Effects of single bolus injection of pimobendan on cardiac function, hemodynamics, and heart rate variability in healthy dogs

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ผลของยาพิโมเบนแดนแบบฉีดเข้าหลอดเลือดดำต่อการทำงานของหัวใจ การไหลเวียนโลหิต และความแปรปรวนของอัตราการเต้นของหัวใจในสุนัขที่มีสุขภาพดี

นายปุณณวิช พิชญไพบูลย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปุณณวิช พิชญไพบูลย์ : ผลของยาพิโมเบนแดนแบบฉีดเข้าหลอดเลือดดำต่อการทำงานของหัวใจ การไหลเวียนโลหิต และความแปรปรวนของอัตราการเต้นของหัวใจในสุนัขที่มีสุขภาพดี. (Effects of single bolus injection of pimobendan on cardiac function, hemodynamics, and heart rate variability in healthy dogs) อ.ที่ปรึกษาหลัก : ผศ. ดร.อนุศักดิ์ กิจถาวรรัตน์

ยาพิโมเบนแดน (pimobendan) เป็นยาที่ถูกแนะนำให้ใช้ในการรักษาโรคหัวใจล้มเหลวในทางสัตวแพทย์มานานกว่าทศวรรษ และในช่วงที่ผ่านมายาพิโมเบนแดนในรูปแบบฉีดเข้าหลอดเลือดดำถูกนำออกขายสู่ตลาด อย่างไรก็ตามข้อมูลเกี่ยวกับผลของยาในรูปแบบฉีดเข้าหลอดเลือดดำต่อ คลื่นไฟฟ้าหัวใจ (electrocardiography) การทำงานของหัวใจ (cardiac functions) การไหลเวียนโลหิต (hemodynamics) และความแปรปรวนของอัตราการเต้นของหัวใจ (heart rate variability) รวมไปถึง เภสัชจลนศาสตร์ (Pharmacokinetics) ของยานี้ในรูปแบบฉีดยังไม่เป็นที่ทราบแน่ชัด วัตถุประสงค์ของการทดลองนี้คือ เพื่อศึกษาผลของยาพิโมเบนแดนแบบฉีดเข้าหลอดเลือดดำต่อ การไหลเวียนโลหิต, การทำงานของหัวใจ, คลื่นไฟฟ้าหัวใจ และความแปรปรวนของอัตราการเต้นของหัวใจ รวมไปถึงคุณสมบัติทางเภสัชจลนศาสตร์ของยา และสารเมทาบอไลท์ที่ออกออกฤทธิ์ในสุนัขที่มีสุขภาพดี ในการทดลองนี้สุนัขจำนวน ๙ ตัวถูกวางยาสลบและทำการผ่าตัดเพื่อใส่อุปกรณ์เพื่อวัดการทำงานของหัวใจห้องล่างซ้าย (left ventricle) การไหลเวียนโลหิต และคลื่นไฟฟ้าหัวใจ หลังจากนั้นจึงฉีดยาพิโมเบนแดนขนาด ๐.๑๕ มิลลิกรัม/กิโลกรัมเข้าทางหลอดเลือดดำแล้วบันทึกผลของยาที่เกิดขึ้นอย่างต่อเนื่องเป็นเวลา ๒ ชั่วโมงหลังฉีด แล้วทำการวัดความเข้มข้นของยากับสารเมทาบอไลท์ที่ออกฤทธิ์ด้วยวิธี ลิควิดโครมาโทกราฟี-แมสส์สเปกโทรเมเตอร์ (liquid chromatography mass spectrometry) ในการศึกษาผลของยานี้ต่อความแปรปรวนของอัตราการเต้นของหัวใจ สุนัขจำนวน ๗ ตัวถูกติดอุปกรณ์เพื่อบันทึกคลื่นไฟฟ้าหัวใจ และฉีดยาพิโมเบนแดนเข้าหลอดเลือดดำขนาดเดียวกันกับการทดลองก่อนหน้าแก่สุนัขจำนวน ๗ ตัว หลังจากนั้นคลื่นไฟฟ้าหัวใจของสุนัขจะถูกบันทึกต่อไปเป็นเวลา ๓ ชั่วโมง ผลจากการศึกษาพบว่า พิโมเบนแดนแบบฉีดเข้าหลอดเลือดดำทำให้หัวใจเต้นเร็วขึ้นร่วมกับทำให้ช่วง พี-คิว (PQ interval) สั้นลง นอกจากนั้นยังพบว่ายานี้ช่วยทำให้หัวใจสูบฉีดเลือดได้ดีขึ้นภายในเวลา ๑๐ ถึง ๒๐ นาทีหลังฉีดยา และยังพบว่ายานี้ลดความต้านทานของหลอดเลือดภายในร่างกาย และที่ปอดได้อย่างมีนัยสำคัญ จากการศึกษาคุณสมบัติทางเภสัชจลนศาสตร์ของยาและ สารเมทาบอไลท์พบว่า ตัวยาและสารเมทาบอไลท์มีความเข้มข้นในเลือดสูงสุดที่ ๘๓.๗ และ ๓๐ ไมโครกรัม/ลิตร ตามลำดับ ในขณะที่เมทาโบไลท์ใช้เวลา ๐.๓๓ ชั่วโมงในการเพิ่มความเข้มข้นจนถึงจุดสูงสุดในเลือด และปริมาตรกระจายตัวของยานี้ กับสารเมทาบอไลท์ คือ ๘.๙ และ ๗.๑ ลิตร/กิโลกรัมตามลำดับ และยังพบว่าค่าครึ่งชีวิตของระดับยาในกระแสเลือดของยานี้ กับสารเมทาบอไลท์คือ ๑ และ ๒.๘ ชั่วโมง ตามลำดับ ค่าการกำจัดยาของยานี้ และสารเมทาโบไลท์มีค่า ๕.๘ และ ๒.๒ ลิตร/กิโลกรัม/ชั่วโมง ตามลำดับ ส่วนการศึกษาผลของยานี้ต่อความแปรปรวนของอัตราการเต้นของหัวใจในสุนัขที่ไม่ได้วางยาสลบพบว่า ยานี้เพิ่มความแปรปรวนของอัตราการเต้นของหัวใจซึ่งอาจตีความได้ว่ายานี้เพิ่มการสั่งการของระบบประสาทอัตโนวัติต่อหัวใจ โดยเฉพาะระบบประสาท พาราซิมพาเทติก โดยสรุปแล้วยานี้ช่วยเพิ่ม การทำงานของหัวใจ การไหลเวียนโลหิต ภายในระยะเวลาอันสั้นหลังฉีด นอกจากนี้ยังช่วยเพิ่มการทำงานของระบบประสาทอัตโนวัติ ซึ่งคาดว่าน่าจะเป็นประโยชน์ต่อสุนัขที่ป่วยเป็นโรคหัวใจล้มเหลว

สาขาวิชา	สรีรวิทยาการสัตว์	ลายมือชื่อนิสิต .....
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Poonavit Pichayapaiboon : Effects of single bolus injection of pimobendan on cardiac function, hemodynamics, and heart rate variability in healthy dogs. Advisor: Asst. Prof. ANUSAK KIJTAWORNRAT, Ph.D.

In veterinary medicine, pimobendan has been recommended for management of dogs with congestive heart failure for decades. Recently, an intravenous injectable solution of this drug has been launched. However, the effects of this preparation on electrocardiography, cardiac functions, hemodynamics, heart rate variability (HRV), and the pharmacokinetics (PK) in dogs are still limited. Therefore, the objective of this investigation is to investigate the hemodynamics, cardiac functions, electrocardiographic parameters, the PK profiles and its active metabolite, O-desmethyl pimobendan (ODMP), as well as its effect on HRV of a single bolus pimobendan in healthy dogs. Nine dogs were anesthetized and instrumented to obtained parameters of left ventricular functions, hemodynamics, and electrocardiograms. Bolus pimobendan (0.15 mg/kg, IV) was given to all dogs and the effects were monitored for 2 h. Plasma concentrations of pimobendan and ODMP were quantified using LC-MS/MS. Seven of those dogs were instrumented with Holter monitoring for obtaining heart rate variability while they were conscious and clam. Bolus pimobendan (0.15 mg/kg, IV) was given to all dogs and the effects were monitored for 3 h. The results revealed that single bolus pimobendan fastened the heart rate while the PQ interval was shortened. Furthermore, the results also indicated that pimobendan significantly improves both cardiac contraction and relaxation within 10 to 20 mins after injection. In addition, pimobendan significantly reduced both systemic- and pulmonic-vascular resistances. The C_{max} of pimobendan and ODMP were 83.7 and 30 ug/L, respectively, while the T_{max} of ODMP was 0.33 h. The volume of distribution of pimobendan and ODMP were 8.9 and 7.1 L/kg, respectively. The half-life of pimobendan and ODMP were 1 h and 2.8 h, respectively. The clearance of pimobendan and ODMP were 5.8 and 2.2 L/kg/h, respectively. In conscious dogs, single bolus pimobendan augmented the cardiac autonomic nervous activity inferred from an increasing of heart rate variability. Moreover, the results also suggested that pimobendan tended to intensify parasympathetic activity more than the sympathetic branch. In conclusion, intravenous pimobendan improves cardiac functions and hemodynamics shortly after injection and enhances a cardiac autonomic nervous activity, which may be a great benefit for dogs with congestive heart failure.

Field of Study:	Animal Physiology	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....

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Poonavit Pichayapaiboon

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Abbreviations

ACVIM	American College of Veterinary Internal Medicine
AMP	Adenosine monophosphate
AoP	Aortic pressure
AUC	Area under the curve
AV node	Atrioventricular node
SA node	Sinoatrial node
$I_{ca,L}$	L-type calcium channel
cAMP	Cyclic adenosine monophosphate
CBC	Complete blood count
cGMP	Cyclic guanosine monophosphate
CHF	Congestive heart failure
CI	Contractility index
CLs	Systemic clearance of the drug
C_{max}	Maximum plasma concentration of the drug
CO	Cardiac output
Cyp	Cytochrome
DCM	Dilated cardiomyopathy
dP/dt_{min}	the maximal rate of fall of the left ventricular pressure
ECG	Electrocardiography
HCN	hyperpolarization-activated cyclic-nucleotide gated
HF	High frequency spectrum of frequency-domains of HRV

HR	Heart rate
HRV	Heart rate variability
I_f	Funny current
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LF	Low frequency spectrum of frequency-domain of HRV
LF/HF	The ratio of LF to HF
LLOQ	The lower limit of quantification
LV	Left ventricle
LVEDP	Left ventricular end-diastolic pressure
LVP	Left ventricular pressure
dP/dt_{max}	Maximal rate of rise of the left ventricular pressure
MRT	Mean residence time
MVO_2	Myocardial oxygen consumption
NNA	The mean of intervals between consecutive normal R waves of ECG (NN intervals)
NSAID	Non-steroidal anti-inflammatory drugs
ODMP	O-desmethyl pimobendan
PAP	Pulmonary arterial pressure
PCWP	pulmonary capillary wedge pressure
PDE	Phosphodiesterase
PDEi	Phosphodiesterase inhibitors
PKA	Protein kinase A

pNN50 ms	The percentage of successive normal RR intervals exceeding 50
PNS	Parasympathetic nervous system
ANS	Autonomic nervous system
PVR	Pulmonary vascular resistance
RAP	Right atrial pressure
rMSSD	The square root of the mean of the squares of the differences between successive normal to normal RR intervals
SBP	Systolic blood pressure
SC	Subcutaneous injection
SDNN index	Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording
SDNN	Standard deviation of NN intervals
SEM	Standard error of mean
SNS	Sympathetic nervous system
SVR	Systemic vascular resistance
$t_{1/2}$	Half-life of the drug
Tau	Left ventricular constant time relaxation
T_{max}	The time that the drug spends to reach its maximum plasma concentration
TnC	Troponin C
TP	Total power of all spectrum of frequency-domains of HRV
Vd	Volume of distribution

Chapter 1 Introduction

Pimobendan, a benzimidazole-pyridazinone derivative, is widely used for treatment of both asymptomatic and symptomatic canine congestive heart failure (CHF) (Smith et al., 2005; Keene et al., 2019). It acts as the calcium sensitizer and phosphodiesterase III inhibitor which has two major effects on cardiovascular system (Bell et al., 2016). Firstly, it increases the affinity of the troponin C to calcium ions. Therefore, myocardial contraction will increase without rising myocardial oxygen consumption (MVO_2) (Boyle and Leech, 2012). Secondly, pimobendan has a mild vasodilation effect resulting in a reduction of afterload (i.e., systemic blood pressure). Formerly, several clinical trials have supported the use of pimobendan in veterinary medicine especially dogs with myxomatous mitral valve disease (MMVD) stage C and D (Haggstrom et al., 2008; Atkins et al., 2009) and CHF due to dilated cardiomyopathy (DCM) (Lombard et al., 2006). Therfore, the previous guidelines for the diagnosis and treatment of canine chronic valvular heart disease has recommended the use of pimobendan in addition to angiotensin converting enzyme inhibitors and diuretics for

treatment of canine with CHF (Atkins and Haggstrom, 2012). However, newer clinical trials have supported the use of pimobendan in asymptomatic MMVD (Boswood et al., 2016; Boswood et al., 2018).

These newer clinical trials lead to the update guidelines for the diagnosis and treatment of MMVD in dogs published by the American College of Veterinary Internal Medicine (ACVIM) for the use of pimobendan in dogs with MMVD stage B2 (Keene et al., 2019).

Previously, pimobendan is supplied in the form of capsule or chewable tablet. In dogs, pimobendan may takes 2-4 hours to reach the maximum effect when given orally (Yata et al., 2016), which is not appropriate for emergency cases of acute CHF. Currently, injectable pimobendan is available in some countries (e.g. United Kingdom, Australia). However, limited data are available in dogs. The previous literature reports that, escalating concentrations of intravenous pimobendan (10, 20, 40 μg/kg/min; 15 min for each dose) increased heart rate (HR), cardiac output (CO), left ventricular contractility implied by an increased in the maximum rate of rise of

the left ventricular pressure (LVP) (dP/dt_{max}) (Pagel et al., 1996). In addition, it decreases the left ventricular end-diastolic pressure (LVEDP) and systemic vascular resistance (SVR). Recently, a single bolus of pimobendan has been investigated in healthy dogs (Hori et al., 2019). The authors have investigated pimobendan effects in anesthetized dogs for one hour and found that it increased dP/dt_{max} while it decreased LVEDP. There was no effect on the maximum rate of fall of the LVP (dP/dt_{min}) and HR. It has been known that dogs with CHF succumb from pulmonary edema developed when pulmonary capillary wedge pressure (PCWP) is elevated and die (Guazzi and Borlaug, 2012). In addition, dogs with MMVD (symptomatic stage) demonstrate parasympathetic withdrawal and/or sympathetic over activation during the development of heart failure and related to the severity of MMVD (Rasmussen et al., 2012). While most of the studies focus on the cardiac function of dogs in response to intravenous pimobendan, no data available for the effects of pimobendan on PCWP and heart rate variability (HRV).

Objectives of Study:

1. To investigate the hemodynamics, cardiac functions, and electrocardiographic parameters after a single bolus intravenous injection of pimobendan in anesthetized healthy dogs.
2. To investigate the pharmacokinetic profiles of a single bolus intravenous injection of pimobendan and its active metabolite O-desmethyl pimobendan in healthy dogs.
3. To investigate the heart rate variability of a single bolus intravenous injection of pimobendan in conscious healthy dogs.

Research Questions:

1. What are the effects of a single bolus intravenous pimobendan injection on cardiac output, systemic vascular resistance and pulmonary capillary wedge pressure in anesthetized healthy dogs?
2. What are the pharmacokinetic profiles of a single bolus intravenous pimobendan and its active metabolite O-desmethyl pimobendan in healthy dogs?

3. What are the effects of a single bolus intravenous pimobendan injection on the heart rate variability in conscious healthy dogs?

Research Hypothesis:

1. Intravenous pimobendan injection will not only increase cardiac output but also reduce systemic vascular resistance and pulmonary capillary wedge pressure in anesthetized healthy dogs.
2. The pharmacokinetic profiles of single bolus intravenous pimobendan should fits to a non-compartment model of compartment model pharmacokinetics.
3. Intravenous pimobendan injection will enhance cardiac autonomic nervous system in conscious healthy dogs.

Chapter 2 Literature review

Phosphodiesterase

Phosphodiesterases (PDE) are the enzymes that hydrolyze the phosphodiester bonds in cyclic nucleotide such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), secondary-messengers for intracellular signal transduction (Knight and Yan, 2012). The PDE superfamily is classified into 11 families of isozymes based on their structures, localizations, and substrates (Shafiee-Nick et al., 2017) (Table 1).

Table 1. superfamily, substrate and localizations of PDE.

Families	Substrates	Locations
PDE1	cAMP/cGMP	heart, vascular smooth muscle, lung and platelets
PDE2	cAMP/cGMP	heart, brain, platelets, adrenal cells and endothelial cells
PDE3	cAMP/cGMP	heart, vascular smooth muscle, kidney, oocyte, hepatocyte
PDE4	cAMP	heart, vascular smooth muscle, brain, immune system
PDE5	cGMP	heart, aortic smooth muscle, lung, skeletal muscle, platelet
PDE6	cGMP	Retina
PDE7	cAMP	heart, brain, skeletal muscle and pancreas
PDE8	cAMP	heart, brain, kidney, skeletal muscle, testes and ovary
PDE9	cGMP	heart, kidney, brain
PDE10	cAMP/cGMP	brain, pineal gland and testes
PDE11	cAMP/cGMP	brain, pituitary gland, liver

Modified from Shafiee-Nick and colleagues (Shafiee-Nick et al., 2017).

PDE 1, 2, 3, 4, 5, 7, 8, and 9 are identified in the cardiac myocyte (Keravis and Lugnier, 2012). In the failing heart, the concentrations of secondary-messengers are diminished due to reduction of synthesis, which alters a normal regulation of cardiac function and leads to several pathological processes including cardiomyocyte dysfunction and cardiac remodeling. Therefore, a pharmacological inhibition of PDE is crucial in the patients with heart disease to restore a normal regulation (Kim and Kass, 2017).

Mechanisms of action of phosphodiesterase inhibitor

A phosphodiesterase inhibitor (PDEi) are the drugs that prevent a degradation of the intracellular secondary messengers (cAMP and cGMP) by inhibit PDE. These drugs have a wide -range of action depend on the isozymes that inhibited by the drugs (Schudt et al., 2011). PDE-3 inhibitors such as amrinone, milrinone, pimobendan and levosimendan are recommended to be used in the treatment of CHF (Boswell-Smith et al., 2006), since the drugs show a several benefits in CHF patient including an improvement in symptoms, quality of life and reduction of cardiac related-death (Pashkovetsky et al., 2019). In veterinary medicine, pimobendan is widely used

because several clinical trials have supported the benefit of using pimobendan in dogs with and without CHF (Fuentes et al., 2002; Haggstrom et al., 2008; Boswood et al., 2016).

Pimobnedan, an inodilator, which has effects on both cardiac contraction (positive inotrope) and peripheral resistance (vasodilator). The medicine is also classified as non-catecholamine and non-glycoside positive inotropic drug. This drug neither increases energy consumption of myocyte nor augments proarrhythmic property (Lake-Bakaar et al., 2015), which is more appropriate for the cardiovascular disease patients than previous positive inotropic agents. Pimobendan increases the affinity of troponin C to calcium ions and troponin I (Schlecht et al., 2016), which leads to the opening of the myosin binding site on actin filaments. Once the biding sites are available, more myosins bind to actin filaments and have more power stroke. Furthermore, positive inotropic effect is also achieved by inhibition of phosphodiesterase 3 enzyme (PDE-3). Normally, cAMP activates protein kinase A (PKA), which phosphorylates protein that takes responsibility of the concentration of intracellular calcium ions such as ryanodine receptors and L-type calcium channels.

After activation of PKA, cAMP will be converted to AMP by PDE-3 (Boswell-Smith et al., 2006). Once the enzyme is inhibited, the concentrations of intracellular calcium ions increase and bind to troponin C. From both pathways, the myocytes have a strengthened contraction force, which antagonizes the decreasing cardiac output from the disease. In healthy heart, pimobendan acts on both pathways. At peripheral arteries, pimobendan has a different effect from the myocytes, although the enzyme that is inhibited is the same enzyme as that of myocytes. The increasing cAMP causes relaxation of vascular smooth muscles around the vessels, which reduces workload of heart (Boyle and Leech, 2012).

Physicochemical properties of pimobendan

Pimobemdan is classify as a phenylbenzimidazoles, the benzimidazole skeleton with a phenyl group at imidazole ring (Figure 1A). The IUPAC name and chemical formula of this compound are 6-[2-(4-methoxyphenyl)-1H-1,3-benzodiazol-5-yl]-5-methyl-2,3,4,5-tetrahydropyridazin-3-one and $C_{19}H_{18}N_4O_2$, respectively (Wishart et al., 2006). The physiochemical of this drug were summarized in table 2.

This drug is known as a substrate of cytochrome P450 2A1, since the drug is demethylated and converted into an active metabolite, O-desmethy-pimobendan (Figure 1B) by the enzyme in phase 1 metabolism (Takahashi and Endoh, 2001). In phase 2 of metabolism, the active metabolite is conjugated with glucuronic acid and sulfonic acid to increase solubility in water then the product of these reaction is excreted in bile and feces (Figure 2)

Table 2. The summary of physicochemical properties of pimobendan

Properties	Value
Molecular weight	334.4 g/mol
Water Solubility	0.0147 mg/mL
Partition coefficient (Log P)	3.35
Acid dissociation constant (pKa)	11.17
Physiological Charge	0

Modified from Wishart et al., 2006

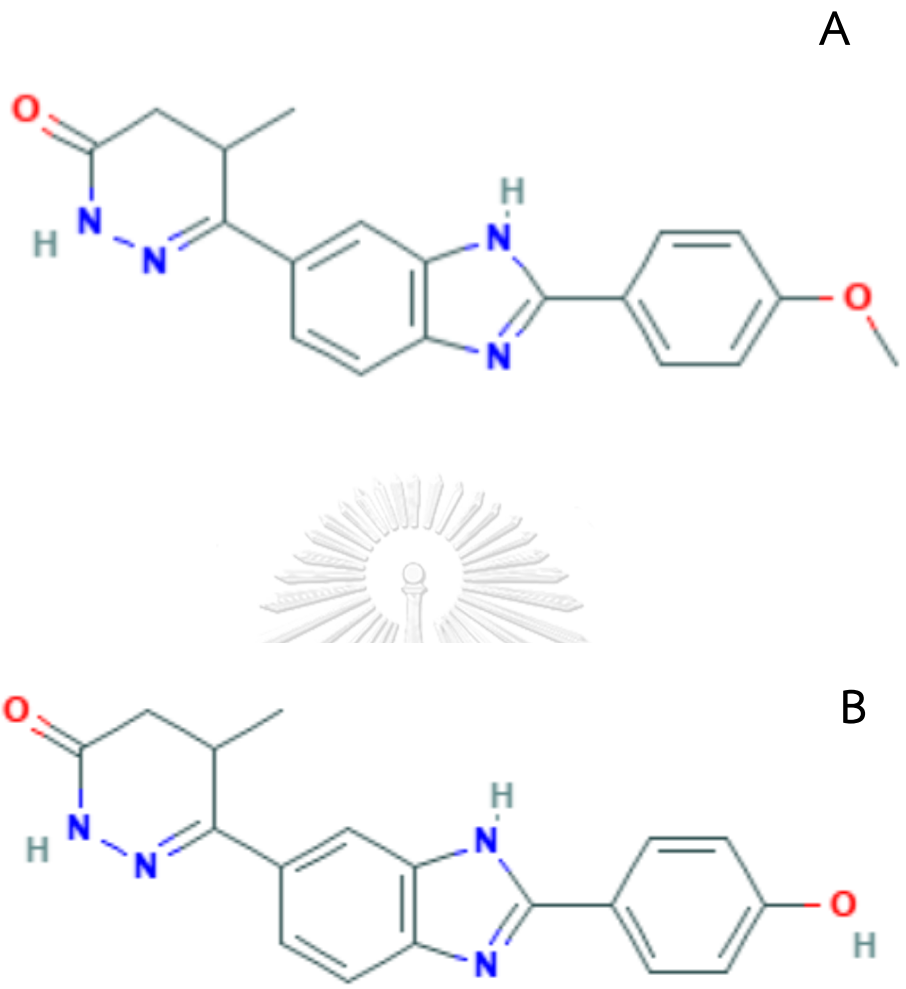


Figure 1. A. molecular structure of pimobendan, B. molecular structure of ODMP from PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 4823, Pimobendan; [cited 2020 Dec. 30]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Pimobendan

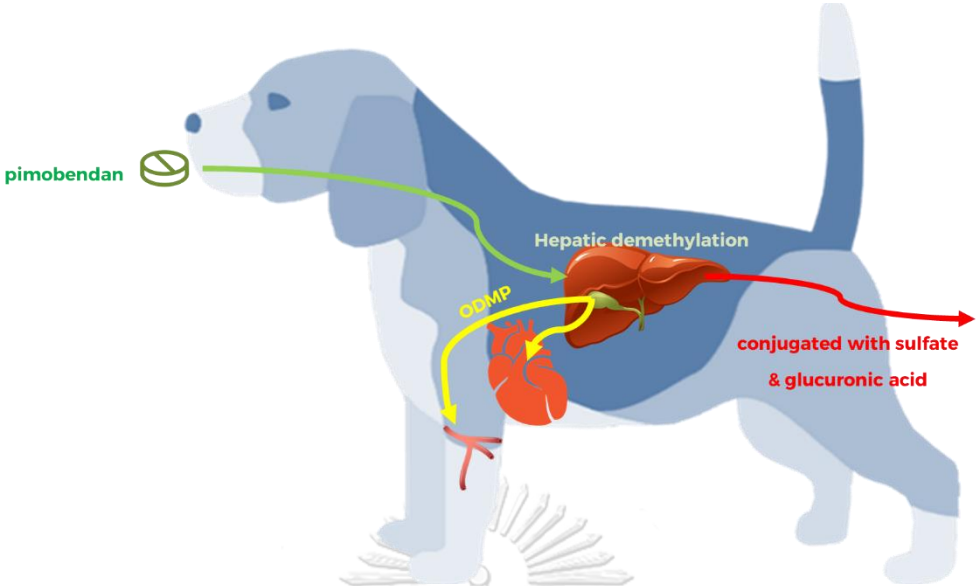


Figure 2. pharmacokinetic of oral pimobendan in dogs.

Pharmacokinetics of pimobendan

The effect of oral pimobendan on the cardiovascular system is a dose-dependent manner, which can be adjusted up to 0.5 mg/kg twice a day (approximately 12 hours apart). Previous study suggests that the bioavailability of this drug is 70% in healthy dogs (Bell et al., 2016). After the drug enters blood stream, it will be oxidized by hepatic demethylation reaction. The product of this reaction is O-desmethyl pimobendan (ODMP) , which is an active metabolite of this drug (Takahashi and Endoh, 2001). Furthermore, it has been shown that this metabolite has more potent effect than the parent compound on both myocardium and

vasculature (Boyle and Leech, 2012). After oral administration, the time to maximum plasma concentration (t_{max}) of pimobendan and its active metabolite in dogs are 2-3 hours after administrated. In the circulatory system, more than 90% of ODMP and its precursor bind to plasma albumin. In dogs, the active metabolites are excreted via feces, after conjugated with sulfate or glucuronic acid. In healthy dogs, the half-life ($t_{1/2}$) of the drug and its metabolite are 0.9 ± 0.2 hours and 1.6 ± 0.3 hours respectively (Yata et al., 2016).

Since the drug is an intravenous injectable form, it reaches its maximum plasma concentration immediately after injected (Table 2). This may have a benefit for the emergency treatment in dogs with congestive heart failure. Furthermore, in this preparation the drug does not affect by the first pass effect or bioavailability (Table 3). However, like the oral preparation, this drug needs hepatic demethylation to become an active metabolite. The half-life of the drug in this preparation is 0.4 ± 0.1 hour (Bell et al., 2016). Interestingly, the half-life of its active metabolite is not studied yet.

Table 3. Pharmacokinetic parameters of pimobendan in blood after oral administration at 0.25 mg/kg and after intravenous administration at 0.125 mg/kg using an aqueous solution pimobendan, in ten healthy dogs. Values are expressed as Mean ± SD.

Pharmacokinetic parameters	Oral administration	Intravenous injection
Dose (mg/kg)	0.25	0.125
$t_{1/2}$(min)	37.4 ± 8.5	28.8 ± 6.9
F (%)	69 ± 16	100
T_{max} (min)	38.5 ± 15	Immediately after injected

$t_{1/2}$: terminal elimination half-life, F: bioavailability, T_{max}: time to maximum blood concentration. Modified from Bell and colleagues (Bell et al., 2016).

Effect of pimobendan in dogs

It has been reported that pimobendan improves a physical fitness and quality of life in dogs with MMVD stage B1 (Iwanuk et al., 2019) and extends the subclinical period of the dogs with MMVD stage B2 (Boswood et al., 2016). Furthermore, it has been proved that pimobendan can delay a time to an onset of the CHF for DCM dogs (Summerfield et al., 2012).

In clinical stage, pimobendan improves the cardiac function with a balance effect of vasodilation and cardiac contractility, which are a great benefit for the CHF dogs (Smith et al., 2005). Previous study indicates that adding pimobendan with conventional therapy (furosemide, angiotensin converting enzyme inhibitor and digoxin) improves the clinical signs and increases a survival time of CHF dogs secondary to MMVD (Lombard et al., 2006) as well as DCM (Fuentes et al., 2002). Additionally, it has been reported that pimobendan does not have a proarrhythmic property when prescribed at the recommended dose in small breed dogs (Lake-Bakaar et al., 2015) and Doberman pinchers (Summerfield et al., 2012).

Cardiac autonomic nervous system assessment

The cardiac functions are regulated by sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) (Gordan et al., 2015). However, the activity of autonomic nervous system (ANS) cannot be assessed directly. Therefore, ANS can be indirectly assessed by a measurement of heart rate variability (HRV), the beat-to-beat variation of heart rate (Billman, 2011).

In veterinary medicine, previous publications reported that the HRV is a very sensitive tool to detecting and prognosis both MMVD (Rasmussen et al., 2012) and dilated cardiomyopathy (DCM) (Pereira et al., 2008) in dogs. In sick dogs, diminished HRV may results from the increasing heart rate due to overstimulation of SNS (Pirintr et al., 2017).

Chapter 3 Materials and Methods

Study design

This study is a prospective cross-over study that divided into two parts. In each part a healthy beagle dogs were injected with intravenous single bolus pimonbendan. The washout period between experiments was at least 5.5 times of terminal elimination half-life of pimobendan to prevent residual effect of the drug (Chow, 2014).

Part I: To investigate the hemodynamics, cardiac functions, electrocardiographic parameters and pharmacokinetic profiles of both pimobendan and its active metabolite, O-desmethyl pimobendan, after a single bolus intravenous injection of pimobendan in healthy dogs in healthy dogs

Materials and methods

Approval

This study was approved by the Institutional Animal Care and Use Committee of Chulalongkorn University Laboratory Animal Center (protocol number 1873019).

All animal procedures were performed in compliance with Animals for Scientific Purpose Act (A.D. 2015) and followed the guidelines outlined by *the Guide for the Care and Use of Laboratory Animals* (NRC, 2011)

Animals

Nine healthy mature Beagles (male 5; female 4) (*Canis familiaris*) were purchased from the commercial breeder and were housed in a dog run maintained at a temperature 22±1°C, a relative humidity of 50±20% and a 12:12 h light:dark cycle. Physical examination, lead II electrocardiography (ECG), complete blood count (CBC) and blood chemistry profiles including blood urea nitrogen, serum creatinine, serum alkaline phosphatase, serum alanine aminotransferase and serum aspartate aminotransferase were performed to evaluate healthy status in all dogs before beginning of the experiment. Dogs were excluded from the study if there was an evidence of clinically important systemic or cardiovascular diseases upon initial assessment.

The experimental procedures

On the experimental day, dogs were given carprofen (4 mg/kg, SC) and cephazolin (25 mg/kg, SC). Then a bolus of propofol (4-6 mg/kg, IV) was injected followed by endotracheal tube intubation. The animals were ventilated mechanically with a 100% O_2 via volume-cycled ventilator at a rate of 8 to 12 breaths per minute and a tidal volume of approximately 20 mL/kg (Veterinary Anesthesia Ventilators Model 2000TM, the Hallowell EMC©, Pittsfield, MA), sustaining the arterial partial pressure of CO_2 between 35 and 45 mmHg and that of O_2 greater than 90 mmHg. Body temperature was maintained at 36.5–37°C by heat therapy pump (T/Pump® Model TP-500, Gaymar©, Orchard Park, NY). A surgical plane of anesthesia was maintained by inhalation of isoflurane and the end-tidal inhalant concentration was maintained between 1.4–1.6%.

Each animal was shaved and scrubbed at the surgical areas and was prepared by aseptic technique (left jugular area). All catheterization procedures were performed under fluoroscopic guidance. A Mikro-Tip catheter pressure transducer (5Fr, Millar, Inc., Houston, TX, USA) was inserted into the left carotid artery and

advanced to the left ventricle for measuring left ventricular pressure. A 5 Fr thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted into the left jugular vein and advanced into the pulmonary artery to permit simultaneously continuous monitoring of right atrial (RAP) and pulmonary arterial (PAP) pressures and intermittent determination of cardiac output (CO) using thermodilution technique and pulmonary capillary wedge pressure (PCWP). A lead II electrocardiogram was obtained. After stabilization, approximately 30 min, baseline data were recorded. Then, single bolus of pimobendan (0.15 mg/kg) was intravenously injected with an observation period of 2 h after injection. The ECG and pressures were recorded throughout the experiment with an IOX system (EMKA Technologies, Paris, France) and stored in a hard drive for further analysis.

All parameters were analyzed at baseline, 10, 20, 30, 60, 120 mins after the beginning of injection (Figure 3). At the end of experiment (i.e., after 2 h of pimobendan administration), all vessels were sutured with 6–0 monofilament nonabsorbable polypropylene suture materials. Tissues and muscles were sutured with absorbable 3–0 suture materials. Skin was closed with monofilament polyamide

suture. Carprofen (4 mg/kg; SID) and cefazolin (25 mg/kg, BID) were administered orally for 3 days and 7 days, respectively.

Blood collections

At the baseline, 3 mL of blood samples were collected from the dogs. After intravenous pimobendan injection, the blood samples were collected at 2, 5, 10, 20, 30, 60, 120, 180, 360, 1440 mins (Figure 3). Each blood sample was collected from the intravenous catheter and stored in lithium heparin containing tube. The samples were placed on the ice and were centrifuged at 3,000xg and 4°C for 10 mins within 1 h after collection to separate plasma from red blood cell. After centrifugation, the samples were stored at -20°C until the time of analysis.

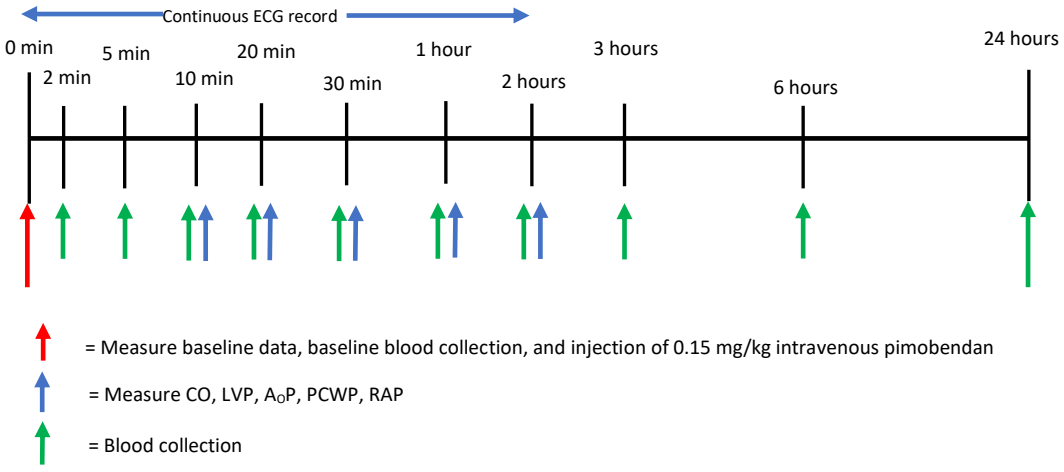


Figure 3 Study timeline.

Sample preparation

The plasma samples were thawed at 25°C for 15 mins then 50 μL of each sample was mixed with an absolute methanol containing 100 ng/mL of glycyrrhizin as internal standard. Then the solutions were centrifuged at 10,000xg for 10 mins. After centrifuged, the supernatant was collected for further analyzed by Liquid chromatography tandem mass spectrometry (LC-MS/MS) while the sediments were discarded.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis

The Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was modified from previous study (Junsaeng et al., 2019). The prepared samples were injected into LC-MS/MS [Nexera Ultra High–Performance Liquid Chromatography and 8060 triple quadrupole mass spectrometers (Shimadzu Co., Ltd.)]. The stationary phase in the current study was Synergi Fusion–RP C18 column (Phenomenex Inc.). Formic acid (0.2%) in absolute methanol and water was used as a mobile phase. The gradient of the mobile phase began with 10% methanol from 0 min to 0.5 mins. Then, the concentration of methanol was elevated to 90% at 0.5 - 1.5 mins and

remained at 90% until 3.0 min after the samples were injected. At 3.0 – 4.0 mins the gradient was reduced to 10% then stayed at 10% until 5.0 mins after injections.

Data analysis

Cardiac output (CO)

The cardiac output was measured with thermodilution method and was calculated with a modified Stewart-Hamilton indicator dilution equation (Armengol et al., 1981). When the cold saline was injected into right ventricle, the changing of temperature in pulmonary artery was detected by temperature sensor of cardiac output machine. Then, the thermodilution curve was plotted. The cardiac output is the area under the curve, which was integrated by the CO machine. In this study, the CO measurement was performed 3 times for each time-point. The data were excluded, if there were more than 10% difference between each value. Then the mean value of each time-point was calculated.

Resistance (R)

Resistance was determined by Ohm's law (Hennig, 1992).

R = $(P_i - P_o)/Q$

R = Resistance

Pi - Po = the pressure gradient of the vessel

Q = blood flow

Systemic vascular resistance (SVR) is the resistance to blood flow of all the vascular systems, excluding the pulmonary vasculature.

SVR = (AoP – RAP)/CO

SVR = systemic vascular resistance

AoP = aortic pressure

RAP = right atrial pressure

CO = cardiac output

Pulmonary vascular resistance (PVR) refers to the resistance in the pulmonary vasculature against right ventricle.

PVR = (PAP – PCWP)/CO

PVR = pulmonary vascular resistance

PAP = pulmonary arterial pressure

PCWP = pulmonary capillary wedge pressure

CO = cardiac output

Left ventricular pressure (LVP)

Inotropic parameters

The dP/dt_{max} is the maximal rate of rise of the left ventricular pressure (Hamlin and del Rio, 2012) (Figure 4).

Contractility index (CI) is a ratio of maximal rate of rise of the left ventricular pressure and the left ventricular pressure at that point.

CI = contractility index

CI = $(dP/dt_{max})/P$

dP/dt_{max} = maximal rate of rise of the left ventricular pressure

P = the pressure of left ventricular pressure at that point

Lusitropic parameters

The dP/dt_{min} is the maximal rate of fall of the left ventricular pressure (Figure 4).

Isovolumic relaxation time constant (τ, Tau) is the exponential decline of ventricular pressure during isovolumic relaxation that is calculated from Glantz method (Raff and Glantz, 1981).

$P(t) = P0^{e-t/\tau E} + P\alpha$

P = pressure at time t,

P0 = amplitude constant

τE = Glantz relaxation constant

$P\alpha$ = non zero asymptote due to pleural and pericardial pressure

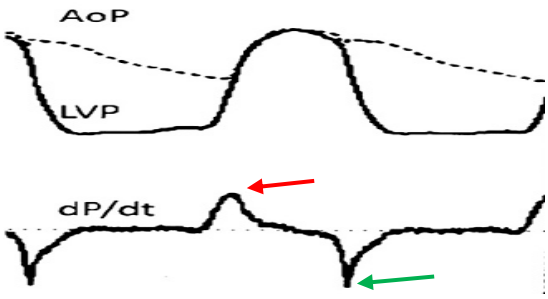


Figure 4 Representative of canine left ventricular pressure (LVP) demonstrating the maximal rate of rise of the LVP (dP/dt_{max}, red arrow), the maximal rate of fall of the LVP (dP/dt_{min}, green arrow) (Hamlin and del Rio, 2012).

Electrocardiogram (ECG)

The ECG derived parameters [HR, RR interval, PQ interval, QRS duration, QT interval (Figure 5) and Van de water's rate-corrected QT interval were measured offline with the aid of pattern recognition software (ECG Auto; EMKA Technologies, Paris, France).

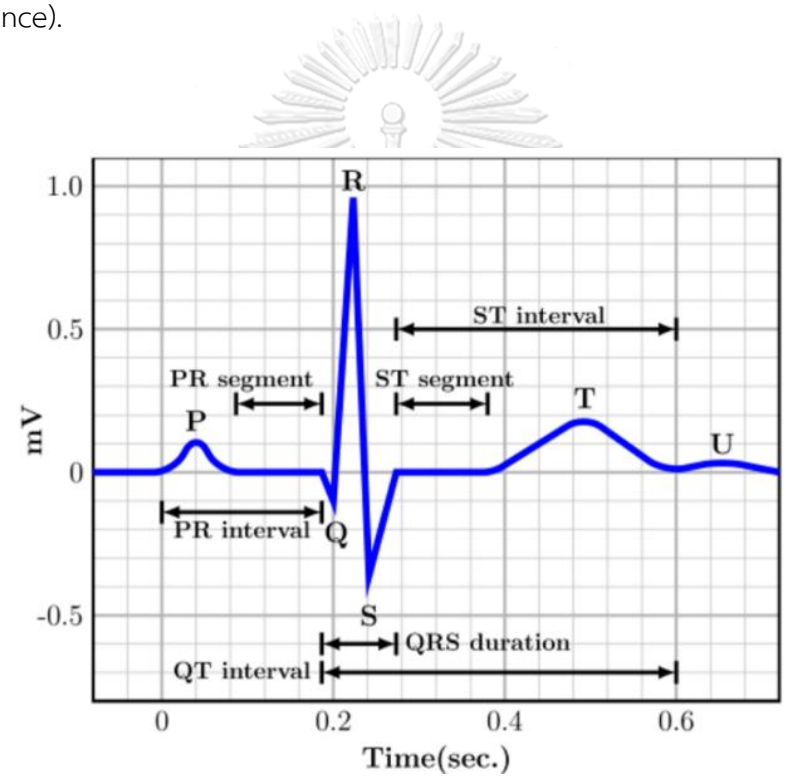


Figure 5. shows normal ECG pattern, PQ interval, QRS duration, and QT interval, respectively (Chen et al., 2014).

Van de water's rate-corrected QT interval was calculated from the following equation (Van de Water et al., 1989).

QTc = QT – 0.087 (RR-1000)

QTc = corrected QT interval

QT = QT interval from ECG

RR = RR interval from ECG

The ECG tracings were also inspected and counted manually for ventricular arrhythmia. The abnormal beats were reported as a number of ventricular arrhythmias. Ventricular tachyarrhythmias are consisted of a large group of abnormal cardiac rhythms including single premature ventricular complexes, sustained monomorphic ventricular tachycardia (VT), polymorphic VT and ventricular fibrillation. These arrhythmias are defined as tachycardia with bizarre and widening QRS complex (>120 ms) (Dresen and Ferguson, 2018).

Pharmacokinetics profiles of intravenous pimobendan and ODMP

The pharmacokinetic profiles of single bolus intravenous pimobendan and its active metabolite O-desmethyl pimobendan were calculated by non-compartment with a commercially available software (PK solution 2.0) The pharmacokinetic parameters were reported as showed in Table 4.

Table 4. Pharmacokinetic parameters and their definitions

PK Parameters	Unit	Definitions
C_{max}	ug/L	The maximum plasma concentration of the compound after administration
T_{max}	h	The time that the drug spends to reach its maximum plasma concentration
AUC_{0-t}	Ug·h/L	An area under the curve of the drug plasma concentration-time, which indicates a total exposure of the body to the compound during a specific period.
AUC_{0-inf}	Ug·h/L	Similar to AUC_{0-t}, but this parameter calculates the area under the curve from an extrapolate plot from 0-specific period to 0-infinity time points
MRT	h	The total mean time that each molecule of the drug uses to absorption, distribution, metabolism, and excretion.
Vd	L/kg	The theoretical volume that contains the total amount of a drug.
CL_s	L/kg/h	The ability of the body to irreversible removal the compound out of the body.
$t_{1/2}$	h^{-1}	The time that the plasma concentration of the compound takes to decrease by 50%.

Modified from (Fan and de Lannoy, 2014)

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analyses were performed with commercially available software. Normal distribution of continuous data was assessed with the Shapiro-wilk test. Comparisons were made for each parameter after drug administration versus baseline. One-way ANOVA with repeated measures design were used to determine the statistically significant among timepoints. Dunnett's test was used as post hoc analysis.

<u>Part II:</u> To investigate the heart rate variability of a single bolus intravenous injection of pimobendan in conscious healthy dogs.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of Chulalongkorn University Laboratory Animal Center (protocol number 1873019).

The experimental procedure

Before the experimental day, the dogs were acclimatized to experimental procedure and were trained to be attached with a Holter monitoring device for two weeks. The Holter monitor (Fukuda Denshi Co., Ltd., Japan) with standard 7 ECG electrodes were attached to the dogs while they were conscious. The continuous ECG were recorded and stored on an SD card for further analysis of heart rate (HR) and heart rate variability (HRV). After 1 hour of baseline recording, dogs were given a single bolus pimobendan intravenously (0.15 mg/kg) and the Holter monitoring were recorded for 3 h after drug administration (Figure 6).

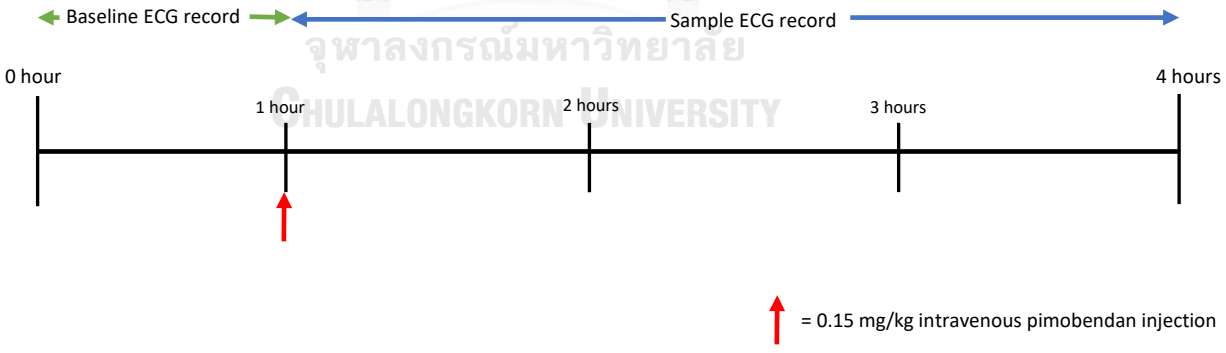
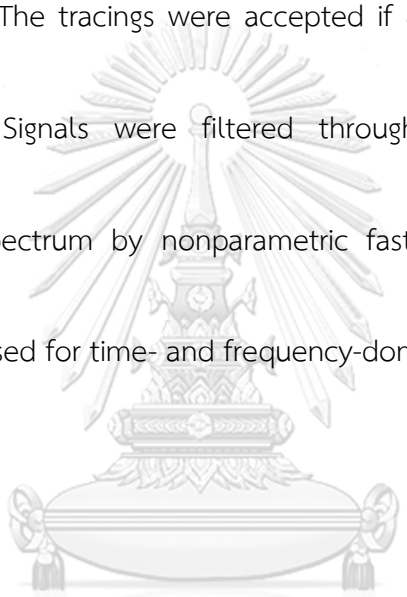


Figure 6. Study timeline.

Data analysis

The HRV were analysed using SCM-510 Holter software (Fukuda Denshi Co., Ltd., Japan). All QRS complexes from ECG were automatically analysed by the software then they were manually inspected for correct RR intervals by a single experienced operator. The tracings were accepted if 85% or more of raw R waves were normal beats. Signals were filtered through a Hamming window and transformed into a spectrum by nonparametric fast Fourier transformation. HRV parameters were analysed for time- and frequency-domains.



Frequency domain of HRV was analyzed for 4 parameters including total power (TP), low frequency (LF), high frequency (HF) and the ratio of low frequency to high frequency (LF/HF) from 512 consecutive RR intervals (Table 5).

Table 5 shows frequency-domain parameters and definitions.

Parameters	unit	Descriptions
Total power (TP)	ms^2	An absolute power of total spectrum of frequency domain (0–0.5 Hz) indicates the total amount of HRV.
Low frequency (LF)	ms^2	The LF band (0 .0 4 –0 .1 5 Hz) can be produced by sympathetic nervous system (SNS), parasympathetic nervous system (PNS) and blood pressure regulation via baroreceptors.
High frequency (HF)	ms^2	The HF or respiratory band (0.15–0.50 Hz), this band is mainly produced by the PNS.
The ratio of low frequency to high frequency (LF/HF)	-	This ratio indicates the sympathovagal balance.

Modified from Shaffer and colleagues (Shaffer and Ginsberg, 2017).

In this study, 4-time domain parameters of HRV, including NNA, SDNN, pNN50 and rMSSD were analysed. Data were calculated for 10-minute interval. The averaged results from 6 calculations were used as a surrogate for each timepoints (Table 6).

Table 6 shows time-domain parameters and definitions.

Parameters	unit	descriptions
NNA	ms	The mean an interval between consecutive normal R waves of ECG (NN intervals). This parameter indicates heart rates.
SDNN	ms	Standard deviation of NN intervals. Both SNS and PNS activity affect to SDNN. This parameter correlated with LF band power and total power.
SDNN index	ms	Mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording. Mainly indicates an autonomic influence on HR
pNN50	%	The percentage of successive normal RR intervals exceeding 50 ms. This parameter is highly correlated with PNS activity, rMSSD and HF power.
rMSSD	ms	The square root of the mean of the squares of the differences between successive normal to normal RR intervals. This parameter indicates the PNS activity along with pNN50 and HF power.

Modified from Shaffer and colleagues (Shaffer and Ginsberg, 2017).

Statistical analysis

Data were presented as mean ± SEM. Statistical analyses were performed with commercially available software. Normal distribution of continuous data was assessed with the Shapiro-wilk test. Comparisons were made for each parameter after drug administration versus baseline. One-way ANOVA with repeated measures design were used to determine the statistically significant among time-points. The Dunnett test was used as post hoc analysis.

Chapter 4 Results

1. The result of signalment, physical examination and complete blood profile of the dogs

In the current study, physical examination, complete blood count and blood chemistry profile were performed prior to the experiment. The physical examinations and blood collection were performed by single experienced veterinarian. The data were showed in the Table 7.

Table 7. The value of physical examination and hematology parameters of the beagle dogs. Data were presented as Mean ± SD

Parameters	Value	Reference range
Signalment		
Age (month)	7 ± 0	-
BW (Kg)	10.4 ± 0.3	-
Sex (female/male)	4/5	-
Physical examination		
BCS	3 ± 0	-
HR (bpm)	131 ± 23	-
Rhythm (SR/A)	9/0	-
RR (bpm)	28 ± 1	-
LS (CL/CK)	9/0	-
Blood chemistry profile		
BUN (mg/dL)	16.5 ± 0.5	7 – 26
Crea (mg/dL)	0.6 ± 0.0	0.6 – 1.4
ALT (Unit/L)	28.3 ± 0.7	4 - 91
ALP (Unit/L)	78.8 ± 2.9	3 – 60

Table 7 (cont). The value of physical examination and haematology parameters of the beagle dogs. Data were presented as Mean ± SD

Complete blood count	Value	Reference range
WBC (10^3/μL)	11.9 ± 0.4	5.4 – 15.3
Neu (%)	52 ± 1.1	51 - 84
Lymph (%)	29.1 ± 0.6	8 - 38
Mon (%)	5.8 ± 0.6	1 - 9
Eos (%)	3 ± 0.2	0 - 9
Bas (%)	0.2 ± 0.0	0 - 1
RBC (10^6/μL)	7.5 ± 0.1	5.2 - 8.06
HCT (%)	48.2 ± 0.7	29.8 – 57.5
Plat (10^3/μL)	183.5 ± 19.6	160 - 525

BW: Body weight; BCS: Body condition score; HR: Heart rate; SR: Sinus rhythm; A: arrhythmia; RR: Respiratory rate; LS: Lung sound; CL: Clear; CK: Crackle; WBC: Total white blood cells; Neu: Neutrophil; Lymph: Lymphocyte; Mon: Monocyte; Eos: Eosinophil; Bas: Basophil; RBC: Red blood cells; HCT: Haematocrit; Plat: Platelet; BUN: Blood urea nitrogen; Crea: Creatinine; ALT: Alanine transaminase; ALP: Alkaline Phosphatase. From hematology and clinical chemistry unit, Small Animal Hospital Faculty of Veterinary Science, Chulalongkorn University.

2. Effect of single bolus pimobendan on ECG parameters in healthy anesthetized dogs

In the current study, the PQ interval was gradually shortened while the HR was gradually increased after administration of intravenous pimobendan and became significantly shortened at 20 minutes ($p < 0.05$) when compared with baseline and remained significant until the end of the experiment. However, the QRS duration, QT interval and corrected QT interval were not altered through the study period (Figure 7.)

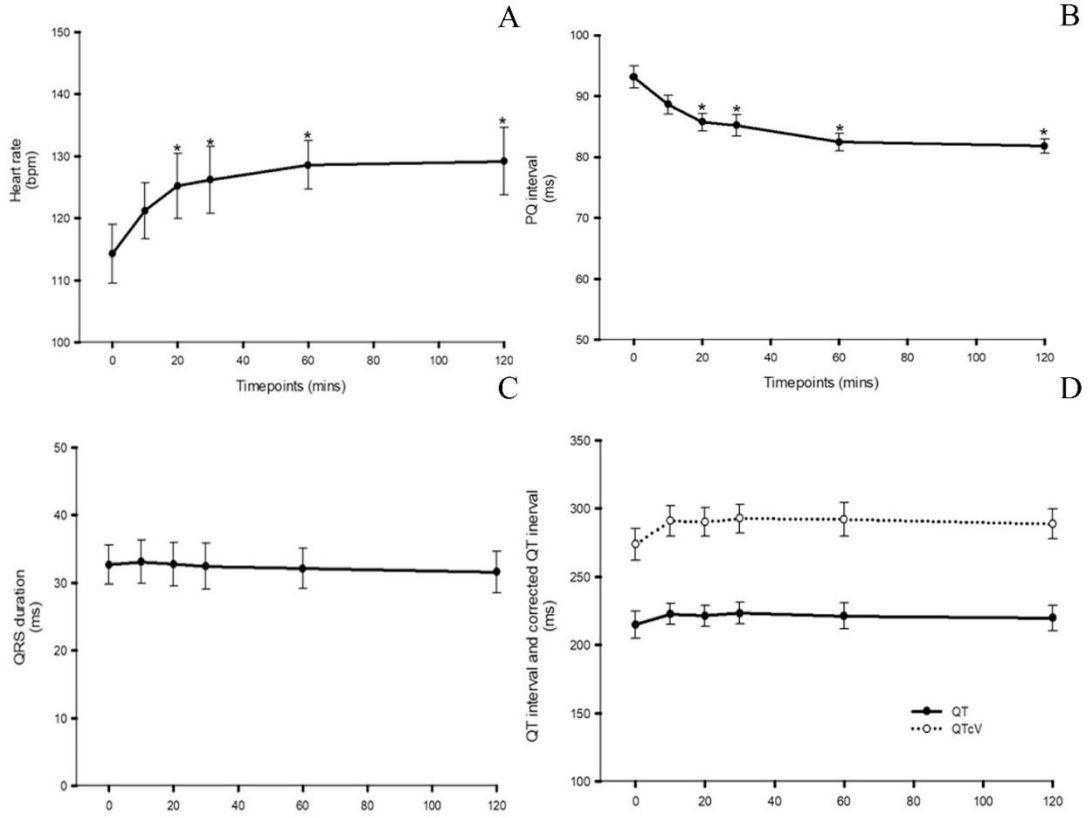


Figure 7. The effect of single bolus intravenous pimobendan at 0.15 mg/kg on electrocardiographic parameters. (A). HR, (B). PQ interval, (C). QRS duration, (D). QT interval and corrected QT interval, *: $p < 0.05$.

3. Effect of single bolus intravenous pimobendan on cardiac functions and hemodynamic parameters in healthy anesthetized dogs

Inotropic and lusitropic properties of left ventricle were inferred from parameters obtained from the left ventricular pressure (Figure 8). In response to single bolus pimobendan, dP/dt_{max} and CI were significantly increased at 10 min (43% when compared with baseline; $p < 0.01$) and 20 min after injection (39% when compared with baseline; $p < 0.05$). These inotropic parameters were continued to increase until the end of experiment. In response to single bolus pimobendan, dP/dt_{min} and Tau were decreased

significantly at 10 min after injection (-22.1% and -26.9% when compared with baseline; $p < 0.01$, respectively). These lusitropic indices were continuously decreased until the end of experiment.

Acute effects of single bolus pimobendan on hemodynamics in anesthetized dogs were shown in Figure 9 and 10. Figure 9 revealed plots of CO (A), SBP (B), SVR (C) and PVR (D) versus timepoints obtained during before and after bolus injection of pimobendan in anesthetized dogs. When dogs were given pimobendan,

CO was continuously increased from baseline, and the change became significant at 30 min (40.9% when compared with baseline; $p < 0.05$) and continued to increase until the end of experiment (77.3% when compared with baseline; $p < 0.05$). Systolic blood pressure was significantly increased at 10 min (11.6% when compared with baseline; $p < 0.01$) and continue to increase until the end of experiment (31.1% when compared with baseline). Systemic vascular resistance was gradually decreased after injection and become significantly decreased at 10 min (-9.3% when compared with baseline; $p < 0.05$) and continued to decrease until the end of experiment while PVR also gradually decreased and reached a significant level ($p < 0.05$) at 20 min (-19.8% when compared to baseline) after injection and remain significant trough the observation period. Figure 10 revealed plots of LVEDP (A), RAP (B), PAP (C) and PCWP (D) versus timepoints obtained at before and after bolus injection of pimobendan in anesthetized dogs. Notice that RAP was significantly reduced at 10 min whereas LVEDP was significantly decreased at 20 min after injection (-24.2% and -54.5% when compared with baseline, respectively; $p < 0.05$) and continue to decrease until the end of experiment. The PAP and PCWP was significantly decreased at 60 min [-7.6%

(p<0.05) and -16.4% (p<0.01), respectively] and continue to decrease at 120 min [-10.0% (p<0.05) and -22.4% (p<0.01), respectively].

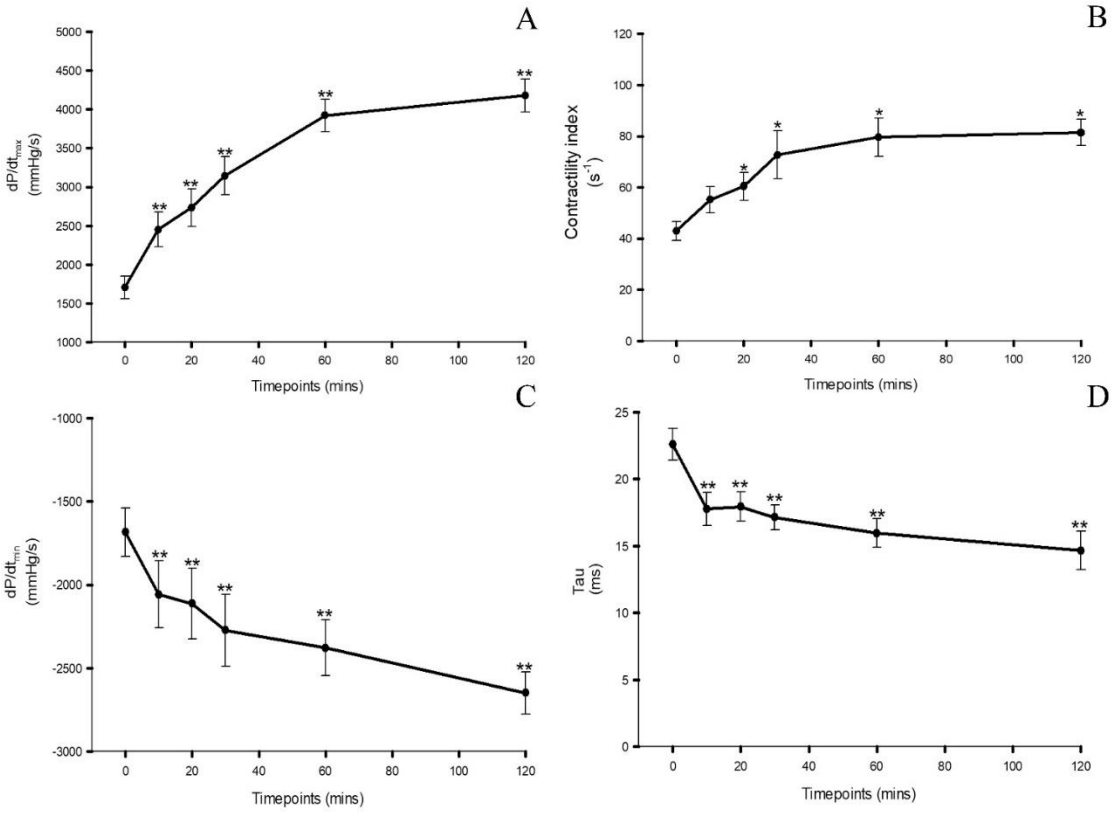


Figure 8. shows acute effects of single bolus pimobendan on left ventricular functions in anesthetized dogs. Values are presented as mean ± standard error of mean. A) $dP/dt_{max,}$ B) Contractility index, C) Tau and D) dP/dt_{min} obtained before and after bolus injection of pimobendan in anesthetized dogs, *p <0.05, **p <0.01.

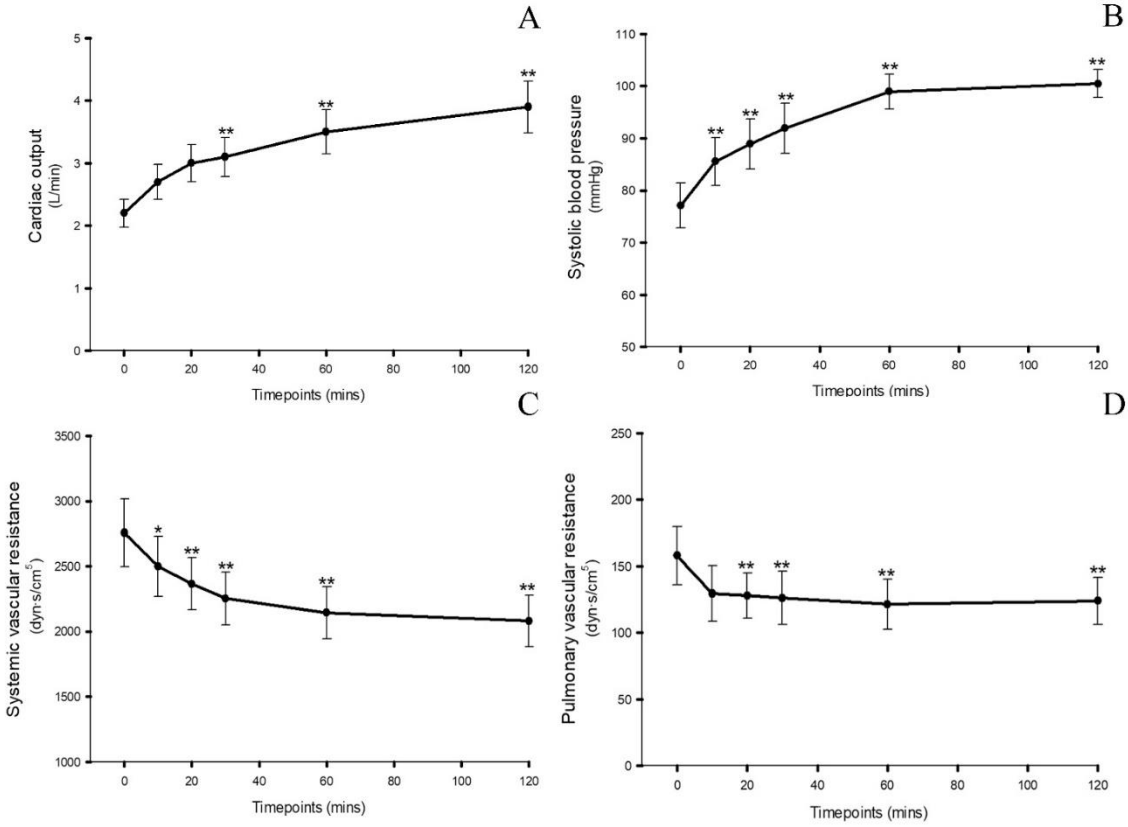


Figure 9. shows acute effects of single bolus pimobendan on hemodynamics in anesthetized dogs. Values are presented as mean ± standard error of mean. A) Cardiac output, B) Systolic blood pressure, C) Systemic vascular resistance and D) Pulmonary vascular resistance obtained before and after bolus injection of pimobendan in anesthetized dogs, *p <0.05, **p <0.01.

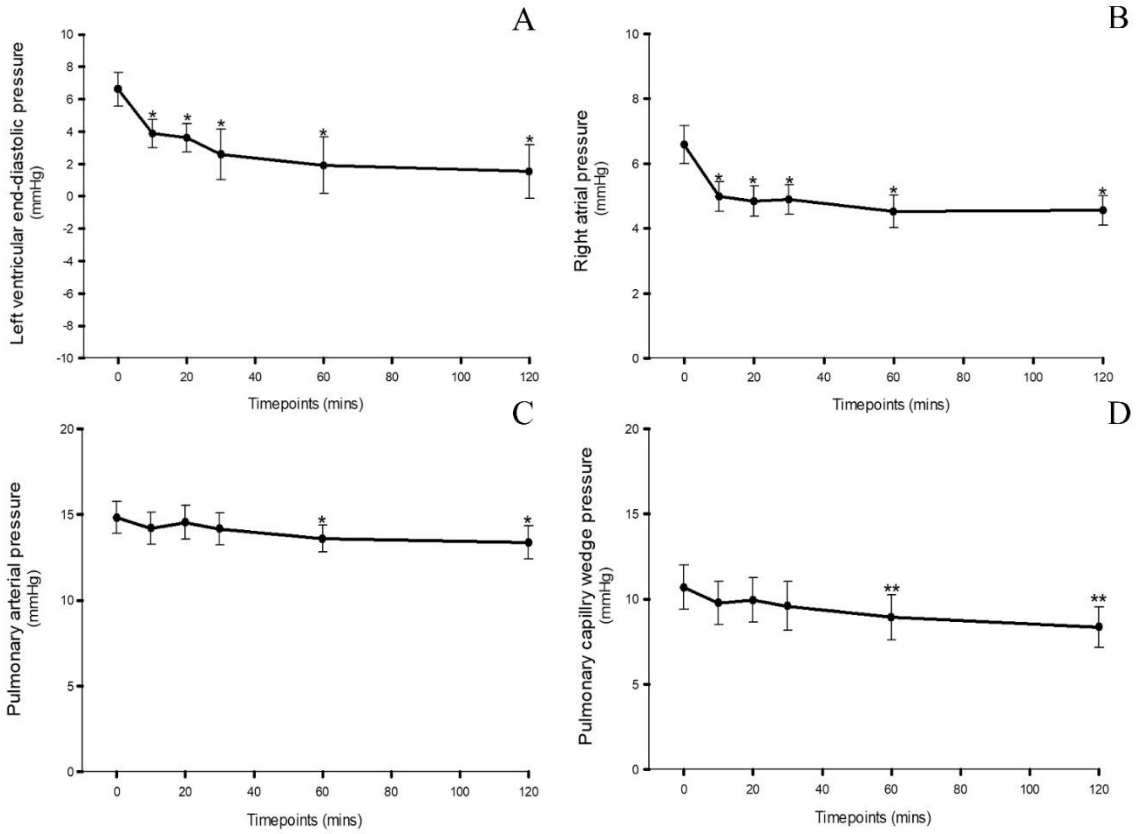


Figure 10. shows acute effects of single bolus pimobendan on hemodynamics in anesthetized dogs. Values are presented as mean ± standard error of mean. A) LVEDP, B) RAP, C) PAP and D) PCWP obtained before and after bolus injection of pimobendan in anesthetized dogs, *p <0.05, **p <0.01.

4. The pharmacokinetic properties of intravenous pimobendan and O-desmethyl pimobendan (ODMP)

In this study, the retention times of pimobendan and its active metabolite, O-desmethyl pimobendan (ODMP) were 2.120 and 1.577 mins, respectively while the retention time of the internal standard was 2.054 mins. In addition, the mass-to-charge ratio (m/z) of pimobendan, ODMP and internal standard were 335/319, 321.10/305.05 and 821.25/350.90 m/z, respectively. Furthermore, the lower limit of the LC-MS/MS to detect both pimobendan and ODMP were 0.09 μg/L. Moreover, the standard curves for pimobendan and ODMP showed a reliable linearity range from 0.09 – 100 μg/L and 0.09 -200 μg/L, respectively (R^2 > 0.99).

The mean plasma concentrations versus time profile of single bolus intravenous pimobendan and ODMP were plotted in linear (Figure 11) and semi-logarithm scale (Figure 12). The plasma concentrations of pimobendan were below lower limit of quantification (LLOQ) from 6 hours after injection while the plasma concentrations of ODMP were lower than LLOQ at 24 hours after injection of the

parent compound. The summary of pharmacokinetic profiles of both pimobendan and ODMP were presented in Table 8.

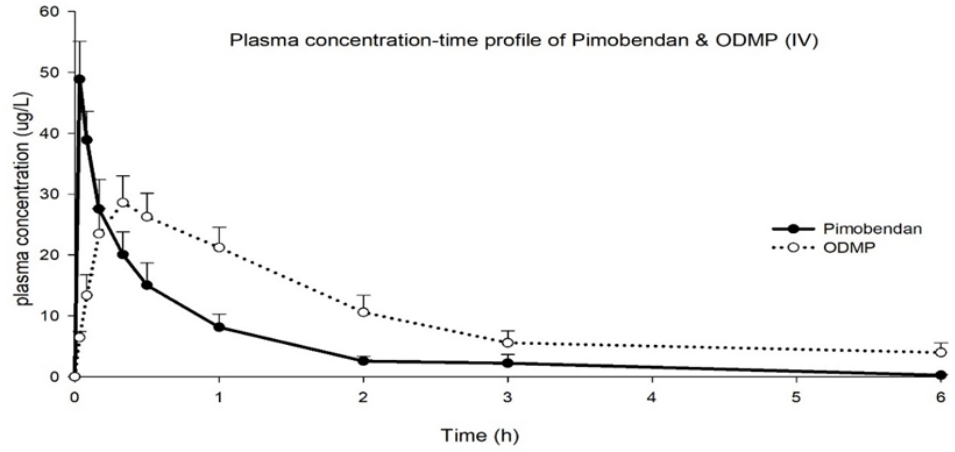


Figure 11. the linear-scale of plasma concentration-time profile of pimobendan and ODMP

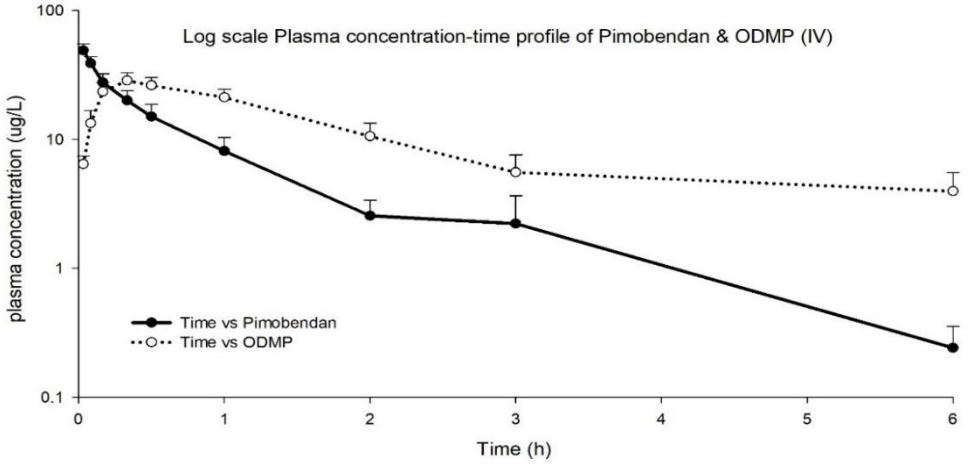


Figure 12. the semi-logarithm scale of plasma concentration-time profile of pimobendan and ODMP

Table 8. Pharmacokinetic profiles of single bolus intravenous pimobendan at 0.15 mg/kg and O-desmethyl pimobendan in healthy dogs. Data were presented as Mean ± SEM.

Parameters	Pimobendan	O-desmethyl pimobendan
C_{max} (μg/L)	83.66 ± 18.76	30 ± 3.5
T_{max} (h)	0	0.33 ± 0.05
AUC_{0-t} (μg-h/L)	30.9 ± 6.9	66.4 ± 9.58
AUC_{0-inf} (μg-h/L)	31.3 ± 6.95	88.4 ± 18.2
MRT (h)	2.2 ± 0.24	4.9 ± 0.9
Vd (L/kg)	8.9 ± 2.0	7.1 ± 0.7
CL (L/kg/h)	5.8 ± 0.9	2.2 ± 0.6
$t_{1/2}$ (h)	1.0 ± 0.2	2.8 ± 0.6

C_{max}, maximum plasma concentration; T_{max}, time to maximum concentration; AUC, area under the concentration-vs. -time curve; MRT, mean residence time; Vd, volume of distribution; CL, clearance; $t_{1/2}$, disappearance half-life.

5. Effect of single bolus intravenous pimobendan on frequency domain of heart rate variability in conscious healthy dogs

In this study, the frequency domain of heart rate variability parameters consisted of low frequency spectrum (LF), high frequency spectrum (HF), total power spectrum (TP) and the ratio of low frequency spectrum to high frequency spectrum (LF/HF). Nevertheless, through the observation period of 4 hour the LF/HF ratio did not significantly altered (Figure 13).

At 1 hour after injection, most frequency domain parameters of HRV including LF, HF and TP were slightly increased, nonetheless these differentiations did not reach the significant level.

At 2 hours after injection, LF, HF and TP were significantly increased ($p < 0.05$) when compared to the baseline. The LF, HF and TP were increased by 49%, 81% and 56%, respectively, when compared to the baseline value.

At 3 hours after injection, all frequency domain parameters of HRV significantly increased ($p < 0.05$). The LF, HF and TP were increased by 49%, 95% and 68%, respectively when compared to the baseline value.

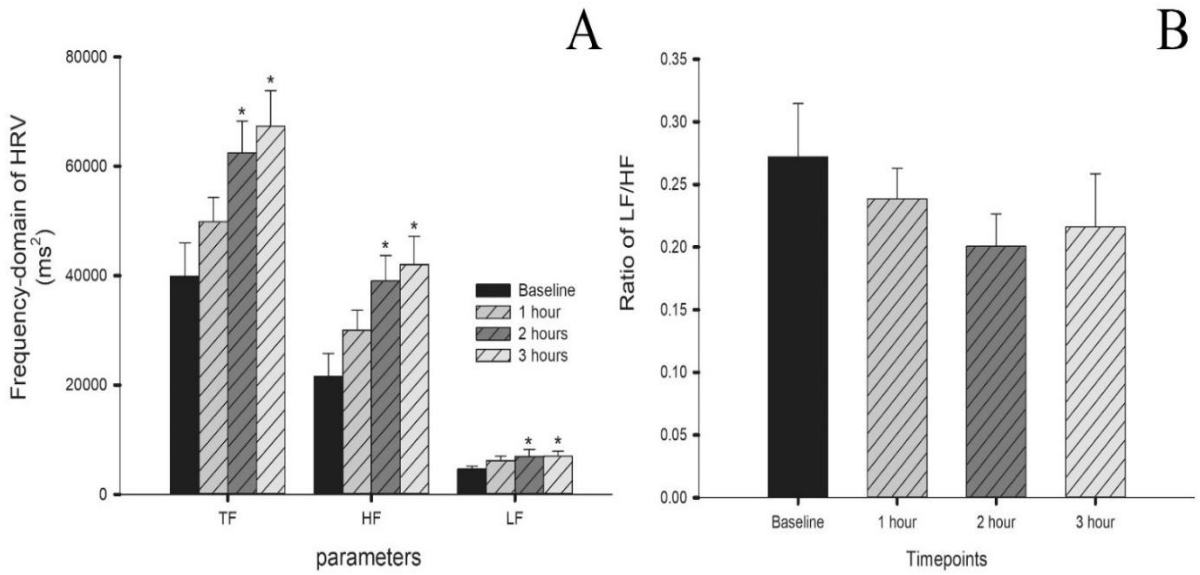


Figure 13. The effect of single bolus intravenous pimobendan at 0.15 mg/kg on the frequency-domains of heart rate variability. (A) TF: total frequency spectrum, HF: high frequency spectrum, LF: low frequency spectrum. (B) the ratio of LF to HF, *p <0.05.

6. Effect of single bolus intravenous pimobendan on time domains of heart rate variability in conscious healthy dogs

In the current study, time domain parameters included a mean of an interval between consecutive normal R waves of ECG (NNA), Standard deviation of NN intervals (SDNN), mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording (SDNN index), the percentage of successive normal RR intervals exceeding 50 ms (pNN50) and the square root of the mean of the squares of the differences between successive normal to normal RR intervals

(rMSSD). All parameters demonstrated a significant alteration after injection of intravenous pimobendan (Figure 14).

At 1 hour after injection, all time domain parameters of HRV except for rMSSD were significantly increased ($p < 0.05$) when compare to the baseline value. The NNA, SDNN, SDNN index and pNN50 were increased by 13.8%, 30.8%, 33.7% and 22.2%, respectively.

At 2 hours after administration of intravenous pimobendan, the alterations of all time-domain parameters of HRV reached a significant level ($p < 0.05$) when compared to the baseline value. NNA, SDNN, SDNN index, pNN50 and rMSSD were augmented by 12.2%, 45.4%, 38.2%, 18.5% and 30%, respectively. At the last hour of the observation period, all time-domain parameters of HRV were elevated significantly ($p < 0.05$) through the study period when compared to the baseline data. The NNA, SDNN, SDNN index, pNN50 and rMSSD were augmented by 17.9%, 37.8%, 40.5%, 20.3% and 41%, respectively.

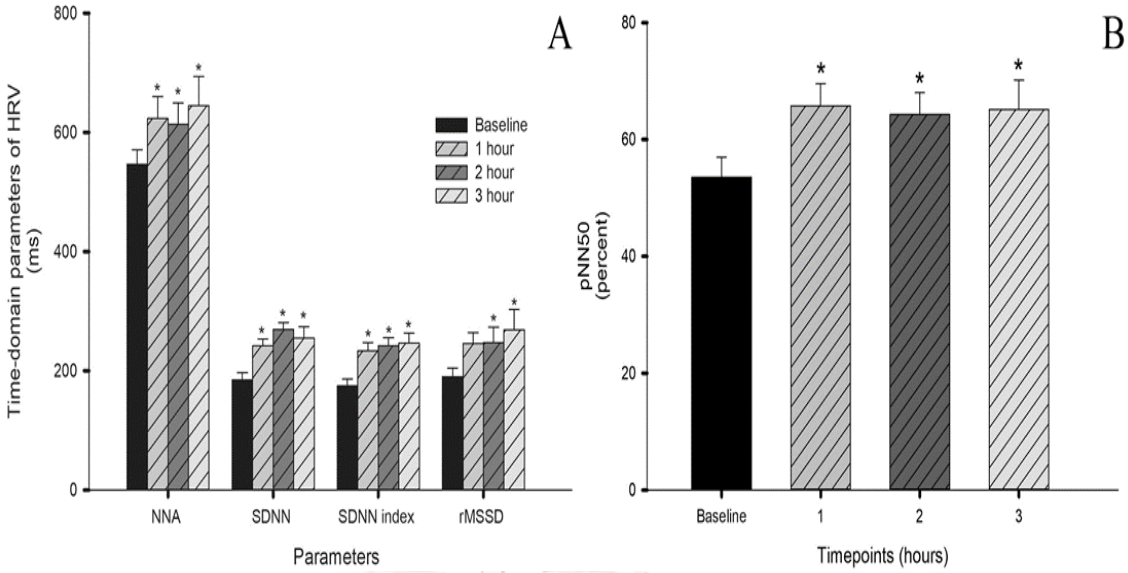


Figure 14. The effect of single bolus intravenous pimobendan at 0.15 mg/kg on the time-domain of heart rate variability. (A) NNA: a mean of an interval between consecutive normal R waves of ECG, SDNN: Standard deviation of NN intervals, SDNN index: mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording, rMSSD: square root of the mean of the squares of the differences between successive normal to normal RR intervals. (B) pNN50: the percentage of successive normal RR intervals exceeding 50 ms, *p <0.05.

Chapter 5 Discussion

signalment, physical examination and complete blood profile of the dogs

in the current study, the result of signalment, physical examination and complete blood profile of the dogs are within normal limits excepted the alkaline phosphatase, which mildly elevated. However, since this enzyme has a several sauce including biliary tract, placenta, kidney, intestine and bones (Sharma et al., 2014), it has been reported that in young adult this enzyme can be slightly elevated from the osteoclastic and osteoblastic activities (Brenten et al., 2016). Therefore, the elevation of ALP in the current study is considered as a physiological elevation, which did not affect the healthy status of the dogs.

2. the effect of intravenous pimobendan on electrocardiographic parameters in healthy anesthetized dogs

In this study, a single bolus pimobendan 0.15 mg/kg caused PQ duration shorten to values lesser than obtained during baseline while the heart rate was increased. The PQ duration, the time that impulse uses for conduction in the intra-atrium and the delay within the atrioventricular node (AV), is known to be heart rate

dependent (Malik et al., 2008; Soliman and Rautaharju, 2011). Thus, this abbreviation of PQ interval can be partly explained by the relationship between heart rate and PQ interval. Our findings on the duration of PQ interval are also consistent with previous published data in dogs (Hanton and Rabemampianina, 2006; Soloviev et al., 2006). A previous study in beagle dogs instrumented with radiotelemetry showed that PR interval demonstrates an almost linear inverse relationship with HR (Sidebotham and Doughty, 2007). In addition, the shortening of PQ duration may be attributed to activation of the L-type calcium current ($I_{Ca,L}$) by pimobendan. It has been shown previously in the rat ventricular myocytes that phosphodiesterase III (PDE-3) and phosphodiesterase IV (PDE-4) inhibitors are the dominant phosphodiesterase (PDE) subtypes that enhance $I_{Ca,L}$ (Verde et al., 1999), suggesting that acute effect of pimobendan at 0.15 mg/kg may significantly alter calcium channel. The elevating HR can be a result form an increasing of cAMP, the intracellular secondary messenger due to an inhibition of PDE-3. The increasing of cAMP could activates more hyperpolarization-activated, cyclic-nucleotide gated (HCN) and L-type calcium channel in the SA node, which responsible for funny current (I_f) and $I_{Ca,L}$, respectively

(DiFrancesco, 2010). Therefore, the slope of phase 4 of the pacemaker cell action potential would be steeper and reached the threshold easier (Larsson, 2010). As a result, the action potential of the pacemaker cells could be generated more frequent.

3. the effect of intravenous pimobendan on cardiac function and hemodynamics in healthy anesthetized dogs

Previous studies in anesthetized and conscious dogs showed that various doses of intravenous pimobendan had increased cardiac contractility as evaluated by left ventricular dP/dt_{max} (van Meel and Diederen, 1989; Pagel et al., 1996; Hori et al., 2019), slope of the LV end-systolic pressure-volume relation (Ohte et al., 1997). Similarly, the left ventricular contractility of our study was increased significantly since the first measurement timepoint as assessed by dP/dt_{max}. It has been known that the dP/dt_{max} is affected by loading conditions, HR, and myocardial contractility (Hamlin and Rio, 2012) whereas the contractility index (CI) is considered to be less load-dependent (Kijtawornrat, 2013). In our study, the dP/dt_{max} achieves statistical significance at 10 min after injection while the CI became significant later (20 min

after injection). This result was supported by the previous study in telemetry beagle which demonstrated that dP/dt_{max} is a robust, reliable and sensitive index for assessing inotropic effects of drug when the loading and heart rate are constant (Guth et al., 2015). The increase in LV contractility in our study could be explained by the inhibition of PDE-3 and the sensitization of troponin C (TnC) to intracellular calcium (Solaro et al., 1989).

The increase in LV contraction resulted in an increased CO. The systolic BP in our study is also increased. In general, SBP determined by SV, stiffness of aorta, and diastolic BP. The increased in SBP in our study may be due to an increase in SV which supported by the study of van Meael and Diederen in which the SV was increased about 59% in response to pimobendan injection (van Meel and Diederen, 1989). Since blood pressure is a product of CO and systemic vascular resistance (SVR), an elevation of CO must overcome a reduction of SVR observed in the current study. In our study, systemic vascular resistance (SVR) was remarkably decreased at 10 min. The SVR was mainly determined by the diameter of the blood vessels. Hence, pimobendane and its active metabolite have been known to possess PDE-3

inhibitory effects which may dilate the resistant vessels. The PVR was significantly reduced at 20min after injection of intravenous pimobendan. This may be a result from an increasing of CO from positive inotropic effect of the drug. Since the resistance has a reciprocal relationship with CO (Hennig, 1992).

The current study also demonstrated that pimobendan decreases LVEDP and PCWP after bolus injection. The effect of pimobendan injection on a reduction of LVEDP is consistent with previous study in anesthetized dogs in which escalating doses of pimobendan (10, 20, 40 µg/kg/min) decrease LVEDP in a dose-dependent manner (Pagel et al., 1996). In addition, the effect of pimobendan injection on a reduction of PCWP is consistent with a study in anesthetized dogs given escalating concentration of pimobendan (10, 30, 100, and 300 µg/kg) in which the PCWP was declined up to -84% at the highest dose (van Meel and Diederen, 1989). The ability of intravenous pimobendan to reduce both LVEDP and PCWP suggesting the beneficial of its use in case of congestive heart failure in which the LVEDP and PCWP are elevated due to left ventricular failure resulting in pulmonary oedema (Sidebotham and Doughty, 2007).

In this study, effect of pimobendan on cardiac relaxation was assessed by dP/dt_{min} and Tau. These indices are known to occur during isovolumetric relaxation not after ventricle had filled completely (Burkhoff et al., 2005). The left ventricular dP/dt_{min} is determined by lusitrope, reduction in heart rate, diastolic systemic arterial pressure, and structural properties of myocardium and constriction of the pericardium or pericardial effusion (Garcia et al., 2000). In response to intravenous pimobendan, dP/dt_{min} decreased (more negative) as well as the Tau. The observed change in Tau (i.e. positive lusitrope) may result from inhibitory effect of pimobendan on PDE-3 which accelerates phosphorylation of phospholamban; therefore, more calcium is resequestrated through the sarco/endoplasmic reticulum Ca^{2+}-ATPase channel resulted in speed up relaxation (Honerjäger and Nawrath, 1992). This result is also in accordance with previous studies in which Tau was significantly decreased and dP/dt_{min} was significantly increased when pimobendan was given to anesthetized dogs (Hata et al., 1992; Ohte et al., 1997).

4. the Pharmacokinetic profile of intravenous pimobendan and its active metabolite O-desmethyl pimobendan (ODMP)

Oral pimobendan has been recommended for management of CHF dogs for more than a decade (Atkins et al., 2009) and the pharmacokinetics of this drug were investigated in several species including humans, pigs, dogs and cats (Duncker et al., 1987; Böhm et al., 1991; Endoh et al., 1991; Westfall et al., 1992). However, since the intravenous injectable pimobendan was newly developed, the pharmacokinetic data of this preparation is still be limited. To the author knowledge, the pharmacokinetic profiles of pimobendan at a recommended dose from the manufacturer (0.15 mg/kg; intravenously) has not been investigated yet.

The volume of distribution (Vd) of pimobendan and ODMP in the current study are 8.9 and 7.1 L/kg. The Vd of pimobendan demonstrated in the package insert was 2.6 L/kg while the plasma binding protein is 93%. This could be due to differences in the sensitivity of instrument for detection of pimobendan and ODMP, the study design, the signalment of the dogs, the samples in each experiment. Moreover, the information obtained from our study was performed in dogs under

anesthesia for at least 2 h which may also affect to the pharmacokinetic properties of the current study. According to the package insert, the plasma elimination half-life of pimobendan is 0.4 ± 0.1 h with the clearance of 90 ± 19 mL/min/kg and a short mean residence time of 0.5 ± 0.1 h. The current study reveals the clearance of pimobendan as 5.8 ± 0.9 L/kg/h which is similar to that of package insert while the half-life of pimobendan is quite different from that of package insert. It has been known that pimobendan is a substrate for cytochrome P450 1A2 (CYP 1a2), the non-steroidal anti-inflammatory drug (NSAID) that used during surgical procedure in the current study may alter elimination duration and other pharmacokinetic parameters of pimobendan (Karjalainen et al., 2008). Furthermore, the pervious publication suggests that generalized anesthesia may prolong the time-course of pharmacokinetic parameters (Gambús and Trocóniz, 2015).

In the current study, the hemodynamics and cardiac function mainly altered at 10-20 mins after injection. Our result was similar to a previous publication in humans, which the cardiovascular effect of pimobendan is poorly related with the

plasma concentration of the drug (Hagemeijer et al., 1989). On the other hand, it has been reported that in healthy beagle dogs, ODMP, an active metabolite of pimobendan achieved its maximal plasma concentration in 15 mins after injection of the parent compound (Schneider et al., 1997). Accordingly, the cardiovascular effect of intravenous pimobendan that had been observed in our study could be the effect of the metabolite compound rather than the parent drug. Additionally, several previous publications suggested that ODMP is more potent as PDE-3i than the pimobendan in several species including human, pig, guinea pig and dogs (Duncker et al., 1987; Böhm et al., 1991; Endoh et al., 1991; Westfall et al., 1992). Even though, the parent drug possessed calcium sensitizing effect, which is a great advantage in CHF cases (Hanzlicek et al., 2012). Moreover, it has been proved that in myocyte form the failing human heart, the positive inotropic effect of pimobendan is still intact whereas the positive inotropic effect of ODMP is blunted (Böhm et al., 1991). Identically, in dogs with pacing-induced heart failure, pimobendan also increases cardiac contractility while the effect of ODMP on cardiac contraction is diminished (van Meel and Diederen, 1989).

5. the effect of intravenous pimobendan on heart rate variability in healthy conscious dogs

To our knowledge, this is the first study that assessed the acute effect of single bolus pimobendan on both time- and frequency-domain indices of HRV in conscious, healthy dogs. The significant finding of the current study is that intravenous pimobendan enhances HRV. The improved parasympathetic modulation in conscious dogs in the present study supported by increased SDNN, SDNN index, pNN50 and rMSSD of time domains parameters and LF, HF and TP values of frequency domains parameters of HRV. The HF reflects predominantly parasympathetic activity while the LF represents both sympathetic and parasympathetic tones. These changes of frequency domain indices were correlated with the time domain parameters. The increase in SDNN, a parameter that correlated with TP, reflects an enhancement of standard deviation of the NN interval implying an augmentation of parasympathetic tone. In the present study, pNN50 and rMSSD was significantly increased in dogs after bolus pimobendan injection which related to

increased HF suggesting the withdrawal of sympathetic tone and/or enhancement of parasympathetic tone in those dogs.

In veterinary medicine, several studies in dogs with MMVD demonstrated that MMVD dogs have impaired cardiac autonomic activities (i.e. increased sympathetic tone, decreased parasympathetic tone) (Haggstrom et al., 1996; Oliveira et al., 2012; Rasmussen et al., 2012; Oliveira et al., 2014; Pirintr et al., 2017). This imbalance of ANS was continuously observed in the advanced stage of CHF (Oliveira et al., 2014). This could be implied that intravenous pimobendan in CHF dogs are not only improved cardiac function and hemodynamics but also improved cardiac autonomic nervous system which beneficial to the patients.

6. Limitation

Since it has been reported that a general anesthesia could be lethal for the CHF patients due to the cardiac and respiratory depression (Saraswat, 2015), the healthy dogs were used as a subject of the experiment. Therefore, the result in the current investigation may differ from the clinical settings.

Previous study suggests that in heart failure dogs, the cAMP mediated effect of the drug and its active metabolite is diminished (Böhm et al., 1991), which can be explained by a reduction of cAMP production from a desensitizing of β1- adrenergic receptor (Lohse et al., 1996). Furthermore, it has been reported that the ration of β1 and β2 – adrenergic receptor is reduced from 75%/25% in healthy heart to 50%/50% in failing heart (Brodde et al., 1986). Since it has been known that the β2 – adrenergic receptor activates both stimulate G-protein and inhibitory G-protein (Kilts et al., 2000), an increasing of β2 – adrenergic receptor may decrease a cAMP production and may leads to the diminished effect of the drug. Nevertheless, pimobendan is both calcium sensitizer and PDE-3i, the effects of this drug on PDE3 inhibition may diminished but the calcium sensitizing effect is still intact. In conclusion, in CHF dogs, pimobendan may depends on its calcium sensitizing effect rather than PDE-3i effects. The result in healthy canine model may differ from the CHF dogs in cAMP mediate effects.

Chapter 6 Conclusion

In summary, our investigation provides a novel information of the effect of a single bolus intravenous pimobendan and its metabolite at recommended dose in healthy dogs. Firstly, we demonstrate the effect of the drug on cardiac functions and hemodynamics in anesthetized healthy dogs. The drug improves both systolic and diastolic function of the heart, which can be inferred by dP/dt_{max}, CI, dP/dt_{min}, and Tau, respectively. Interestingly, pimobendan also reduces both SVR and PVR and enhances forward flow, which can be indicated by an increasing of CO.

Furthermore, our study not only reveals a novel information of pharmacokinetics profile of pimobendan but also the pharmacokinetic profile of ODMP, an active metabolite of pimobendan. The result shows that pharmacokinetic profile of the parent drugs is more concise than its metabolite. For example, pimobendan achieved its C_{max} immediately after injection and had a mean $t_{1/2}$ of 1.06 h whereas the metabolite achieved its C_{max} at 20 mins after injection of the parent drug with a mean $t_{1/2}$ of 2.81 h.

Additionally, our study provides a novel information of the effect of single bolus intravenous pimobendan on HRV in conscious healthy dogs. Our result indicates that pimobendan increases both frequency-domain and time-domain of HRV. This evidence shows that pimobendan increases an activity of autonomic innervation on cardiac tissue. In addition, the result also suggested that pimobendan tend to increase a parasympathetic branch of ANS more than a sympathetic branch.

Taken together, our acquired data informs that single bolus intravenous pimobendan enhances both cardiac functions and hemodynamic, which can be observed mainly at the time that ODMP achieved its C_{max}. Moreover, pimobendan also increase cardiac autonomic tone especially parasympathetic tone. Therefore, these effect of single bolus intravenous pimobendan at 0.15 mg/kg should be a great benefit in CHF patient. Nevertheless, more research is still needed to clarify the effect of this drug in the CHF dogs in the clinical setting.

Appendices

The effect of intravenous pimobendan in anesthethized healthy dogs

The effect of a single bolus intravenous pimobendan at 0.15mg/kg in ECG parameter, cardiac function, and hemodynamics shown in table 9, 10, and 11, respectively.

Table 9. the effect of intravenous pimobendan at 0.15 mg/kg on the electrocardiographic parameters. Data were presented as Mean ± SEM

ECG parameters	Baseline	10 min	20 min	30 min	60 min	120 min
PQ (ms)	93 ± 1.8	89 ± 1.5	86 ± 1.5*	85 ± 1.8*	82 ± 1.6*	82 ± 1.3*
QRS (ms)	32.7 ± 2.8	33.1 ± 3.2	32.8 ± 3.3	32.5 ± 3.3	32.1 ± 2.9	31.6 ± 3.0
QT (ms)	215 ± 9.9	223 ± 7.6	221 ± 7.5	223 ± 8.0	221 ± 9.4	220 ± 9.3
QTc (ms)	274 ± 11.5	291 ± 11	290 ± 10	293 ± 10	292 ± 12	289 ± 11
HR (bpm)	114 ± 4.7	121 ± 4.5	125 ± 5.2*	126 ± 5.3*	129 ± 3.8*	128 ±5.9*

PQ: PQ interval, QRS: QRS duration, QT: QT interval, QTc: corrected QT interval (Van der water's method), ms: millisecond, bpm: beat per minute, *: $p < 0.05$.

Table 10. the effect of single bolus intravenous pimobendan on LV parameters and cardiac functions in anesthetized healthy dogs. Data are presented as Mean ± SEM

parameters	Baseline	10 min	20 min	30 min	60 min	120 min
LVESP (mmHg)	77 ± 4.3	86 ±4.6**	89 ± 4.7**	92 ± 4.8**	99 ± 3.3**	101 ± 2.6**
LVEDP (mmHg)	6.6 ± 1	3.9 ± 0.8	3.6 ± 0.8*	2.6 ± 1.5*	1.9 ± 1.7*	1.5 ± 1.6*
dP/dt_{max} (mmHg/s)	1,708 ± 144	2,450 ± 223**	2,734 ± 241**	3,146 ± 243**	3,923 ± 209**	4,178 ± 210**
dP/dt_{min} (mmHg/s)	-1,685 ± 145	-2,057 ± 200**	-2,113 ± 213**	-2,272 ± 215**	-2,379 ± 167**	-2,648 ± 125**
CI (s^1)	43 ± 3.6	55 ± 5.1	60 ± 5.4*	73 ± 9.3*	80 ± 7.5*	81 ± 5.1*
$Tau_{(/e)}$ (ms)	22.6 ± 1.1	17.8 ± 1.2**	17.9 ± 1.0**	17.1 ± 0.9**	16 ± 1.0**	14.7 ± 1.4**
CO (L)	2.2 ± 0.2	2.7 ± 0.3	3.0 ± 0.3	3.1 ± 0.3*	3.5 ± 0.4*	3.9 ± 0.4*

LVESP: Left ventricular end-systolic pressure, LVEDP: Left ventricular end-diastolic pressure, dP/dt_{max}: maximum rate of rise of the left ventricular pressure, dP/dt_{min}: the maximum rate of fall of the left ventricular pressure, CI: contractility index, $Tau_{(/e)}$: left ventricular relaxation time constant, CO: cardiac output, *: p-value <0.05, **: p-value <0.01.

Table.11 the effect of single bolus intravenous pimobendan on hemodynamic parameters in anesthetized healthy dogs. Data are presented as Mean ± SEM

parameters	Baseline	10 min	20 min	30 min	60 min	120 min
RAP (mmHg)	6.6 ± 0.6	5.0 ± 0.4*	4.8 ± 0.4*	4.9 ± 0.4*	4.5 ± 0.5*	4.6 ± 0.4*
PAP (mmHg)	17 ± 2.3	16.1 ± 2.1	16.6 ± 2.2	16.2 ± 2.2	15.7 ± 2.2*	15.3 ± 2.1*
PCWP (mmHg)	10.7 ± 1.3	9.7 ± 1.2	9.9 ± 1.3	9.5 ± 1.4	8.94 ± 1.3*	8.3 ± 1.2*
SVR (dynes - s/cm^{-5})	2,756 ± 118	2,499 ± 103*	2,365 ± 89**	2,253 ± 90**	2,143 ± 90**	2,079 ± 89**
PVR (dynes-s/cm^{-5})	166 ± 24.4	144 ± 19.9	133 ± 19.4*	127 ± 23.7*	117 ± 22*	114 ± 18*

RAP: Right atrial pressure, PAP: pulmonary arterial pressure, PCWP: pulmonary capillary wedge pressure, SVR: systemic vascular resistance, PVR: pulmonic vascular resistance. *: p-value <0.05, **: p-value <0.01.

Calculation of pharmacokinetic parameters

In part one, the pharmacokinetic parameters of intravenous pimobendan and its active metabolite were calculated according to the following equations by PK solution 2.0 (Figure 15)

$$AUC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i + C_{i+1})$$

$$V = \frac{FD}{AUC_{\infty}\lambda_z}$$

$$AUC_{\infty} = AUC_{(0-t)} + \frac{C_n}{\lambda_z}$$

$$CL = \frac{FD}{AUC_{\infty}}$$

$$t_{½} = \frac{0.693 \cdot V}{CL}$$

$$MRT = \frac{AUMC_{\infty}}{AUC_{\infty}}$$

Figure 15. the equation of pharmacokinetic parameters in this study. t: time points, C: concentration of the drug, λ_z: Elimination rate constant, $AUC_{(0-t)}$: Trapezoid calculation of AUC using observed data points only, AUC_{∞}: Total AUC computed by combining $AUC_{(0-t)}$ with an extrapolated value, MRT: Mean Residence Time calculated using trapezoid area calculations extrapolated to infinity, $AUMC_{\infty}$: calculation of total

area under the first moment curve (plot of C•t vs t) by combining trapezoid calculation of AUMC(0-t) and extrapolated area. V: volume of distribution, CL: Systemic clearance based on trapezoid AUC_{∞}, $t_{1/2}$: Half-life based on Vd and CL. Modified from PK Solutions 2.0 User Guide.

The effects of intravenous pimobendan on HRV in conscious healthy dogs

The effect of single bolus intravenous pimobendan at 0.15 mg/kg on frequency-domain and time-domain of HRV in conscious healthy dogs are shown in table 12 and 13, respectively.

Table 12. the effect of single bolus intravenous pimobendan on frequency-domain parameters of HRV in conscious healthy dogs. Data are presented as Mean ± SEM.

Parameters	Baseline	1 hour	2 hours	3 hours
TP (ms^2)	39,901 ± 2,178	49,841 ± 1,587	62,491 ± 2,068*	67,294 ± 2,324*
LF (ms^2)	4,651 ± 190	6,189 ± 278	6,934 ± 465*	6,965 ± 330*
HF (ms^2)	21,520 ± 1,516	30,056 ± 1,282	39,082 ± 1,656*	42,019 ± 1,840*
LF/HF	0.27 ± 0.02	0.24 ± 0.01	0.2 ± 0.01	0.22 ± 0.02

TP: total power spectrum, LF: low frequency spectrum, HF: high frequency spectrum, and LF/HF: the ratio of low frequency to high frequency, *: p-value <0.05

Table 13. the effect of single bolus intravenous pimobendan on time-domain parameters of HRV in conscious healthy dogs. Data are presented as Mean ± SEM.

Parameters	Baseline	1 hour	2 hours	3 hours
NNA (ms)	547 ± 24	623 ± 37*	614 ± 35*	645 ± 49*
SDNN (ms)	185 ± 12	242 ± 12*	269 ± 12*	255 ± 19*
SDNN index (ms)	175 ± 11	234 ± 14*	242 ± 13*	246 ± 17*
pNN50 (%)	54 ± 3	66 ± 4*	64 ± 4*	65 ± 5*
rMSSD (ms)	190 ± 15	245 ± 19	247 ± 26*	268 ± 34*

NNA: mean of an interval between consecutive normal R waves of ECG, SDNN: standard deviation of NN intervals, SDNN Index: mean of the standard deviations of all the NN intervals for each 5 min segment of a 24 h HRV recording, pNN50: the percentage of successive normal RR intervals exceeding 50 ms, rMSSD: the square root of the mean of the squares of the differences between successive normal to normal RR intervals, * p-value <0.05

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