



# Pharmacokinetics of single dose sildenafil orally administered in canine models of chronic embolic pulmonary hypertension

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**ABSTRACT.** Information regarding the pharmacokinetics of oral sildenafil in dogs with pulmonary hypertension is limited. In this study, we examined the pharmacokinetics of oral sildenafil in a canine model of chronic embolic pulmonary hypertension (CEPH). The CEPH model was developed by repeatedly injecting microspheres into the pulmonary arteries. The pharmacokinetics of oral sildenafil at 1, 2 and 4 mg/kg was evaluated using four dogs with pulmonary hypertension in the fasted state. The plasma concentrations of sildenafil were determined using high-performance liquid chromatography, and pharmacokinetic parameters were calculated using a noncompartmental analysis. Sildenafil was well tolerated in this study. Proportional increments in the maximum plasma concentration and area under the curve extrapolated to infinity at drug doses of 1, 2 and 4 mg/kg were detected using a power model analysis. No significant differences were observed among the three doses in the time to maximum plasma concentration. The mean residence time and elimination half-life were slightly but significantly higher at a dose of 4 mg/kg than at a dose of 1 mg/kg.

**KEY WORDS:** dog, microsphere, pharmacokinetics, pulmonary hypertension, sildenafil

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Pulmonary hypertension (PH) is a progressive disease characterised by the persistent elevation of pulmonary arterial pressure (PAP), ultimately leading to right ventricular failure (ie. reduction in cardiac output [CO] and systemic congestion) and death [4, 12]. PH can be classified as pre- or post-capillary PH, or it can be identified on the basis of the causative disease process. There are generally five categories for this condition: 1) pulmonary arterial hypertension, 2) pulmonary venous hypertension, 3) pulmonary disease or hypoxia, 4) thromboembolic disease and 5) miscellaneous disease [3, 8, 11, 14, 23, 25]. In veterinary medicine, PH is mainly diagnosed using systolic PAP (sPAP) >30 mmHg determined by considering estimates of tricuspid regurgitation velocity obtained via echocardiography, whereas right heart catheterization (RHC) remains the gold standard for detecting PH, generally using the following parameters: sPAP >30 mmHg and mean PAP (mPAP) >20 mm Hg [26].

In dogs with PH, abnormalities or imbalances in multiple signalling pathways such as nitric oxide, endothelin and prostanoid signalling are described, and these pathological changes result in medial hypertrophy, intimal proliferation and decreased pulmonary vascular compliance, leading to further elevation of pulmonary arterial pressure [12]. Treatment for PH aims to improve the underlying causes, and a specific agent targeting the aforementioned abnormalities or imbalances in the multiple signalling pathways mentioned above is administered with the progression of PH. Sildenafil, a highly selective PDE-5 inhibitor, is used to block the inactivation of cGMP by PDE-5, which leads to pulmonary arterial vasodilation [16]. Several studies have demonstrated the benefits of sildenafil in dogs with PH caused by different pathogeneses such as respiratory disease and left heart disease [3, 5, 13, 14, 17, 20, 27, 28].

Alterations in the pharmacokinetic properties of drugs are observed in human patients with heart failure. Several factors are known to influence the pharmacokinetics of drugs in patients with cardiac disease. Gut wall dysfunction may lead to impaired absorption, which is also influenced by reduced blood flow to the gastrointestinal tract [29], contributing to both chronic inflammation and malnutrition. Conversely, reduced blood flow to central organs and peripheral tissue may lead to altered drug distribution [9]. In addition to absorption and distribution, hypoperfusion of organs in chronic heart failure may also influence drug

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elimination by either the liver or kidneys [21].

However, to the best of our knowledge, no report has described the pharmacokinetic properties of sildenafil in dogs with PH, and basic information about the effect of PH on the pharmacokinetics of sildenafil is scarce. In addition, the dose proportionality of sildenafil in dogs with PH has not been evaluated despite the use of wide-ranging doses (approximately 1.0–4.0 mg/kg) in previous studies reporting the benefits of sildenafil in dogs with PH. Therefore, the present study examined the pharmacokinetic properties of oral sildenafil in canine models of chronic PH.

## MATERIALS AND METHODS

This study was performed using four female beagle dogs that were considered clinically healthy based on a physical examination, complete blood count, biochemistry, echocardiography, electrocardiography and blood pressure measured using an oscillometric method. The dogs were housed individually in cages and fed commercial dry food twice daily with free access to water. We followed the Guidelines for Institutional Laboratory Animal Care and Use at the Nippon Veterinary and Life Science University (Approval number 29S-24).

### *Right heart catheterization and creation of chronic embolic PH (CEPH) model*

The dogs were anesthetized with propofol (6 mg/kg, IV) and intubated, which was maintained using a continuous-rate infusion of propofol (0.1–0.2 mg/kg/min, IV) under 100% oxygen. The anesthetized dogs were positioned in left or right lateral recumbency. A 5-Fr introducer sheath (Medikit, Tokyo, Japan) was percutaneously inserted through the right or left jugular vein, and then a 4-Fr balloon wedge pressure catheter (Harmac Medical Products, Buffalo, NY, USA) or 5-Fr thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted and advanced into the pulmonary artery. The fluid-filled catheter was connected to pressure transducers to monitor pulmonary artery pressure. The transducer was zeroed at the level of the right heart and recalibrated before each set of measurements. The catheter location was confirmed by detecting the typical pressure wave of this artery. After advancing the catheter into the pulmonary artery, the infusion of propofol was stopped. The dogs were allowed to recover from anesthesia, and sufficient recovery time (60–90 min) until the dogs could walk without assistance was allotted. Then, the conscious dogs were again restrained in left or right lateral recumbency for hemodynamic measurements. Hemodynamic measurements, including those of sPAP, mPAP and diastolic pulmonary artery pressure (dPAP), right atrial pressure (RAP), pulmonary artery wedge pressure (PAWP) and CO, were performed before (baseline) and after creating the CEPH models. CO was calculated as the average of three measurements obtained using a thermodilution technique with an injection of 3 ml of normal saline. Systemic artery pressure (SAP) recordings were obtained by the oscillometric method (Ramsey Medical Inc., Tampa, FL, USA). SAP measurements were performed simultaneously with RHC. Pulmonary (PVR) and systemic vascular resistance (SVR) was calculated using the following formulae: 1) PVR (Wood units)=(mPAP [mmHg]–PAWP [mmHg])/CO (l/min), 2) SVR (Wood units)=(mean SAP [mmHg] –RAP [mmHg])/CO (l/min).

CEPH was induced according to a modified version of a previously described method [18, 24]. Briefly, 300- $\mu$ m microspheres (GE Healthcare, Chicago, IL, USA) were injected into the pulmonary artery through the 4-Fr catheter monitoring PAP. The number of microspheres infused in each treatment was adjusted to increase sPAP to approximately 40–50 mm Hg. After each procedure, the sheath and catheter were removed from the jugular vein. As partial recovery of PAP between each injection day was consistently observed as described previously, this treatment was repeated approximately once a week until sPAP and mPAP prior to microsphere injection exceeded 30 and 20 mmHg, respectively [24]. Then, at least one month was allotted to confirm that sPAP and mPAP exceeded 30 and 20 mmHg, respectively, without further injection.

### *Pharmacokinetic study*

Plasma pharmacokinetics following the oral administration of sildenafil (Viagra, 50 mg/tablet; Pfizer) at 1, 2 and 4 mg/kg was compared in CEPH dogs (5.0  $\pm$  3.3 years, 10.3  $\pm$  1.4 kg) with a washout period of 1 week. After each washout period, the dogs were randomly assigned to receive one of the remaining doses. This continued until each dog had been given all doses. All dogs were fasted overnight before dosing, and food was supplied 4 hr after sildenafil administration. Immediately before administration, the sildenafil tablet was ground to prepare the correct dose calculated from the ratio of the tablet mass and contained sildenafil amount. The tablet mass was measured using an electronic scale. Dogs were monitored at each blood collection for signs of cutaneous flushing, vomiting and diarrhoea.

### *Blood sampling and storage*

Blood samples (5 ml) were collected into EDTA tubes via a venous catheter placed in the cephalic vein before administration and 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 480 and 720 min after administration. The tubes were immediately centrifuged at 4°C and 1,000  $\times$  g for 10 min. Plasma was separated and frozen at –80°C until analysis. Sildenafil concentrations were measured within 1 week of sampling.

### *Determination of sildenafil concentrations in canine plasma samples*

Sildenafil concentrations were determined using high-performance liquid chromatography (HPLC) as reported in our previous study [1]. Briefly, the plasma sample (500  $\mu$ l) was spiked with 50  $\mu$ l of the internal standard (IS) solution (2  $\mu$ g/ml butylparaben) and 200  $\mu$ l of 5 mol/l NaOH. After vortexing for 30 sec, the mixture was combined with 3 ml of ethyl acetate via vortexing for

3 min and centrifuged at  $1,000 \times g$  for 10 min. The supernatant was separated and evaporated under a stream of nitrogen gas at  $40^\circ\text{C}$ . Then,  $140 \mu\text{l}$  of the mobile phase, consisting of acetonitrile and 30 mmol/l potassium dihydrogen phosphate buffer solution at a ratio of 47:53, were added to dissolve the residue and centrifuged at  $21,880 \times g$  for 10 min at  $4^\circ\text{C}$ . The supernatant ( $80 \mu\text{l}$ ) was then injected into a C18 Inertsil ODS2 HPLC column ( $150 \times 4.6 \text{ mm}$ ;  $5 \mu\text{m}$  particle size; GL Sciences Inc., Japan). The flow rate was set at 1 ml/min, sildenafil and IS were isocratically eluted from the column with retention times of 7.03 and 9.22 min, respectively, at temperature of  $25^\circ\text{C}$ . The wavelength of the detector was set at 230 nm.

Sildenafil was quantified using a calibration curve ranging from 5 to 5,000 ng/ml. The limit of quantification was identified as 5 ng/ml, which was the lowest concentration of the calibration curve. The calibration curve was accepted when the linear coefficient of determination exceeded 0.999 and the calibration curve concentrations could be back-calculated to within 15% of the true concentration of the standard. Intraday and interday variability were assessed using reference standards at 5, 50 and 500 ng/ml. For intraday variability, the accuracy and precision were 100–107% and 2.4–5.6%, respectively. For interday variability, the accuracy and precision were 92–115% and 8.6–9.2%, respectively.

### Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated using a noncompartmental analysis (Excel 2013; Microsoft, Redmond, WA). The maximum plasma concentration ( $C_{\text{max}}$ ) and time ( $T_{\text{max}}$ ) to reach  $C_{\text{max}}$  were obtained from the plasma concentration vs. the time curve. The area under the plasma concentration vs. the time curve to the final measured concentration ( $\text{AUC}_{\text{fin}}$ ) was calculated using the linear trapezoidal method. The elimination rate constant ( $k_{\text{el}}$ ) was determined using the log-linear slope of the terminal phase. The area under the plasma concentration vs. the time curve extrapolated to infinity ( $\text{AUC}_{\text{inf}}$ ) was calculated using  $\text{AUC}_{\text{fin}}$  and  $k_{\text{el}}$  by the following formula:  $\text{AUC}_{\text{fin}} + C_{\text{fin}}/k_{\text{el}}$ , where  $C_{\text{fin}}$ =sildenafil concentration at final point. Likewise, the area under the moment curve to the final measured values ( $\text{AUMC}_{\text{fin}}$ ) was calculated to obtain the area under the moment curve extrapolated to infinity ( $\text{AUMC}_{\text{inf}}$ ). The mean residence time (MRT) was calculated as  $\text{AUMC}_{\text{inf}}/\text{AUC}_{\text{inf}}$ . The elimination half-life ( $t_{1/2}$ ) was calculated as  $\ln 2/k_{\text{el}}$ . In addition, the apparent clearance (CL/F) and apparent volume of distribution (V/F) were determined according to the following equations:  $\text{CL}/F = \text{dose}/\text{AUC}_{\text{inf}}$  and  $V/F = (\text{CL}/F)/k_{\text{el}}$ , respectively.

The statistical analysis was performed using commercial software (SPSS Statistics 24.0; IBM, Armonk, NY, USA). Data are presented as the mean  $\pm$  standard deviation.  $P < 0.05$  was considered statistically significant. The normality of data was assessed using the Shapiro–Wilk test. A paired *t*-test or Wilcoxon's signed-rank test was used to compare the hemodynamic parameters between the baseline and CEPH conditions. Power model analysis was utilised to examine dose proportionality based on previous reports [1, 7, 10]. Briefly,  $C_{\text{max}}$  and  $\text{AUC}_{\text{inf}}$  were fit to the model  $Y = \alpha \times \text{Dose}^\beta$ . This equation can be expressed as  $\log(Y) = \log(\alpha) + \beta \times \log(\text{Dose})$ , where  $Y = C_{\text{max}}$  or  $\text{AUC}_{\text{inf}}$ ,  $\log(\alpha)$  is the intercept and  $\beta$  is the slope of the equation.  $\alpha$  is dependent on other terms in the model, and a value of 1 for  $\beta$  indicates perfect dose proportionality. The point estimation of  $\beta$  was conducted, and a 90% confidence interval (CI) was determined. Dose proportionality was confirmed if the 90% CI on the estimate of  $\beta$  included 1 and the test for the lack of fit based on analysis of variance was not significant ( $P > 0.05$ ). For  $T_{\text{max}}$ , MRT,  $t_{1/2}$ , CL/F and V/F, the Friedman test followed by Bonferroni's multiple comparison test was used to compare the pharmacokinetic parameters among the three sildenafil doses.

## RESULTS

### Creation of the CEPH model

We needed 8–15 months (22–42 injection times) to create our preferred CEPH model. sPAP and mPAP in all dogs included in this study exceeded 30 and 20 mmHg, respectively, and fulfilled the criterion of CEPH. The hemodynamic characteristics of dogs with CEPH in this study are shown in Table 1. CO decreased, and PVR and SVR increased significantly in animals with CEPH. HR, SAP, PAWP and RAP remained unchanged.

### Pharmacokinetic analysis

Oral sildenafil was well tolerated, and no adverse effects were observed. The plasma concentration profiles for the three different doses of sildenafil are shown in Fig. 1. Table 2 shows the pharmacokinetic parameters for these three doses. The absorption of sildenafil was rapid (approximately 1 hr) after all doses. The power model analysis illustrated that the 90% CIs for  $\beta$  of  $C_{\text{max}}$  (mean=0.81, 90% CI=0.62–1.00) and  $\text{AUC}_{\text{inf}}$  (mean=0.94, 90% CI=0.65–1.23) both included 1. Consequently,  $C_{\text{max}}$  and  $\text{AUC}_{\text{inf}}$  proportionately increased over the dose range of 1–4 mg/kg.  $C_{\text{max}}$  and  $\text{AUC}_{\text{inf}}$  obtained from healthy dogs in which sildenafil was administered at 1–4 mg/kg has also been included in Table 3 for comparison [1]. In dogs with CEPH, MRT and  $t_{1/2}$  increased slightly but significantly at 4 mg/kg (MRT=5.4  $\pm$  0.3 hr,  $t_{1/2}$ =3.5  $\pm$  0.3 hr) compared to those (MRT=4.0  $\pm$  0.7 hr,  $t_{1/2}$ =2.6  $\pm$  0.5 hr) obtained at 1 mg/kg. No significant differences were observed among the three doses for  $T_{\text{max}}$ , CL/F and V/F.

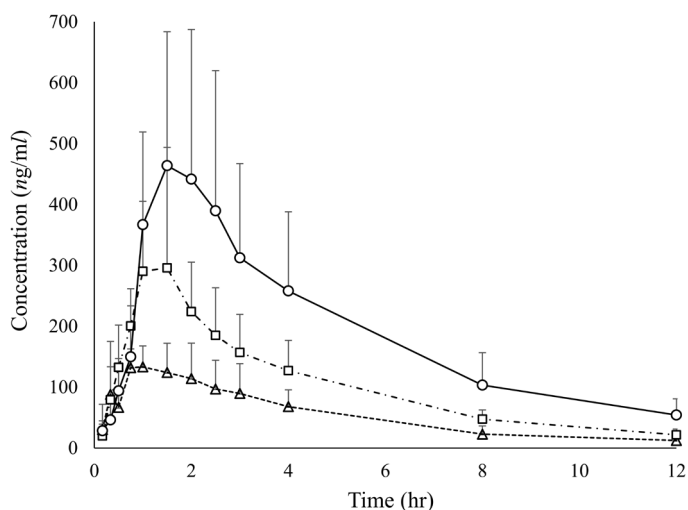
## DISCUSSION

In veterinary medicine, sildenafil is administered to animals with PH resulting from various pathogenesises, and the drug has been found to improve clinical signs and quality of life and decrease PH severity based on echocardiography [3, 5, 13, 14, 17, 20, 27, 28]. However, basic information related to the pharmacokinetics of sildenafil in dogs with PH is scarce, and to our knowledge, this is the first report describing the pharmacokinetics of oral sildenafil in dogs with PH.

**Table 1.** Hemodynamic parameters (mean ± standard deviation [SD]) at baseline and chronic embolic pulmonary hypertension (CEPH)

	Baseline	CEPH
	Mean ± SD	Mean ± SD
HR (bpm)	117 ± 8	99 ± 23
sPAP (mmHg)	17.1 ± 4.1	37.4 ± 3.8 <sup>a)</sup>
mPAP (mmHg)	12.7 ± 2.5	31.5 ± 3.0 <sup>a)</sup>
dPAP (mmHg)	8.9 ± 1.8	25.4 ± 2.5 <sup>a)</sup>
sSAP (mmHg)	145 ± 10	148 ± 15
mSAP (mmHg)	118 ± 9	116 ± 15
dSAP (mmHg)	91 ± 4	96 ± 13
PAWP (mmHg)	5.3 ± 1.2	5.5 ± 0.4
RAP (mmHg)	2.2 ± 0.4	4.2 ± 1.3
CO (ml/min)	1,544.5 ± 581.1	1,120.5 ± 350.6 <sup>a)</sup>
PVR (Wood units)	5.1 ± 1.3	25.1 ± 9.2 <sup>a)</sup>
SVR (Wood units)	80.9 ± 21.7	106.9 ± 32.8 <sup>a)</sup>

a)  $P < 0.05$  vs. Baseline. HR, heart rate; sPAP, systolic pulmonary artery pressure; mPAP, mean pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; sSAP, systolic systemic artery pressure; mSAP, mean systemic artery pressure; dSAP, diastolic systemic artery pressure; PAWP, pulmonary artery wedge pressure; RAP, right atrial pressure; CO, cardiac output; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance.



**Fig. 1.** Plasma concentration profiles (mean and standard deviation) of sildenafil following oral administration at 1 (triangle), 2 (quadrangle) and 4 (circle) mg/kg in dogs with chronic embolic pulmonary hypertension (each  $n=4$ ).

**Table 2.** Pharmacokinetic parameters (mean ± standard deviation [SD]) following the oral administration of sildenafil at 1, 2 and 4 mg/kg in dogs with chronic embolic pulmonary hypertension (each  $n=4$ )

Pharmacokinetic Parameter	1 mg/kg	2 mg/kg	4 mg/kg
	Mean ± SD	Mean ± SD	Mean ± SD
$C_{max}$ (ng/ml)	154.5 ± 41.9	352.5 ± 192.3	500.7 ± 216.6
$T_{max}$ (hr)	1.0 ± 0.7	1.1 ± 0.2	1.5 ± 0.4
$AUC_{inf}$ (ng·hr/ml)	659.7 ± 282.5	1,313 ± 517.9	2,512.4 ± 1,185.9
AUC extrap (%)	8.0 ± 4.4	6.8 ± 1.3	10.3 ± 1.6
$AUMC_{inf}$ (ng·hr <sup>2</sup> /ml)	2,805.5 ± 1,580.8	5,962.7 ± 2,273.2	13,734.5 ± 6,842.0
MRT (hr)	4.0 ± 0.7	4.6 ± 0.3	5.4 ± 0.3 <sup>a)</sup>
$t_{1/2}$ (hr)	2.6 ± 0.5	3.0 ± 0.2	3.5 ± 0.3 <sup>a)</sup>
CL/F (l/hr/kg)	1.8 ± 0.8	1.7 ± 0.6	2.0 ± 1.1
V/F (l/kg)	6.2 ± 1.5	7.4 ± 2.8	10.1 ± 6.4

a)  $P < 0.05$  vs. 1 mg/kg.  $C_{max}$ , maximum plasma concentration;  $T_{max}$ , time to maximum plasma concentration;  $AUC_{inf}$ , area under the curve from time 0 to infinity; AUC extrap, percent of the AUC extrapolated to infinity;  $AUMC_{inf}$ , area under the moment curve extrapolated to infinity; MRT, mean residence time;  $t_{1/2}$ , elimination half-life; CL/F, apparent plasma clearance per bioavailability; V/F, apparent volume of distribution per bioavailability.

**Table 3.**  $C_{max}$ ,  $AUC_{inf}$  and 90% confidence interval (CI) of the estimate of  $\beta$  following the oral administration of sildenafil at 1, 2 and 4 mg/kg in dogs with chronic embolic pulmonary hypertension (CEPH)

	$C_{max}$ (ng/ml)			$\beta$	90%CI	$AUC_{inf}$ (ng·hr/ml)			$\beta$	90%CI
	1 mg/kg	2 mg/kg	4 mg/kg			1 mg/kg	2 mg/kg	4 mg/kg		
CEPH ( $n=4$ )	154.5	352.5	500.7	0.81	0.62–1.00	659.7	1,313.0	2,512.4	0.94	0.65–1.23
Healthy ( $n=5$ ) <sup>a)</sup>	174.9	416.0	1,111.4	1.33	1.17–1.49	791.6	1,593.0	4,319.6	1.23	1.07–1.39

a) For reference,  $C_{max}$ ,  $AUC_{inf}$  and 90%CI of the estimate of  $\beta$  in healthy dogs (each  $n=5$ ) were taken from a previous study [1] in which the same assay as performed in the present study was utilised.  $C_{max}$ , maximum plasma concentration;  $AUC_{inf}$ , area under the curve from time 0 to infinity.

Throughout all studies, blood sampling time is thought to be adequate because AUC extrap is generally lower than 10%. Sildenafil was well tolerated, and no dogs displayed apparent adverse effects such as cutaneous flushing, vomit and diarrhoea [22]. This finding might ensure the safety of this drug at high doses in dogs with PH, although we could not evaluate all adverse effects, and further investigation of the safety of sildenafil is needed, especially regarding long-term effects associated with repeated dosing.

In this study, we observed proportionality for the pharmacokinetics of sildenafil in dogs with CEPH via power model analysis. Although we cannot directly compare our results with previous findings, our current data and published report using healthy dogs reveal that  $C_{\max}$  and  $AUC_{\text{inf}}$  increase proportionately in dogs with CEPH with increasing dosage, whereas those in healthy dogs increased at a faster than expected rate [1]. Consequently, it is likely that the non-proportionality of sildenafil observed in healthy dogs disappeared in animals with CEPH. In healthy dogs, as we discussed in our previous study, non-proportional increment of  $C_{\max}$  and  $AUC_{\text{inf}}$  at high dose are observed due to saturation of the metabolism in the liver because sildenafil absorbed normally into the body through the gastrointestinal tract is mainly metabolized by hepatic microsomal isoenzymes [1]. In human medicine, hypoperfusion of the gastrointestinal tract is usually observed in patients with heart failure, who exhibit reduced CO, and the absorption of most drugs is dependent on gastrointestinal blood flow [15]. Thus, in CEPH models administered sildenafil at high dose, amount of the drug absorbed from gastrointestinal tract might fall below the limit of metabolism in the liver, which leads to lower  $C_{\max}$  and  $AUC_{\text{inf}}$  compared to those of healthy dogs, consequently the disappearance of non-proportionality, though we could not obtain the absolute bioavailability of oral sildenafil at each dose.

Concerning the elimination of sildenafil in our CEPH model, a previous report in humans indicated that our finding of slightly but significantly higher MRT and  $t_{1/2}$  at the highest dose might be attributable to reduced systemic clearance resulting from decreased CO due to CEPH [21]. However, the extent of this decrement in the elimination rate is unlikely to be pharmacokinetically and clinically significant because even prolonged MRT and  $t_{1/2}$  at 4 mg/kg were equivalent to those obtained from healthy dogs in a previous study [2]. It generally takes  $5\text{--}6 \times t_{1/2}$  hours to reach a steady state. On the precondition that the absorption and elimination is proportional to the drug concentration and repeated administration has little influence on clearance, steady state of sildenafil in PH dogs is achieved after approximate 15–18 hr of administration, irrespective of the dosing interval. Consequently, considering  $t_{1/2}$  of sildenafil, thrice-daily administration could enhance the effect of treatment relative to once- or twice-daily treatment in dogs with PH though we should note that it may be difficult to predict the drug concentration in a steady state because the pharmacokinetics of the drug might depend on normal or pathological condition of dogs.

In the current study, the major hemodynamic alteration in CEPH models was reduced CO opposed to systemic congestion. Thus, dogs with cardiac disease in which reduced CO is the major hemodynamic alteration might exhibit similar changes in the pharmacokinetic characteristics of sildenafil as described previously. However, in clinical practice, left-sided heart disease is a common cause of PH in dogs [4]. In this pathological condition, it is believed that fluid retention resulting from neurohormonal activation such as the sympathetic and renin–angiotensin–aldosterone systems progresses before developing PH [6]. Consequently, systemic congestion appears to occur relatively quickly in PH models compared with the findings in CEPH models. Chronic congestion also influences pharmacokinetic properties by inducing the impairment of liver function, leading to a decreased elimination rate and increased drug exposure [9]. Therefore, further studies regarding the pharmacokinetics of sildenafil in PH patients with systemic congestion are warranted to validate the use of this drug.

This study had several limitations. First, we could not obtain the intravenous pharmacokinetic parameters of sildenafil at each dose because an injectable formulation was not available at the time of the study. Thus, we could not calculate the absolute bioavailability of oral sildenafil at each dose, necessitating the use of CL/F and V/F. Second, we utilized electronic scale to prepare the needed dose of sildenafil. Therefore, the dosing of sildenafil might not be sufficiently accurate, which might cause wide variability. However, in veterinary medicine, partitioning tablets is often needed to prescribe the correct dose. Furthermore, in human medicine, it is reported that sildenafil tablet is stable when it is ground to powder [19]. Finally, the number of dogs included here was small, thus we might have missed the statistical significance.

In conclusion, in dogs with CEPH,  $C_{\max}$  and  $AUC_{\text{inf}}$  of sildenafil increased proportionally at doses ranging from 1 to 4 mg/kg, whereas elimination of the drug was in line with the observations in healthy dogs.

CONFLICT OF INTEREST. We have no conflicts of interest.

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