Studies on Therapeutic Effects of Tyrosine Kinase Inhibitors for Pulmonary Hypertension in Dogs

（犬の肺高血圧症に対するチロシンキナーゼ阻害薬の治療効果に関する研究）

2022

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General Introduction

Pulmonary hypertension (PH) is defined as systolic pulmonary artery pressure (sPA) of >30 mmHg or mean pulmonary arterial pressure (mPAP) of >20 mmHg. PH is one of the predictors of mortality and refractory cases in human medicine and often results in a poor prognosis (Humbert et al., 2004). The prognosis in dogs with PH is also poor, with median survival duration of 3–91 days after diagnosis (Johnson et al., 1999; Bach et al., 2006). It is caused by pulmonary arterial vasoconstriction and vascular remodelling (Mandegar et al., 2004). Several extracellular and intracellular signalling abnormalities including platelet-derived growth factor (PDGF) receptors and c-Kit receptors have been implicated in this remodelling (Mandegar et al., 2004; Schermuly et al., 2005; Rabinovitch, 2008; Montani et al., 2011). Moreover, it has been suggested that overexpressed PDGF and its receptors may play a pathogenic role in the development of human PH, and that 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., 2018b), 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, thrombocytopenia, and anemia (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 at a lower dose (approximately one-third of an

antineoplastic dose) (Leong and Hikasa, 2018; Leong et al., 2018b). 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 cases of this type, imatinib therapy in combination with other agents specific, or with other agents for PH may be required.

Sildenafil is a selective phosphodiesterase type 5 (PDE5) inhibitor that relaxes vascular smooth muscle cells by increasing cyclic guanosine monophosphate and lowers pulmonary vascular resistance (Lewis et al., 2007). It decreases sPA and improve clinical signs in PH dogs (Bach et al., 2006; Brown et al., 2010; Kellihan and Stepien, 2012). However, it has also been reported that sildenafil did not significantly or only slightly reduce sPA in PH dogs, even though it improved clinical signs (Kellum and Stepien, 2007; Saetang and Surachetpong, 2020). Therefore, it may be important to further investigate combination therapies of sildenafil and other drugs to ensure that the sPA in dogs with PH is reduced adequately. It has been reported that a combination of low-dosage imatinib and sildenafil was effective in treating PH in rats (Jasiska-Stroschein et al., 2015). Additive effects in improvement of PH may be caused by pharmacodynamic and pharmacokinetic drug–drug interactions between imatinib and sildenafil. These include a potential mechanism resulting from both agents being metabolised by the CYP3A4 enzyme (Jasiska-Stroschein et al., 2015). Pharmacokinetic interactions between imatinib and sildenafil in patients with severe PH have also shown that sildenafil concentrations increased an average of 64% in the presence of imatinib without an increased risk of liver toxicity (Renard et al., 2015). Therefore, a combination of low-dosage imatinib and sildenafil may be a better regimen for treating PH in dogs.

Masitinib is a veterinary drug that has been approved to treat canine mast cell tumors (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 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 remodeling and the increased vasoconstriction. However, no published reports on the therapeutic effect of masitinib for PH in dogs are available.

Therefore, this study was conducted to investigate the therapeutic effects of tyrosine kinase inhibitors, imatinib and masitinib for pulmonary hypertension in dogs. In chapter 1, the study was aimed to investigate the therapeutic effects of low-dosage imatinib in combination with sildenafil in dogs with PH due to chronic degenerative mitral valve disease (CDMVD) or ventricular septal defect (VSD) with Eisenmenger's syndrome. In chapter 2, the study aimed to investigate the efficacy of low-dose masitinib therapy for canine PH caused by advanced mitral insufficiency and heartworm disease.

Chapter 1

Therapeutic effects of sildenafil combined with low-dosage imatinib on pulmonary hypertension in dogs

Introduction

Pulmonary hypertension (PH) is defined as systolic pulmonary artery pressure (sPA) of >30 mmHg or mean pulmonary arterial pressure (mPAP) of >20 mmHg. PH is one of the predictors of mortality and refractory cases in human medicine and often results in a poor prognosis (Humbert et al., 2004). The prognosis in dogs with PH is also poor, with median survival duration of 3–91 days after diagnosis (Johnson et al., 1999; Bach et al., 2006). It is caused by pulmonary arterial vasoconstriction and vascular remodelling (Mandegar et al., 2004). Several extracellular and intracellular signalling abnormalities including platelet-derived growth factor (PDGF) receptors and c-Kit receptors have been implicated in this remodelling (Mandegar et al., 2004; Schermuly et al., 2005; Rabinovitch, 2008; Montani et al., 2011). Imatinib, a tyrosine kinase inhibitor targeting PDGF and c-Kit receptors, reverses pulmonary vascular remodelling (Schermuly et al., 2005; Leong et al., 2018b) and exhibits pulmonary vasodilatory effects in rats (Maihöfer et al., 2017). Using high doses of imatinib in humans with PH has yielded controversial outcomes (Ghofrani et al., 2010) and has reported side effects such as nausea, thrombocytopenia and anaemia (Frost et al., 2015). Observed side effects including neutropenia and vomiting have also been reported in dogs treated with a neoplastic dose (10–12 mg/kg) (Bonkobara, 2015). However, it has been also reported that imatinib in lower dosages reduces pulmonary fibrosis more effectively than at higher dosages in mouse models (Vuorinen et al., 2007) and reverses pulmonary arterial remodelling in PH model rats (Leong et al., 2018b). Therapy with low-dosage imatinib improved clinical symptoms and echocardiographic outcomes without noticeable adverse effects in dogs (Arita et al., 2013; Leong et al., 2018a) and humans (Hatano et al., 2010) with PH. In contrast, it has been suggested that resistance to imatinib treatment might occur in PH patients as a result of the relationship between the apoptosis and

plasma PDGF levels (Nakamura et al., 2012). In cases of this type, imatinib therapy in combination with other agents specific for PH may be required.

Sildenafil is a selective phosphodiesterase type 5 inhibitor that relaxes vascular smooth muscle cells by increasing cyclic guanosine monophosphate and lowers pulmonary vascular resistance (Lewis et al., 2007). It decreases sPA and improve clinical signs in PH dogs (Bach et al., 2006; Brown et al., 2010; Kellihan and Stepien, 2012). However, it has also been reported that sildenafil did not significantly or only slightly reduce sPA in PH dogs, even though it improved clinical signs (Kellum and Stepien, 2007; Saetang and Surachetpong, 2020). Therefore, it may be important to further investigate combination therapies of sildenafil and other drugs to ensure that the sPA in dogs with PH is reduced adequately.

It has been reported that a combination of low-dosage imatinib and sildenafil was effective in treating PH in rats (Jasiska-Stroschein et al., 2015). Additive effects in improvement of PH may be caused by pharmacodynamic and pharmacokinetic drug–drug interactions between imatinib and sildenafil. These include a potential mechanism resulting from both agents being metabolised by the CYP3A4 enzyme (Jasiska-Stroschein et al., 2015). Pharmacokinetic interactions between imatinib and sildenafil in patients with severe PH have also shown that sildenafil concentrations increased an average of 64% in the presence of imatinib without an increased risk of liver toxicity (Renard et al., 2015). Therefore, a combination of low-dosage imatinib and sildenafil may be a better regimen for treating PH in dogs. The purpose of the present study was to investigate the therapeutic effects of low-dosage imatinib in combination with sildenafil in five dogs with PH due to chronic degenerative mitral valve disease (CDMVD) or ventricular septal defect (VSD) with Eisenmenger's syndrome.

Materials and Methods

Animals

Five client-owned dogs with PH were prospectively recruited at the Sahashi Veterinary Hospital (Hyogo, Japan). Informed consent was obtained from each dog owner. The diagnosis of PH was defined as sPA of >30 mmHg or mPAP of >20 mmHg. Patient signalment, aetiology of PH, clinical findings, the International Small Animal Cardiac Health Council (ISACHC) severity, duration of treatments and outcome of the five participating dogs are summarised in Table 1. Each case was classified as ISACHC IIIa or IIIb and was in the exacerbation phase of chronic heart failure.

Medications

All of the dogs had been previously treated with a polypharmaceutical approach that included alacepril (1.1–3.0 mg/kg, PO, q12h), pimobendan (0.17–0.37 mg/kg, PO, q12h) and furosemide (1.5–2.0 mg/kg, PO, q12h) for periods ranging from 1 month to 2 years. In all dogs with severe PH, low-dosage imatinib mesylate (Glivec; Novartis Pharma, Tokyo), 3 mg/kg, PO, q24h, was initially administered. Before and after imatinib administration, all dogs continued to receive previous medications without changes. From day 53 to day 168 following initial imatinib administration, the PH and clinical signs of the dogs worsened and sildenafil (Levatio; Viatris, Tokyo) administration at a low-dosage (0.5 mg/kg, PO, q12h) was started in combination with a low-dosage of imatinib. Examinations were performed prior to imatinib administration (pre-0; day 0), 1 and 3 months after imatinib administration, before sildenafil + imatinib administration (pre-1) and 1 and 3 months after administration of both agents.

Clinical evaluations

Clinical signs such as cough, exercise intolerance, syncope, ascitic fluid buildup and peripheral oedema were assessed before and after the administration of imatinib and sildenafil (Table 2). The degree of clinical symptom was assessed using the scoring method previously utilised in dogs (Arita et al., 2013). The total score was calculated as the sum of the four scores: cough, exercise intolerance, syncope and ascites-oedema.

Haematological and blood biochemical examinations

Blood cell counts were determined using an automatic hemocytometer (pocH-100iV; Sysmex Corporation, Hyogo, Japan). Blood urea nitrogen (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 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).

Radiography, echocardiography and various circulation parameters

Vertebral heart size (VHS) was measured using the right lateral radiograph of dogs. Mean blood pressure (MBP) were measured using the oscillometric method with a noninvasive 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. Heart rate (HR) was calculated from the preceding R-to-R interval on the electrocardiogram. The key echocardiographic parameters listed in Table 3 were

measured using transthoracic two-dimensional, M-mode and pulsed, continuous wave and tissue Doppler echocardiography, as reported previously (Arita et al. 2013). In the apical 5-chamber view, a pulsed-wave sample volume was placed just under the aortic valve and the cross-sectional area of the left ventricular (LV) outflow tract, aortic ejection flow velocity and time velocity integral were measured, and stroke volume (SV) was calculated. Cardiac output (CO) was calculated as SV × HR. The estimated sPA was calculated by adding the estimated right atrial pressure (10 mmHg) to the systolic right ventricle-to-right atrial pressure gradient, using the modified Bernoulli equation: 4 × maximum systolic tricuspid regurgitation velocity $(TRmax)^2$. The estimated mPAP was calculated using the following equations: 4 × (the early diastolic pulmonary regurgitation velocity)^2. Left atrium/aorta (LA/Ao) and right atrium/aorta (RA/Ao) ratios were selected as indices of LA and RA pressure load, respectively. Tei index was chosen to evaluate both systolic and diastolic functions of the LV or right ventricle (RV). The LV fractional shortening, ratio of peak velocity of early diastolic transmitral flow wave to peak velocity of early diastolic mitral annular motion (E/Em) and tricuspid annular plane systolic excursion (TAPSE) were selected to evaluate LV contractility, LV dilatation function and RV contractility, respectively. Each parameter was measured at least three times and the average value was then utilised as the recorded data. All measurements and follow-up examinations on each dog were performed by the same investigator.

Statistical analysis

Data were analysed using statistical software (Prism 7.0, GraphPad, CA). All Data, except for the score data, were tested for normality using the Shapiro–Wilk test. The paired *t*-test or Wilcoxon signed rank test was used for comparison between pre-data and data after imatinib or

imatinib + sildenafil administration. For clinical score data, the Wilcoxon signed rank test was used for the comparison. The significance level for each analysis was at $P < 0.05$.

Results

Clinical scores

Clinical scores are shown in Table 2. After imatinib administration, the cough, exercise intolerance and syncope scores tended to be lower ($P = 0.062$) at 1 month compared with pre-0. The total score was significantly ($P < 0.05$) lower at 1 month after imatinib compared with that at pre-0. After imatinib + sildenafil administration, cough score was significantly ($P < 0.05$) lower at 1 month compared with that at pre-1. Syncope score tended to be lower ($P = 0.062$) at 1 month compared with that at pre-1. Ascites was observed at pre-0 and pre-1 in case 2 only, but both disappeared 1–3 months after imatinib or imatinib + sildenafil administration. The total score was significantly ($P < 0.05$) lower at 1 month after imatinib + sildenafil administration compared with that at pre-0 or pre-1.

Radiographic, echocardiographic and circulation variables

The results are shown in Table 3. The elevated sPA (mean 93.0 mmHg) at pre-0 decreased significantly ($P < 0.05$) at 1 month (mean 59.0 mmHg; decrease of 37%) after imatinib administration. Moreover, the sPA decreased significantly ($P < 0.05$) at 1 and 3 months (mean 52.3 and 65.8 mmHg, respectively) after imatinib + sildenafil administration compared with pre-0 or pre-1 value (mean 100.3 mmHg). The largest decrease in sPA was seen at 1 month after imatinib + sildenafil administration (44% and 48% decrease from pre-0 and pre-1 values). The mPAP also decreased significantly ($P < 0.05$) at 1 month after imatinib + sildenafil administration compared with pre-1 value.

The VHS, left atrium (LA) diameter, right atrium/aorta (RA/Ao) ratio, right ventricular (RV) Tei index, maximum tricuspid regurgitation velocity (TRmax), end-diastolic pulmonary

regurgitation maximum velocity (PRmax) all decreased significantly ($P < 0.05$) at 1 month and/or 3 months after imatinib administration compared with the pre-0 data, whereas the stroke volume (SV), cardiac output (CO) and tricuspid annular plane systolic excursion (TAPSE) values increased significantly ($P < 0.05$–0.01) at 1 month after imatinib administration. The other parameters did not significantly change at 1 and 3 months after imatinib administration compared with the pre-0 data.

After imatinib + sildenafil administration, the HR, LA and right atrium (RA) diameters, LA/Ao and RA/Ao ratios, RV Tei index and TRmax all decreased significantly ($P < 0.05$–0.01) at 1 month and/or 3 months compared with the pre-0 or pre-1 data, whereas the aortic ejection flow velocity (AEV) and TAPSE increased significantly. The VHS and PRmax tended to decrease ($P = 0.069$–0.084) at 1 month and/or 3 months compared with the pre-1 data, whereas CO tended to increase ($P = 0.063$) at 1 month. The other parameters did not significantly change at 1 and 3 months after imatinib + sildenafil administration compared with the pre-0 or pre-1 data.

Haematological and blood biochemical variables

The NT-proBNP concentration was elevated at pre-0, but decreased significantly ($P < 0.01$) at 1 month after imatinib administration (Table 4). At 1 and 3 months after imatinib + sildenafil administration, NT-proBNP concentration did not significantly increase from pre-0 and pre-1 levels. The PCV; white and red blood cell counts; BUN and CRE concentrations and AST, ALT and ALP activities were not significantly affected by imatinib or imatinib + sildenafil therapies.

Table 1. Summary of patient signalment, aetiology, history and clinical findings. and outcomes in 5 dogs.

Case	**1**	**2**	**3**	**4**	**5**
Breed	Pomeranian	Chihuahua	Chihuahua	Maltese	Miniature Dachshund
Age (y)	13	11	11	9	0.5
Sex	Male castrated	Male castrated	Male intact	Male castrated	Female intact
BW (kg)	3.3	3.3	2.1	5.4	3.7
Etiology of PH	Pulmonary venous PH due to MI	Pulmonary venous PH due to MI	Pulmonary venous PH due to MI	Pulmonary venous PH due to MI	Pulmonary arterial PH due to VSD
Clinical signs	Syncope, cough, exercise intolerance	Cough, exercise intolerance, ascites	Syncope, cough, exercise intolerance	Syncope, cough, exercise intolerance	Syncope, exercise intolerance
Auscultation findings	Grade V/VI left murmur, grade III/VI right murmur	Grade V/VI left murmur, grade III/VI right murmur	Grade IV/VI left murmur, grade III/VI right murmur	Grade IV/VI left murmur, grade III/VI right murmur	Grade I/VI left murmur
Thoracic radiograph findings	VHS:12.9, left atrial enlargement, bronchial compression, perihilar pulmonary edema	VHS:15.0, left atrial enlargement, bronchial compression, perihilar pulmonary edema	VHS:13.7, left atrial enlargement, bronchial compression, perihilar pulmonary edema	VHS:14.5, left atrial enlargement, bronchial compression, perihilar pulmonary edema	VHS:9.8, right heart enlargement, dilation of main pulmonary artery
ISACHC severity	Ⅲa	Ⅲb	Ⅲb	Ⅲa	Ⅲa
ACVIM stage	C	D	C	C	—
History of medications	Alacepril (1.8 mg/kg, q12h), pimobendan (0.37 mg/kg, q12h), furosemide (2.0 mg/kg, q12h) for 2 y	Alacepril (1.8 mg/kg, q12h), pimobendan (0.37 mg/kg, q12h), furosemide (1.5 mg/kg, q12h) for 9 mo	Alacepril (3.0 mg/kg, q12h), pimobendan (0.30 mg/kg, q12h), furosemide (2.0 mg/kg, q12h) for 1 y	Alacepril (1.1 mg/kg, q12h), pimobendan (0.37 mg/kg, q12h), furosemide (2.0 mg/kg, q12h) for 1 y	Pimobendan (0.17 mg/kg, q12h) for 1 mo
Imatinib therapy (day)	167	168	53	113	138
Imatinib+sildenafil therapy (day)	186	249	95	975	2301

PH, pulmonary hypertension; ISACHC, International Small Animal Cardiac Health Council; ACVIM, American College of Veterinary Internal Medicine; MI, mitral valve insufficiency; VSD, ventricular septal defect；VHS, vertebrae heart size.

Table 2. Clinical score before and after imatinib or imatinib + sildenafil administration in 5 dogs.

Variables	After imatinib (months)			After imatinib + sildenafil (months)		
	Pre-0 (n = 5)	1 (n = 5)	3 (n = 4)	Pre-1 (n = 5)	1 (n = 5)	3 (n = 4)
Cough score	3 (0–3)[a]	1 (0–2)	1.5 (0–3)	3 (1–3)	2 (0–2)†	2 (0–2)
Exercise intolerance score	2 (1–2)	1 (0–1)	1 (1–1)	2 (1–2)	1 (1–2)	1 (1–2)
Syncope score	2 (0–2)	0 (0–1)	0 (0–1)	2 (0–2)	0 (0–1)	1 (0–2)
Ascites and oedema score	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–2)	0 (0–0)	0 (0–0)
Total score	6.5 (2–7)	2 (0–3)*	2 (1–4)	6.5 (2–7)	2.5 (0–5)*†	3 (1–5)

Pre-0 = immediately before imatinib administration; Pre-1 = immediately before imatinib + sildenafil administration; [a] Median (minimum-maximum). 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 (2–6 times per week), and 3 = severe (every day); ascites and oedema score 0 = none, 1 = positive for ascites and oedema, which decreases after imatinib or imatinib + sildenafil administration, and 2 = positive for ascites and oedema, which does not change or is exacerbated after imatinib or imatinib + sildenafil administration. * $P < 0.05$, significantly different from the Pre-0; † $P < 0.05$, significantly different from the Pre-1.

Table 3. Radiographic, echocardiographic and circulation variables before and after imatinib or imatinib + sildenafil administration.

Variables	After imatinib (months)			After imatinib + sildenafil (months)		
	Pre-0 (n = 5)	1 (n = 5)	3 (n = 4)	Pre-1 (n = 5)	1 (n = 5)	3 (n = 4)
HR (beats/min)	151 ± 22	137 ± 26	143 ± 29	160 ± 17	134 ± 15†	150 ± 7
MBP (mmHg)	123 ± 19	123 ± 18	106 ± 22	114 ± 22	134 ± 27	125 ± 14
VHS	13.2 ± 2.1	12.7 ± 1.7	12.6 ± 2.3*	13.4 ± 1.7	12.8 ± 2.0	12.8 ± 1.9
LA (mm)	24.6 ± 5.9	24.4 ± 7.8	23.5 ± 6.8*	23.6 ± 7.4	21.1 ± 8.6	21.2 ± 6.1*
RA (mm)	17.5 ± 6.8	12.6 ± 4.1	15.4 ± 3.7	18.4 ± 4.1	14.9 ± 4.1††	16.5 ± 3.6
LA/Ao	2.57 ± 1.14	2.32 ± 0.84	2.20 ± 0.91	2.39 ± 1.01	2.10 ± 0.89†	2.05 ± 0.78
RA/Ao	1.90 ± 0.63	1.14 ± 0.29*	1.33 ± 0.25	1.63 ± 0.19	1.24 ± 0.15††	1.46 ± 0.28
FS (%)	42.5 ± 12.4	46.0 ± 12.8	49.1 ± 8.2	50.3 ± 3.2	44.5 ± 11.5	51.5 ± 7.6
EF (%)	77.8 ± 17.2	79.7 ± 16.4	84.9 ± 7.8	86.0 ± 4.3	78.9 ± 14.2	87.1 ± 6.0
LVIDdN	2.0 ± 0.5	2.3 ± 0.7	1.9 ± 0.7	2.0 ± 0.5	2.0 ± 0.5	1.8 ± 0.7
PV flow (cm/s)	88.8 ± 36.7	94.6 ± 35.3	86.6 ± 28.2	90.2 ± 32.3	89.2 ± 34.9	89.0 ± 27.1
E/A	1.19 ± 0.24	1.11 ± 0.64	1.59 ± 0.91	1.30 ± 0.63	1.42 ± 1.04	0.87 ± 0.22
DT_E (ms)	124 ± 29	128 ± 44	97 ± 30	110 ± 32	129 ± 29	100 ± 32
AEV (cm/s)	98.4 ± 16.2	91.9 ± 16.9	99.4 ± 10.7	90.3 ± 15.7	105.2 ± 22.0†	113.7 ± 23.5
VTI (cm)	7.5 ± 1.1	8.9 ± 1.9	8.2 ± 1.6	8.4 ± 2.2	8.2 ± 2.1	8.5 ± 1.4
SV (mL)	4.84 ± 1.57	7.50 ± 2.64*	5.00 ± 1.60	4.00 ± 0.96	7.12 ± 4.72	4.38 ± 2.04
CO (L/min)	0.672 ± 0.248	1.157 ± 0.424*	0.747 ± 0.196	0.548 ± 0.148	1.029 ± 0.717	0.676 ± 0.396
LV Tei	0.400 ± 0.260	0.293 ± 0.218	0.437 ± 0.179	0.533 ± 0.187	0.372 ± 0.245	0.501 ± 0.282
RV Tei	0.586 ± 0.090	0.327 ± 0.105*	0.438 ± 0.254	0.554 ± 0.263	0.256 ± 0.172*†	0.382 ± 0.125*
MRmax (cm/s)	644 ± 76	579 ± 50	573 ± 61	574 ± 60	604 ± 89	639 ± 83
TRmax (cm/s)[a]	455 ± 22	348 ± 54*	355 ± 90	475 ± 26	316 ± 81*†	370 ± 57†
PRmax (cm/s)[b]	358 ± 131	342 ± 130*	337 ± 121	383 ± 142	284 ± 159	223 ± 33
Em/Am	0.89 ± 0.32	0.80 ± 0.15	0.70 ± 0.09	0.79 ± 0.26	0.74 ± 0.28	0.73 ± 0.20
E/Em	10.6 ± 4.3	10.2 ± 4.1	14.6 ± 6.3	13.1 ± 5.5	11.6 ± 2.8	8.9 ± 4.5†
TAPSE (mm)	9.3 ± 3.6	13.9 ± 5.2**	10.9 ± 5.4	6.8 ± 2.3*	13.3 ± 5.2**†	12.4 ± 5.8†
sPA (mmHg)[a]	93.0 ± 8.2	59.0 ± 14.5*	61.5 ± 22	100.3 ± 10.0	52.3 ± 21.0*†	65.8 ± 16.4*†
mPAP (mmHg)[b]	54.6 ± 37.4	50.2 ± 35.6	48.3 ± 32.6	56.2 ± 45.8	40.1 ± 41.0†	46.3 ± 33.3

Each value represents as mean ± SD; Pre-0 = immediately before imatinib administration; Pre-1 = immediately before imatinib + sildenafil administration; HR = heart rate; MBP = mean blood pressure; VHS = radiographic vertebrae heart size; LA = left atrium; RA = right atrium; LA/Ao = left atrium/aorta; RA/Ao = right atrium/aorta; FS = left ventricular fractional shortening; EF = left ventricular ejection fraction; LVIDdN = normalized end-diastolic left ventricular inner dimension; PV flow = pulmonary valve flow velocity; E/A = peak velocity of early diastolic transmitral flow wave/peak velocity of late transmitral flow wave; DT_E = deceleration time of early diastolic transmitral flow; AEV = aortic ejection flow velocity; VTI = velocity time integral; SV = stroke volume; CO = cardiac output; LV Tei = left ventricular Tei index; RV Tei = right ventricular Tei index; MRmax = maximum systolic mitral regurgitation velocity; TRmax = maximum tricuspid regurgitation velocity; PRmax = end-diastolic pulmonary regurgitation maximum velocity; Em/Am = peak velocity of early diastolic mitral annular motion/peak velocity of diastolic mitral annular motion; TAPSE = tricuspid annular plane systolic excursion; sPA = systolic pulmonary arterial pressure; mPAP = mean pulmonary arterial pressure; [a] n = 3–4; [b] n = 2–3; * $P < 0.05$, ** $P < 0.01$, significantly different from the Pre-0; [†] $P < 0.05$, [††] $P < 0.01$, significantly different from the Pre-1.

Table 4. Haematological, blood biochemical and cardiac biomarker variables before and after imatinib or imatinib + sildenafil administration.

Variables	Reference range	After imatinib (months)			After imatinib + sildenafil (months)		
		Pre-0 (n = 5)	1 (n = 5)	3 (n = 4)	Pre-1 (n = 5)	1 (n = 5)	3 (n = 4)
White blood cells ($\times 10^3/mm^3$)	6−17	16.6 ± 12.9	12.0 ± 2.5	11.2 ± 2.2	9.2 ± 4.3	11.9 ± 1.0	16.3 ± 6.8
Red blood cells ($\times 10^4/mm^3$)	550−850	710 ± 169	734 ± 138	729 ± 61	765 ± 257	772 ± 255	814 ± 230
Packed cell volume (%)	37−55	46 ± 10	45 ± 5	48 ± 2	51 ± 12	50 ± 15	51 ± 16
Aspartate aminotransferase (U/L)	10−100	34 ± 9	30 ± 8	33 ± 4	32 ± 14	30 ± 13	27 ± 5
Alanine aminotransferase (U/L)	0−50	53 ± 20	53 ± 21	68 ± 46	79 ± 27	77 ± 29	62 ± 13
Alkaline phosphatase (U/L)	23−212	172 ± 108	151 ± 74	138 ± 53	170 ± 95	170 ± 120	220 ± 89
Blood urea nitrogen (mg/dL)	7−27	26 ± 13	24 ± 4	21 ± 6	22 ± 6	27 ± 12	27 ± 12
Creatinine (mg/dL)	0.5−1.8	0.7 ± 0.1	0.5 ± 0.1	0.6 ±0.1	0.7 ± 0.1	0.6 ± 0.3	0.6 ± 0.4
Serum N-terminal probrain natriuretic peptide (pmol/L)	< 900	3004 ± 1758	2357 ± 1793*	2591 ± 1585	4065 ± 3097	3579 ± 3787	3928 ± 3035

Each value represents as mean ± SD; Pre-0 = immediately before imatinib administration; Pre-1 = immediately before imatinib + sildenafil administration; * $P < 0.01$, significantly different from the Pre-0 value.

Discussion

The rationale for using low-doses of imatinib (3 mg/kg) in dogs has been outlined in previous studies (Arita et al., 2013). Canine case subjects involved in this investigation had more severe PH (estimated sPA = 93.0 ± 8.2 mmHg, mean ± *SD*) than the cases with PH (estimated sPA = 45.7 ± 16.2 and 63.3 ± 24.9 mmHg) in previous studies (Arita et al., 2013; Leong et al., 2018a). This study revealed that low-dosage imatinib reduced sPA and mPAP and improved RV function parameters including RV Tei index, RA/Ao ratio and TAPSE without worsening left ventricular (LV) function, and improved clinical symptoms. These findings agreed with those in a previous study (Arita et al., 2013). These results may be due to the anti-inflammatory, antifibrotic and pulmonary artery antiremodeling actions of imatinib (Schermuly et al., 2005; Leong et al., 2018b), as well as the pulmonary vasodilatory effects of imatinib (Abe et al., 2011; Maihöfer et al., 2017). Additionally, the present study revealed that imatinib administration increased the SV and CO, which may be due to the reduction of RV afterload. This improvement in hemodynamics might suppress excessive secretion and activation of neurohumoral factors and prevent the deterioration of chronic heart failure. In fact, serum NT-proBNP was found to decrease after the administration of imatinib in this study.

In this study, PH and clinical symptoms in all the dogs were found to deteriorate from days 53 to 168 (median 138 days) after initiation of imatinib medication. This period seemed to be slightly shorter than what was found in previous reports (Arita et al., 2013; Leong et al., 2018a). As mentioned above, this may be the result of the canine cases in this study having more severe PH and higher ISACHC severity than the cases in previous studies (Arita et al. 2013; Leong et al., 2018a). Additionally, the up-regulated high plasma PDGF-BB in human patients with PH could be suppressed by low-dose imatinib therapy, suggesting that this event is one of the

determinant factors for its efficacy in treating PH in humans (Hatano et al., 2010). Resistance to imatinib therapy in canine PH may be associated with the changes in plasma PDGF-BB levels, but further investigations are necessary to make this determination. On the other hand, when PH was re-worsened at 53–168 days after imatinib medication, TAPSE at that time (pre-1) was decreased compared with the value before imatinib administration (pre-0). This decrease in TAPSE may be due to the reduction of RV contraction associated with the re-deterioration of PH. In addition, the resistance to imatinib treatment may be involved in this event.

This study revealed that in dogs with PH due to CDMVD, the combined administration of low-dosage sildenafil (0.5 mg/kg, q12h) and imatinib (3 mg/kg, q24h) largely reduced sPA by an average 48% (from average 100.3 mmHg at pre-1 to average 52.3 mmHg at post 1 month) and improved clinical symptoms. In addition, the elevated mPAP due to CDMVD and VSD was reduced by an average 29% (from average 56.2 mmHg at pre-1 to average 40.1 mmHg at post 1 month). The decrease in sPA and/or mPAP after administration of the low-dose sildenafil and imatinib combination observed in our study was apparently greater than the decrease found in previous studies after administration of sildenafil alone at larger dosages (Bach et al., 2006; Kellum and Stepien, 2007; Brown et al., 2010; Saetang and Surachetpong, 2020). Additionally, this combination improved RV function parameters including RA/Ao ratio, RV Tei index and TAPSE, without diminishing LV function parameters including MBP, SV and CO. These results agreed with a report that a combination of low-dosage imatinib and sildenafil was effective in treating PH in rats (Jasiska-Stroschein et al., 2015). These effects may be due to the pulmonary vasodilatory activity through pulmonary vascular relaxation and antiremodeling effects from sildenafil (Lewis et al., 2007; Kiss et al., 2014) and pulmonary vasodilatory activities in addition to inhibition of pulmonary vascular remodeling by imatinib (Abe et al., 2011; Maihöfer et al.,

2017; Leong et al., 2018b). Pharmacokinetic interactions between imatinib and sildenafil might have resulted in additive effects of both agents in this study (Jasiska-Stroschein et al., 2015; Renard et al., 2015). Therefore, this study suggests that the combined use of imatinib and sildenafil would more effectively improve PH and clinical symptoms through their additive interactions.

Median survival time in dogs with severe PH has been previously reported to be 3–91 days (Johnson et al., 1999; Bach et al., 2006). Furthermore, a recent study reported that the median survival time in dogs with PH secondary to CDMVD stage C was 368 days (Udomkiattikul et al., 2022). However, the cases in this study had longer survival time (median 417 days, range 148 to >2439 days after imatinib medication; median 249 days, range 95 to >2301 days after imatinib + sildenafil medication) than those found in previous studies (Johnson et al., 1999; Bach et al., 2006). Thus, the use of a low-dosage imatinib and sildenafil combination may be effective in prolonging the survival of dogs with severe PH.

Regarding the safety of imatinib, it has been reported that in humans, renal dysfunction was observed in some patients who received low-dosage imatinib (100 mg/day) for 24 weeks, but no other adverse events were observed (Hatano et al., 2010). In the present study, no significant changes in the PCV, white and red blood cell counts, BUN and CRE concentrations and AST, ALT and ALP activities were observed during the treatment, as well as no apparent clinical side effects associated with the administration of imatinib + sildenafil were found. These results suggest that a combination of low-dose imatinib and sildenafil can be used safely for long-term therapy in dogs with PH without obvious adverse effects.

In conclusion, the combined administration of low-dose imatinib and sildenafil markedly reduced systolic pulmonary arterial pressure by an average 48% 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 showing the effectiveness of low-dosage imatinib and sildenafil combination for treating dogs with PH caused by CDMVD and VSD. However, further work with larger, placebo-controlled, randomised and blinded studies would be required to determine the long-term efficacy of low-dose imatinib + sildenafil in reducing PH in dogs due to various aetiologies and in improving their clinical symptoms.

Chapter 2

Effectiveness of therapy with low-dosage masitinib on pulmonary hypertension in dogs

Introduction

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., 2018b), 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, thrombocytopenia, and anemia (Frost et al., 2015). Similar adverse effects have been reported in dogs given antineoplastic dose (Bonkobara, 2015). On the other hand, imatinib or sorafenib reversed pulmonary arterial remodeling and right ventricular systolic pressure in PH model rats at a lower dose (approximately one-third of an

antineoplastic dose) (Leong and Hikasa, 2018; Leong et al., 2018b). 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 tumors (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 remodeling and the increased vasoconstriction. However, no published reports on the therapeutic effect of masitinib for PH in dogs are available. The purpose of this 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, hemodynamic, and blood biochemical parameters 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 5 summarizes the patient signalment, etiology 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), orally (PO), q12h), alacepril (1.1–2.5 mg/kg, PO, q12-24h), pimobendan (0.20–0.67 mg/kg, PO, q12h), torasemide (0.13–0.15 mg/kg, PO, q12h), spironolactone (1.6 mg/kg, PO, q12h), amlodipine (0.16 mg/kg, PO, q12h), sildenafil (1.6–2.7 mg/kg BW, PO, q12h), and furosemide (1.5–2.0 mg/kg, PO, q12h) for periods ranging from 1 month to 15 months (Table 5). 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), PO, 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 buildup, and peripheral edema were all assessed before and after masitinib administration (Table 6). 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-edema.

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 −35°C for analysis. Moreover, red blood cell (RBC), white blood cell (WBC) counts, and packed cell volume (PCV) were determined using an automatic hemocytometer (pocH-100iV; Sysmex Corporation, Hyogo, Japan). On the other hand, blood urea nitrogen (BUN) and creatinine (CRE) concentrations and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were measured using an automatic biochemical analyzer (Fuji Dry Chem 4000V; Fujifilm Medical, Tokyo, Japan). Serum C-reactive protein (CRP) was

measured by enzyme inhibitory homogeneous immunoassay using the analyzer 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 analyzer (i-STAT 1 Analyzer; Abbott Point of Care Inc., USA).

Radiography, echocardiography, and various circulation parameters

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 noninvasive 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 parameters 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 centered 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) = $4 \times TRmax^2$), 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 analyzed. 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 analyzed again by the software (Kovács et al., 2015).

Each parameter 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 analyze the data. Except for the score data, all data were tested for normality using the Shapiro-Wilk test. For comparisons between the before-pre and pre-data, as well as between pre-data and data after masitinib administration, the paired *t*-test or Wilcoxon signed rank test was used. For clinical score data, the 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 6 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. 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 7 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 8 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.

Hematological and blood biochemical variables

Table 9 shows the changes of hematological 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.

Table 5. 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	Etiology 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, PO, q12h), pimobendan (0.63 mg/kg BW, PO, q12h), torasemide (0.13 mg/kg BW, PO, q12h), spironolactone (1.6 mg/kg BW, PO, q12h), amlodipine (0.16 mg/kg BW, PO, q12h), sildenafil (1.56 mg/kg BW, PO, q12h), for 3 months	Alive on days 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, PO, q12h), pimobendan (0.30 mg/kg BW, PO, q12h), furosemide (1.5 mg/kg BW, PO, q12h) for 9 months	Death on days 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, PO, q12h), pimobendan (0.27 mg/kg BW, PO, q12h), furosemide (2.0 mg/kg BW, PO, q12h) for 12 months	Death on days 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, PO, q24h), pimobendan (0.39 mg/kg BW, PO, q12h), for 15 months	Alive on days 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, PO, q24h), pimobendan (0.37 mg/kg BW, PO, q12h), furosemide (2.0 mg/kg BW, PO, q12h) for 12 months	Death on days 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 tumor, mastocytoma	Alacepril (1.9 mg/kg BW, PO, q24h), pimobendan (0.20 mg/kg BW, PO, q12h), prednisolone (0.3 mg/kg BW, PO, q24h) for 12 months	Alive on days 728
7	Shiba	12	Female ovariohyste rectomized	7.4	Heartw orm disease	Pulmonary arterial PH due to *Dirofilaria immitis*	Syncope, cough, exercise intolerance, ascites, pleural fluid	Ⅲb	-	Mammary tumor	Pimobendan (0.67 mg/kg BW, PO, q12h), torasemide (0.15 mg/kg BW, PO, q12h), sildenafil (2.7 mg/kg BW, PO, q12h), for 1 month	Alive on days 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; PO, orally.

Table 6. Clinical score before and after masitinib administration in seven dogs.

Variables	After masitinib administration (month)					
	Pre	1	2	3	6	12
	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 4)	(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 edema score 0, none; 1, positive for ascites and edema, which decreases after masitinib administration; and 2, positive for ascites and edema, which does not change or is exacerbated after masitinib administration. [a]$P < 0.05$, [b]$P < 0.01$, significantly different from the pre value.

Table 7. Changes in the systolic pulmonary arterial pressure (mmHg) before and after masitinib administration in seven dogs.

Case	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)
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 8. 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[c]	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[c]
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[c]
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
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[c]	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.

Table 9. Hematological, blood biochemical, and cardiac biomarker variables before and after masitinib administration in seven dogs.

Variables	Reference range	After masitinib administration (month)						
		Before-Pre (n = 7)	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.

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 remodeling, 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-remodeling 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 parameters including RA/Ao ratio, TRmax, RV Tei index, and TAPSE without worsening LV function and rather improved 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 hemodynamics 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 parameters. 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 hemodynamics 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 hematological 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 etiologies.

General Conclusion

In chapter 1, the study aimed to investigate the therapeutic effects of sildenafil combined with imatinib at low dosages on echocardiographic, cardiovascular, haematological and blood biochemical variables and clinical symptoms in dogs with pulmonary arterial hypertension (PH) caused by advanced chronic degenerative mitral valve disease or ventricular septal defect with Eisenmenger's syndrome. Five client-owned dogs with PH were initially administered low-dose imatinib mesylate (3 mg/kg, PO, q24h). The low-dose imatinib improved the right ventricular (RV) function parameters including systolic and mean pulmonary arterial pressures (sPA and mPAP, respectively) and improved clinical symptoms. However, beginning on day 53 to day 168 after imatinib administration, PH and clinical signs returned and worsened. Additionally, sildenafil at a low-dosage (0.5 mg/kg, PO, q12h) was administered in combination with low-dosage imatinib. The combined administration of low-dose imatinib and sildenafil greatly reduced sPA and mPAP values, improved RV function variables without worsening left ventricular function at 1 and 3 months with an improvement of clinical symptoms and resulted in longer survival times. This was the first study showing the effectiveness of a low-dose imatinib and sildenafil combination for treating PH dogs. This combination may be effective to prolong the survival of dogs with severe PH.

In chapter 2, this study aimed to investigate the efficacy of long-term masitinib therapy at low doses on echocardiographic, cardiovascular, hematological, and blood biochemical parameters, 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 parameters 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 parameters. These findings suggested 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.

Abstract

Pulmonary hypertension (PH) is defined as systolic pulmonary artery pressure (sPA) of >30 mmHg or mean pulmonary arterial pressure (mPAP) of >20 mmHg. The prognosis in dogs with PH is poor. It is caused by pulmonary arterial vasoconstriction and vascular remodeling. Several extracellular and intracellular signalling abnormalities including platelet-derived growth factor (PDGF) receptors and c-Kit receptors have been implicated in this remodeling. Imatinib, tyrosine kinase inhibitor targeting PDGF and c-Kit receptors, reverse pulmonary vascular remodeling in PH model rats by inhibiting the mitogen-activated protein kinase (MAPK) pathway, and also have pulmonary vasodilatory effects. Using high doses of imatinib in humans with PH has yielded mixed results with side effects including nausea, thrombocytopenia and anemia. Similar adverse effects have been reported in dogs given antineoplastic doses. Imatinib reversed pulmonary arterial remodeling and right ventricular systolic pressure in PH model rats at a lower dose (approximately one-third of an antineoplastic dose). Low-dose imatinib therapy improved clinical symptoms and echocardiographic outcomes without noticeable adverse effects in dogs with PH. However, resistance to imatinib treatment occurs in PH patients. In cases of this type, imatinib therapy in combination with other agents specific, or with other agents for PH may be required.

Sildenafil is a selective phosphodiesterase type 5 (PDE5) inhibitor that relaxes vascular smooth muscle cells and lowers pulmonary vascular resistance. It decreases sPA, and improve clinical signs in PH dogs. However, it has also been reported that sildenafil did not significantly or only slightly reduce sPA in PH dogs, even though it improved clinical signs. Therefore, it may be important to further investigate combination therapies of sildenafil and other drugs to ensure that sPA in dogs with PH is reduced adequately. It has been reported that a combination of low-

dosage imatinib and sildenafil was effective in treating PH in rats. Additive effects in improvement of PH may be caused by pharmacodynamic and pharmacokinetic drug–drug interactions between imatinib and sildenafil. A combination of low-dosage imatinib and sildenafil may be a better regimen for treating PH in dogs.

Masitinib is a veterinary drug that has been approved to treat canine mast cell tumors. 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. Masitinib has also been reported to be safer than imatinib. Moreover, masitinib elicits stronger cardiopulmonary preventive properties than tadalafil, via dual inhibition of the MAPK pathway and PDE5, and long-term therapy with a lower dose of masitinib reduces PH severity and improves survival. Therefore, a lower dose of masitinib could be used in PH therapy to target both cardiopulmonary remodeling and the increased vasoconstriction. However, no published reports on the therapeutic effect of masitinib for PH in dogs are available.

Therefore, this study was conducted to investigate the therapeutic effects of tyrosine kinase inhibitors, imatinib and masitinib for pulmonary hypertension in dogs. The study in chapter 1 aimed to investigate the therapeutic effects of low-dosage imatinib in combination with sildenafil in dogs with PH due to chronic degenerative mitral valve disease (CDMVD) or ventricular septal defect (VSD) with Eisenmenger's syndrome. In chapter 2, the study aimed to investigate the efficacy of low-dose masitinib therapy for canine PH caused by advanced mitral insufficiency and heartworm disease.

In chapter 1, the therapeutic effects of sildenafil combined with imatinib at low dosages on echocardiographic, cardiovascular, hematological and blood biochemical variables and clinical symptoms were evaluated in dogs with PH caused by advanced CDMVD or VSD with

Eisenmenger's syndrome. Five client-owned dogs with PH were initially administered low-dose imatinib mesylate (3 mg/kg, orally, q24h). The low-dose imatinib improved the right ventricular (RV) function parameters including estimated sPA and mPAP, and improved clinical symptoms. However, beginning on day 53 to day 168 after imatinib administration, PH and clinical signs returned and worsened. Additionally, sildenafil at a low-dosage (0.5 mg/kg, orally, q12h) was administered in combination with low-dosage imatinib. The combined administration of low-dose imatinib and sildenafil greatly reduced the estimated sPA and mPAP values, improved RV function variables without worsening left ventricular function (LV) at 1 and 3 months with an improvement of clinical symptoms and resulted in longer survival times. This was the first study showing the effectiveness of a low-dose imatinib and sildenafil combination for treating PH dogs. This combination may be effective to prolong the survival of dogs with severe PH.

In chapter 2, the effects of long-term masitinib therapy at low doses on echocardiographic, cardiovascular, hematological and blood biochemical parameters and clinical symptoms were investigated in dogs with PH caused by advanced CDMVD or heartworm disease. Seven client-owned dogs with severe PH were recruited prospectively and given low-dose masitinib orally, 3 mg/kg (approximately one-fourth of the recommended antineoplastic dosage), q24h, for 123 days to 928 days. Examinations were performed prior to masitinib administration, as well as 1, 2, 3, 6, and 12 months later. At 1 month to 12 months, low-dose masitinib significantly reduced the estimated sPA, and dramatically improved clinical symptoms. Low-dose masitinib treatment improved RV function parameters such as right atrium/aorta ratio, maximum tricuspid regurgitation velocity, RV Tei index, and tricuspid annular plane systolic excursion, without worsening LV function parameters. These findings suggested 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.

In conclusion, the present study revealed that the combined administration of low-dose imatinib and sildenafil markedly reduced the estimated sPA and improved RV function and clinical symptoms in dogs with severe PH caused by CDMVD and VSD. In addition, this study revealed that masitinib at low dosage significantly reduced sPA and improved RV function and clinical symptoms in dogs with severe PH caused by CDMVD and heartworm disease. Although larger, placebo-controlled, randomized, and blinded studies may be needed to determine the long-term efficacy of low-dose imatinib and sildenafil combination or low-dose masitinib in dogs with PH caused by a variety of etiologies, this study provided new information on the effectiveness of tyrosine kinase inhibitors, imatinib and masitinib for treating PH dogs.

Acknowledgements

I would like to thank my doctoral course supervisor Professor Dr. Yoshiaki Hikasa (Tottori University) for his guidance and support throughout my graduate student years, and for the critical reviewing of the manuscript. He is a talented and brilliant scientist and a great mentor, from whom I have learned a great deal.

I am grateful to Professor Dr. Tomohiro Imagawa (Tottori University) and Associate Professor Dr. Naohito Nishii (Gifu University) for their guidance as assistant supervisors during my study and for the review of this doctoral thesis.

I am also grateful to my wife, Dr. Miwako Sahashi for a variety of support in performing the study with data collection. I also wish to thank the dog's owners for their involvement in the study.

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