Medicine
Faculty of Agriculture, Tottori University
4-101 Koyama-minami, Tottori 680-8553, Tottori, Japan

Phone: +81 857 31 5431
E-mail: hikasa@tottori-u.ac.jp
http://actavet.vfu.cz/

at a lower dose (approximately one-third of an antineoplastic dose) (Leong and Hikasa 2018; Leong et al. 2018). Low-dose imatinib therapy improved clinical symptoms and echocardiographic outcomes without noticeable adverse effects in dogs (Arita et al. 2013) and humans (Hatano et al. 2010) with PH. However, resistance to imatinib treatment occurs in PH patients due to the relationship between apoptosis and plasma PDGF levels (Nakamura et al. 2012). In these cases, therapy with other agents may be required.

Masitinib is a veterinary drug that has been approved to treat canine mast cell tumours (Marech et al. 2014). Further, masitinib has a higher affinity for PDGF receptor beta and c-Kit receptors than imatinib, but it lacks activity against cardiotoxic breakpoint cluster region-Abelson kinase (Soria et al. 2009). Masitinib has also been reported to be safer than imatinib (Dubreuil et al. 2009). Moreover, masitinib elicits stronger cardiopulmonary preventive properties than tadalafil, via dual inhibition of the MAPK pathway and phosphodiesterase type 5 (PDE5), and long-term therapy with a lower dose of masitinib reduces PH severity and improves survival (Leong and Hikasa 2019). Therefore, a lower dose of masitinib could be used in PH therapy to target both cardiopulmonary remodelling and the increased vasoconstriction. However, no published reports on the therapeutic effect of masitinib for PH in dogs are available. The purpose of this pilot study was to examine the efficacy of low-dose masitinib therapy for canine PH caused by advanced mitral insufficiency and heartworm disease. Clinical manifestations and radiographic, echocardiographic, haemodynamic, and blood biochemical indicators were all evaluated.

Materials and Methods

Animals

At the Sahashi Veterinary Hospital (Hyogo, Japan), seven client-owned dogs with PH were recruited prospectively. Each dog owner provided informed consent. Ethical approval from a committee was not required. Data for all dogs were obtained from September 2019 to March 2022. The diagnosis of PH was defined as sPA of > 30 mmHg calculated using the modified Bernoulli equation and estimated right atrial pressure (Reinero et al. 2020). Six dogs developed PH as a result of chronic degenerative mitral valve disease (CDMVD). One dog had PH due to heartworm disease. Table 1 summarizes the patient signalment, aetiology of PH, clinical findings, the International Small Animal Cardiac Health Council (ISACHC) severity and American College of Veterinary Internal Medicine (ACVIM) stage, duration of treatments, and outcome of the seven participating dogs. Each case was classified as ISACHC IIIa or IIIb and ACVIM stage C or D (Keene et al. 2019).

Medications

All of the dogs had been previously treated with a polypharmaceutical approach that included benazepril (0.63 mg/kg body weight [BW], *per os* [p.o.], q12h), alacepril (1.1–2.5 mg/kg, p.o., q12-24h), pimobendan (0.20–0.67 mg/kg, p.o., q12h), torasemide (0.13–0.15 mg/kg, p.o., q12h), spironolactone (1.6 mg/kg, p.o., q12h), amlodipine (0.16 mg/kg, p.o., q12h), sildenafil (1.6–2.7 mg/kg BW, p.o., q12h), and furosemide (1.5–2.0 mg/kg, p.o., q12h) for periods ranging from 1 month to 15 months (Table 1). All dogs had some complications. Case 6 was treated with prednisolone for mastocytoma. In all dogs with severe PH, a low-dosage masitinib (Masivet 50 mg, AB Science, France), 3 mg/kg (approximately one-fourth of recommended antineoplastic dosage), p.o., q24h, was initially administered. Before and after masitinib administration, all dogs received their previous medications as usual. Examinations for full data collection were performed on 15–90 days before masitinib administration (before-pre), immediately before (pre; day 0), 1, 2, 3, 6, and 12 months after masitinib administration.

Clinical evaluations

Cough, exercise intolerance, syncope, ascitic fluid build-up, and peripheral oedema were all assessed before and after masitinib administration (Table 2). The severity of clinical symptoms was determined using the scoring method previously used in dogs (Arita et al. 2013). The total score was computed by adding the four scores: cough, exercise intolerance, syncope, and ascites-oedema.

Hematological and blood biochemical examinations

Blood (5 ml) was drawn from the jugular vein of each dog. A 1.0 ml volume was mixed with ethylenediaminetetraacetic acid for blood cell counts, and a 1.0 ml volume was mixed with heparin for plasma biochemical examination. The remaining 3.0 ml volume was transferred to a tube for serum collection. After centrifugation, plasma or serum was separated and stored at −35 °C for analysis. Moreover, red blood cell (RBC), white blood cell (WBC) counts, and packed cell volume (PCV) were determined using an automatic haemocytometer (pocH-100iV; Sysmex Corporation, Hyogo, Japan). On the other hand, blood urea nitrogen

Table 1. Summary of patient signalment, medication history, clinical findings, and outcomes after masitinib therapy in seven dogs with pulmonary hypertension.

Case	Breed	Age (y)	Sex	BW (kg)	Cardiac failure	Aetiology of PH	Clinical signs	ISACHC	ACVIM	Complications	History of medications	Outcome after masitinib therapy
1	Italian greyhound	10	Female ovariohyste rectomized	8	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance, ascites	IIIb	D	Choledocholit hiasis, Cervical hernia	Benazepril (0.63 mg/kg BW, p.o., q12h), pimobendan (0.63 mg/kg BW, p.o., q12h), torasemide (0.13 mg/kg BW,p.o., q12h), spironolactone (1.6 mg/kg BW, p.o., q12h), amlodipine (0.16 mg/kg BW, p.o., q12h), sildenafil (1.56 mg/kg BW, p.o., q12h), for 3 months	Alive on day 928
2	Toy poodle	14	Male intact	4.2	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance	IIIa	C	Tracheal collapse	Alacepril (1.42 mg/kg BW, p.o., q12h), pimobendan (0.30 mg/kg BW, p.o., q12h), furosemide (1.5 mg/kg BW, p.o., q12h) for 9 months	Death on day 138
3	Chihuahua	13	Male intact	2.4	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance	IIIa	C	Gallbladder myxocele	Alacepril (2.5 mg/kg BW, p.o., q12h), pimobendan (0.27 mg/kg BW, p.o., q12h), furosemide (2.0 mg/kg BW, p.o., q12h) for 12 months	Death on day 123
4	Toy poodle	10	Female intact	3.2	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance	IIIa	C	Cataract, lens luxation	Alacepril (1.8 mg/kg BW, p.o., q24h), pimobendan (0.39 mg/kg BW, p.o., q12h), for 15 months	Alive on day 590
5	Miniature pinscher	14	Female ovariohyste rectomized	4.5	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance	IIIa	C	Renal failure	Alacepril (1.1 mg/kg BW, p.o., q24h), pimobendan (0.37 mg/kg BW, p.o., q12h), furosemide (2.0 mg/kg BW, p.o., q12h) for 12 months	Death on day 199
6	Papillon	14	Female intact	6.3	MI, TI	Pulmonary venous PH due to MI	Syncope, cough, exercise intolerance	IIIa	C	Renal failure, mammary tumour, mastocytoma	Alacepril (1.9 mg/kg BW, p.o., q24h), pimobendan (0.20 mg/kg BW, p.o., q12h), prednisolone (0.3 mg/kg BW, p.o., q24h) for 12 months	Alive on day 728
7	Shiba	12	Female ovariohyste rectomized	7.4	Heartworm disease	Pulmonary arterial PH due to *Dirofilaria immitis*	Syncope, cough, exercise intolerance, ascites, pleural fluid	IIIb	-	Mammary tumour	Pimobendan (0.67 mg/kg BW, p.o., q12h), torasemide (0.15 mg/kg BW, p.o., q12h), sildenafil (2.7 mg/kg BW, p.o., q12h), for 1 month	Alive on day 380

BW - body weight; PH - pulmonary hypertension; ISACHC - severity classification by International Small Animal Cardiac Health Council; ACVIM - severity stage by American College of Veterinary Internal Medicine; MI - mitral valve insufficiency; TI - tricuspid valve insufficiency; p.o. - orally

Table 2. Clinical score before and after masitinib administration in seven dogs.

Variables	Pre (n = 7)	After masitinib administration (month) 1 (n = 7)	2 (n = 7)	3 (n = 7)	6 (n = 4)	12 (n = 4)
Cough score	1 (1–4)	1 (0–2)	1 (0–2)	1 (1–2)	1.5 (1–2)	1.5 (1–2)
Exercise intolerance score	1 (1–2)	1 (0–1)[a]	1 (0–1)[a]	1 (0–1)[a]	0 (0–1)	1 (0–1)
Syncope score	1 (0–1)	0 (0–1)[a]	0 (0–0)[a]	0 (0–0)[a]	0 (0–0)	0 (0–0)
Ascites and edema score	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
Total score	4 (3–8)	2 (1–4)[b]	2 (1–3)[b]	2 (1–3)[b]	1.5 (1–3)	2 (2–3)

Values were expressed as median (minimum–maximum). Pre - immediately before masitinib administration (day 0). Each score was based on the scoring method reported previously (Arita et al. 2013) as follows: cough score 0, none; 1, mild; 2, moderate; and 3, severe; exercise intolerance score 0, none; 1, mild; and 2, severe; syncope score 0, none; 1, mild (once per week); 2, moderate (two to six times per week); and 3, severe (every day); ascites and oedema score 0, none; 1, positive for ascites and oedema, which decreases after masitinib administration; and 2, positive for ascites and oedema, which does not change or is exacerbated after masitinib administration.
[a]$P < 0.05$, [b]$P < 0.01$, significantly different from the pre value

(BUN) and creatinine (CRE) concentrations and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were measured using an automatic biochemical analyser (Fuji Dry Chem 4000V; Fujifilm Medical, Tokyo, Japan). Serum C-reactive protein (CRP) was measured by enzyme inhibitory homogeneous immunoassay using the analyser above. Serum N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration was measured using an enzyme-linked immunosorbent assay at a reference laboratory (IDEXX Laboratories, Tokyo, Japan). Plasma atrial natriuretic peptide (ANP) was measured using an enzyme-linked immunosorbent assay at a reference laboratory (Fujifilm Vet Laboratories, Tokyo, Japan). Cardiac troponin I (cTNI) was measured using an automatic biochemical analyser (i-STAT 1 Analyzer; Abbott Point of Care Inc., USA).

Radiography, echocardiography, and various circulation indicators

The right lateral radiograph of dogs was used to measure vertebral heart size (VHS) and vertebral left atrial size (VLAS) (Mikawa et al. 2020). The cardiothoracic ratio (CTR) was measured using the ventral-dorsal radiograph of dogs. Systolic blood pressure (SBP), diastolic blood pressure, and mean blood pressure (MAP) were measured using the oscillometric method with a non-invasive blood pressure monitor (petMAP graphic; Ramsey Medical Inc., USA) attached to the right forelimb. Echocardiography was conducted using a digital ultrasonography system (Arietta 70; Hitachi, Tokyo, Japan) with a 5-MHz probe. The preceding R-to-R interval on the electrocardiogram was used to calculate the heart rate (HR). The following echocardiographic indicators were measured as previously reported (Arita et al. 2013): transthoracic two-dimensional, M-mode, and pulsed, continuous wave and tissue Doppler echocardiography were performed with dogs in right or left lateral recumbency.

The left atrium/aorta (LA/Ao) and right atrium/aorta (RA/Ao) ratios were measured at the aortic or pulmonary artery level from the right or left parasternal short-axis view. By using the M-mode method, LV fractional shortening (FS), LV ejection fraction (EF), interventricular septum thickness at end-diastole (IVSd), LV internal dimension at end-diastole (LVIDd), LV posterior wall thickness at end-diastole (LVPWd), interventricular septum thickness at end-systole (IVSs), LV internal dimension at end-systole (LVIDs), and LV posterior wall thickness at end-systole (LVPWs) were measured in the LV short-axis view. Normalized end-diastolic left ventricular inner dimension (LVIDdN) was also calculated. In addition, tricuspid annular plane systolic excursion (TAPSE) was measured with the M-mode method of the lateral aspect of the tricuspid valve annulus centred on the RV in an "off-axis" left parasternal apical four-chamber view (Pariaut et al. 2012). TAPSE value was averaged from 3 beats out of stable records of 3 consecutive beats or more.

By using pulsed Doppler echocardiography in left lateral recumbency, the early diastolic transmitral flow (E) wave, late transmitral flow (A) wave, ratio of peak velocity of E to peak velocity of A (E/A), and deceleration time of the E wave (DT_E) were recorded in the left apical four-chamber view. In the apical five-chamber view, pulmonary valve flow velocity (PVV) was measured. Then, a pulsed-wave sample volume was placed just under the aortic valve and the cross-sectional area of the LV outflow tract, aortic ejection flow velocity (AEV) and time velocity integral were measured, and stroke volume (SV) and cardiac output (CO) were calculated. The CO was calculated as SV × HR. Although HR was calculated based on the R-to-R interval, the average value of the five preceding R-to-R intervals was used to calculate HR for correction of changes in the R-to-R interval due to sinus arrhythmia. Time (a) from the end of the LV active and late inflow to the initiation of the early and passive re-inflow was measured using the left apical four-chamber view. Time (b) from the onset to the offset of the LV ejection flow was measured using the apical five-chamber view. The LV Tei index was calculated as Tei index = (a − b)/b (Tei 1995). Likewise, the right ventricular (RV) Tei index was determined from the time (a) of the end of the RV tricuspid inflow to the initiation of re-inflow in the apical four-chamber view and time (b) of the onset to the offset of the RV ejection flow in the apical short-axis view.

By using continuous wave Doppler echocardiography, the maximum systolic mitral regurgitation velocity (MRmax) was measured in the left apical four-chamber view. Moreover, the maximum systolic tricuspid regurgitation velocity (TRmax) was measured in the left and right apical four-chamber views and the left aortic short-axis view, and the highest value was used. Using the modified Bernoulli equation (pressure difference (ΔP) = 4 × $TRmax^2$), the sPA was calculated by adding the estimated right atrial pressure (10 mmHg) to the systolic right ventricle-to-right atrial pressure gradient (Vazquez de Prada et al. 1987).

By using tissue Doppler imaging, the mitral annular velocity wave was recorded based on the medial aspect of the mitral valve annulus in the left apical four-chamber view. The peak velocity of early diastolic mitral annular motion (Em) and the peak velocity of the late diastolic mitral annular motion (Am) were measured, and the ratio of Em to Am (Em/Am) was calculated. Additionally, the ratio of E to Em (E/Em) was calculated. To calculate global longitudinal strain (GLS), three different long-axis loops from each dog and three cardiac cycles from each loop were analysed. To calculate global circumferential strain (GCS), the same repetition of measurements was performed using the short-axis recordings. After manual delineation of the endocardial border on the end-diastolic frame, the software automatically divided the region of interest to six segments and tracked them throughout the cardiac cycles. In case of low tracking quality, the tracing was corrected manually and analysed again by the software (Kovács et al. 2015).

Each indicator was measured at least three times, and the average value was then utilized as the recorded data. All measurements and follow-up examinations on each dog were performed by the same investigator.

Statistical analysis

Statistical software (Prism 7.0, GraphPad, CA) was used to analyse the data. Except for the score data, all data were tested for normality using Shapiro-Wilk test. For comparisons between the before-pre and pre-data, as well as between pre-data and data after masitinib administration, paired *t*-test or Wilcoxon signed rank test was used. For clinical score data, Wilcoxon signed rank test was used for the comparison. The significance level for each analysis was at $P < 0.05$.

Results

Case descriptions and clinical scores

Before starting masitinib, all cases had a severe cough, exercise intolerance, and syncope. Ascites and pleural fluid were present in cases 1 and 6. Table 2 displays the clinical scores. When compared to the pre-masitinib period (day 0), exercise intolerance and syncope scores were significantly ($P < 0.05$) lower at 1–3 months. Cough score was lower in 1 and 2 months after masitinib administration, but not significantly ($P = 0.063$). Ascites in cases 1 and 6 disappeared 1–3 months after masitinib administration. Moreover, the total score was significantly ($P < 0.01$) lower at 1–3 months after masitinib compared with the pre. On day 385–928 after masitinib administration, four dogs (cases 1, 4, 6, and 7) had exercise intolerance and a slight cough but survived with no exacerbation of clinical symptoms.

Table 3. Changes in the systolic pulmonary arterial pressure (mmHg) before and after masitinib administration in seven dogs.

		After masitinib administration (month)					
Case	Before-pre (n = 7)	Pre (n = 7)	1 (n = 7)	2 (n = 7)	3 (n = 7)	6 (n = 4)	12 (n = 4)
1	67.7	85.5	30.5	51.0	56.0	29.5	49.8
2	59.0	75.8	43.0	66.8	38.7	–	–
3	58.7	83.9	46.9	58.6	61.8	–	–
4	59.3	108.7	32.6	49.3	54.4	40.7	71.2
5	80.9	100.0	89.0	136.0	114.6	–	–
6	34.2	122.3	42.6	76.4	56.0	81.9	54.8
7	97.9	152.8	115.0	144.9	83.9	64.6	37.2
Mean ± SD	64.5 ± 20.0	104.1 ± 26.8[a]	57.1 ± 32.1[c]	83.3 ± 40.2	66.5 ± 25.1[b]	54.2 ± 23.6[c]	55.3 ± 14.1[b]

Before-pre - 15–90 days before masitinib administration; Pre - immediately before masitinib administration (day 0); SD - standard deviation

[a]$P < 0.01$ - significantly different from the before-pre value; [b]$P < 0.05$, [c]$P < 0.01$ - significantly different from the pre value

Table 4. Radiographic, echocardiographic, and circulation variables before and after masitinib administration in seven dogs.

Variables	Before-pre (n = 7)	After masitinib administration (month)					
		Pre (n = 7)	1(n = 7)	2 (n = 7)	3 (n = 7)	6 (n = 4)	12 (n = 4)
Body weight (BW; kg)	5.3 ± 2.2	5.1 ± 2.1	5.2 ± 2.2	5.3 ± 2.4	5.2 ± 2.4	6.2 ± 2.5	6.5 ± 2.9
Heart rate (HR; beats/min)	126 ± 22	159 ± 14[b]	118 ± 20[d]	106 ± 17[d]	130 ±39[e]	123 ± 18[c]	121 ± 13[c]
Systolic blood pressure (SBP; mmHg)	160 ± 9	171 ± 32	152 ± 17	163 ± 31	176 ± 25	162 ± 38	158 ± 29
Diastolic blood pressure (DBP; mmHg)	78 ± 10	90 ± 18	82 ± 14	86 ± 16	91 ± 12	87 ± 15	98 ± 36
Mean blood pressure (MBP; mmHg)	103 ± 15	127 ± 24[a]	101 ±16[d]	114 ± 23	119 ± 11	116 ± 18	133 ± 28
Vertebral heart size (VHS)	9.8 ± 1.4	11.8 ± 1.0[b]	11.4 ± 0.9	11.7 ± 0.9	11.9 ± 1.0	11.1 ± 1.3	10.9 ± 0.9[e]
Vertebral left atrial size (VLAS)	2.3 ± 0.43	3.0 ± 0.4[b]	2.7 ± 0.4[c]	2.6 ± 0.5[d]	2.7 ± 0.4[c]	2.6 ± 0.4[d]	2.6 ± 0.4[d]
Cardiothoracic ratio (CTR; %)	49.2 ± 6.8	53.8 ± 8.2[a]	50.3 ± 6.1	48.9 ± 4.5	50.7 ± 4.5	45.6 ± 3.9	49.8 ± 5.5
LA/Ao	1.73 ± 0.46	1.92 ± 0.37	1.74 ± 0.29	1.82 ± 0.38	1.77 ± 0.33	1.67 ± 0.25	1.58 ± 0.2
RA/Ao	1.34 ± 0.31	1.47 ± 0.15	1.31 ± 0.16[c]	1.23 ± 0.10[c]	1.24 ± 0.11[c]	1.08 ± 0.21	1.11 ± 0.14
Left ventricular fractional shortening (FS; %)	51.0 ± 12.2	52.8 ± 9.0	55.8 ± 6.4	57.7 ± 7.4	53.8 ± 12.4	45.4 ± 10.2	53.4 ± 7.0[e]
Left ventricular ejection fraction (EF; %)	86.8 ± 8.6	88.5 ± 6.3	90.0 ± 3.7	93.0 ± 4.8[c]	88.0 ± 9.2	82.4 ± 9.2	89.3 ± 4.8
Interventricular septum thickness at end-diastole (IVSd; mm)	5.2 ± 0.67	6.0 ± 1.4	6.3 ± 0.89	6.3 ± 0.8	7.0 ± 1.3	6.0 ± 1.0	6.2 ± 1.3
Left ventricular inner dimension at end-diastole (LVIDd; mm)	26.8 ± 11.9	29.1 ± 9.0	28.5 ± 10.5	28.0 ± 6.1	29.6 ± 8.4	34.4 ± 7.6	32.5 ± 10.3
Left ventricular posterior wall thickness at end-diastole (LVPWd; mm)	5.1 ± 0.9	5.5 ± 1.6	5.6 ± 0.8	5.7 ± 0.6	6.1 ± 1.5	5.5 ± 0.4	6.3 ± 1.9
Interventricular septum thickness at end-systole (IVSs; mm)	9.1 ± 3.0	10.5 ± 2.9	9.6 ± 2.6	11.0 ± 1.6	10.5 ± 2.3	8.7 ± 1.7	10.5 ± 2.0
Left ventricular internal dimension at end-systole (LVIDs; mm)	12.9 ± 6.7	13.9 ± 5.6	13.3 ± 6.5	12.0 ± 4.0	14.0 ± 7.5	18.9 ± 6.0	15.3 ± 5.6
Left ventricular posterior wall thickness at end-systole (LVPWs; mm)	9.0 ± 2.4	9.5 ± 1.9	8.8 ± 1.3	9.9 ± 1.7	10.2 ± 2.0	8.9 ± 2.5	10.1 ± 3.9
Normalized end-diastolic left ventricular inner dimension (LVIDdN)	1.67 ± 0.61	1.84 ± 0.45	1.80 ± 0.53	1.74 ± 0.28	1.83 ± 0.34	2.01 ± 0.34	1.95 ± 0.45
Peak velocity of early diastolic transmitral flow (E; cm/s)	105.7 ± 35.9	114.9 ± 33.5	102.1 ± 22.8	99.3 ± 24.54	105.9 ± 23.1	98.8 ± 13.9	88.7 ± 21.0
Peak velocity of late transmitral flow (A; cm/s)	85.7 ± 12.6	87.6 ± 14.1	98.2 ± 26.8	89.6 ± 23.1	86.7 ± 14.9	84.8 ± 50.0	89.9 ± 15.2
E/A	1.2 ± 0.3	1.3 ± 0.5	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	0.91 ± 0.17	1.01 ± 0.21
Deceleration time of early diastolic transmitral flow (DT_E; ms)	101 ± 39	114 ± 31	120 ± 29	86 ± 37	125 ± 27	124 ± 19	141 ± 46
Peak velocity of early diastolic mitral annular motion (Em; cm/s)	8.8 ± 2.1	8.8 ± 1.5	8.9 ± 1.7	8.3 ± 1.5	8.9 ± 2.6	8.6 ± 1.9	7.8 ± 2.4
Peak velocity of diastolic mitral annular motion (Am; cm/s)	10.5 ± 1.6	12.1 ± 3.9	11.3 ± 2.0	9.9 ± 1.9	10.5 ± 1.8	9.3 ± 1.1	9.4 ± 1.8
Em/Am	0.85 ± 0.28	0.78 ± 0.33	0.79 ± 0.11	0.85 ± 0.15	0.85 ± 0.19	0.84 ± 0.30	0.84 ± 0.28
E/Em	12.6 ± 2.5	13.0 ± 3.2	11.5 ± 1.1	11.8 ± 2.5	12.2 ± 1.5	11.3 ± 2.0	11.8 ± 1.0
Aortic ejection flow velocity (AEV; cm/s)	89.5 ± 16.0	89.8 ± 5.6	104.2 ± 24.0	113.8 ± 9.2[c]	102.4 ± 12.1	95.4 ± 8.2	112.0 ± 30.6
Time velocity integral (TVI; cm)	6.5 ± 1.4	8.0 ± 1.1	9.1 ± 2.5	10.1 ± 2.8	9.1 ± 1.3	9.2 ± 1.7	11.1 ± 2.9

Table 4. Radiographic, echocardiographic, and circulation variables before and after masitinib administration in seven dogs.

Variables	Before-pre (n = 7)	Pre (n = 7)	After masitinib administration (month) 1(n = 7)	2 (n = 7)	3 (n = 7)	6 (n = 4)	12 (n = 4)
Stroke volume (SV; ml)	6.1 ± 2.7	7.3 ± 4.7	8.2 ± 3.8	8.5 ± 2.4	9.9 ± 3.9	10.0 ± 4.0	7.9 ± 5.0
Cardiac output (CO; l/min)	0.76 ± 0.40	0.77 ± 0.28	1.12 ± 0.57[c]	1.10 ± 0.34[c]	1.45 ± 0.89[a]	1.42 ± 0.91	1.32 ± 0.55
Pulmonary valve flow velocity (PVV; cm/s)	77.1 ± 13.8	90.4 ± 32.3	96.9 ± 24.3	93.5 ± 20.1	88.6 ± 9.1	69.4 ± 38.7	94.5 ± 8.4
Maximum systolic mitral regurgitation velocity (MRmax; cm/s)	606 ± 78	599 ± 58	600 ± 53	606 ± 59	627 ± 53	591 ± 37	589 ± 64
Maximum tricuspid regurgitation velocity (TRmax; cm/s)	372 ± 70	468 ± 68[a]	323 ± 118[d]	402 ± 117	327 ± 148[c]	342 ± 92[d]	341 ± 49[c]
Left ventricular Tei index (LV Tei)	0.300 ± 0.152	0.310 ± 0.173	0.428 ± 0.239	0.294 ± 0.150	0.294 ± 0.131	0.36 ± 0.152	0.186 ±0.075
Right ventricular Tei index (RV Tei)	0.300 ± 0.133	0.507 ± 0.073[b]	0.287 ± 0.171[d]	0.302 ± 0.166[c]	0.291 ± 0.065[d]	0.397 ± 0.251	0.341 ± 0.140
Tricuspid annular plane systolic excursion (TAPSE; mm)	14.2 ± 4.2	10.3 ± 2.0	12.3 ± 2.8[c]	13.4 ± 5.5	15.0 ± 3.3[c]	15.4 ± 8.1	14.5 ± 5.4
Global circumferential strain (GCS; %)	−7.7 ± 4.7	−6.6 ± 2.1	−9.2 ± 2.8[c]	−9.4 ± 2.9[c]	−9.7 ± 2.7[c]	−9.80 ± 2.4	−9.46 ± 1.15
Global longitudinal strain (GLS; %)	−24.0 ± 8.1	−15.7 ± 3.9[a]	−22. 7± 7.4[c]	−19.6 ± 5.2	−22.9 ± 5.7	−26.4 ± 2.7[d]	−22.2 ± 10.4

Values are expressed as mean ± standard deviation.
Before-pre - 15–90 days before masitinib administration; Pre - immediately before masitinib administration (day 0)
[a]$P < 0.05$, [b]$P < 0.01$ - significantly different from the before-pre value; [c]$P < 0.05$, [d]$P < 0.01$ - significantly different from the pre value

On day 138, case 2 died of congestive heart failure (CHF). On day 123, case 3 died as a result of gallbladder mucocele rupture. On day 199, case 5 died from CHF with chronic renal failure. After masitinib administration, the median survival time (range) was > 380 days (123 to > 928 days).

Radiographic, echocardiographic, and circulation variables

Table 3 shows the changes in sPA values for all dogs. During general therapeutic drug treatment, sPA increased significantly ($P < 0.01$) from 15 to 90 days before masitinib administration to the pre (day 0). The elevated sPA (mean 104.1 mmHg) in the pre decreased markedly and significantly ($P < 0.05$ or 0.01) at 1, 3, 6, and 12 months (mean 57.1, 66.5, 54.2, and 55.3 mmHg, respectively; 45%, 36%, 48%, and 47% decrease from the pre-value, respectively) after masitinib administration.

Table 4 shows the other results for radiographic, echocardiographic, and circulation variables analyses. The HR, MBP, VHS, VLAS, CTR, TRmax, and RV Tei index increased significantly ($P < 0.05$ or 0.01) at the pre-compared with the before-pre and the absolute value of GLS decreased significantly ($P < 0.05$) at the pre. The TAPSE tended to decrease at the pre-compared with the before-pre-value, but not significantly (P = 0.056). After masitinib administration, HR, MAP, VLAS, RA/Ao, TRmax, and RV Tei index decreased significantly ($P < 0.05$ or 0.01) at 1, 2, 3, 6, and/or 12 months, respectively, compared with the pre (day 0), whereas CO, TAPSE, absolute values of GCS, and GLS increased significantly at 1, 2, 3, and/or 6 months. Other parameters such as BW, SBP, DAP, VHS, CTR, LA/Ao, FS, EF, IVSd, LVIDd, LVPWd, IVSs, LVIDs, LVPWs, LVIDdN, E wave, A-wave, E/A, DT_E, Em/Am, E/Em, AEV, VTI, SV, PVV, MRmax, and LV Tei index did not significantly change ($P > 0.05$) at each time after masitinib administration compared with the pre.

Table 5. Hematological, blood biochemical, and cardiac biomarker variables before and after masitinib administration in seven dogs

Variables	Reference range	Before-pre (n = 7)	After masitinib administration (month) Pre (n = 7)	1 (n = 7)	2 (n = 7)	3 (n = 7)	6 (n = 4)	12 (n = 4)
White blood cells (WBC; $\times 10^3/mm^3$)	6–17	8.8 ± 5.1	12.4 ± 5.3[b]	9.4 ± 1.9	9.2 ± 3.3	10.9 ± 6.9	10.5 ± 3.9	11.8 ± 5.1
Red blood cells (RBC; $\times 10^4/mm^3$)	550–850	695 ± 104	713 ± 165	745 ± 110	675 ± 303	708 ± 101	665 ± 112	667 ± 128
Packed cell volume (PCV; %)	37–55	44 ± 7	44 ± 10	46 ± 7	46 ± 8	44 ± 7	46.1 ± 8.3	44.1 ± 6.6
Aspartate aminotransferase (AST; U/l)	10–100	96 ± 35	136 ± 101	258 ± 291	203 ± 188	270 ± 335	334 ± 441	112 ± 69
Alanine aminotransferase (ALT; U/l)	17–50	33 ± 11	29 ± 4	45 ± 18	41 ± 21	66 ± 83	43 ± 22	35 ± 13
Alkaline phosphatase (ALP; U/l)	23–212	234 ± 285	332 ± 487	269 ± 239	235 ± 195	235 ± 195	550 ± 523	291 ± 465
Blood urea nitrogen (BUN; mg/dl)	7–27	29 ± 11	33 ± 18	35 ± 8	34 ± 11	29 ± 13	30 ± 11	33 ± 10
Creatinine (CRE; mg/dl)	0.5–1.8	0.9 ± 0.4	0.9 ± 0.3	1.0 ± 0.4	1.2 ± 0.4	0.9 ± 0.4	0.9 ± 0.2	0.9 ± 0.3
C-reactive protein (CRP; mg/dl)	<0.7	0.7 ± 0.3	0.9 ± 0.7	1.2 ± 0.6	1.3 ± 1.2	0.7 ± 0.4	2.4 ± 3.1	1.7 ± 1.6
Serum N-terminal probrain natriuretic peptide (NT-proBNP; pmol/l)	<900	2405 ± 1099	2955 ± 1327[a]	2478 ± 1016	2041 ± 1237	2084 ± 1210	3263 ± 3141	2438 ± 864
Atrial natriuretic peptides (ANP; pg/ml)	<106	118 ± 46	282 ±194[a]	224 ± 178	216 ± 151	230 ±162	189 ±180	161 ± 58
Cardiac troponin I (cTNI; ng/ml)	<0.06	0.03 ± 0.02	0.17 ± 0.28	0.07 ± 0.06	0.04 ± 0.03	0.06 ± 0.03	0.06 ± 0.04	0.11 ± 0.11

Values are expressed as mean ± standard deviation
Before-pre - 15–90 days before masitinib administration; Pre - immediately before masitinib administration (day 0)
[a]$P < 0.05$, [b]$P < 0.01$ - significantly different from the before-pre value

Haematological and blood biochemical variables

Table 5 shows the changes of haematological and blood biochemical variables. The WBC, NT-proBNP, and ANP increased significantly ($P < 0.05$ or 0.01) at the pre- compared with the before-pre. After masitinib administration, both NT-proBNP and ANP tended to decrease at 2 and 3 months after masitinib therapy compared with pre, but not significantly (NT-proBNP at 3 months, $P = 0.065$; ANP at 2 months, $P = 0.061$). In addition, the WBC, RBC, PCV, AST, ALT, ALP, BUN, CRE, CRP, and cTnI values did not significantly change ($P > 0.05$) after masitinib administration. All cases tolerated well to low-dose masitinib, with no apparent adverse effects by its administration.

Discussion

Previous studies have shown that low-dose imatinib therapy, which is approximately 1/6 to 1/3 of the antineoplastic dosage, improves clinical symptoms and echocardiographic outcomes without noticeable adverse effects in humans (Hatano et al. 2010) and dogs (Arita et al. 2013) with PH. A dosage of 12.5 mg/kg/day of masitinib has been used as an antineoplastic dosage in dogs (Marech et al. 2014). In monocrotaline-induced PH model rats, it has been reported that treatment with a low-dose of masitinib (approximately 1/3 of the antineoplastic dosage) resulted in significantly decreased RV systolic pressure and hypertrophy,

as well as pulmonary vascular remodelling, comparable to a high antineoplastic dosage (Leong and Hikasa 2019). Based on these findings, the dosage of masitinib in the present study was set at 3 mg/kg/day, which is approximately 1/4 of the antineoplastic dosage recommended for dogs. The present study was the first to show that giving dogs with chronic heart failure low-dose masitinib for 123–928 days resulted in significant and noticeable improvement of PH.

To treat PH in dogs, angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, prostacyclins, PDE3 inhibitors, or PDE5 inhibitors are commonly used (Atkinson et al. 2009; Brown et al. 2010; Reinero et al. 2020); however, in some cases, the use of these multiple drugs does not prevent the worsening of symptoms. Cases tested in this study had worsening PH despite standard treatments such as ACE and PDE5 inhibitors. This study discovered that an additional administration of a low-dose masitinib (3 mg/kg/day) greatly reduced sPA by an average of 45% (from average 104.1 mmHg at the pre to average 57.1 mmHg at post 1 month) and improved clinical symptoms in all cases of seven dogs with PH caused by CDMVD or heartworm disease. The decrease in sPA observed in this study was similar to that observed on imatinib therapy in a previous study (Arita et al. 2013), despite the fact that case subjects involved in this investigation had more severe PH (sPA = 104.1 ± 26.8 mmHg, mean ± SD) than the cases with PH (sPA = 63.3 ± 24.9 mmHg) in a previous study (Arita et al. 2013). These findings could be attributed to the anti-remodelling actions of the pulmonary artery of masitinib as well as the pulmonary vasodilatory effects (Soria et al. 2009; Leong and Hikasa 2019).

The present study also revealed that administration of low-dose masitinib improved RV function indicators including RA/Ao ratio, TRmax, RV Tei index, and TAPSE without worsening LV function and rather improving LV function and cardiac performance, as indicated by increases in CO, GCS, and GLS. The improvement of RV function may be due to the reduction in RV afterload, RA pressure, and peripheral venous pressure caused by the decrease in sPA. These effects could have improved clinical symptoms, such as decreased ascites and resolution of syncope attacks. The improvement in haemodynamics may suppress excessive secretion and activation of neurohumoral factors and prevent the deterioration of chronic heart failure. In fact, in this study, both NT-proBNP and ANP tended to decrease after masitinib administration. On the other hand, the reduction of RV afterload by masitinib administration may induce LV preload. However, in the present study, VLAS significantly reduced, and LA/Ao ratio showed a decreasing trend after masitinib administration, without the worsening in E/Em and other LV function indicators. Additionally, both GLS and GCS, which are indices of LV contractility, and CO increased significantly after masitinib administration. Therefore, masitinib may have pulmonary venous relaxation effect similar to the effect of imatinib reported earlier (Maihöfer et al. 2017), which may result in lower LA pressure and increased LV return.

In dogs with severe CDMVD, increased LVIDd and increased HR and LA/Ao have been reported to be fate-predictive factors (Borgarelli et al. 2008). In the present study, HR and LVIDd were not increased after masitinib therapy, with a decrease or a decreasing trend of VLAS and LA/Ao. Furthermore, elevated NT-proBNP and ANP in dogs with cardiac disorders have been shown to be prognostic (Greco et al. 2003; Serres et al. 2009; Moonarmart et al. 2010). In the present study, NT-proBNP and ANP decreased after masitinib therapy. These findings imply that treatment with masitinib may improve prognosis by lowering sPA, thereby improving haemodynamics and protecting cardiac function by suppressing sympathetic nerve activity. Previously, the median survival time in dogs with severe PH was reported to be 3–91 days (Johnson et al. 1999; Bach et al. 2006). The cases in this study had a longer survival time (median > 380 days, range 123 to > 928 days) after masitinib medication. Thus, low-dose masitinib may be effective in prolonging the survival of dogs with severe PH. However, the small sample size is

a limitation of this study, and the efficacy of masitinib needs to be evaluated in a larger number of PH cases.

Regarding the safety of masitinib, it has been reported that masitinib lacks activity against cardiotoxic breakpoint cluster region-Abelson kinase, and its use is safer than that of imatinib (Soria et al. 2009; Dubreuil et al. 2009). In the present study, all cases tolerated well to low-dose masitinib. During masitinib treatment, no significant changes in the haematological or blood biochemical variables indicative of kidney and liver damage were observed, and no apparent clinical side effects associated with the administration of masitinib were discovered. These results imply that low-dose masitinib can be used safely for long-term therapy in dogs with PH without causing obvious side effects.

In conclusion, low-dose masitinib significantly reduced sPA by an average of 45% at 1 month and improved RV function and clinical symptoms in dogs with severe PH. To the best of our knowledge, this is the first report indicating the efficacy of low-dosage masitinib for treating dogs with PH caused by CDMVD and heartworm disease. However, larger, placebo-controlled, randomized, and blinded studies are needed to determine the long-term efficacy of low-dose masitinib in dogs with PH caused by a variety of aetiologies.

Acknowledgements

The Japan Society for the Promotion of Science provided funding for this study through a Grant-in-Aid for Scientific Research (C) (no. 18K05993; to Y. Hikasa). We thank the dogs' owners for their involvement in the study.

References

Arita S, Arita N, Hikasa Y 2013: Therapeutic effect of low-dose imatinib on pulmonary arterial hypertension in dogs. Can Vet J **54**: 255-261

Atkinson KJ, Fine DM, Thombs LA, Gorelick JJ, Durham HE 2009: Evaluation of pimobendan and N-terminal probrain natriuretic peptide in the treatment of pulmonary hypertension secondary to degenerative mitral valve disease in dogs. J Vet Intern Med **23**: 1190-1196

Bach JF, Rozanski EA, MacGregor J, Betkowski JM, Rush JE 2006: Retrospective evaluation of sildenafil citrate as a therapy for pulmonary hypertension in dogs. J Vet Intern Med **20**: 1132-1135

Barst RJ 2005: PDGF signaling in pulmonary arterial hypertension. J Clin Invest **115**: 2691-2694

Bonkobara M 2015: Dysregulation of tyrosine kinases and use of imatinib in small animal practice. Vet J **205**: 180-188

Borgarelli M, Savarino P, Crosara S, Santilli RA, Chiavegato D, Poggi M, Bellino C, La Rosa G, Zanatta R, Haggstrom J, Tarducci A 2008: Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. J Vet Intern Med **22**: 120-128

Brown AJ, Davison E, Sleeper MM 2010: Clinical efficacy of sildenafil in treatment of pulmonary arterial hypertension in dogs. J Vet Intern Med **24**: 850-854

Dubreuil P, Letard S, Ciufolini M, Gros L, Humbert M, Castéran N, Borge L, Hajem B, Lermet A, Sippl W, Voisset E, Arock M, Auclair C, Leventhal PS, Mansfield CD, Moussy A, Hermine O 2009: Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. PLoS One **4**: e7258

Frost AE, Barst RJ, Hoeper MM, Chang HJ, Frantz RP, Fukumoto Y, Galié N, Hassoun PM, Klose H, Matsubara H, Morrell NW, Peacock AJ, Pfeifer M, Simonneau G, Tapson VF, Torres F, Dario Vizza C, Lawrence D, Yang W, Felser JM, Quinn DA, Ghofrani HA 2015: Long-term safety and efficacy of imatinib in pulmonary arterial hypertension. J Heart Lung Transplant **34**: 1366-1375

Ghofrani HA, Morrell NW, Hoeper MM, Olschewski H, Peacock AJ, Barst RJ, Shapiro S, Golpon H, Toshner M, Grimminger F, Pascoe S 2010: Imatinib in pulmonary arterial hypertension patients with inadequate response to established therapy. Am J Respir Crit Care Med **182**: 1171-1177

Greco DS, Biller B, Van Liew CH 2003: Measurement of plasma atrial natriuretic peptide as an indicator of prognosis in dogs with cardiac disease. Can Vet J **44**: 293-297

Hatano M, Yao A, Shiga T, Kinugawa K, Hirata Y, Nagai R 2010: Imatinib mesylate has the potential to exert its efficacy by down-regulating the plasma concentration of platelet-derived growth factor in patients with pulmonary arterial hypertension. Int Heart J **51**: 272-276

Humbert M, Sitbon O, Simonneau G 2004: Treatment of pulmonary arterial hypertension. N Engl J Med **351**: 1425-1436

Johnson L, Boon J, Orton EC 1999: Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992-1996. J Vet Intern Med **13**: 440-447

Keene BW, Atkins CE, Bonagura JD, Fox PR, Häggström J, Fuentes VL, Oyama MA, Rush JE, Stepien R, Uechi M 2019: ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med **33**: 1127-1140

Kellihan HB, Stepien RL 2010: Pulmonary hypertension in dogs: diagnosis and therapy. Vet Clin North Am Small Anim Pract **40**: 623-641

Kovács A, Oláh A, Lux Á, Mátyás C, Németh BT, Kellermayer D, Ruppert M, Török M, Szabó L, Meltzer A, Assabiny A, Birtalan E, Merkely B, Radovits T 2015: Strain and strain rate by speckle-tracking echocardiography correlate with pressure-volume loop-derived contractility indices in a rat model of athlete's heart. Am J Physiol Heart Circ Physiol **308**: H743-H748

Leong ZP, Hikasa Y 2018: Effects of toceranib compared with sorafenib on monocrotaline-induced pulmonary arterial hypertension and cardiopulmonary remodeling in rats. Vascul Pharmacol **110**: 31-41

Leong ZP, Hikasa Y 2019: Effects of masitinib compared with tadalafil for the treatment of monocrotaline-induced pulmonary arterial hypertension in rats. Vascul Pharmacol **122-123**: 106599

Leong ZP, Okida A, Higuchi M, Yamano Y, Hikasa Y 2018: Reversal effects of low-dose imatinib compared with sunitinib on monocrotaline-induced pulmonary and right ventricular remodeling in rats. Vascul Pharmacol **100**: 41-50

Mandegar M, Fung YC, Huang W, Remillard CV, Rubin LJ, Yuan JX 2004: Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. Microvasc Res **68**: 75-103

Maihöfer NA, Suleiman S, Dreymüller D, Manley PW, Rossaint R, Uhlig S, Martin C, Rieg AD 2017: Imatinib relaxes the pulmonary venous bed of guinea pigs. Respir Res **18**: 32

Marech I, Patruno R, Zizzo N, Gadaleta C, Introna M, Zito AF, Gadaleta CD, Ranieri G 2014: Masitinib (AB1010), from canine tumor model to human clinical development: where we are? Crit Rev Oncol Hematol **91**: 98-111

Mikawa S, Nagakawa M, Ogi H, Akabane R, Koyama Y, Sakatani A, Ogawa M, Miyakawa H, Shigemoto J, Tokuriki T, Toda N, Miyagawa Y, Takemura N 2020: Use of vertebral left atrial size for staging of dogs with myxomatous valve disease. J Vet Cardiol **30**: 92-99

Montani D, Perros F, Gambaryan N, Girerd B, Dorfmuller P, Price LC, Huertas A, Hammad H, Lambrecht B, Simonneau G, Launay JM, Cohen-Kaminsky S, Humbert M 2011: C-kit-positive cells accumulate in remodeled vessels of idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med **184**: 116-123

Moonarmart W, Boswood A, Luis Fuentes V, Brodbelt D, Souttar K, Elliott J 2010: N-terminal pro B-type natriuretic peptide and left ventricular diameter independently predict mortality in dogs with mitral valve disease. J Small Anim Pract **51**: 84-96

Nakamura K, Akagi S, Ogawa A, Kusano KF, Matsubara H, Miura D, Fuke S, Nishii N, Nagase S, Kohno K, Morita H, Oto T, Yamanaka R, Otsuka F, Miura A, Yutani C, Ohe T, Ito H 2012: Pro-apoptotic effects of imatinib on PDGF-stimulated pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension. Int J Cardiol **159**: 100-106

Pariaut R, Saelinger C, Strickland KN, Beaufrère H, Reynolds CA, Vila J 2012: Tricuspid annular plane systolic excursion (TAPSE) in dogs: reference values and impact of pulmonary hypertension. J Vet Intern Med **26**: 1148-1154

Perros F, Montani D, Dorfmüller P, Durand-Gasselin I, Tcherakian C, Le Pavec J, Mazmanian M, Fadel E, Mussot S, Mercier O, Hervé P, Emilie D, Eddahibi S, Simonneau G, Souza R, Humbert M 2008: Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med **178**: 81-88

Rabinovitch M 2008: Molecular pathogenesis of pulmonary arterial hypertension. J Clin Invest **118**: 2372-2379

Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, Williams K, Abbott J, Borgarelli M, Scansen BA 2020: ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med **34**: 549-573

Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, Sydykov A, Lai YJ, Weissmann N, Seeger W, Grimminger F 2005: Reversal of experimental pulmonary hypertension by PDGF inhibition. J Clin Invest **115**: 2811-2821

Serres F, Pouchelon JL, Poujol L, Lefebvre HP, Trumel C, Daste T, Sampedrano CC, Gouni V, Tissier R, Hawa G, Chetboul V 2009: Plasma N-terminal pro-B-type natriuretic peptide concentration helps to predict survival in dogs with symptomatic degenerative mitral valve disease regardless of and in combination with the initial clinical status at admission. J Vet Cardiol **11**: 103-121

Soria JC, Massard C, Magné N, Bader T, Mansfield CD, Blay JY, Bui BN, Moussy A, Hermine O, Armand JP 2009: Phase 1 dose-escalation study of oral tyrosine kinase inhibitor masitinib in advanced and/or metastatic solid cancers. Eur J Cancer **45**: 2333-2341

Stepien RL 2009: Pulmonary arterial hypertension secondary to chronic left-sided cardiac dysfunction in dogs. J Small Anim Pract **50 Suppl 1**: 34-43

Tei C 1995: New non-invasive index for combined systolic and diastolic ventricular function. J Cardiol **26**: 135-136

Vazquez de Prada JA, Ruano J, Martin-Duran R, Larman M, Zueco J, Ortiz de Murua JA, Torres A, Figueroa A 1987: Noninvasive determination of pulmonary arterial systolic pressure by continuous wave Doppler. Int J Cardiol **16**: 177-184