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Acute effect of ivabradine on heart rate and myocardial oxygen consumption in dogs with asymptomatic mitral valve degeneration

Prapawadee PIRINTR[1)], Vudhiporn LIMPRASUTR[2)], Nakkawee SAENGKLUB[3)], Parnpradub PAVINADOL[1)], Napat YAPAO[1)], Natthakarn LIMVANICHARAT[1)], Hathaisiri KUECHAROEN[1)], and Anusak KIJTAWORNRAT[1,4)]

[1)]Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri Dunant Road, Pathumwan, Bangkok 10330, Thailand
[2)]Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand
[3)]Department of Physiology, Faculty of Pharmacy, Mahidol University, 447 Sri Ayudhya Road, Rajathevi, Bangkok 10400, Thailand
[4)]Research clusters: research study and testing of drug's effect related to cardiovascular system in laboratory animal, Chulalongkorn University, 39 Henri Dunant Road, Pathumwan, Bangkok 10330, Thailand

Abstract: Degenerative mitral valve disease (DMVD) is a common cardiac disease in geriatric dogs characterized by the degeneration of the mitral valve, leading to decreased cardiac output and activation of the sympathetic and renin-angiotensin-aldosterone system. This disease results in an increased resting heart rate (HR) and myocardial oxygen consumption (MVO_2). A recent publication demonstrated that dogs with asymptomatic DMVD had a significantly higher HR and systemic blood pressure (BP) than age-matched control dogs. This higher HR will eventually contribute to increased MVO_2. This study aimed to determine the effects of a single oral dose of ivabradine on the HR, MVO_2 as assessed by the rate-pressure product, and BP in dogs with asymptomatic DMVD. Seven beagles with naturally occurring DMVD were instrumented by the Holter recorder and an oscillometric device to measure electrocardiogram and BP for 24 and 12 h, respectively. Each dog was randomly subjected to receive either placebo or ivabradine (0.5, 1.0 and 2.0 mg/kg). The results revealed that oral administration of ivabradine significantly decreased the HR and rate-pressure product in a dose-dependent manner without adverse effects. The highest dose of 2.0 mg/kg significantly reduced systolic and mean BP. Therefore, the findings imply that a single oral ivabradine administration at a dose of 1.0 mg/kg is suitable for dogs with asymptomatic DMVD to reduce the HR and MVO_2 without marked effects on BP. This may potentially make ivabradine promising for management of an elevated HR in DMVD dogs.

Key words: dog, heart rate, ivabradine, mitral valve degeneration, myocardial oxygen consumption

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Address corresponding: A. Kijtawornrat, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri Dunant Rd, Pathumwan, Bangkok 10330, Thailand

Supplementary Tables: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/expanim

Introduction

Degenerative mitral valve disease (DMVD) is a common cardiac disease in geriatric, small-breed dogs. It is characterized mostly by the degeneration of the mitral valve [20, 25]. This pathological lesion results in systolic mitral regurgitation (MR) and the systolic murmur can be best heard at the left apex. Several compensatory mechanisms, including the renin-angiotensin-aldosterone system (RAAS) and baroreceptor reflex are activated to compensate for reduced cardiac output. Consequently, cardiac output increases at a cost of an elevated heart rate (HR). An increased resting HR results in elevated myocardial oxygen consumption (MVO_2), which may lead to development of congestive heart failure [9, 34]. A recent clinical trial in patients with heart failure (HF) also suggested that an increased HR is a predictor of adverse cardiovascular outcomes as well as a risk factor for cardiovascular disease in healthy men and women [4, 10].

According to the American College of Veterinary Internal Medicine (ACVIM), DMVD can be divided into four classes: asymptomatic class A and B and symptomatic class C and D [1]. While there is a consensus on pharmacological treatment of symptomatic DMVD, the management of asymptomatic DMVD is still unclear. A recent publication demonstrated that dogs with asymptomatic DVMD had a significantly higher HR and systemic blood pressure than age-matched control dogs [26]. An elevated HR and systemic blood pressure will contribute to increased MVO_2 [30]. In addition, coronary blood flow and myocardial perfusion are reduced while the left ventricle end diastolic pressure is increased [7]. These factors may aggravate the decompensation of the heart disease. Therefore, a reduction of the heart rate may be useful in managing increased MVO_2 in dogs with asymptomatic DMVD.

Ivabradine is a relatively new agent that has been developed and has demonstrated a pure HR reduction and MVO_2 reduction in normal, exercising conscious dogs [8]. Ivabradine has been approved for clinical use in patients with coronary artery disease and ischemic heart disease by the European Medicines Agency since 2005 and by the United States Food and Drug Administration since 2015, but no veterinary-labeled ivabradine is currently approved for using in dogs and cats [5, 13, 29].

Although reducing the HR lowers MVO_2, the effect of ivabradine on the HR and MVO_2 remains unknown in asymptomatic DMVD dogs. This study was designed to determine the effects of a single oral dose of ivabradine on the HR, MVO_2 assessed by the rate-pressure product (RPP), and systemic blood pressure in dogs with asymptomatic DMVD. The hypothesis of the current study was that ivabradine will reduce the HR and MVO_2 without significant change in systemic blood pressure.

Materials and Methods

Approvals

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Chulalongkorn University Laboratory Animal Center (CULAC), Bangkok, Thailand (protocol number: 1673003). All experimental animal procedures were performed in compliance with CULAC IACUC regulation, Animals for Scientific Purposes Act (A.D. 2015) and followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals [22].

Animals

Seven beagles (*Canis familiaris*) of both genders (two males, five females) were transferred from a breeding colony of the Department of Obstetric Gynecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University. They were housed in a group from the time of arrival to the end of the study in a dog run maintained at a temperature between 19°C and 23°C, a relative humidity between 30% and 70%, and a 12-h:12-h dark: light cycle. All animals received commercial chow twice daily, and water was provided *ad libitum* in stainless steel containers. Physical examination, routine lead II electrocardiogram (ECG) recording, 2D, M-mode and Doppler echocardiography, thoracic radiograph, complete blood cell count, and blood chemistry analyses were performed to evaluate the health status in all dogs, and to confirm that all dogs were in ACVIM class B1 or B2 (i.e. dogs presented with mitral regurgitation with or without structural changes but no clinical sign) before beginning the experiment (Supplementary Table 1 and 2). None of the dogs were on any pharmacological treatment.

Study design and experimental procedures

Repeated and placebo-controlled studies were performed in this study. Each dog underwent four study periods, each continued for 24 h, and was randomized

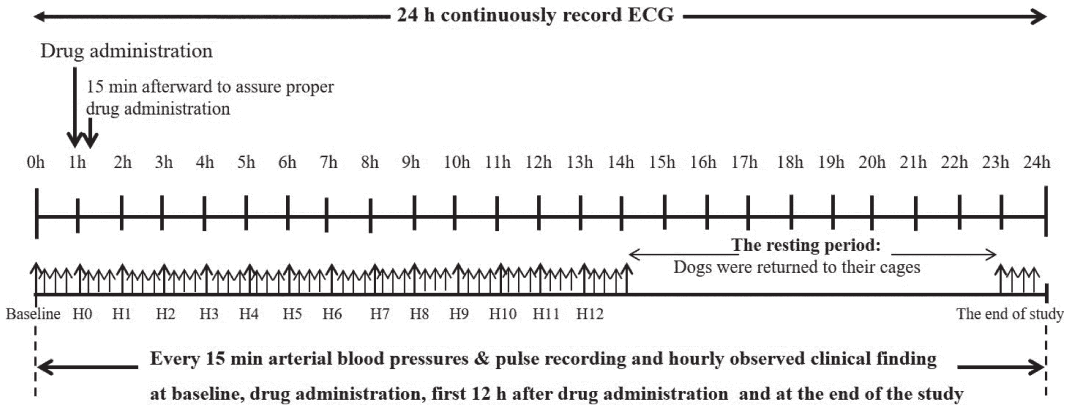

Fig. 1. Study timeline. h=hour after holter recording; H=hour after receiving placebo or ivabradine (0.5, 1.0 or 2.0 mg/kg).

to receive either one dose of ivabradine (0.5, 1.0 and 2.0 mg/kg) or placebo. The washout period between treatments was at least 2 days as described in a previous publication [7]. Experimental procedures were started after at least 2 h of fasting. All dogs were removed from their cages to a quiet study room and were attached to a continuous ECG recording instrument (Fukuda Denshi Co., Ltd., Tokyo, Japan) as described previously [26]. Baseline recordings of arterial blood pressure including SBP, DBP and MBP were performed prior to administration of placebo or ivabradine using an indirect blood pressure method, an oscillometric device (petMAP^{TM}, CardioCommand, Inc., Florida, USA), which has been validated and described previously [37]. Thereafter, dogs were orally given placebo (i.e. a meat ball) or ivabradine (i.e. a meat ball containing the pills). The feeding behavior of the dogs was monitored for at least 15 min afterward to ensure drug administration. The dogs were left in the room with examiners who took the BP measurement. The arterial blood pressure and pulse rate were recorded every 15 min for 12 h, consecutively. The clinical observation after drug administration was monitored hourly for the first 12 h as well. At 12 h after drug administration, the dogs were returned to their home cages. At 24 h after drug administration, the dogs were brought back to the study room to record the last BP, and the Holter monitor was removed (Fig. 1).

Data analysis

The 24-h continuous ECG was recorded and stored on an SD card for further analysis of hourly heart rate using SCM-510 Holter software (Fukuda Denshi Co., Ltd., Japan). All QRS complexes from the 24-h ECG were automatically analyzed by the program and it was carefully manually inspected for the RR intervals by an experienced operator.

The hourly systolic, diastolic and mean arterial blood pressure were obtained from an average of 15-min-interval BP measurements using an oscillometric device. Every 15 min, five consecutive measurements of blood pressure were performed and an average of three consistent BP measurements was used. Myocardial oxygen consumption was calculated from the rate-pressure product (RPP), which is SBP multiplied by HR [7, 23].

The frequency of arrhythmia was reported as a percentage (i.e. numbers of abnormal beats per numbers of total beats during the entire 24 h period of recording multiply by 100). Supraventricular complexes (SVPC) are defined as the premature complexes originated from the atria or tissue above the ventricle other than the sinus node cells (i.e. atrial premature complex, junctional premature complex). Ventricular premature complexes (VPC) are defined as the premature complexes originated from the ventricle. Sinus pause (i.e. sinus arrest) is characterized by a transiently ceases of impulse from sinus node more than 2 s.

Statistical analysis

All numerical data were presented as mean ± standard error of mean (SEM). In each figure, the adjusted baseline of each parameter was plotted against collected time points. A normality test (D'Agostino-Pearson omnibus test) was performed to determine whether data were normally distributed. The HR, blood pressure and RPP were compared among treatment groups (placebo and ivabradine 0.5, 1.0 and 2.0 mg/kg) and time points (base-

line, 1–12 h and 24 h) using two-way ANOVA with repeated measures followed by Dunnett's post-hoc analysis. In all cases, a P value <0.05 was considered statistically significant. Statistical analyses were performed using commercially available software.

Results

The complete blood cell count and blood chemical profiles of all seven dogs were within normal limits. The thoracic radiograph and echocardiography revealed that all dogs were in ACVIM class B2 (i.e. dogs presented with mitral regurgitation and structural changes but no clinical sign) (see Supplementary Table 2). The HR, systemic blood pressure and RPP were obtained at all time points indicated in the protocol (Fig. 1). No major adverse effects were observed in this study.

Effects of ivabradine on the HR

The baseline HR for placebo and ivabradine (0.5, 1.0 and 2.0 mg/kg) groups was 139 ± 6.4 bpm, 146 ± 4.7 bpm, 140 ± 4.2 bpm and 144 ± 5.2 bpm, respectively. Figure 2 shows the baselines of the adjusted HR for all groups. It was clear that ivabradine reduced HR in a dose-dependent manner. The HR of the placebo group was not different among time points. After dogs received ivabradine at 0.5 mg/kg, the HR was significantly decreased 3 h after administration (P<0.05) and remained significantly lower until 12 h after administration; however, all these time points did not differ from the placebo group evaluated at the same time points. When compared with baselines, ivabradine 1.0 mg/kg significantly reduced the HR 3 h after administration (P<0.05), while ivabradine 2.0 mg/kg significantly lowered the HR 1 h after administration (P<0.05). In both groups, the HR remained at the decreased levels until 12 h after administration. When the ivabradine groups (1.0 or 2.0 mg/kg) were compared with the placebo group at the same time points, ivabradine 1.0 mg/kg significantly reduced the HR 7 to 10 h after (P<0.05) while ivabradine 2.0 mg/kg significantly reduced the HR 3 to 12 h after administration (P<0.05).

The average HR recorded for 24 h after administration was decreased for the three doses of ivabradine. The mean 24-h HR after ivabradine administration of 1.0 mg/kg (117 ± 2.1 bpm) and 2.0 mg/kg (112 ± 1.9 bpm) was significantly lower than that of the placebo (124 ± 1.7 bpm; P<0.05). However, no significant differences were found between the average 24-h HR after ivabradine administration (0.5 mg/kg; 123 ± 2.3 bpm) and the placebo. No dog in any groups had a minimal instantaneous HR below 60 bpm.

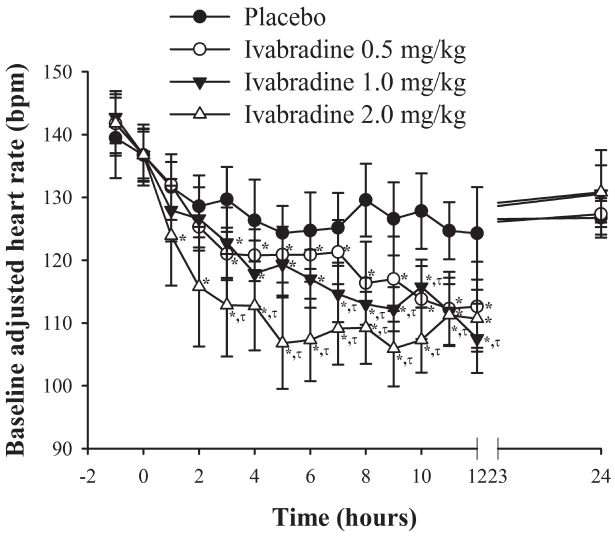

Fig. 2. Plots of baseline adjusted heart rate against time (hours) before and after receiving placebo or ivabradine (0.5, 1.0 or 2.0 mg/kg, orally) in dogs with degenerative mitral valve disease (N=7). * indicates P<0.05 when compared with baseline (0 hour) in the same group. $^{\tau}$ indicates P<0.05 when compared with placebo group at the same time point.

Effects of ivabradine on systemic blood pressure

Figures 3a–c demonstrates the baseline adjusted SBP, MBP and DBP, respectively, for all groups. The baseline SBP of the placebo and ivabradine (0.5, 1.0 and 2.0 mg/kg) groups were 155 ± 6.6 mmHg, 154 ± 8.6 mmHg, 167 ± 5.8 mmHg and 168 ± 12.4 mmHg, respectively (Fig. 3a). The SBP in the groups receiving ivabradine at 0.5 and 1.0 mg/kg was not altered, whereas that in the ivabradine 2.0 mg/kg group was significantly reduced at 5 and 6 h after administration when compared with its baseline or the placebo group at the same time points (P<0.05). The baseline MBP of the placebo and ivabradine (0.5, 1.0 and 2.0 mg/kg) groups were 112 ± 4.8 mmHg, 113 ± 5.5 mmHg, 119 ± 4.2 mmHg and 119 ± 11.8 mmHg, respectively (Fig. 3b). Dogs receiving placebo and ivabradine responded similarly as shown by their SBP, but the MBP even with ivabradine (2.0 mg/kg) was not different from the baseline. The baseline DBP of the placebo and ivabradine (0.5, 1.0 and 2.0 mg/kg) groups were 91 ± 4.5 mmHg, 91 ± 3.9 mmHg, 96 ± 4.2 mmHg and 106 ± 5.8 mmHg, respectively (Fig. 3c).

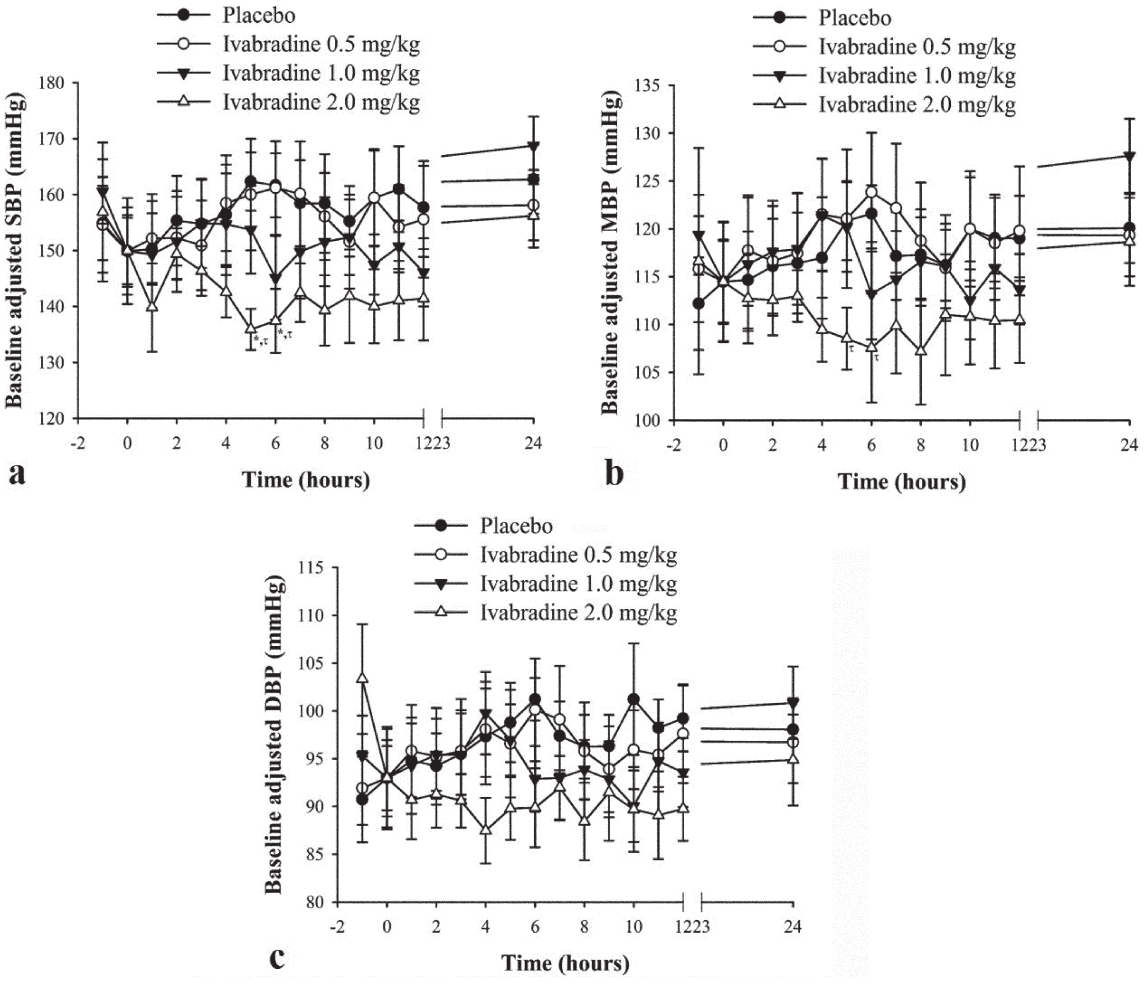

Fig. 3. Plots of baseline adjusted systolic blood pressure (SBP; a), mean blood pressure (MBP; b) and diastolic blood pressure (DBP; c) against time (hours) before and after receiving placebo or ivabradine (0.5, 1.0 or 2.0 mg/kg, orally) in dogs with degenerative mitral valve disease (N=7). * indicates $P<0.05$ when compared with baseline (0 hour) in the same group. $^{\tau}$ indicates $P<0.05$ when compared with placebo group at the same time point.

There was no difference among the four groups.

Effects of ivabradine on rate pressure product

The baseline RPP of the placebo and ivabradine (0.5, 1.0 and 2.0 mg/kg) groups were 21,813 ± 1,887 bpm.mmHg, 22,773 ± 1,715 bpm.mmHg, 23,327 ± 1,098 bpm.mmHg and 24,263 ± 1,912 bpm.mmHg, respectively. Figure 4 shows the baseline adjusted RPP for all groups. It can be noticed that ivabradine administration significantly reduced RPP in a dose-dependent manner ($P<0.05$). The RPP for the placebo and ivabradine (0.5 mg/kg) groups were not different between group except for the RPP at 11 h after administration in which it was significantly lowered in ivabradine group ($P<0.05$). When compared between among ivabradine groups with placebo, RPP for the ivabradine (1.0 and 2.0 mg/kg) groups was significantly lowered 6 h and 5 h after administration, respectively, and the decreased levels were maintained until 12 h after administration.

Effects of ivabradine on 24 h electrocardiogram

Supraventricular premature complexes were found in approximately 0.001% (2 beat out of 173,875 normal beats) to 0.117% (221 beats out of 189,289 normal beats) in four out of seven dogs, both before and after receiving placebo and ivabradine. Ventricular premature complexes were found in approximately 0.001% (1 beat out of 192,639 normal beats) to 0.061% (114 beats out of 187,895 normal beats) in three out of seven dogs, both before and after receiving placebo and ivabradine. One

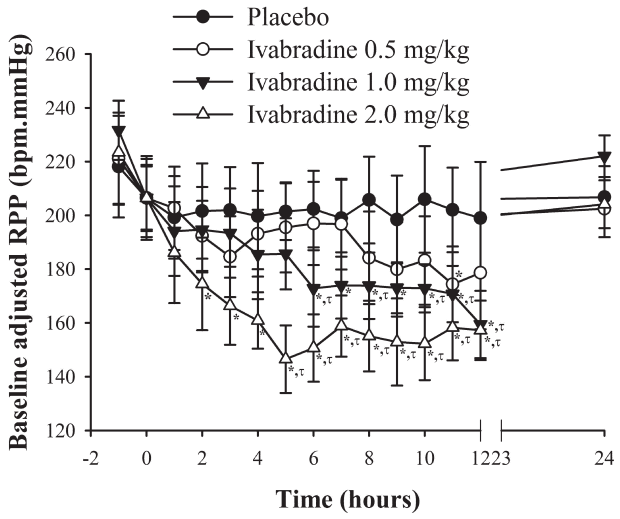

Fig. 4. Plots of baseline adjusted rate pressure product (RPP) against time (hours) before and after receiving placebo or ivabradine (0.5, 1.0 or 2.0 mg/kg, orally) in dogs with degenerative mitral valve disease (N=7). The scale of RPP was divided by 100. * indicates $P<0.05$ when compared with baseline (0 hour) in the same group. $^{\tau}$ indicates $P<0.05$ when compared with placebo group at the same time point.

dog receiving ivabradine 2.0 mg/kg had an increase in% of VPC beyond normal limits (from 0.003% at baseline to 26% at 2 mg/kg). The incidence of sinus pause observed during dogs were given placebo was varied from 0 to 20 episodes in 24 h recording period. At the highest dose, the incidence of sinus pause was varied from 0 to 28 episodes in 24 h recording period. No other arrhythmias were observed during the study period.

Discussion

The results of this study in dogs with DMVD demonstrated that ivabradine when given orally at 1.0 mg/kg significantly decreased the HR and RPP (i.e. reduced MVO_2) without adverse effects on blood pressure. It also did not induce supraventricular and ventricular arrhythmias.

Ivabradine is a hyperpolarization-activated cyclic nucleotide (HCN) channel blocker, which acts on the SA node to reduce the HR by reducing the slope of the diastolic potential of pacemaker cells [11, 35]. The slope of diastolic potential is regulated by the funny current (I_f) which is a voltage-gated, time-dependent mixed Na^+ and K^+ inward current that activated by hyperpolarization of membrane potentials and intracellular cyclic AMP (cAMP). Activation of the I_f current leads to increase membrane permeability to Na^+ and K^+ causing a less negative membrane potential which increases the slope of diastolic depolarization (phase 4 of action potential). Because it is a selective HCN blocker, most studies have indicated that ivabradine possesses neither direct negative nor positive inotropic, dromotropic nor lusitropic effects, determined by tests both in humans and animals [2, 6, 15, 31, 32]. Interestingly, a recent study in cats with hypertrophic cardiomyopathy showed that intravenous ivabradine administration possessed slightly negative inotropic and lusitropic effects [28]. The present study did not investigate those effects; therefore, it may be necessary to investigate further to elucidate the inotropic and lusitropic effects of ivabradine in dogs with DMVD.

In the present study, the highest dose of ivabradine (2.0 mg/kg) reduced the HR and blood pressure, thus lowering the RPP more than with the other two doses (0.5 and 1.0 mg/kg). This is consistent with a previous study in which ivabradine exerted a dose-dependent effect [7]. In addition, another study indicated that intravenous ivabradine administration (0.25, 0.5 and 1.0 mg/kg) demonstrated a dose-dependent reduction not only in normal conscious dogs but also in exercise-induced tachycardia dogs [8]. It has been known that BP is determined by HR, stroke volume, and total peripheral resistance. Ivabradine possesses a pure funny channel blocker without any effect on cardiac contractility and vascular smooth muscle [11]. Therefore, the highest dose of ivabradine (2 mg/kg) in the current study causes markedly decreased HR and leads to a falling of BP.

To the best of our knowledge, there is no pharmacokinetic study of ivabradine in dogs with DMVD. In normal dogs, the oral ivabradine administration was rapidly absorbed at roughly 40% of bioavailability. The plasma protein binding was roughly 50–70%. The peak plasma concentration achieved 1 h after administration was approximately 1 L/kg volume of distribution at a steady state. In addition, the elimination of ivabradine mainly occurred via hepatic circulation, and its main half-life was less than 2 h [12]. The hemodynamics of DVMD dogs are different from healthy dogs [24]; therefore, further pharmacokinetic investigation of ivabradine should be conducted in various stages of DMVD dogs to ensure the proper dose.

Previous studies in normal cats with HCM indicated that ivabradine was clinically well tolerated without undesired side effects [3, 7, 27]. In the present study,

ivabradine was also clinically well tolerated in dogs with DMVD. The oral dose of 1 mg/kg was the maximum dose used in the current study that significantly reduced the HR and RPP without lowering SBP or increasing numbers of arrhythmic beats per 24 h. In the current study, one dog showed increased numbers of VPCs after receiving ivabradine at 2.0 mg/kg. The effect of ivabradine on arrhythmia induction was unclear. The literature has suggested that the risk of atrial fibrillation (AF) when using ivabradine was about 1 in 10,000 patients. However, studies conducted in dogs with age-related AF and in dogs with vagal nerve stimulation demonstrated that ivabradine did not increase the risk of AF in those dogs [18, 36]. A recent study in patients with decompensated HF indicated that ivabradine was effective in reducing the VPCs induced by low and medium doses of dobutamine infusion [21]. Furthermore, studies conducted in failing heart mice and rats with acute myocardial infarction (MI) revealed that ivabradine was effective in preventing β-adrenergic stimulation-induced abnormal automaticity or partially preventing the proarrhythmic effects of MI [16, 19].

In humans, several clinical trials have demonstrated that there was a benefit of HR reduction in cardiovascular disease because the clinical outcomes were improved [14, 33]. The HR is an independent factor for cardiovascular diseases and is mainly related to the MVO_2 [8, 10]. In the present study, MVO_2 was decreased in response to oral ivabradine administration in a dose-dependent manner along with HR reduction. This finding agreed with previous studies in resting and exercising dogs, in which the reduction of the HR by ivabradine led to decreased MVO_2 and to an increased diastolic time interval [8]. MVO_2 decreased by ivabradine improved the balance between oxygen demand and supply during cardiovascular diseases whereas increased diastolic time improved myocardial calcium cycling [17]. Both mechanisms may be beneficial to dogs with DMVD.

In conclusion, the current study indicated that ivabradine can be safely used in dogs with DMVD at 1.0 mg/kg, PO. A long-term treatment should be performed to ensure the safety. In addition, further study in symptomatic DMVD dogs should be conducted to ensure our findings.

Study limitations: This study did not measure BP in detail from the 12th to 24th h after ivabradine administration. The lack of the data on BP during that period may not confound with the outcome of this study because the BP trend in Fig. 3 began to stabilize from the 5th h after dosing.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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