The Veterinary Journal ■■ (2014) ■■–■■

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension

Yasutomo Hori [a,*], Chigusa Kondo [a], Maho Matsui [a], Maki Yamagishi [a], Shozo Okano [b], Seishiro Chikazawa [a], Kazutaka Kanai [a], Fumio Hoshi [a], Naoyuki Itoh [a]

[a] Laboratory of Small Animal Internal Medicine, School of Veterinary Medicine, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, Japan
[b] Laboratory of Small Animal Surgery 2, School of Veterinary Medicine, Kitasato University, 23-35-1 Higashi, Towada, Aomori 034-8628, Japan

ARTICLE INFO

Article history:
Accepted 9 August 2014

Keywords:
Dog
Phosphodiesterase type 5 (PDE5) inhibitor
Pulmonary arterial hypertension
Tadalafil
Hemodynamic

ABSTRACT

Phosphodiesterase type 5 (PDE5) inhibitors are used for treating pulmonary arterial hypertension (PAH) in dogs. The long-acting PDE5 inhibitor tadalafil was recently approved for treatment of PAH in humans. Basic information related to the pharmacological and hemodynamic effects of tadalafil in dogs is scarce. In this study, the hemodynamic effects of tadalafil after intravenous (IV) and oral administration were investigated in a healthy vasoconstrictive PAH Beagle dog model induced by U46619, a thromboxane A_2 mimetic. Six healthy Beagle dogs were anesthetized with propofol and maintained with isoflurane. Fluid-filled catheters were placed into the descending aorta to measure systemic arterial pressure and in the pulmonary artery to measure pulmonary arterial pressure (PAP). U46619 was infused via the cephalic vein to induce PAH.

IV infusion of U46619 significantly elevated PAP from baseline in a dose-dependent manner. U46619-elevated PAP and pulmonary vascular resistance was significantly attenuated by the simultaneous infusion of tadalafil at 100 and 200 μg/kg/h. Likewise, oral administration of tadalafil at 1.0, 2.0, and 4.0 mg/kg significantly attenuated U46619-elevated PAP in a dose-dependent manner. U46619-elevated systolic and mean PAP decreased significantly 1 h after oral tadalafil administration at 4.0 mg/kg, and this effect was maintained for 6 h. In conclusion, tadalafil had a pharmacological effect in dogs and IV infusion of tadalafil induced pulmonary arterial relaxation, while oral administration of tadalafil decreased PAP. These results suggest that tadalafil may offer a new therapeutic option for treating dogs with PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by chronic elevation in pulmonary arterial pressure (PAP). In veterinary medicine, PAH in dogs is defined by a systolic/mean PAP of >30/20 mmHg at rest (Johnson, 1999; Johnson et al., 1999). PAH can occur secondary to multiple abnormalities of the pulmonary or cardiovascular systems, such as left-sided heart failure, left-to-right cardiac shunting, heartworm disease, and pulmonary disease (Johnson, 1999; Glaus et al., 2004; Uchide and Saida, 2005; Bach et al., 2006; Zabka et al., 2006; Kellum and Stepien, 2007; Seibert et al., 2010; Nakamura et al., 2011). In severe cases, PAH can lead to right-sided heart failure and death (Johnson, 1999; Johnson et al., 1999; Zabka et al., 2006).

The pathogenesis of PAH involves an imbalance between pulmonary vasoconstriction and vasodilation, which leads to increases in PAP (Humbert et al., 2004; Pietra et al., 2004). Thromboxane (TX)A_2 is a potent autacoid that induces platelet aggregation and vasoconstriction, and its plasma levels are increased in animals and humans with PAH (Christman et al., 1992; Adatia et al., 1993). In dogs, an intravenous (IV) infusion of U46619, a TXA_2-mimetic agent, induces pulmonary arterial constriction (Yamada et al., 1998; Gong et al., 2000; Tamura et al., 2001).

Three classes of pulmonary artery-specific vasodilators have been approved for the treatment of PAH in humans, namely, prostaglandin I_2 (prostacyclin), endothelin receptor antagonists, and phosphodiesterase type 5 (PDE5) inhibitors. Of these, PDE5 inhibitors, including sildenafil, vardenafil, and tadalafil, are currently available in human medicine (Galiè et al., 2009; Anderson and Nawarskas, 2010). Sildenafil has a short half-life of 4–5 h and thus requires dosing three times daily (Supuran et al., 2006; Falk et al., 2010). In veterinary medicine, sildenafil is the most frequently used PDE5 inhibitor for treatment of PAH. It reportedly improves

* Corresponding author. Tel.: +81 176 249301.
E-mail address: hori@vmas.kitasato-u.ac.jp (Y. Hori).

http://dx.doi.org/10.1016/j.tvjl.2014.08.009
1090-0233/

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009

symptoms and clinical outcomes in dogs with PAH (Bach et al., 2006; Kellum and Stepien, 2007; Brown et al., 2010; Nakamura et al., 2011).

In contrast, tadalafil has a longer half-life of 17.5 h that allows for once-daily dosing (Supuran et al., 2006; Arif and Poon, 2011); it is currently approved for the treatment of PAH and erectile dysfunction in humans (Porst et al., 2003; Skoumal et al., 2004). Tadalafil may present a new therapeutic option for PAH in dogs because of its less frequent dosing and more sustained benefits. However, only one case report has described the use of tadalafil for the treatment of PAH in dogs (Serres et al., 2006). Basic information on the pharmacological and hemodynamic effects of tadalafil as a treatment for PAH in dogs remains scarce. In the present study, to clarify its pulmonary hemodynamic action, we investigated the effects of tadalafil on PAP and vascular resistance in healthy dogs with U46619-induced acute vasoconstrictive PAH.

Materials and methods

Animals

This study was approved by the Institutional Animal Care and Use Committee for Kitasato University (approval number 13-123, 5 December 2013).

Six Beagle dogs were used in this study (3 males, 3 females; aged 4–5 years; weight 10–13 kg). All dogs were determined to be healthy according to the results of complete physical and echocardiography examinations. The dogs were housed individually in cages and fed commercial dry food with free access to water.

Drugs

U46619 (9,11-dideoxy-11a,9a-epoxymethanoprostaglandin $F_{2\alpha}$; U46619, Cayman) was dissolved in ethanol according to the manufacturer's instructions. Tadalafil (Tadalafil, Acanthus Research) was dissolved in dimethyl sulfoxide (DMSO) for IV infusion or purchased for oral administration (Adcirca 20 mg). Immediately before examination, each drug was diluted in sterile saline to a final concentration of 50 μg/mL (for U46619) and 0.2 mg/mL (for tadalafil), respectively.

Anesthesia

The dogs were sedated with butorphanol (0.2 mg/kg IV), diazepam (0.5 mg/kg SC) and atropine (0.025 mg/kg SC); anesthetized with propofol (6.0 mg/kg IV); and intubated. Anesthesia was maintained using a mixture of 2.0–3.0% isoflurane and oxygen. The end-tidal partial pressure of carbon dioxide in arterial blood ($PaCO_2$) and hemoglobin saturation level of oxygen (SPO_2) were monitored and maintained between 35–45 mmHg and 95–100%, respectively. Heart rate was monitored using electrocardiography (COLIN BP-608, Nihon Kohden). Respiratory rate was maintained between 10 and 20 breaths/min. Fluid loss was replaced by IV infusion of Ringer's solution, and the total fluid volume was adjusted to 60 mL/kg/day. Following the examinations, the dogs were allowed to recover from anesthesia.

Hemodynamic measurements

The anesthetized dogs were positioned in left lateral recumbency. A 3-Fr fluid-filled catheter was placed via the left femoral artery into the descending aorta to measure systolic, mean, and diastolic systemic arterial pressure (SAP). A 4-Fr fluid-filled catheter or a Swan–Ganz catheter (5 Fr thermodilution catheter, TC-504, Nihon Kohden) was inserted through the jugular vein and advanced into the pulmonary artery to measure the systolic, mean, and diastolic PAP. Similarly, mean right ventricular pressure (RVP) and mean central venous pressure (CVP) were also measured.

Mean pulmonary capillary wedge pressure (PCWP) and cardiac output (CO) were measured using a Swan–Ganz catheter. CO was calculated as the average of the three measurements obtained by thermodilution (CCOM monitor, CO-203, Terumo) with an injection of 5 mL of normal saline kept at 4 °C (Gong et al., 2000; Tamura et al., 2001).

Pulmonary vascular resistance (PVR), systemic vascular resistance (SVR), and PVR to SVR ratio (Rp/Rs ratio) were calculated using the following formulae (Tamura et al., 2001):

$$PVR = (\text{mean PAP} - \text{mean PCWP})/CO$$

$$SVR = (\text{mean SAP} - \text{mean CVP})/CO$$

$$Rp/Rs\ ratio = PVR/SVR$$

All pressures were measured using fluid-filled transducers (TruWave Disposable Pressure Transducer, Nihon Kohden) and recorded by polygraphy (Omniace RT 3200, NEC). After completing the procedures, a 20- to 30-min equilibration period was allowed to stabilize hemodynamics. Heart rate, SPO_2 and blood pressures were recorded initially as a baseline.

Study protocols

Study 1

The infusion rates of U46619 were established based on previous reports (Yamada et al., 1998; Tamura et al., 2001). U46619 was infused at rates of 0.1, 0.3, 0.6 and 0.9 μg/kg/min for 10–20 min via the cephalic vein. SAP and PAP were measured under anesthesia.

Study 2

After pretreatment with IV U46619 (0.9 μg/kg/min) for 10 min, tadalafil was infused simultaneously at rates of 50, 100, and 200 μg/kg/h for 10 min. The infusion rate of U46619 was maintained until the end of tadalafil infusion. This study was conducted with a crossover design. The order of tadalafil administration (50, 100, and 200 μg/kg/h) was randomly assigned to each dog. After completing the first procedure, a stabilization period of at least 30 min was provided to re-establish a stable baseline condition. The dogs were then randomly assigned to receive one of the remaining treatments, and each dog received each of the three doses. SAP was measured using a fluid-filled catheter and PAP, mean RVP, mean CVP, mean PCWP and CO were measured with a Swan–Ganz catheter under anesthesia. SVR, PVR, and the Rp/Rs ratio were calculated.

Study 3

The dose-dependent effects of oral tadalafil were determined. The dogs were divided into placebo, U46619, and tadalafil groups. A single dose of placebo (for the placebo and U46619 groups) was administered orally. Tadalafil was administered orally at 1.0, 2.0, and 4.0 mg/kg in the tadalafil group. PAP was measured under anesthesia 3 h after administration.

Study 4

The time-dependent effects of oral tadalafil were determined 1, 2, 3, and 6 h after oral administration. The dogs were divided into three groups, namely, placebo, U46619 and tadalafil. A single dose of placebo (for the placebo and U46619 groups) was administered orally. Similarly, tadalafil at 4.0 mg/kg was administered orally to the tadalafil group. PAP was measured under anesthesia.

In Studies 3 and 4, U46619 (0.9 μg/kg/min) was simultaneously infused for 10 min at each time point in the U46619 and tadalafil groups. Sterile saline was administered for 10 min in the control group. Both studies were conducted with a crossover design. Each drug was given randomly, and the interval between treatments was at least 3 days. After each washout period, the dogs were then randomly assigned to receive one of the remaining treatments. This continued until each dog had received all treatments.

Statistical analyses

Normality of data was assessed with the Kolmogorov–Smirnov test. Data are presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the results among the groups in Studies 1, 2, and 3. In Study 4, one-factor repeated measures ANOVA was used to compare the time-dependent changes in heart rate and PAP with the baseline values. Two-way ANOVA was used to compare the time-dependent changes in PAP among the groups. The significance of the differences between the mean values of the each condition was tested with Tukey's multiple-comparison test. $P < 0.05$ was considered statistically significant.

Results

As baseline values before the induction of anesthesia, mean heart rate was 115 ± 29 beats/min, respiratory rate 41 ± 33 breaths/min and body temperature 38.7 ± 0.2 °C.

The dose-dependent hemodynamic effects of IV U46619 in Study 1 are shown in Fig. 1. SPO_2 remained unchanged during the study (baseline; 97.5 ± 1.5%, 0.1 μg/kg/min; 97.5 ± 1.5%, 0.3 μg/kg/min; 98.2 ± 0.8%, 0.6 μg/kg/min; 98.3 ± 0.8%, 0.9 μg/kg/min; 98.3 ± 1.4%). Compared with baseline, the heart rate and SAP did not change with IV infusion of U46619. In contrast, IV infusion of U46619 changed the PAP in a dose-dependent manner from baseline. Systolic PAP was significantly higher than baseline at U46619 infusion rates of 0.6 and 0.9 μg/kg/min. The mean and diastolic PAP were also significantly higher than baseline at infusion rates of 0.3, 0.6 and 0.9 μg/kg/min.

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009

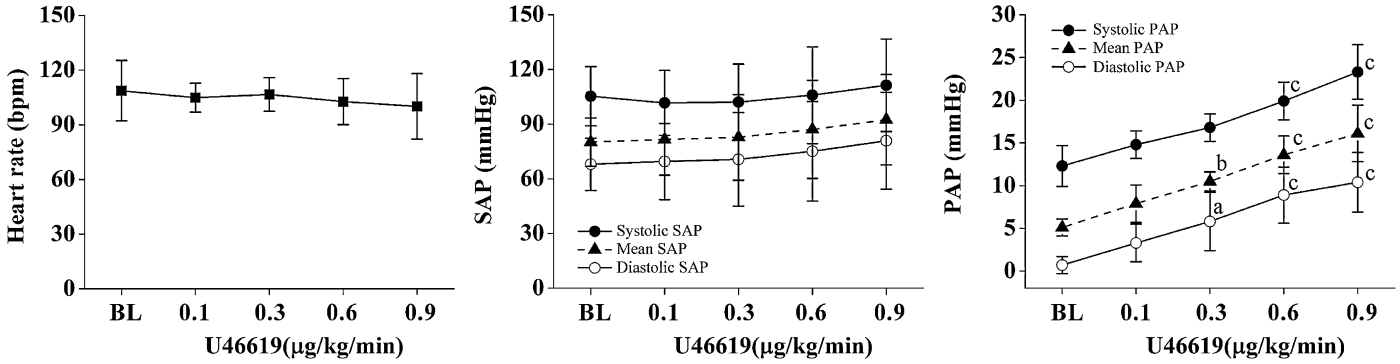

Fig. 1. Dose-dependent hemodynamic effects of U46619 with intravenous administration. Data are shown as means ± standard deviation (SD). SAP, systemic arterial pressure; PAP, pulmonary arterial pressure. [a] $P < 0.05$ vs. baseline, [b] $P < 0.01$ vs. baseline, [c] $P < 0.001$ vs. baseline.

The hemodynamic effects of simultaneous infusion of tadalafil and U46619 were investigated in Study 2. U46619-elevated systolic, mean and diastolic PAP were slightly lower with simultaneous infusion of tadalafil at 50 μg/kg/h. In contrast, U46619-elevated systolic, mean and diastolic PAP were significantly lower with simultaneous infusion of tadalafil at 100 and 200 μg/kg/h (Fig. 2). Changes in the other hemodynamic measurements are shown in Table 1. The heart rate did not change with U46619 and/or any dose of tadalafil. The SAP increased with U46619, but the change was not statistically significant. SAP did not change with simultaneous infusion of any dose of tadalafil. U46619 significantly increased the RVP, CVP and PCWP from baseline, but these parameters were not changed by simultaneous infusion of tadalafil. The CO decreased with U46619, but the change was not statistically significant; CO did not change with simultaneous infusion of any dose of tadalafil. As a result, although U46619 significantly increased PVR and SVR from baseline, simultaneous infusion of tadalafil at 50, 100 and 200 μg/kg/h resulted in a significantly lower PVR than that seen with

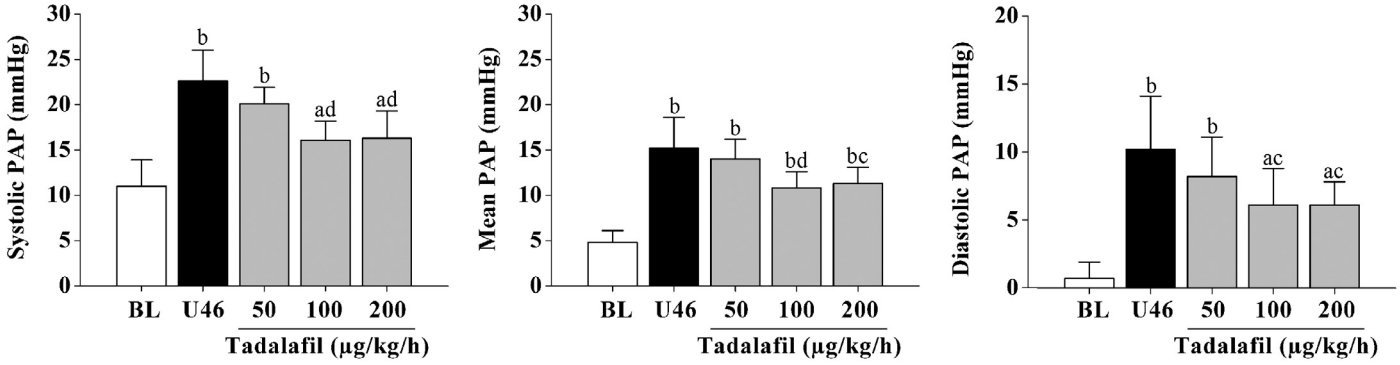

Fig. 2. Changes in pulmonary arterial pressure (PAP) with simultaneous infusion of tadalafil and U46619. Data are shown as means ± standard deviation (SD). BL, baseline; U46, U46619. [a] $P < 0.01$ vs. baseline, [b] $P < 0.001$ vs. baseline, [c] $P < 0.05$ vs. U46619, [d] $P < 0.01$ vs. U46619.

Table 1
Hemodynamic effects of intravenous infusion of tadalafil and U46619.

	Baseline	U46619	U46619 + tadalafil		
		0.9 μg/kg/min	50 μg/kg/h	100 μg/kg/h	200 μg/kg/h
Heart rate (bpm)	116 ± 21	104 ± 18	102 ± 18	102 ± 19	99 ± 19
SPO_2 (%)	97.5 ± 1.5	97.5 ± 1.5	98.2 ± 0.8	98.3 ± 0.8	98.3 ± 1.4
Systolic SAP (mmHg)	96 ± 22	108 ± 22	105 ± 13	105 ± 13	111 ± 17
Mean SAP (mmHg)	68 ± 13	82 ± 13	82 ± 12	82 ± 5	88 ± 13
Diastolic SAP (mmHg)	53 ± 10	74 ± 9	73 ± 11	73 ± 4	83 ± 5
Mean RVP (mmHg)	3.4 ± 3.5	12.4 ± 3.4[c]	8.9 ± 2.8[a]	8.7 ± 2.8[a]	8.4 ± 3.1[a]
Mean CVP (mmHg)	–0.1 ± 1.7	3.7 ± 2.1[b]	3.3 ± 2.1[a]	3.7 ± 2.2[b]	3.3 ± 2.2[a]
Mean PCWP (mmHg)	1.8 ± 2.9	8.7 ± 4.3[b]	8.3 ± 4.0[a]	6.3 ± 3.5	6.3 ± 3.4
CO (L/min)	1.7 ± 0.6	1.1 ± 0.4	1.2 ± 0.5	1.1 ± 0.4	1.2 ± 0.4
SVR (dyn/s/cm^{-5})	49.4 ± 16.3	71.8 ± 30.6[a]	84.4 ± 39.0[a]	87.0 ± 28.2[a]	88.1 ± 29.1[a]

Data are shown as means ± standard deviation (SD). Baseline represents data before drug administration under anesthesia in each group. SAP, systemic arterial pressure; RVP, right ventricular pressure; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; SVR, systemic vascular pressure.
[a] $P < 0.05$ vs. baseline.
[b] $P < 0.01$ vs. baseline.
[c] $P < 0.001$ vs. baseline.

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009

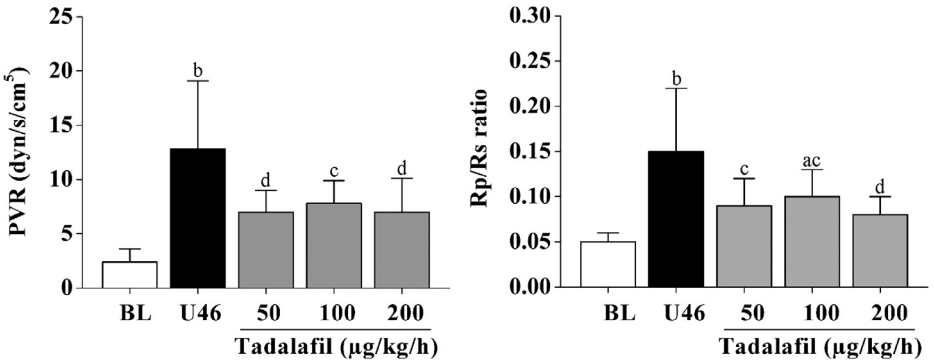

Fig. 3. Changes in pulmonary vascular resistance (PVR) with simultaneous infusion of tadalafil and U46619. Data are shown as means ± standard deviation (SD). BL, baseline; U46, U46619; Rp/Rs ratio, pulmonary resistance to systemic arterial resistance ratio. [a] $P < 0.01$ vs. baseline, [b] $P < 0.001$ vs. baseline, [c] $P < 0.05$ vs. U46619, [d] $P < 0.01$ vs. U46619.

U46619 (Fig. 3). Similarly, the Rp/Rs ratio was significantly higher than baseline with U46619 and significantly lower with simultaneous infusion of tadalafil than that seen with U46619 (Fig. 3).

The dose-dependent effects on PAP 3 h after a single oral administration of tadalafil were investigated in Study 3 (Fig. 4, Table 2). Oral administration of tadalafil alone did not change the systolic, mean or diastolic PAP or heart rate compared with placebo. U46619-elevated systolic, mean, and diastolic PAP did not change by oral administration of tadalafil at 1.0 mg/kg. In contrast, the U46619-elevated systolic, mean, and diastolic PAP were significantly lower with prior oral administration of tadalafil at 2.0 and 4.0 mg/kg.

Based on the results of study 3, the time-dependent effects on PAP after a single oral administration of tadalafil were investigated in Study 4 (Fig. 5, Table 3). Neither U46619 infusion nor oral tadalafil changed the heart rate from baseline. U46619 significantly elevated the systolic, mean and diastolic PAP for 6 h compared to baseline. Despite prior administration of tadalafil at 4.0 mg/kg, the U46619 infusion significantly elevated the systolic, mean and diastolic PAP from 1 to 6 h. However, oral tadalafil administration significantly decreased U46619-elevated systolic and diastolic PAP at 3 and 6 h compared to the U46619 group. Similarly, oral tadalafil significantly decreased U46619-elevated mean PAP at 1 h and until 6 h.

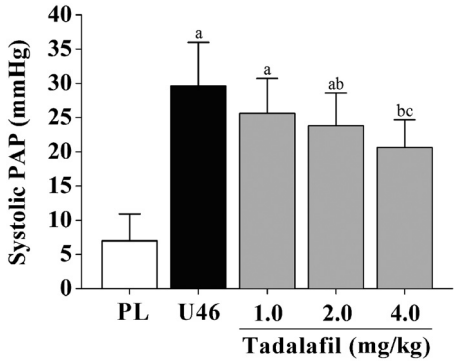

Fig. 4. Dose-dependent changes in systolic pulmonary arterial pressure (PAP) after oral administration of tadalafil. Data are shown as means ± standard deviation (SD). U46619 was infused at a rate of 0.9 μg/kg/min for 10 min at 3 h after oral administration of each drug. U46, U46619; PL, placebo. [a] $P < 0.001$ vs. control, [b] $P < 0.05$ vs. U46619, [c] $P < 0.001$ vs. U46619.

Discussion

The pathogenesis of PAH is poorly understood, but an imbalance between vasoconstriction and vasodilation contributes to the development of PVR (Humbert et al., 2004; Pietra et al., 2004; Kellihan and Stepien, 2012). For example, TXA_2 is a potent autacoid that induces platelet aggregation and vasoconstriction, and there is experimental and clinical evidence that its plasma levels are increased in animals and humans with PAH (Christman et al., 1992; Adatia et al., 1993).

U46619, a TXA_2-mimetic agent, increased PVR in isolated canine lungs (Stephenson et al., 1998), indicating that U46619 directly stimulates vasoconstriction of the pulmonary artery. In the present study, an IV infusion of U46619 significantly elevated PAP in a dose-dependent manner. In addition, U46619 at a rate of 0.9 μg/kg/min significantly elevated PVR and particularly the Rp/Rs ratio from baseline suggesting that U46619 stimulates pulmonary arterial constriction in dogs and that the pulmonary artery is a main target of TXA_2 (Yamada et al., 1998; Gong et al., 2000; Tamura et al., 2001).

In addition to its effect on the pulmonary artery, U46619 insignificantly elevated SAP, significantly elevated PCWP and SVR and insignificantly decreased CO. Similar to our results, a previous study

Table 2
Dose-dependent effects on heart rate and pulmonary arterial pressure (PAP) after oral administration of tadalafil.

	Control	U46619	Tadalafil			U46619 + tadalafil		
		0.9 μg/kg/min	1 mg/kg	2 mg/kg	4 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg
Heart rate (bpm)	116 ± 21	111 ± 23	111 ± 23	111 ± 24	117 ± 21	107 ± 24	109 ± 19	112 ± 13
Systolic PAP (mmHg)	7.0 ± 3.9	29.6 ± 6.4[b]	8.7 ± 3.4	9.6 ± 3.5	9.1 ± 2.7	25.6 ± 5.1[b]	23.8 ± 4.8[b,c]	20.6 ± 4.1[b,d]
Mean PAP (mmHg)	4.4 ± 2.8	21.4 ± 2.5[b]	3.3 ± 1.7	5.1 ± 2.5	4.2 ± 2.3	17.3 ± 1.6[b]	16.6 ± 2.5[b,c]	14.9 ± 2.8[a,d]
Diastolic PAP (mmHg)	1.8 ± 2.3	15.2 ± 2.8[b]	0.7 ± 1.4	1.6 ± 1.3	–0.1 ± 2.2	12.4 ± 0.8[b]	11.5 ± 3.2[b]	9.7 ± 2.7[b,c]

Data are shown as means ± standard deviation (SD). bpm, beats/min.
[a] $P < 0.01$ vs. control.
[b] $P < 0.001$ vs. control.
[c] $P < 0.05$ vs. U46619.
[d] $P < 0.001$ vs. U46619.

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009

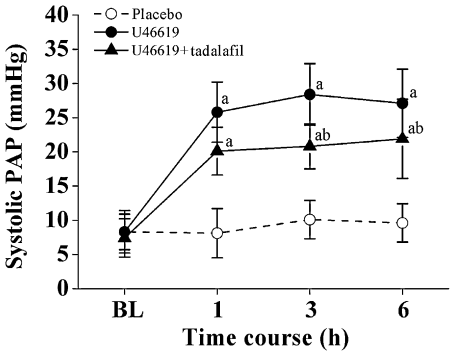

Fig. 5. Time-dependent changes in systolic pulmonary arterial pressure (PAP) after oral administration of tadalafil. Data are shown as means ± standard deviation (SD). BL, baseline. Baseline represents data before drug administration under anesthesia in each group. [a] $P < 0.001$ vs. baseline, [b] $P < 0.01$ vs. U46619.

demonstrated that the SAPs, SVR and PCWP increased following infusion of U46619 as CO decreases (Gong et al., 2000). U46619 produced concentration-dependent contractions in canine isolated coronary, mesenteric and femoral arteries, which were inhibited by a TXA_2 antagonist (Matsuzaki et al., 1992). These results indicate that U46619 stimulates systemic arterial constriction in addition to its effect on the pulmonary artery. U46619-elevated systemic arterial constriction and decreased CO might lead to an increase in PCWP.

Tadalafil, a specific PDE5 inhibitor, has greater affinity for PDE5 compared to other PDE5 inhibitors (Corbin and Francis, 2002; Francis and Corbin, 2003) and induces a significant reduction in PAP and PVR in patients with PAH (Ghofrani et al., 2004; Mukhopadhyay et al., 2006). PDE5 is located primarily in the corpora cavernosa of the penis and pulmonary arterial smooth muscle (Senzaki et al., 2001). Its main role is to degrade cyclic guanosine monophosphate (cGMP) in these tissues. PDE5 inhibition increases cGMP bioavailability, which causes smooth muscle relaxation and decreased vasomotor tone (Francis and Corbin, 1999; Lincoln et al., 2001). In the present study, IV infusion of tadalafil significantly decreased the U46619-elevated PAP in a dose-dependent manner. In addition, the U46619-elevated PVR and Rp/Rs ratio were significantly lower with simultaneous infusion of tadalafil without significant changes in SAP and SVR. The results indicate that tadalafil has a pharmacological effect in dogs and that it induces pulmonary arterial but not systemic arterial relaxation.

Tadalafil is widely used to treat humans with PAH and erectile dysfunction (Porst et al., 2003; Skoumal et al., 2004). Previous clinical studies in humans reported that tadalafil improved 6-min walk test results and quality of life (Galiè et al., 2009; Pepke-Zaba et al., 2009). In veterinary medicine, sildenafil is generally used for dogs with PAH. This agent reportedly improves clinical signs and outcomes in dogs with PAH (Bach et al., 2006; Kellum and Stepien, 2007; Brown et al., 2010; Nakamura et al., 2011). In contrast, tadalafil has infrequently been used in dogs with PAH. One case report of a 7-day treatment with oral tadalafil (1 mg/kg) in a dog showed improvement in clinical signs such as cyanosis, syncope, and tachypnea, and decreased systolic PAP from 122 to 96 mmHg as estimated by continuous-wave Doppler echocardiography of the tricuspid jet (Serres et al., 2006). However, no detailed information regarding the hemodynamic efficacy of oral tadalafil in dogs is available. In the present study, we have demonstrated that oral tadalafil at 2 and 4 mg/kg decreases U46619-elevated PAP in dogs without significant changes in heart rate, whereas tadalafil alone did not affect PAP. Our results suggest that oral administration of tadalafil induces a reduction in PAP in dogs.

Pharmacokinetic studies of tadalafil in humans report an 81% bioavailability, a maximum drug concentration time (Tmax) of 2 h, and a 17.5 h half-life (Francis and Corbin, 2003). Indeed, tadalafil is absorbed within 20 min after oral administration and shows a peak effect 70–90 min after oral administration in humans (Ghofrani et al., 2004; Forgue et al., 2006; Mukhopadhyay et al., 2006). In contrast, pharmacokinetic data for tadalafil in dogs remain scarce. The current study demonstrated that oral tadalafil at a dosage of 4 mg/kg showed a maximum effect. Thus, we deliberately chose a high dose to induce a marked hemodynamic effect and determine the time-dependent effects of tadalafil. Oral tadalafil significantly decreased U46619-elevated mean PAP at 1 h, and this effect was maintained for 6 h. These results indicate that oral administration of tadalafil in dogs was efficacious within 1 h and that the effect is maintained for at least 6 h. However, further studies are required to clarify the pharmacokinetics of tadalafil in dogs.

Our study has several limitations. We could not exclude the possibility that general anesthesia may have modulated the hemodynamic effects of tadalafil. Although oral tadalafil at 2 and 4 mg/kg lowered the U46619-induced increase in PAP, the optimum clinical dosage remains unknown. Reportedly, common side effects in humans, including headache, facial flushing, nasal congestion, dyspepsia, and transient visual impairment, have been associated with tadalafil administration (Supuran et al., 2006). The current study did not evaluate the potential chronic effects and side effects of tadalafil in dogs.

Table 3
Time-dependent effects on heart rate and pulmonary arterial pressure (PAP) after oral administration of tadalafil.

Heart rate (bpm)	Baseline	1 h	3 h	6 h
Control	118 ± 27	116 ± 28	103 ± 34	106 ± 29
U46619	117 ± 25	112 ± 18	110 ± 20	100 ± 25
U46619 + tadalafil	116 ± 20	119 ± 13	108 ± 19	117 ± 24
Mean PAP (mmHg)	**Baseline**	**1 h**	**3 h**	**6 h**
Control	3.9 ± 2.3	3.7 ± 2.7	5.1 ± 2.3	4.1 ± 1.6
U46619	4.4 ± 2.6	19.3 ± 3.0[a]	20.7 ± 3.1[a]	20.2 ± 2.1[a]
U46619 + tadalafil	3.2 ± 2.1	14.9 ± 3.1[a,b]	14.8 ± 3.0[a,b]	15.4 ± 3.5[a,b]
Diastolic PAP (mmHg)	**Baseline**	**1 h**	**3 h**	**6 h**
Control	0.9 ± 1.2	0.9 ± 1.4	1.3 ± 2.0	0.6 ± 0.9
U46619	1.3 ± 2.0	13.9 ± 3.2[a]	13.8 ± 4.1[a]	14.7 ± 2.7[a]
U46619 + tadalafil	0.4 ± 1.0	10.4 ± 3.3[a]	8.8 ± 3.2[a,b]	10.7 ± 2.9[a,b]

Data are shown as means ± standard deviation (SD). Baseline represents data before drug administration under anesthesia in each group. bpm, beats/min.
[a] $P < 0.001$ vs. baseline at the same time point.
[b] $P < 0.05$ vs. U46619 at the same time point.

Conclusions

IV infusion of tadalafil decreased U46619-elevated PAP and PVR in dogs without changes in SAP and SVR. Oral administration of tadalafil alone did not change PAP, but decreased U46619-elevated PAP 1 h after oral administration and the effect was maintained for 6 h. The results suggest that tadalafil improved U46619-induced pulmonary arterial constriction, leading to pulmonary arterial relaxation.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009

References

Adatia, I., Barrow, S.E., Stratton, P.D., Miall-Allen, V.M., Ritter, J.M., Haworth, S.G., 1993. Thromboxane A2 and prostacyclin biosynthesis in children and adolescents with pulmonary vascular disease. Circulation 88, 2117–2122.

Anderson, J.R., Nawarskas, J.J., 2010. Pharmacotherapeutic management of pulmonary arterial hypertension. Cardiology in Review 18, 148–162.

Arif, S.A., Poon, H., 2011. Tadalafil: A long-acting phosphodiesterase-5 inhibitor for the treatment of pulmonary arterial hypertension. Clinical Therapeutics 33, 993–1004.

Bach, J.F., Rozanski, E.A., MacGregor, J., Betkowski, J.M., Rush, J.E., 2006. Retrospective evaluation of sildenafil citrate as a therapy for pulmonary hypertension in dogs. Journal of Veterinary Internal Medicine 20, 1132–1135.

Brown, A.J., Davison, E., Sleeper, M.M., 2010. Clinical efficacy of sildenafil in treatment of pulmonary arterial hypertension in dogs. Journal of Veterinary Internal Medicine 24, 850–854.

Christman, B.W., McPherson, C.D., Newman, J.H., King, G.A., Bernard, G.R., Groves, B.M., Loyd, J.E., 1992. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. The New England Journal of Medicine 327, 70–75.

Corbin, J.D., Francis, S.H., 2002. Pharmacology of phosphodiesterase-5 inhibitors. International Journal of Clinical Practice 56, 453–459.

Falk, J.A., Philip, K.J., Schwarz, E.R., 2010. The emergence of oral tadalafil as a once-daily treatment for pulmonary arterial hypertension. Vascular Health and Risk Management 6, 273–280.

Forgue, S.T., Patterson, B.E., Bedding, A.W., Payne, C.D., Phillips, D.L., Wrishko, R.E., Mitchell, M.I., 2006. Tadalafil pharmacokinetics in healthy subjects. British Journal of Clinical Pharmacology 61, 280–288.

Francis, S.H., Corbin, J.D., 1999. Cyclic nucleotide-dependent protein kinases: Intracellular receptors for cAMP and cGMP action. Critical Reviews in Clinical Laboratory Sciences 36, 275–328.

Francis, S.H., Corbin, J.D., 2003. Molecular mechanisms and pharmacokinetics of phosphodiesterase-5 antagonists. Current Urology Reports 4, 457–465.

Galiè, N., Brundage, B.H., Ghofrani, H.A., Oudiz, R.J., Simonneau, G., Safdar, Z., Shapiro, S., White, R.J., Chan, M., Beardsworth, A., et al., 2009. Tadalafil therapy for pulmonary arterial hypertension. Circulation 119, 2894–2903.

Ghofrani, H.A., Voswinckel, R., Reichenberger, F., Olschewski, H., Haredza, P., Karadas, B., Schermuly, R.T., Weissmann, N., Seeger, W., Grimminger, F., 2004. Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension: A randomized prospective study. Journal of the American College of Cardiology 44, 1488–1496.

Glaus, T.M., Soldati, G., Maurer, R., Ehrensperger, F., 2004. Clinical and pathological characterisation of primary pulmonary hypertension in a dog. Veterinary Record 154, 786–789.

Gong, F., Shiraishi, H., Kikuchi, Y., Hoshina, M., Ichihashi, K., Sato, Y., Momoi, M.Y., 2000. Inhalation of nebulized nitroglycerin in dogs with experimental pulmonary hypertension induced by U46619. Pediatrics International 42, 255–258.

Humbert, M., Morrell, N.W., Archer, S.L., Stenmark, K.R., MacLean, M.R., Lang, I.M., Christman, B.W., Weir, E.K., Eickelberg, O., Voelkel, N.F., et al., 2004. Cellular and molecular pathobiology of pulmonary arterial hypertension. Journal of the American College of Cardiology 43, S13–S24.

Johnson, L., 1999. Diagnosis of pulmonary hypertension. Clinical Techniques in Small Animal Practice 14, 231–236.

Johnson, L., Boon, J., Orton, E.C., 1999. Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992–1996. Journal of Veterinary Internal Medicine 13, 440–447.

Kellihan, H.B., Stepien, R.L., 2012. Pulmonary hypertension in canine degenerative mitral valve disease. Journal of Veterinary Cardiology 14, 149–164.

Kellum, H.B., Stepien, R.L., 2007. Sildenafil citrate therapy in 22 dogs with pulmonary hypertension. Journal of Veterinary Internal Medicine 21, 1258–1264.

Lincoln, T.M., Dey, N., Sellak, H., 2001. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: From the regulation of tone to gene expression. Journal of Applied Physiology 91, 1421–1430.

Matsuzaki, T., Noguchi, K., Nakasone, J., Uezu, K., Higuchi, M., Sakanashi, M., 1992. Effects of the new thromboxane A2 antagonist vapiprost on isolated canine blood vessels. Arzneimittel-Forschung 42, 1318–1322.

Mukhopadhyay, S., Sharma, M., Ramakrishnan, S., Yusuf, J., Gupta, M.D., Bhamri, N., Trehan, V., Tyagi, S., 2006. Phosphodiesterase-5 inhibitor in Eisenmenger syndrome: A preliminary observational study. Circulation 114, 1807–1810.

Nakamura, K., Yamasaki, M., Ohta, H., Sasaki, N., Murakami, M., Bandula Kumara, W.R., Takiguchi, M., 2011. Effects of sildenafil citrate on five dogs with Eisenmenger's syndrome. Journal of Small Animal Practice 52, 595–598.

Pepke-Zaba, J., Beardsworth, A., Chan, M., Angalakuditi, M., 2009. Tadalafil therapy and health-related quality of life in pulmonary arterial hypertension. Current Medical Research and Opinion 25, 2479–2485.

Pietra, G.G., Capron, F., Stewart, S., Leone, O., Humbert, M., Robbins, I.M., Reid, L.M., Tuder, R.M., 2004. Pathologic assessment of vasculopathies in pulmonary hypertension. Journal of the American College of Cardiology 43, S25–S32.

Porst, H., Padma-Nathan, H., Giuliano, F., Anglin, G., Varanese, L., Rosen, R., 2003. Efficacy of tadalafil for the treatment of erectile dysfunction at 24 and 36 hours after dosing: A randomized controlled trial. Urology 62, 121–125.

Seibert, R.L., Maisenbacher, H.W., 3rd, Prosek, R., Adin, D.B., Arsenault, W.G., Estrada, A.H., 2010. Successful closure of left-to-right patent ductus arteriosus in three dogs with concurrent pulmonary hypertension. Journal of Veterinary Cardiology 12, 67–73.

Senzaki, H., Smith, C.J., Juang, G.J., Isoda, T., Mayer, S.P., Ohler, A., Paolocci, N., Tomaselli, G.F., Hare, J.M., Kass, D.A., 2001. Cardiac phosphodiesterase 5 (cGMP-specific) modulates beta-adrenergic signaling in vivo and is down-regulated in heart failure. Federation of American Societies for Experimental Biology Journal 15, 1718–1726.

Serres, F., Nicolle, A.P., Tissier, R., Gouni, V., Pouchelon, J.L., Chetboul, V., 2006. Efficacy of oral tadalafil, a new long-acting phosphodiesterase-5 inhibitor, for the short-term treatment of pulmonary arterial hypertension in a dog. Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine 53, 129–133.

Skoumal, R., Chen, J., Kula, K., Breza, J., Calomfirescu, N., Basson, B.R., Kopernicky, V., 2004. Efficacy and treatment satisfaction with on-demand tadalafil (Cialis) in men with erectile dysfunction. European Urology 46, 362–369.

Stephenson, A.H., Sprague, R.S., Lonigro, A.J., 1998. 5,6-Epoxyeicosatrienoic acid reduces increases in pulmonary vascular resistance in the dog. The American Journal of Physiology 275, H100–H109.

Supuran, C.T., Mastrolorenzo, A., Barbaro, G., Scozzafava, A., 2006. Phosphodiesterase 5 inhibitors – Drug design and differentiation based on selectivity, pharmacokinetic and efficacy profiles. Current Pharmaceutical Design 12, 3459–3465.

Tamura, M., Kurumatani, H., Matsushita, T., 2001. Comparative effects of beraprost, a stable analogue of prostacyclin, with PGE(1), nitroglycerin and nifedipine on canine model of vasoconstrictive pulmonary hypertension. Prostaglandins, Leukotrienes, and Essential Fatty Acids 64, 197–202.

Uchide, T., Saida, K., 2005. Elevated endothelin-1 expression in dogs with heartworm disease. The Journal of Veterinary Medical Science 67, 1155–1161.

Yamada, T., Yukioka, H., Hayashi, M., Asada, A., Inoue, M., 1998. Effects of inhaled nitric oxide on platelet-activating factor-induced pulmonary hypertension in dogs. Acta Anaesthesiologica Scandinavica 42, 358–368.

Zabka, T.S., Campbell, F.E., Wilson, D.W., 2006. Pulmonary arteriopathy and idiopathic pulmonary arterial hypertension in six dogs. Veterinary Pathology 43, 510–522.

Please cite this article in press as: Yasutomo Hori, et al., Effect of the phosphodiesterase type 5 inhibitor tadalafil on pulmonary hemodynamics in a canine model of pulmonary hypertension, The Veterinary Journal (2014), doi: 10.1016/j.tvjl.2014.08.009