ศักยภาพของยาโดรนดาโรนในการควบคุมการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่นในสุนัข

นางสาวนรรฆวี แสงกลับ



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาสรีรวิทยาการสัตว์ ภาควิชาสรีรวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

# POTENTIAL FOR DRONEDARONE TO CONTROL ATRIAL FIBRILLATION IN DOGS

Miss Nakkawee Saengklub


A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Animal Physiology
Department of Veterinary Physiology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2015

| | |
|---|---|
| Thesis Title | POTENTIAL FOR DRONEDARONE TO CONTROL ATRIAL FIBRILLATION IN DOGS |
| By | Miss Nakkawee Saengklub |
| Field of Study | Animal Physiology |
| Thesis Advisor | Assistant Professor Dr. Anusak Kijtawornrat, D.V.M., Ph.D. |

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Veterinary Science
(Professor Dr. Roongroje Thanawongnuwech, D.V.M., M.Sc., Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Dr. Sarinee Kalandakanond thongsong, D.V.M., Ph.D.)

.....Thesis Advisor
(Assistant Professor Dr. Anusak Kijtawornrat, D.V.M., Ph.D.)

.....Examiner
(Assistant Professor Dr. Suwanakiet Sawangkoon, D.V.M., Ph.D.)

.....Examiner
(Assistant Professor Dr. Sirilak Surachetpong, D.V.M., Ph.D.)

.....External Examiner
(Dr. Soontaree Petchdee, D.V.M., Ph.D.)

นรรฆวี แสงกลับ : ศักยภาพของยาโดรนดาโรนในการควบคุมการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่นในสุนัข (POTENTIAL FOR DRONEDARONE TO CONTROL ATRIAL FIBRILLATION IN DOGS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. น.สพ. ดร. อนุศักดิ์ กิจถาวรรัตน์, 108 หน้า.

การศึกษานี้มีสมมุติฐาน คือ ยาโดรนดาโรนเปลี่ยนแปลงคุณสมบัติสรีรวิทยาทางไฟฟ้าของหัวใจห้องบน ส่งผลในการป้องกันการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่น โดยไม่มีผลอันไม่พึงประสงค์ต่อการบีบตัวและการคลายตัวของหัวใจในโมเดลสุนัขที่เหนี่ยวนำให้เกิดภาวะนี้ เพื่อทดสอบสมมุติฐานดังกล่าว จึงทำการแบ่งการศึกษาออกเป็น 3 ส่วน โดยส่วนที่ 1 ได้ทำการศึกษาผลแบบเฉียบพลันของการให้ยาโดรนดาโรนในขนาดต่างๆ (ขนาด 0.5 1.0 และ 2.5 มก./กก. ทางหลอดเลือดดำเป็นเวลา 15 นาทีในแต่ละขนาด) ต่อคลื่นไฟฟ้าหัวใจ โลหิตพลศาสตร์และการทำงานของหัวใจในสุนัขสุขภาพดีที่ได้รับการวางยาสลบ พบว่าพารามิเตอร์ต่างๆ ไม่มีการเปลี่ยนแปลงในกลุ่มที่ให้สื่อของยา ยาโดรนดาโรนขนาด 2.5 มก./กก. ทำให้ระยะพีคิวยืดยาวออกอย่างมีนัยสำคัญทางสถิติ ($P<0.01$) ปริมาตรเลือดที่ออกจากหัวใจใน 1 นาทีลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.01$) และแรงต้านทานการไหลของหลอดเลือดในระบบเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.01$) ยาโดรนดาโรนทำให้การบีบตัวของหัวใจแย่ลงและประสิทธิภาพการคลายตัวลดลง ขนาดของยาที่ให้เข้าหลอดเลือดได้ถูกนำไปประเมินขนาดของยาที่จะให้โดยการป้อนทางปาก ในการศึกษาส่วนที่ 2 ได้ทำการศึกษาผลแบบเรื้อรังของการป้อนยาโดรนดาโรนในขนาด 20 มก./กก. วันละ 2 ครั้ง ที่มีต่อการบีบตัวและการคลายตัวของหัวใจ ความดันโลหิต และค่าคลื่นไฟฟ้าหัวใจในสุนัขสุขภาพดีที่ฝังเทเลเมทรี และอุปกรณ์อื่นๆ ที่ใช้ในการวัดความดันโลหิตและปริมาตรของโลหิตในหัวใจ พบว่ายาโดรนดาโรนไม่มีผลต่อการบีบตัวและการคลายตัวของหัวใจ ในขณะที่การยืดยาวออกของระยะพีคิวเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.001$) และความดันโลหิตลดลงอย่างมีนัยสำคัญ ($P<0.05$) การศึกษาส่วนที่ 3 ทำการศึกษาประสิทธิภาพของยาโดรนดาโรนในการลดการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่นในโมเดลสุนัขที่เหนี่ยวนำให้เกิดภาวะนี้ ด้วยการกระตุ้นหัวใจด้วยไฟฟ้า (20 โวลต์ 40 เฮิรตซ์) ที่บริเวณหัวใจห้องบนขวาอย่างต่อเนื่อง ร่วมกับการให้ยาฟีนิลเอฟรีนขนาด 2 ไมโครกรัม/กก./นาที เป็นเวลา 20 นาที เมื่อสุนัขฟื้น ป้อนยาโดรนดาโรนขนาด 20 มก./กก. วันละ 2 ครั้ง เป็นเวลา 7 วัน และทำการวางยาสลบเพื่อกระตุ้นให้เกิดภาวะดังกล่าวอีกครั้ง พบว่าภายหลังการให้ยาโดรนดาโรน ระยะเออีอาร์พีได้ยืดยาวออกมากกว่าระยะเอพีดีทำให้เกิดพีอาร์อาร์ นอกจากนี้ระยะของการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่นนั้นลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) สรุปได้ว่า การป้อนยาโดรนดาโรนในขนาด 20 มก./กก. วันละ 2 ครั้ง ในสุนัขที่ไม่ได้รับการวางยาสลบ ทำให้การส่งสัญญาณในหัวใจช้าลง และความดันโลหิตลดลง ในขณะที่การให้ยาโดรนดาโรนขนาดสูง เข้าทางหลอดเลือดดำ ทำให้การบีบตัวและการคลายตัวของหัวใจลดลง นอกจากนี้การป้อนยาโดรนดาโรนสามารถลดระยะเวลาการเกิดภาวะหัวใจห้องบนเต้นแบบฟิบริลเลชั่นได้โดยกลไกที่เรียกว่า พีอาร์อาร์

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558

# # 5475403431 : MAJOR ANIMAL PHYSIOLOGY
KEYWORDS: ATRIAL FIBRILLATION / CONTRACTION / DOG / DRONEDARONE / POST-REPOLARIZATION REFRACTORINESS

NAKKAWEE SAENGKLUB: POTENTIAL FOR DRONEDARONE TO CONTROL ATRIAL FIBRILLATION IN DOGS. ADVISOR: ASST. PROF. DR. ANUSAK KIJTAWORNRAT, D.V.M., Ph.D., 108 pp.

The present study was hypothesized that dronedarone changed electrophysiological properties of atria resulted in prevention of atrial fibrillation (AF) without adverse effects on inotropy and lusitropy in dog model of sustained AF. In order to test the hypothesis, this study was divided into 3 parts. The aim of part 1 was to determine the acute effects of escalating concentrations of dronedarone (0.5, 1.0 and 2.5 mg/kg, 15 min for each dose) on electrocardiograms (ECG), hemodynamics and cardiac mechanics in healthy anesthetized dogs. All parameters in vehicle-treated dogs were unaltered. Dronedarone at 2.5 mg/kg significantly lengthened PQ interval ($P<0.01$), reduced cardiac output (CO) ($P<0.01$) and increased systemic vascular resistance ($P<0.01$). Dronedarone also produced negative inotropy and negative lusitropy. Then, the intravenous doses were extrapolated to the oral doses. Part 2 was designed to evaluate the chronic effects of oral dronedarone (20 mg/kg, BID) on cardiac inotropy and lusitropy, blood pressure (BP), and ECG in conscious, healthy dogs instrumented with telemetry units and sono-micrometry crystals to obtain left ventricular pressure-volume relationship, BP and ECG. The results showed that dronedarone had no effect on inotropy and lusitropy while it significantly lengthened PQ interval ($P<0.001$) and lowered MBP ($P<0.05$). Part 3 was intended to assess efficacy of dronedarone on attenuation AF duration in a canine model of sustained AF induced by rapid right atrial pacing (20 V, 40 Hz) simultaneously with infusion of phenylephrine (2μg/kg/min, intravenously) for 20 min. The duration of sustained AF was recorded and the animals were allowed to recover. Dronedarone was given at a dose of 20 mg/kg, BID, PO for 7 days. On the last day, dogs were anesthetized again to record action potential duration (APD) of atrium and atrial effective refractory period (AERP). Then, the AF was induced with the similar procedure. After dronedarone administration, the AERP was significantly lengthened more than APD's, developing a post-repolarization refractoriness (PRR). The duration of sustained AF was also significantly attenuated after receiving dronedarone ($P<0.05$). In conclusion, short-term oral dronedarone administration (20 mg/kg, BID, 7 days) produced negative dromotropy and induced hypotension in conscious dogs while the highest dose of intravenous dronedarone induced negative inotropy and lusitropy. Furthermore, short-term oral dronedarone effectively attenuates duration of AF supported by PRR mechanism.

Department: Veterinary Physiology
Field of Study: Animal Physiology
Academic Year: 2015

Student's Signature .....
Advisor's Signature .....

# ACKNOWLEDGEMENTS

This dissertation could not be successfully completed without the kindness of my advisor. I would like to express my sincere and the deepest appreciation to my kind advisor, Asst. Prof. Dr. Anusak Kijtawornrat, who has the attitude and the substance of a genius of teaching and researching. I am deeply grateful to him for his understanding, encouragement, personal guidance, patience, tolerance and persistent help to me. He consistently motivates me into the researcher path and gives me an opportunity for scholarships. Moreover, he is also inspire me to be a qualified instructor.

I would like to express my sincere thanks to the chairman, Assoc. Prof. Dr. Sarinee Kalandakanond-Thongsong and my thesis committee, Asst. Prof. Dr. Suwanakiet Sawangkoon, Asst. Prof. Dr. Sirilak Surachetpong and Dr. Soontaree Petchdee, for their valuable comments, suggestions and corrections of this dissertation.

I sincerely thank to all teachers in the program of Animal Physiology, Faculty of Veterinary Science, Chulalongkorn University for all their kindness. Moreover, I would like to thanks staffs in the department of Veterinary Physiology for all their help in providing facilities and materials for my study and experiment. I would like to special thanks for Drs. Carlos del Rio and Yukie Ueyama at QTest Labs for their help with equipment set up, and also thanks to Dr. Youngblood and his surgical team for implantation of complicated instrument.

My graduation would not be achieved without best wish from my beloved family, Mr. Saksit, Mrs. Amporn and Dr. Ittisak Saengklub, who help me for everything and always gives me greatest love, willpower and financial support until this study completion. And the last gratefully special thanks to my sincere friends for their help and encouragement.

In addition, I would like to thanks all animals who sacrificed their life for my study.

Finally, my thanks go to the financial support from Ratchadaphisek Sompot endowment fund from Chulalongkorn University (the 72th and 90th Anniversary of Chulalongkorn University Fund (GCUGR1125572059D); Special task Force for Activating Research (GSTAR 56-008-31-001)) and Qtest Labs, LLC. I am most grateful to partly support by Graduate School, Chulalongkorn University to gain a wonderful experience both in research and advance education at QTest labs, USA.

**CONTENTS**

Page

Page

Page

Page

# LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AERP | Atrial effective refractory period |
| AF | Atrial fibrillation |
| AoP | Aortic pressure or arterial blood pressure |
| $APD_{70}$ | Action potential duration at 70 % repolarization of atrial action potential |
| $APD_{90}$ | Action potential duration at 90 % repolarization of atrial action potential |
| BID | Twice a day |
| bpm | Beats per minute |
| CAVB | Complete atrioventricular block |
| CI | Contractility index |
| CO | Cardiac output |
| CV | Conduction velocity |
| DADs | Delayed afterdepolarizations |
| DBP | Diastolic blood pressure |
| DCM | Dilated cardiomyopathy |
| $dP/dt_{max}$ | Maximal rate of rise of the left ventricular pressure during isovolumetric contraction |
| $dP/dt_{min}$ | Maximal rate of fall of the left ventricular pressure during isovolumetric relaxation |
| EA | Effective arterial elastance |
| EADs | Early afterdepolarizations |
| ECGs | Electrocardiograms |
| EDPVR | End-diastolic pressure-volume relationship |
| ERP | Effective refractory period |
| ESPVR | End-systolic pressure-volume relationship |
| HF | Heart failure |
| HR | Heart rate |

| | |
|---|---|
| hr | Hour(s) |
| $I_{Ca,L}$ | Voltage-gated L-type calcium channel |
| $I_{K.Ach}$ | Acetylcholine-activated inward rectifier potassium current |
| $I_{Kr}$ | Rapid component of delayed rectifier potassium channel |
| $I_{KUR}$ | Ultrarapid delayed rectified potassium current |
| $I_{Na}$ | Sodium current or voltage-gated sodium channel |
| IV | Intravenous administration |
| kg | Kilogram(s) |
| l | Liter(s) |
| LV | Left ventricle |
| LVP | Left ventricular pressure |
| MBP | Mean blood pressure |
| µg | Microgram(s) |
| mg | Milligram(s) |
| min | Minute(s) |
| ms | Millisecond(s) |
| P | Pressure at that point |
| PAP | Pulmonary arterial pressure |
| PE | Phenylephrine |
| $P_i$ | Pressure from the inlet of the vessel |
| $P_o$ | Pressure from the outlet of the vessel |
| PO | Oral administration |
| PRR | Post-repolarization refractoriness |
| PRSW | Preload recruitable stroke work |
| PV | Pressure-volume |
| PVA | Pressure-volume area |
| PVL | Pressure-volume loop relationship |
| PVR | Pulmonary vascular resistance |

| | |
|---|---|
| Q | Flow |
| QOL | Quality of life |
| QTc | Corrected QT interval |
| R | Resistance |
| RAP | Right atrial pressure |
| SBP | Systolic blood pressure |
| sec, s | second(s) |
| SV | Stroke volume |
| SVR | Systemic vascular resistance |
| SW | Stroke work |
| Tau | Isovolumic relaxation time constant |
| WL | Wavelength |

# CHAPTER I
# INTRODUCTION

Atrial fibrillation (AF) is a supraventricular arrhythmia characterized by irregularly rapid atrial activity. Consequently, this arrhythmia may lead to decrease cardiac output (CO) and impair mechanical function of the heart and quality of life (QOL) due to inadequate ventricular filling and the accelerated rate of ventricular response. In addition, AF may develop or worsen clinical signs of heart failure (HF) resulted in a higher risk of mortality (Ehrlich et al., 2002; Menaut et al., 2005).

In dogs, AF most commonly develops in association with structural or functional heart diseases such as dilated cardiomyopathy (DCM) and acquired valvular heart disease especially degenerative of mitral valve (Brundel et al., 2005). In large breed dogs (i.e. Irish wolfhound, Newfoundland, Great Dane, and Doberman Pinscher), the occurrence of AF has been reported to be related with underlying heart diseases such as dilated cardiomyopathy (Menaut et al., 2005; Westling et al., 2008). On the other hand, the prevalence of AF in small breed dogs has been found to be related with degenerative mitral valve disease (Brundel et al., 2005). AF may occur in healthy dogs (known as lone AF) especially in large breeds such as Irish wolfhound, Mastiff, Newfoundland, and Rottweiler (Menaut et al., 2005; Westling et al., 2008).

The main objectives of treatment AF in dogs are to improve cardiac function and quality of life. This can be done by either rate control or rhythm control. The treatment goal of AF in veterinary medicine is preferential for rate control by maintaining ventricular rate that optimizes CO even AF still goes on (Saunders et al., 2009). Digitalis glycosides, beta-adrenergic receptor blockers, and calcium channel blockers are common drugs that may be used for controlling ventricular rate in AF patients (Tamariz and Bass, 2004). Although the reduction of ventricular rate may be the reasonable therapeutic goal, some literatures suggested that the conversion to sinus rhythm might be the better goal for managing AF (Crijns, 2005). AF in dogs can be restored to sinus rhythm by means of either electrical cardioversion or using

antiarrhythmic drugs. Previous retrospective studies reported that the success rate of biphasic transthoracic cardioversion in affected dogs was achieved between 92.3 % (36/39) to 93.2 % (41/44) (Bright et al., 2005; Bright and zumBrunnen, 2008). Unfortunately, AF has been reported to recur shortly after the cardioversion. Several studies in dogs with AF also reported that a few drugs such as amiodarone, quinidine, diltiazem, and verapamil can convert AF to sinus rhythm (Oyama and Prosek, 2006; Saunders et al., 2006). Among those drugs, amiodarone appears to have higher success conversion rate (Saunders et al., 2006).

Amiodarone is the most widely used antiarrhythmic drugs for treatment of AF in both humans and dogs (Saunders et al., 2006). It possesses high efficacy and low proarrhythmic profile. Unfortunately, treatment AF with amiodarone was also contributed to serious adverse effects especially extracardiac toxicity. Previous studies in dogs have shown that amiodarone may toxic to lungs, liver, skin, eyes, nervous tissue and thyroid gland (Bicer et al., 2002b; Bicer et al., 2002c; Saunders et al., 2006). Therefore, several new antiarrhythmic drugs have been developed to minimize toxic effect for treatment of AF.

Dronedarone is one of the novel drugs that has similar pharmacological profile with amiodarone while minimizing adverse effects due to lacking of iodine molecules in its structure (Nattel et al., 2002; Naccarelli et al., 2011). In addition, dronedarone contains methylsulfonamide group resulted in a reduction of lipophilicity and decreasing toxic effects. Furthermore, it has half-life of 24 hours which is shorter than amiodarone's (Wegener et al., 2006; Piccini et al., 2009; Zimetbaum, 2009). Dronedarone has been approved by the U.S. Food and Drug Administration for management AF in patients without concurrent severe heart failure (HF) since July 2009 (Touboul et al., 2003; Singh et al., 2007; Kober et al., 2008; Hohnloser et al., 2009). Several large clinical trials in patients with nonpermanent AF demonstrated that dronedarone reduced mortality and hospitalization rates whereas it increased mortality and morbidity in patients with AF and heart failure (Singh et al., 2007; Christiansen et al., 2010a; Torp-Pedersen et al., 2011). Many clinical trials in patients also suggested that dronedarone would rather have better safety profile than amiodarone (Touboul et al., 2003; Singh et al., 2007; Kober et al., 2008; Piccini et al.,

2009). Based on the success of clinical trials of dronedarone in humans, dronedarone would be beneficial for the management of AF in dogs. However, the study of dronedarone for management of AF in anesthetized and/or conscious dogs is limited. Few studies have examined the effect of dronedarone on electrocardiograms in anesthetized dogs (Manning et al., 1995). There is no study of pharmacodynamics especially its effect on hemodynamics and left ventricular geometrics in conscious dogs. Furthermore, its mechanism to prevent the recurrence of AF in dogs remains unelucidated.

Therefore the objectives of this study were as follow:

1. To describe the acute effects of escalating concentration of intravenous dronedarone on electrocardiograms, cardiac electrograms, hemodynamics, and cardiac mechanics in anesthetized healthy dogs
2. To study pharmacodynamics of single and short-term oral doses of dronedarone in conscious healthy dogs instrumented with telemetry units, sono-micrometry crystals and vascular occluder
3. To determine mechanism of chronic dronedarone administration to attenuate the duration of sustained AF in canine model of AF induced by rapid atrial pacing simultaneously with phenylephrine infusion

The hypotheses of this study were as follow:

1. Intravenous dronedarone infusion lengthens PQ interval in a dose dependent manner. When given dronedarone at the highest dose, dronedarone may depress cardiac function and lower blood pressure.
2. Conscious healthy dogs instrumented with telemetry units, sono-micrometry crystals and vascular occluder may tolerate dronedarone when given at 20 mg/kg, BID for at least 7 days. Dronedarone may not interfere cardiac function and blood pressure.
3. Dronedarone at 20 mg/kg, BID, 7 days may attenuate the duration of AF in dog model of sustained AF induced by rapid atrial pacing together with phenylephrine infusion.

This study would provide more information concerning the effects of acute and chronic dronedarone administration in both anesthetized and conscious dogs on electrocardiograms, hemodynamics, and cardiac function. The therapeutic dosage as well as its potency for managing AF in dogs were also evaluated.

# CHAPTER II
# LITERATURE REVIEWS

## A. Atrial fibrillation

Atrial fibrillation (AF) is one of the supraventricular tachyarrhythmias characterized by irregularly rapid atrial electrical activity. In AF, atria only quiver rather than contract leading to impair mechanical function of the heart. In addition, the rapid atrial electrical activity results in an increase rate of ventricular response and diminishes diastolic ventricular filling. Atrial fibrillation is associated with decreased cardiac output, increased risk of death and reduced quality of life (Dukes-Mcewan, 2002). Furthermore, clinical sign of heart failure may be worsen in individuals who depends on the atrial portion of the cardiac output and may develop in those with hypertensive or valvular heart disease (Ehrlich et al., 2002).

In humans, AF can be classified as paroxysmal (duration less than 7 days), persistent (sustained more than 7 days) or permanent (enduring or long-lasting AF, cannot be cardioversion) (European Heart Rhythm et al., 2010; Wann et al., 2011). AF can occur not only with underlying cardiopulmonary diseases but also without those diseases, called lone AF (Kopecky et al., 1987). In canine, AF is an arrhythmia that most commonly found in large and giant breeds. A previous retrospective study found that the prevalence of affected dogs had increased over the year from 5.07/10000 cases in 1969 to 23.31/10000 cases in 2007 (Westling et al., 2008). This may be partly due to an improvement of diagnosis techniques. AF is associated with male more than female. In addition, giant breeds have higher risk to develop AF than small breed dogs (Westling et al., 2008). As with humans, lone AF can occur in healthy dogs (Brundel et al., 2005). Most AF in dogs often occur secondary to heart diseases associated with atrial enlargement such as dilated cardiomyopathy in large and giant breeds and degenerative mitral valve disease in small breeds (Brundel et al., 2005; Westling et al., 2008).

**B. Diagnosis of atrial fibrillation**

AF mainly diagnoses by electrocardiograms (ECGs). The characteristic features of AF on ECG are the presence of irregularly irregular rapid ventricular response, with an absence of normal P wave or the presence of low-frequency irregular oscillations (F waves) (figure 1) (Saunders et al., 2009).

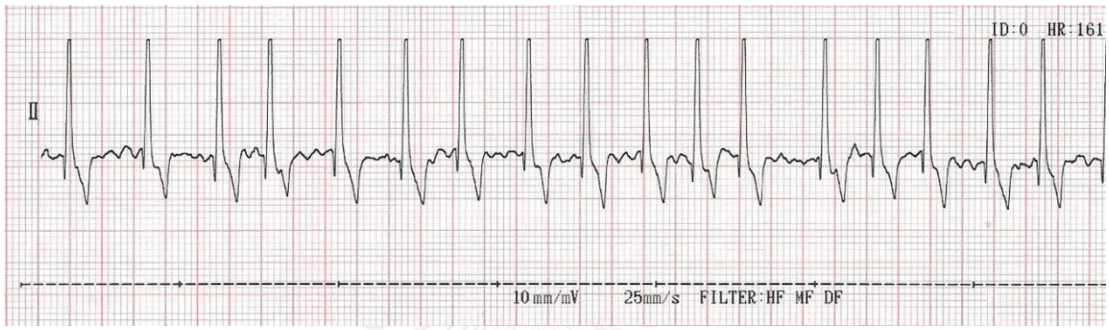


**Figure 1.** Lead II electrocardiograms (ECGs) in a dog demonstrated an irregularly irregular rapid ventricular response without P wave. The paper speed is 25 mm/s and the calibration is 10 mm/mV. The ventricular response rate is 144 beats per min. This tracing was recorded by using the CardiMax FCP-7101 Electrocardiograph, Fukuda Denshi Inc., Redmond, WA, USA.



**C. Mechanisms of atrial fibrillation**

Several theories have been proposed for explanation the mechanism(s) underlying AF. Most of those theories are focused on either triggered activity or re-entry. Rapid spontaneous atrial ectopic foci, a single reentry circuit, and multiple functional reentrant circuits were the earlier principle theories underlying AF (Mayer, 1906; Scherf and Terranova, 1949; Moe et al., 1964). These theories are related to the function of triggers. Recent theories are included AF begets AF and pulmonary vein foci (Wijffels et al., 1995; Haissaguerre et al., 1998). The former theory is associated with atrial remodeling whereas the latter points out a function of triggers for initiating AF.

**1. Re-entry**

In 1906, Mayer was the first person who postulates the concept of re-entry to clarify the mechanism of AF studied in jelly fish (Mayer, 1906). Then the concept was developed by Mines and Garrey (Mines, 1913; Garrey, 1914; Mines, 1914). Basically, fibrillating activity is generated from a single re-entry circuit (figure 2A). In the study of Mines, re-entry mechanism cannot occur unless it consists of all three components: 1) a unidirectional block area, 2) the depolarizing wavefront that repeatedly travels along the pathway, and 3) the termination of reentrant circuit which can be occurred when it was interrupted at any site of path.

In 1920, Lewis postulated new concept called "the circus movement hypothesis of re-entry" also known as "the anatomical re-entry" (Lewis et al., 1920) (figure 2B). Lewis hypothetized that AF could be occurred by the circus movement and this movement relies primarily on the circuit size and the refractory period at a tissue level.

Fifty years later, the concept of ectopic focus and single re-entry circuit was mostly out of favor. In 1962, Moe postulated the multiple functional reentrant circuits as a mechanism of AF (figure 2C) (Moe, 1920). In 1973 and a few years later, Allessie and colleagues (1973) were the first to emphasize the functional re-entry model, the leading circle model (figure 2D). This model suggested that functional re-entry circuits spontaneously involve in the smallest size of circuit (or wavelength) (Allessie et al., 1977). Basically, wavelength (WL) equals to conduction velocity (CV) multiply by effective refractory period (ERP). At a specified conduction velocity, the circuit size equals the distance progressed in the minimum time that sufficient for tissue recovery from refractory period. The circuit cannot have a smaller size than the wavelength, otherwise the depolarizing wavefront will hit with the refractory period of the tissue leading to termination of its own (Veenhuyzen et al., 2004).

**2. Ectopic foci**

In 1909, Rothberger and Winterberg postulated that an ectopic focus can cause AF (figure 2E) (Rothberger and Winterberg, 1909). Several years later, Haissaguerre demonstrated the importance of pulmonary vein foci for initiating arrhythmia (Haissaguerre et al., 1998). AF can be initiated from ectopic foci that locate at left atrial wall and the superior vena cava, although the myocardial pulmonary veins sleeves are the most common source (Doshi et al., 1999; Tsai et al., 2000; Nattel, 2002).

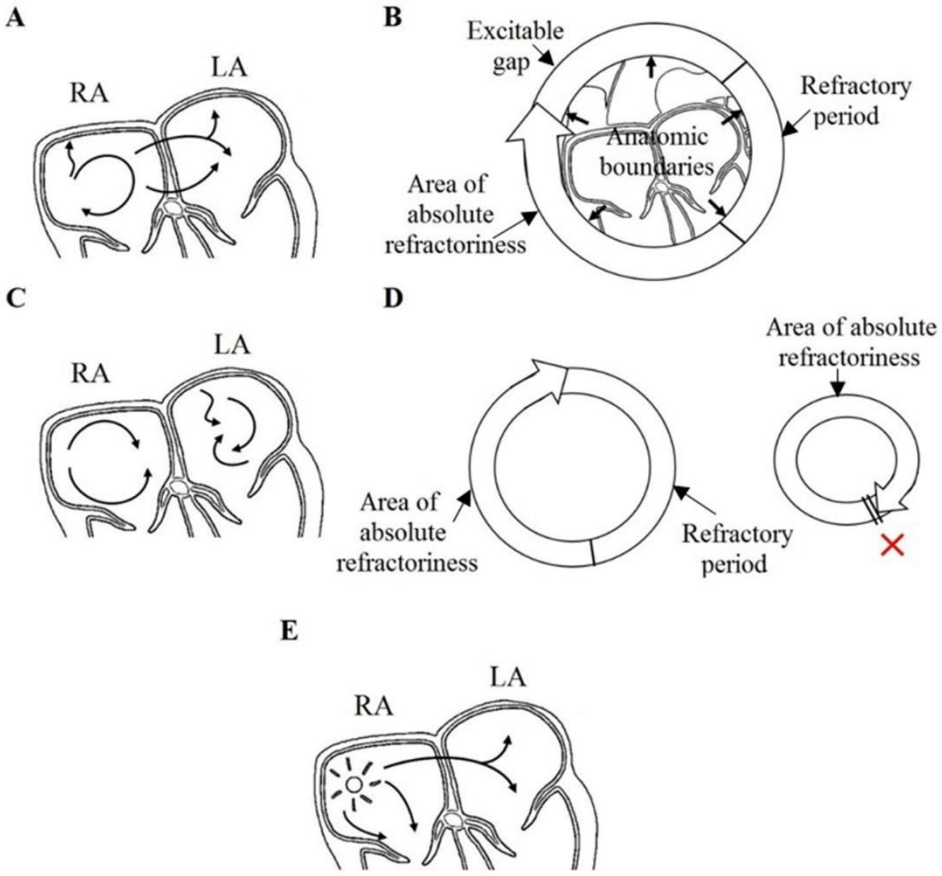


**Figure 2.** Representative drawing of mechanisms of atrial fibrillation (AF); the single re-entry circuit (A), the anatomical re-entry circuit (B), the multiple functional reentrant circuits (C), the leading circle model (D), and the ectopic focus (E). RA = right atrium, LA = left atrium

### 3. AF begets AF

In 1995, the AF begets AF concept resulted from the burst-pacing goat model was introduced which focuses on the initiating AF from the mechanism involved in the atrial adaptation (Wijffels et al., 1995). A fibrillation pacemaker was used and activated when sinus rhythm presented to create sustained AF in goat. Another animal model in which the results were similar to those in burst-pacing goat model was conducted in a canine model of rapid atrial pacing (Morillo et al., 1995). Sustained AF leads to a marked shortening of atrial effective refractory period (known as electrical remodeling) which leads to shorten the wavelength (WL = CV x ERP); therefore, the inducibility and stability of AF were increased. Furthermore, a decrease in the inward $Ca^{2+}$ current which arises from adaptive mechanisms to protect cells from $Ca^{2+}$ overload toxicity during rapid atrial rates also leads to shorten refractory period. Studies performed on animals and humans also suggested that AF involved in atrial contractile dysfunction (contractile remodeling) is response for a decrease in L-type $Ca^{2+}$ current ($I_{Ca,L}$).

Atrial fibrillation may result in ultrastructural changes (structural remodeling). Atrial fibrillation can also result in myofibrillar disarray, myolysis, alterations in mitochondrial structure and changes in nuclear structure (Morillo et al., 1995). Atrial interstitial fibrosis was commonly found to be related with AF, but it could also be associated with aging and other diseases (Allessie et al., 2002). Fibrosis can cause increasing non-uniform anisotropic conduction, thus it leads to enhance the substrate for reentry (Spach and Boineau, 1997). Furthermore, the fibrillating goat model revealed that alterations in distribution of atrial connexins, the gap junction proteins, may promote conduction heterogeneity which is the substrate for the arrhythmias (van der Velden et al., 1998; Takeuchi et al., 2006).

## D. Therapeutic strategies of AF

In humans, rhythm control, ventricular rate control, and anticoagulation are three main objectives for treatment of AF. The purpose of rhythm control is to convert AF to continuing sinus rhythm while the aim of rate control is to optimize ventricular response though AF still goes on. Anticoagulation is aimed to prevent

thromboembolism that may cause a risk of stroke. Therefore, therapeutic strategies of AF can be divided into two categories, non-pharmacotherapy and pharmacotherapy. Electrical cardioversion, surgical ablation, catheter ablation, pacemakers and internal defibrillators have been used for treatment of AF. Several reports demonstrated that some AF patients gained advantage from these non-pharmacotherapies, although distinctively different of successful rate and adverse effects had been suggested (Damiano et al., 2003; Gillinov and McCarthy, 2004).

The most common medications that can be used effectively in managing ventricular rate are beta-adrenergic blockers, L-type calcium channel blockers, and digitalis glycosides (Tamariz and Bass, 2004).

General antiarrhythmic drugs used for rhythm control are class I and III antiarrhythmic drugs (sodium channel blockers and potassium channel blockers, respectively) (Vaughan Williams, 1984). Class I acts by slow conduction and suppress ectopic activity. Class III prolongs refractory periods resulted in suppression of re-entry, but this can enhance early afterdepolarizations (EADs), considered as the proarrhythmic property of drugs (Dobrev and Nattel, 2010). In addition, drugs that act as multichannel blockers have been used for converting AF to sinus rhythm, but there still have reports of adverse effects (Mason and DiMarco, 2009).

Several new compounds have been developed and investigated both *in vitro* and *in vivo* and clinical trials have been launched for management of several types of AF in patients. Atrial-selective compounds are novel strategy for the management of AF since these compounds possess inhibitory effect on ultrarapid delayed rectified potassium current ($I_{KUR}$), acetylcholine-activated inward rectifier potassium current ($I_{K.ACh}$), or connexin-40 (Nattel et al., 2006). In addition, atrial-selective compounds may include agents that state-dependent block sodium current ($I_{Na}$) and agents that rapidly dissociate from sodium channel (Kodama et al., 1999; Burashnikov et al., 2007; Bogdan et al., 2011).

Vernakalant was developed as an atrial selective antiarrhythmic compound. It acts as sodium channel blocker and potassium channel blocker (Fedida et al., 2005). From clinical trials in humans, intravenous vernakalant terminated acute onset of AF (3-72 hours), and sinus rhythm was restored in short AF (3-7 days) but vernakalant was

ineffective to restored sinus rhythm in long-lasting AF (8-45 days) (Roy et al., 2004; Roy et al., 2008). Until now no publication of oral administration of this drug was found.

Furthermore, drugs that possess sodium channel blocker may terminate AF by increasing post-repolarization refractoriness (PRR) at the atria with minimally affected APD. PRR was defined as the extending of effective refractory period (ERP) beyond the action potential duration at 70 % of atrial repolarization ($APD_{70}$) or at 90 % of ventricular repolarization ($APD_{90}$) (Franz and Costard, 1988; Costard et al., 1989; Lee et al., 1989).

Flecainide (class Ic antiarrhythmic drug), ranolazine (antianginal drug), amiodarone (class III antiarrhythmic drug), and dronedarone (class III antiarrhythmic drug) have been demonstrated to block sodium channel led to increase PRR in experimental studies *(in vitro*) and effectively suppressed AF under the studied conditions (Burashnikov et al., 2008; Burashnikov et al., 2010b; Aliot et al., 2011).

In veterinary medicine, the main purpose of AF therapy is to manage clinical signs that may affect quality of life. After AF is diagnosed, the start of treatment relies on the present of underlying cardiac diseases and clinical signs and the hemodynamic conditions. Dogs with acute and severe of hemodynamic problems, such as weakness, collapse, and systemic hypotension are required acute intravenous antiarrhythmic medications. On the other hand, oral medications are adequate for general cases.

AF in dogs can be restored to sinus rhythm by synchronized electrical cardioversion. In two retrospective reports, the success rate of biphasic transthoracic cardioversion was achieved in 92.3 % (36/39) and 93.2 % (41/44) (Bright et al., 2005; Bright and zumBrunnen, 2008). It can be noticed that conversion AF to sinus rhythm succeeds in acute AF and lone AF (or without underlying cardiac diseases) human patients; however, those are uncommon in dogs (Westling et al., 2008; Saunders et al., 2009). Although several antiarrhythmic drugs have been used to restore sinus rhythm in human patients, only amiodarone, diltiazem, quinidine, and verapamil have been reported to convert AF to sinus rhythm in a small number of dogs. Only one and two cases of AF dogs were converted to sinus rhythm by verapamil and quinidine respectively. In a retrospective study of using amiodarone to manage AF in dogs, only

6 AF dogs (out of 17 dogs) were converted to sinus rhythm (Oyama and Prosek, 2006; Saunders et al., 2006).

According to Vaughan Williams classification, amiodarone is mainly a class III antiarrhythmic drug with effects of class I (sodium channel blocker), class II (beta-adrenergic blocker), and class IV (calcium channel blockers). Amiodarone has efficiency for managing AF in both of human patients and dogs (Saunders et al., 2006). In the retrospective study of 17 dogs with AF that were treated with amiodarone, 35 % of dogs were successfully converted sinus rhythm. Chronic treatment of amiodarone has complicated pharmacokinetic and adverse effects that restrict widespread usage. Adverse effects in dogs mostly are extracardiac effects including increasing of hepatic enzyme activity, decreasing of neutrophil, decreasing of platelets, agglutination, corneal deposits, GI disorders, and thyroid dysfunction. (Calvert et al., 2000; Jacobs et al., 2000; Bicer et al., 2002a; Bicer et al., 2002b; Saunders et al., 2006). Recently a novel antiarrhythmic drug, dronedarone, a structurally related to amiodarone has been developed into clinical use.

## E. Dronedarone

Dronedarone is a benzofuran derivative. It is synthetized based on amiodarone molecule (figure 3A), but has some structural changes. In order to avoid adverse effects of amiodarone, iodine molecules were removed to get rid of iodine-related organ toxicity, especially thyroid gland. In addition, a methane-sulphonyl group was added to reduce lipophilicity resulted in decreasing drug accumulated in tissue (figure 3B) (Wegener et al., 2006).

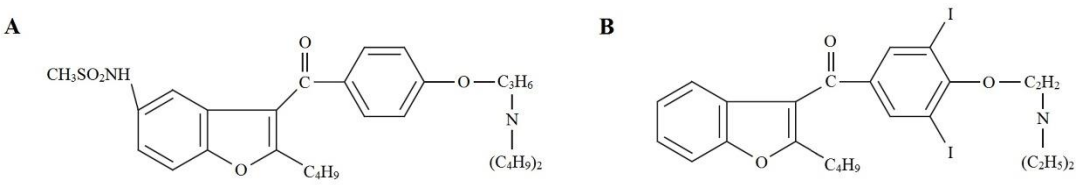


**Figure 3.** Chemical structures of dronedarone (A) and amiodarone (B)

Furthermore, dronedarone has shown an improvement of safety profile together with advantages on the considerable clinical endpoints, such as cardiovascular hospitalization and mortality in AF patients in clinical trials (Hohnloser et al., 2009). From the results of several large clinical trials, dronedarone has been approved to manage AF patients that absent severe heart failure by the U.S. Food and Drug Administration (Touboul et al., 2003; Singh et al., 2007; Kober et al., 2008; Hohnloser et al., 2009; Piccini et al., 2009). In humans, dronedarone has the elimination half-life approximately 24 hr. As a result of high first-past hepatic metabolism of dronedarone, its bioavailability is approximately 15 %. The N-debutyl metabolite is an active metabolite and its pharmacodynamic activity is similar to those of parent drug but less potent than dronedarone approximately 3 - 10 times. In addition, to achieve a sufficient steady-state in plasma, dronedarone has to administer orally two times a day. Plasma concentration of dronedarone reaches the steady state at 84-167 ng/ml after administration for 7-14 days (Patel et al., 2009). The therapeutic dose of dronedarone administered in humans is 800 mg/day (Piccini et al., 2009; Zimetbaum, 2009). The efficacies of that dose in AF patient were alteration in electrocardiograms (heart rate (HR), QT and corrected QT (QTc) intervals), reduction of ventricular response rates and maintenance of sinus rhythm (Singh et al., 2007; Singh and Cingolani, 2010). Adverse effects were rarely found. Gastrointestinal adverse effects such as nausea, vomiting, and diarrhea were most frequently reported and could be a reason for drug discontinuation in humans (Patel et al., 2009). A few of cardiac adverse effects such as bradycardia and QT prolongation were reported (Patel et al., 2009). The pharmacokinetics of dronedarone in dogs have been previously established (Australian Government, 2010; Product Monograph, 2014). The plasma clearance of dronedarone in dog was 1.8-2.4 l/hr/kg after single intravenous administration at dose 1-10 mg/kg (Australian Government, 2010). After oral administration, the absorption rate in dog was 64-95 %. The bioavailability of dronedarone from oral dosage form in dog was 14-22 %. The plasma protein binding of dronedarone and its metabolized in dogs was also investigated. The bounding fraction in plasma was more than 99.5 % and 98 % for dronedarone and its

metabolized, respectively. Dronedarone was extensively metabolized in dog to form N-debutylated metabolite (SR35021) and O-propanoic acid derivative (SR90154). Following oral administration, dronedarone was mainly excreted in feces (72-97 %) with no excretion of unchanged drug (Product Monograph, 2014).

*In vitro* studies reveal heterogeneities of drug responses among left ventricle, right ventricle and Purkinje fibers. Acute effect of dronedarone (10 μM) caused no change in action potential duration (APD) in most of those regions. However, APD at right ventricle was slightly increased in the isolated arterially perfused canine heart (Varro et al., 2001). In canine left ventricular Purkinje fibers, dronedarone (10 μM) lowered the incidence of early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) (Varro et al., 2001). Other studies in canine left ventricular M cells were also showed that dronedarone (30 μM) reduced early afterdepolarizations and triggered activities (Moro et al., 2007). Furthermore, dronedarone (10 μM) had to prevent the induction and termination of persistent Ach-mediated AF in canine coronary-perfused right atrial preparations (Burashnikov et al., 2010a).

In anesthetized dogs, escalating concentrations of intravenous dronedarone (1, 2.5, and 5 mg/kg) had shown electrophysiological effects similar to those of amiodarone (5 mg/kg) (Manning et al., 1995). Effects of dronedarone on QT and QTc intervals have been reported in only a few studies of conscious telemetry healthy dogs and complete AV block dogs (Verduyn et al., 1999; Varro et al., 2001; Patel et al., 2009). It has been suggested that effects of dronedarone *in vivo* studies depend on duration of administration and species of animals (Patel et al., 2009). In dogs with complete atrioventricular block (CAVB), intravenous administration of dronedarone (2.5mg/kg) produced shortening of APD while the sustained oral administration (20 mg/kg, BID, for 3 weeks) lengthened the QTc interval (Verduyn et al., 1999). Similarly, oral administration of dronedarone (20 mg/kg, BID, for 4 weeks) has been demonstrated to increase QTc interval in CAVB dogs (van Opstal et al., 2001). In contrast, chronic oral dronedarone administration (25 mg/kg, BID, for 4 weeks) in normal dogs did not prolong the QTc interval (Varro et al., 2001). In anesthetized dog, intravenous effects of dronedarone (1, 2.5, and 5 mg/kg) produced significant increases in sinus cycle length, effective refractory period (ERP) of atrioventricular node, and Wenckebach cycle length

(Manning et al., 1995). In conscious dogs with healed myocardial infarction, oral administration of dronedarone (10 and 30 mg/kg, for 1 week) reduced heart rate and did not deteriorate left ventricular function estimated by the maximal rate of rise of the left ventricular pressure, $dP/dt_{max}$ (Djandjighian et al., 2000).

**F. Canine telemetry models and pressure-volume loop relationships**

In order to monitor biological parameters (i.e. blood pressure, ECG, left ventricular pressure, body temperature, etc.) in conscious laboratory animals, telemetry has become widely used and was useful to obtain reliable physiological data. Dog telemetry model has been validated for sensitivity and specificity recently and used extensively for monitoring ECG, blood pressure, and left ventricular pressure simultaneously (Chaves et al., 2007). The telemetry system is quite simple but required experience for surgical implantation. The general system composes of radiotransmitter device, one or two fluid-filled catheters, and ECG electrodes for one lead (figure 4). The telemetry system allows continuously recording of those parameters in conscious animal with free movement, less stress from handing and restraint. The system also provides consistent data without anesthesia artifacts. Study of pharmacology and toxicology in drugs can be evaluated and completely naïve to drugs.

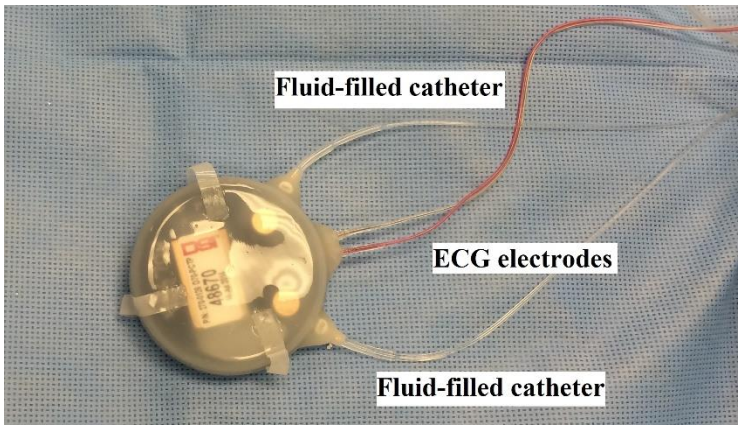


**Figure 4.** A photograph of radiotransmitter device (TL11M3-D70-PCTP, Data Sciences International, St. Paul, MN, USA) with two fluid-filled catheters and a pair of ECG electrodes for one lead.

Cardiac pressure-volume loops are considered to be the gold standard for evaluation of cardiac function (Suga et al., 1973). This method has been used to determine myocardial inotropy and lusitropy, compliance, muscle energetic and other important quantitative measures of function (Suga et al., 1973). Currently, pressure-volume catheter technology is advanced and become the most reliable tool to generate real-time ventricular pressure-volume loops in the intact heart. The advanced catheter together with vascular occluder help to generate a family of pressure-volume loop so that parameters which determine left ventricular function such as stroke volume (SV), stroke work (SW), preload recruitable stroke work (PRSW), contractility index (CI), end-systolic pressure-volume relationship (ESPVR), end-diastolic pressure-volume relationship (EDPVR), pressure-volume area (PVA), and effective arterial elastance (EA) can be obtained (Sarazan et al., 2011; Sarazan, 2014).

### G. Canine models of AF

It has been known that animal models are essential for novel pharmacology development and drug safety evaluation. Several animal models have been used for investigation of AF; however, canine models of AF have been used extensively due to

the similarity of molecular mechanisms of atrial remodeling underlying the atrial fibrillation (Brundel et al., 2002). Canine AF models include rate-related remodeling, atrial structural remodeling, acute atrial insults, and autonomic models (Nishida et al., 2010). Rate-related remodeling or atrial tachycardia can induce electrical remodeling, a substrate for AF. Therefore, antiarrhythmic drugs which may prevent electrical remodeling can be evaluated in this model. Atrial structural remodeling which consists of congestive heart failure model and mitral valve regurgitation model is involved in enhancing fibrosis to promote AF; however, these models require a long preparation period. Acute atrial insults such as atrial stretch, aconitine-induced atrial fibrillation, and acute ischemia produce AF without structural and functional changing at atria; therefore, the preparation time is short when compared with atrial structural remodeling. Vagal nerve stimulation and acetylcholine perfusion are models that involve autonomic modulation which can induce AF by shortening effective refractory period (ERP) and increasing ERP heterogeneity in atria. These autonomic models have been used for screening the potential of antiarrhythmic drugs (Goldberger and Pavelec, 1986; Wijffels et al., 1995; Satoh and Zipes, 1996; Gaspo et al., 1997; Liu and Nattel, 1997; Li et al., 1999; Sinno et al., 2003; Verheule et al., 2004).

Atrial tachycardia induced remodeling by rapid atrial pacing is simple method, but the fibrillation cannot be sustained sufficiently. Kijtawornrat and colleagues (2008) demonstrated that rapid atrial pacing simultaneously with phenylephrine infusion may sustain AF in dogs at least 40 min which is sufficient for testing potential for drugs to treat the AF.

Until now, there is no study of potential for dronedarone to control AF in dogs despite the success of clinical trials of dronedarone in humans. In addition, the pharmacodynamics data in dogs are limited. Furthermore, not only its effect on hemodynamics and left ventricular geometrics in dogs, but also its mechanisms to prevent the recurrence or termination of AF in dogs remain uninvestigated.

# CHAPTER III
# MATERIALS AND METHODS

**In order to test the hypotheses, this study was divided into 3 study parts as follow:**

## Study part 1: Acute effects of intravenous dronedarone on electrocardiograms, hemodynamics, and cardiac functions in anesthetized dogs

### 1. Approvals

This study was approved by the Institutional Animal Care and Use Committee of QTest Labs, LLC, Columbus, Ohio, USA (Protocol number SPD13-032) and by the Institutional Animal Care and Use Committee of Chulalongkorn University (Protocol number 11310043 and 13310024). All experimental animal procedures were performed both at Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, and at QTest Labs and in compliance with QTest IACUC regulation, and followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (2011).

### 2. Animals

Seven healthy mature Beagles of either gender were purchased from Marshall BioResources (North Rose, NY, USA). The animal was housed individually from the time of arrival to the end of study in a dog run maintained at a temperature of 21±2 ℃, a relative humidity of 50±20 %, and a 12 hr:12 hr light:dark cycle. All animals were received commercial chow twice daily, and water was provided *ad libitum* in stainless steel containers.

Three healthy mature mongrel dogs of either gender were donated from dog's colony located at Department of Animal Husbandry, Large Animal Veterinary Teaching Hospital, Nakhonpathom. These animals were housed individually in a dog run with natural light and humidity. All dogs were received commercial chow twice a day and water was provided *ad libitum* in a bowl.

Physical examination, routine lead II ECG recording, complete blood count, and blood chemistry analysis were performed to evaluate healthy status in all dogs before beginning of the experiment. Experimental procedures were started after at least 6 hr period of fasting.

**3. Drug preparation**

Dronedarone (Multaq® 400 mg tablet, Sanofi-Aventis U.S. LLC, Bridge water, NJ, USA) 400 mg was dissolved with polyethylene glycol (PEG 400) (Sigma-Aldrich, St. Louis, MO, USA) and distilled water (2:1) and the mixture was heated on hot plate (<90°C) until all drugs are dissolved. The solution was cooled down at room temperature and filtered with 0.8 µm sterile-syringe filter before given intravenously to the animal. The drug preparation and dose selection were based on our pilot study and a previous publication (Manning et al., 1995).

**4. Experimental procedures**

All dogs were given butorphanol (0.1 mg/kg, IV) 10 min before receiving propofol (4-6 mg/kg, IV, Abbott Laboratories, North Chicago, IL, USA, to effect). Orotracheal intubation was performed and ventilated mechanically with ascending-bellows, volume-cycled, pressure-regulated ventilator. The ventilator was set to deliver a tidal volume of 12-15 ml/kg (maximum allowed pressure, 20 $cmH_2O$) at a rate of 8 to 12 breaths per min, sustaining the end-tidal partial pressure of $CO_2$ between 35 and 45 mmHg and that of $O_2$ greater than 80 mmHg. The endotracheal tube was connected to a circle anesthetic rebreathing circuit, and anesthesia was maintained with isoflurane in oxygen delivered by a use of vaporizer. The end-tidal inhalant concentration was maintained between 1.4-1.6 %. Body temperature was maintained at 36.5-37°C by a warm water heating pump.

Lead II electrocardiograms was obtained (Ponemah 12 lead ECG amplifier, DSI, MN, USA). All catheterization procedures were performed under fluoroscopic guidance. A 5 French thermodilution catheter (Swan-Ganz 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 central venous (RAP) and pulmonary arterial (PAP) pressures and intermittent determination of cardiac output by a use of thermodilution technique. A 5 French pressure-volume (PV) loop admittance catheter (Scisense Inc, London, ON, Canada) was inserted into the left internal carotid artery through catheter introducer and the tip was advanced into the left ventricle (LV). This catheter was used to record pressure-volume relationship. A 6 French fluid-filled catheter was inserted into the left internal carotid artery through other side of catheter introducer to record arterial blood pressure (AoP). A 12 French venous occlusion catheter was inserted into the left femoral vein and positioned at the caudal vena cava for periodic occlusion of the vein to generate a family of pressure-volume loop. A bipolar pacing catheter (5 French Bard® electrophysiology, Lowell, MA, USA) were positioned at the non-coronary cusp of the aortic valves through the right femoral artery via catheter introducer to obtain His-bundle electrogram. IV catheter was inserted into right cephalic vein for drug infusion (figure 5). Each dog was in a stabilized anesthetic state for approximately 30 min before recording baseline data.

After stabilization, vehicle (PEG 400 and distilled water (2:1)) was infused for 15 min, at a rate of 0.33 ml/min. All hemodynamics and left ventricular function were observed for 15 min after the end of vehicle. Then escalating concentrations of dronedarone (0.5, 1, 2.5 mg/kg) were given at the same rate as vehicle for 15 min per each concentration with an observation period of 15 min between each dose. The initial dose was selected because it was found to be a no-effect dose in our preliminary studies. While the ECG and blood pressure were recorded throughout the experiment, parameters were analyzed at 30 min after the beginning of each concentration. Pressure-volume loops were obtained at 30 min after the beginning of infusion as previously described (Kijtawornrat et al., 2014). Briefly, at a given time point after dosing, left ventricular preload was acutely reduced by means of brief (~8-10 beats) caudal vena caval occlusions in order to generate a family of pressure-volume curves; approximately three occlusions were performed at each time point, allowing for hemodynamic recovery between occlusions. The cardiac output (CO) was determined by a modified Stewart-Hamilton indicator dilution equation (Armengol et al., 1981).

Basically, the COM-2 machine integrates area under the curve at the instant of saline injection (5 ml) and terminates integration when the exponential decay reaches a value of about 30 %. The computer then extrapolates the exponential decay to baseline. The CO measurement was performed 3 times for each time-point and the mean value was calculated. The CO and pressure-volume loop relationship were measured at 30 min after injection of each concentration (figure 6).

Before commencing the main experiments, vehicle-treated dogs (n = 2) were performed to establish that there was no difference in ECG, hemodynamic, and cardiac function parameters at each time points for 150 min after stabilization period. The average data of each measurement in vehicle-treated dogs were presented in the same figure as of the dronedarone-treated dogs but none of the standard deviation and standard error of mean was calculated.

Due to a severe cardiac suppression by dronedarone, all animals were euthanized at the end of the experiment while they were under general anesthesia with sodium pentobarbital (200 mg/kg; Somnasol, Butler Animal Health Supply, Dublin, OH, USA) in accordance with American Veterinary Medical Association guidelines (Artwhol et al., 2013).

Because the availability of anesthetic drugs and expensive equipment, the 3 dogs performed at Department of Veterinary Physiology were given with a different anesthetic regimen from dogs performed at QTest Labs. Furthermore, these three dogs were not instrumented to obtain pressure-volume loop relationship and His bundle electrograms. The responses of dog's physiology to drug infusion were also slightly different from the dogs performed at QTest Labs. Therefore, in order to maintain the integrity of the study, the result of these three dogs on electrocardiograms, cardiac function, and hemodynamic were not included in the result section.

Venous blood collections were also collected from a peripheral intravenous catheter placed at either left or right saphenous vein for analysis of dronedarone in plasma at 55-, 85-, 115-, and 145-minute. In order to determine plasma concentration of dronedarone, venous blood samples were collected in EDTA-containing tubes and

centrifuged at 3900g, 10 min, 4°C and plasma samples were stored at -80°C for further analysis. The extraction procedure was performed according to the previous publication (Bolderman et al., 2009). At the day of analysis, standard curve was performed from injections of 5 different concentrations of dronedarone and amiodarone in blank plasma (5, 10, 20, 30 and 40 ug/ml). The concentrations of dronedarone were calculated in reference to standards and internal standards using peak integration and expressed as ng/ml plasma of dog by computer program (ChromQuest 5.0)

Unfortunately, the process of extraction was not reliable and the concentration of dronedarone cannot be detected from all of 3 dogs. Therefore, the result of plasma concentration of dronedarone was not reported.

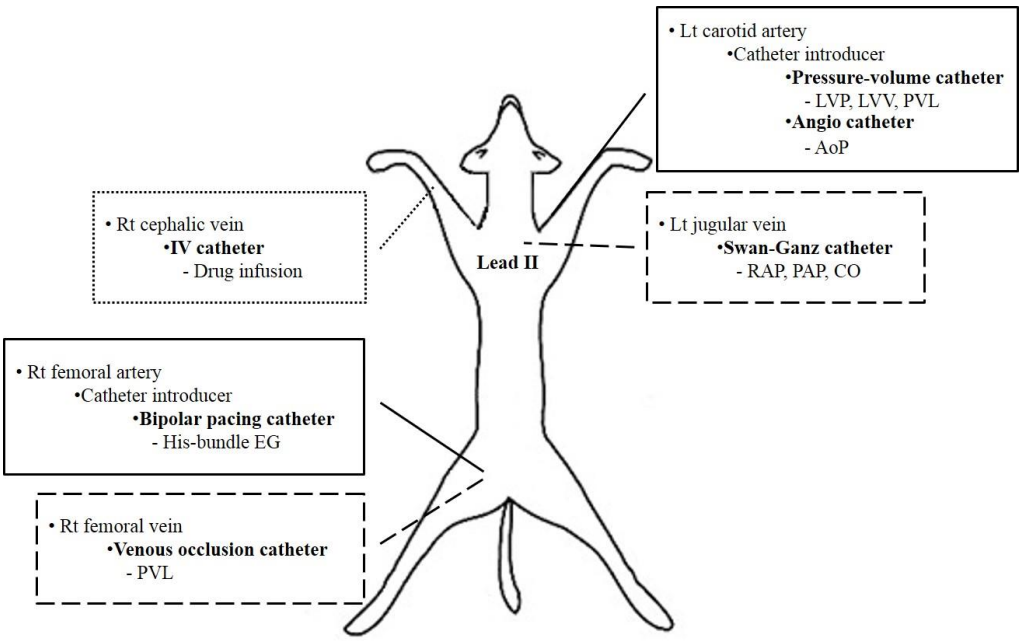


**Figure 5.** Instrumentation for the study acute effects of escalating doses of dronedarone on electrocardiograms (ECGs), hemodynamics, and left ventricular functions in anesthetized dogs. PVL = pressure-volume loop, LVP = left ventricular pressure, LVV = left ventricular volume, AoP = aortic pressure, RAP = right atrial pressure, PAP = pulmonary arterial pressure, CO = cardiac output

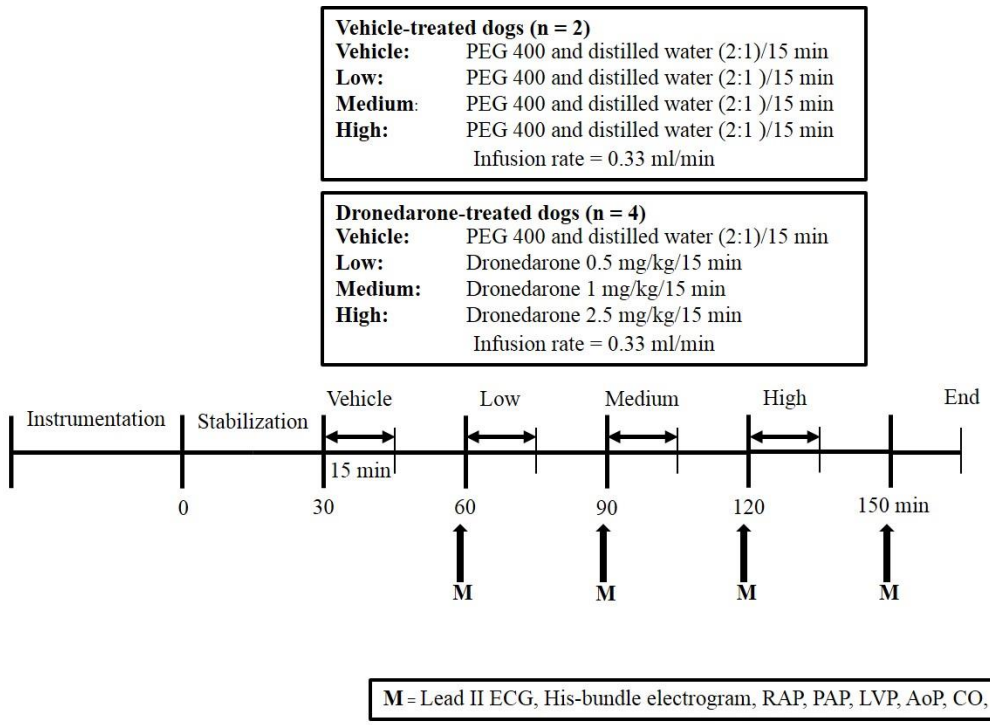


**Figure 6.** Experimental procedure to study acute effects of escalating doses of dronedarone on electrocardiograms (ECGs), hemodynamics, and left ventricular functions in anesthetized dogs. RAP = right atrial pressure, PAP = pulmonary arterial pressure, LVP = left ventricular pressure, AoP = aortic pressure, CO = cardiac output, PVL = pressure-volume loop



## 5. Data analysis

### *5.1 Analysis of electrophysiologic parameters*

Electrocardiographic data were analyzed for rhythm and rate, including P wave, QRS complex, QT, and QTc. His-bundle electrographic data were analyzed, including AH interval (as a measurement of AV nodal conduction time), and HV interval (as a measurement of His-Purkinje's conduction time) (figure 7). Value of each parameter was averaged from cardiac cycles over 30 sec of each time point. Corrected QT interval (QTc interval) was calculated by using Van de Water equation (Van de Water et al., 1989).

$$QTc = QT - 0.087 \times (RR - 1000)$$

QTc = corrected QT interval, QT = QT interval (ms), RR = RR interval (ms)

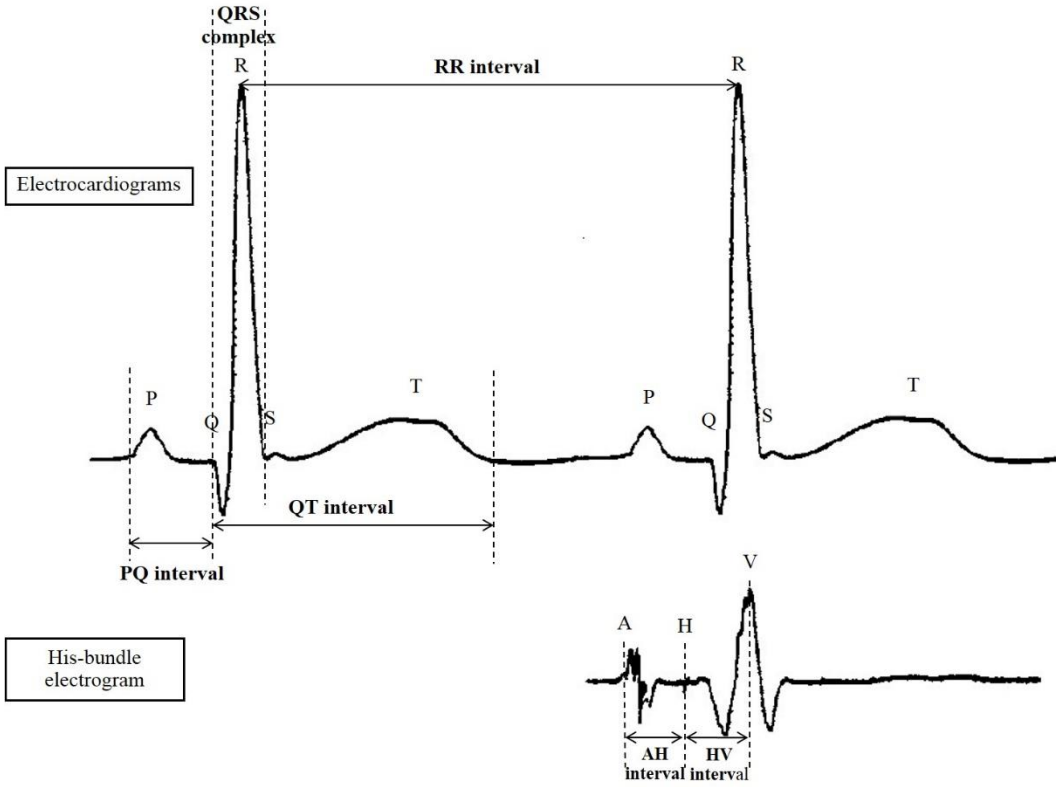


**Figure 7.** Representative electrocardiograms (upper panel) and His-bundle electrogram (lower panel) recorded from isoflurane-anesthetized dogs. P wave = the duration of P wave, PQ interval = the duration of the onset of the P wave to the beginning of the QRS complex, QRS complex = the duration of QRS complex, QT = the duration of the beginning of the QRS complex to the end of the T wave, A wave = the duration of atrial wave, H wave = the duration of His-bundle wave, V wave = the duration of ventricular wave, AH = the AV nodal conduction time, HV = the His-Purkinje's conduction time

*5.2 Analysis of hemodynamic parameters*

**5.2.1 Mean blood pressure (MBP)**

Systolic blood pressure and diastolic blood pressure were collected at a specific time point from AoP (figure 8) and calculated for mean blood pressure (MBP) followed this equation.

$$MAP = DBP + 1/3 (SBP - DBP)$$

MBP = mean blood pressure, DBP = diastolic blood pressure, SBP = systolic blood pressure

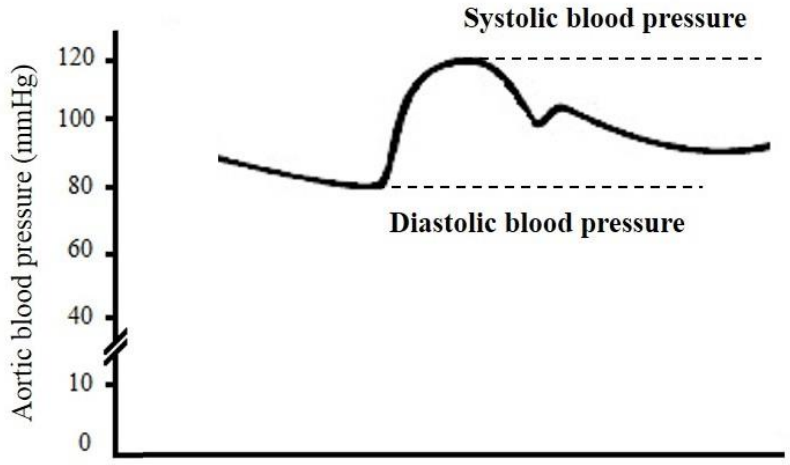


**Figure 8.** Representative of blood pressure tracing recorded at aortic arch showing systolic blood pressure, diastolic blood pressure and pulse pressure obtained from isoflurane-anesthetized dogs.

**5.2.2 Cardiac output (CO)**

The COM-2 thermodilution method for determining cardiac output was calculated by the use of modified Stewart-Hamilton indicator dilution equation (Armengol et al., 1981). Basically, the CO machine integrates area under the curve at the instant of injection and terminates integration when the exponential decay reaches a value of about 30 %. The computer then extrapolates the exponential decay to baseline.

The values of three cardiac output were calculated to obtain an average of cardiac output at each time point.

**5.2.3 Resistance (R)**

Resistance was determined by using Ohm's law, the proportion of the pressure gradient from the inlet (i) of the vessel to the outlet (o) ($P_i$ - $P_o$) to flow (Q); R = ($P_i$ - $P_o$)/Q.

**Systemic vascular resistance (SVR)** refers to the resistance to blood flow presented by all the vascular system, excluding the pulmonary vasculature. SVR was calculated by using the following equation.

$$SVR = 80*(MBP - PAP) / CO$$

SVR = systemic vascular resistance, MBP = mean blood pressure, PAP = pulmonary arterial pressure, CO = cardiac output

**Pulmonary vascular resistance (PVR)** refers to the resistance in the pulmonary vasculature. This is the resistance that the right ventricle must eject blood against. PVR was calculated as the following equation. In our study, pulmonary capillary wedge pressure (PCWP) did not measure; therefore, the RAP was used to calculate the PVR instead of PCWP.

$$PVR = 80*(PAP - RAP) / CO$$

PVR = pulmonary vascular resistance, PAP = pulmonary arterial pressure, RAP = right atrial pressure, CO = cardiac output

*5.3 Analysis of left ventricular pressure*

**5.3.1 Inotropic parameters**

**$dP/dt_{max}$,** the maximal rate of rise of the left ventricular pressure during isovolumetric contraction was obtained from the LV pressure (figure 9).

**Contractility index (CI)** is defined as the ratio of maximal rate of rise of the left ventricular pressure over the left ventricular pressure at that point. The CI was calculated from the following equation

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

CI = contractility index, $dP/dt_{max}$ = maximal rate of rise of the left ventricular pressure, P = the left ventricular pressure at that point

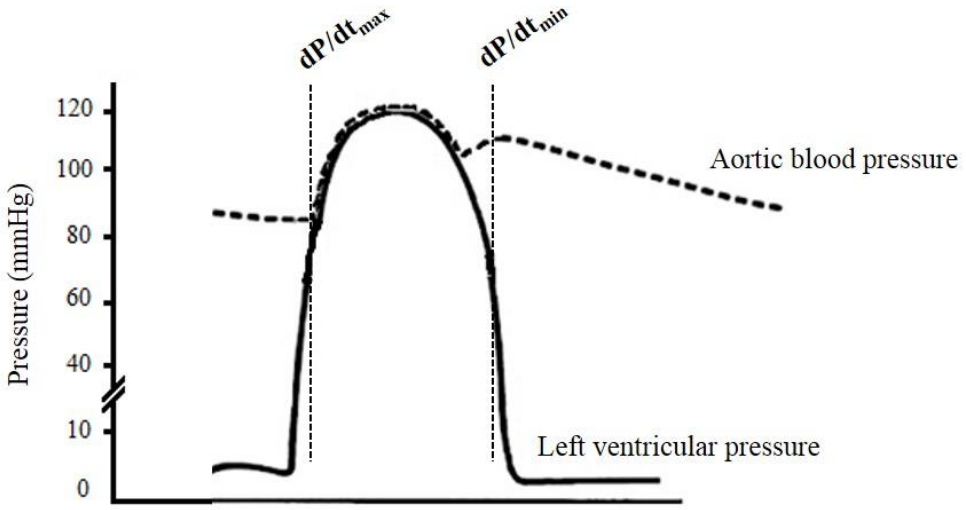


**Figure 9.** Representative left ventricular pressure (LVP) and aortic pressure showing the maximal rate of rise of the LVP ($dP/dt_{max}$) and the maximal rate of fall of the LVP ($dP/dt_{min}$) recorded from isoflurane-anesthetized dogs.

**5.3.2 Lusitropic parameters**

**$dP/dt_{min}$,** the maximal rate of fall of the left ventricular pressure during isovolumetric relaxation was obtained from the LV pressure (figure 9).

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

*5.4 Analysis of pressure-volume loop (PVL) relationship*

Base on the time-varying elastance concept, the left ventricular pressure and volume data were analyzed both on- and off-line in order to generate relationships representing the contractile and energetic state of the myocardium to each dose of vehicle and dronedarone (Suga et al., 1973).

**5.4.1 Preload recruitable stroke work (PRSW)**

PRSW, the slope of the relations between stroke work (SW) and end-diastolic volume (linear) (figure 10), was derived by graphing LV pressure versus LV volume generated during brief periods of venous occlusion.

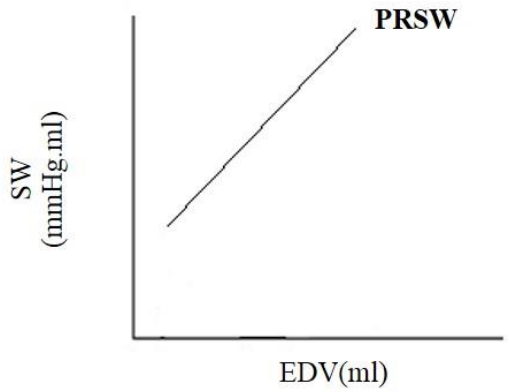


**Figure 10.** A representative drawing of preload recruitable stroke work (PRSW) in isoflurane-anesthetized dogs.

**5.4.2 End-systolic pressure-volume relationship (ESPVR)**

End-systolic pressure-volume relationship (ESPVR), the maximal ventricular development pressure at any given left ventricular volume, was obtained from a family of PV loops and the slope of linear relation of ESPVR was obtained (figure 11).

**5.4.3 End-diastolic pressure-volume relationship (EDPVR)**

End-diastolic pressure-volume relationship (EDPVR), the ventricular passive filling curve, was also obtained from the PV loops and used as an index of LV relaxation (figure 11).

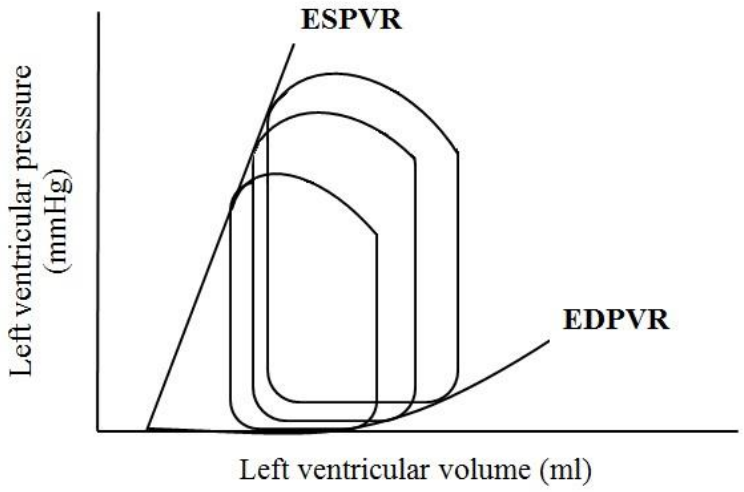


**Figure 11.** Representative drawing of a family of pressure-volume loops demonstrating end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR) in isoflurane-anesthetized dogs.

*Statistical analysis*

Statistical analyses were performed with commercially available software. Data are presented as mean ± standard error of the mean. Comparisons were made for each parameter in dronedarone-treated dogs versus baseline of the same group because none of the parameters in the vehicle-treated dogs changed with time. Differences among time points were determined using one-way ANOVA with repeated measures design. When indicated by a significant F-statistic, specific means were compared by Dunnett's test multiple comparison. Values of $P < 0.05$ were considered significance for all analyses.

**Study part 2: Effects of single and short-term oral dose of dronedarone administration on cardiac functions, blood pressures, and electrocardiograms in conscious telemetry dogs**

**1. Approvals**

This study was approved by the Institutional Animal Care and Use Committee of QTest Labs, LLC, Columbus, OH, USA (SPD13-012 and SPD13-018). All experimental animal procedures were performed in compliance with QTest IACUC regulation, and followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (2011).

**2. Animals**

Six healthy mature Beagles of either gender were purchased from Marshall BioResources (North Rose, NY, USA). They were housed individually from the time of arrival to the end of study in a dog run maintained at a temperature of 21±2 °C, a relative humidity of 50±20 %, and a 12 hr:12 hr light:dark cycle. All animals were received commercial chow twice daily, and water was provided ad libitum in stainless steel containers. Physical examination, routine lead II ECG recording, complete blood count, and blood chemistry analysis were performed to evaluate health status in all

dogs before beginning of the experiment. Surgical procedures were performed after at least 6 hr period of fasting.

**3. Surgical procedures for telemetry instrumentation**

All dogs were given butorphanol (0.1 mg/kg, intravenously) 10 min before receiving propofol (4-6 mg/kg, intravenously, Abbott Laboratories, North Chicago, IL, USA, to effect). Orotracheal intubation was performed and ventilated mechanically with the ascending-bellows, volume-cycled, pressure-regulated ventilator. The ventilator was set to deliver a tidal volume of 12-15 ml/kg (maximum allowed pressure, 20 $cmH_2O$) at a rate of 8 to 12 breaths per min, sustaining the end-tidal partial pressure of $CO_2$ between 35 and 45 mmHg and that of $O_2$ greater than 80 mmHg. The endotracheal tube was connected to a circle anesthetic rebreathing circuit, and anesthesia was maintained with isoflurane in oxygen delivered by a use of vaporizer. The end-tidal inhalant concentration of isoflurane was maintained between 1.4-1.6 %. Body temperature was maintained at 36.5-37$^{\circ}$C by a warm water heating pump blanket. Each animal was shaved and scrubbed at the surgical areas.

In order to determine oral dose, two dogs were surgically implanted with radiotelemetry transmitters (TL11M2-D70-PCT, Data Sciences International, St. Paul, MN, USA), which has systemic arterial blood pressure, heart rate, ECG, and body temperature data collection capabilities. The procedure for implantation has been described previously (Soloviev et al., 2006). In brief, an incision was made on midline of lower abdomen and abdominal cavity was exposed. The body of transmitter was sutured on the left abdominal wall to hold the transmitter body. Another incision was made along the left medial thigh and the femoral artery was exposed by blunt dissection. A blood pressure catheter was passed through the trocar tunneling from abdominal cavity to inguinal area. The femoral artery was ligated distally and incised to insert a blood pressure catheter. The catheter was advanced until the tip resided in the abdominal aorta. Then the catheter was secured by ligation with 3/0 prolene. The two ECG cables (positive and negative) were positioned by using trocar to tunnel each lead to the appropriated region to form transthoracic ECG lead (positive: under

the left side of the last thoracic cage; negative: the base of the right side of the axilla) (figure 12). The silastic insulator was removed from each lead and the exposed wire was coiled into a tight loop. The loop of wire was placed and secured in the muscle using a non-absorbable suture material. All incisions were closed using absorbable sutures and sterile staples on the skin. Butorphanol (0.05-0.4 mg/kg, once a day, subcutaneously; Abbott Laboratories, North Chicago, IL, USA), acepromazine (0.05-0.2 mg/kg, once a day, subcutaneously; Butler Animal Health Supply, Dublin, OH, USA), and cephazolin (15 mg/kg, once a day, subcutaneously; Butler Animal Health Supply, Dublin, OH, USA) were administered for 7 days. All dogs were allowed to recover for 2 weeks and transmitter signals were verified before the beginning of the study.

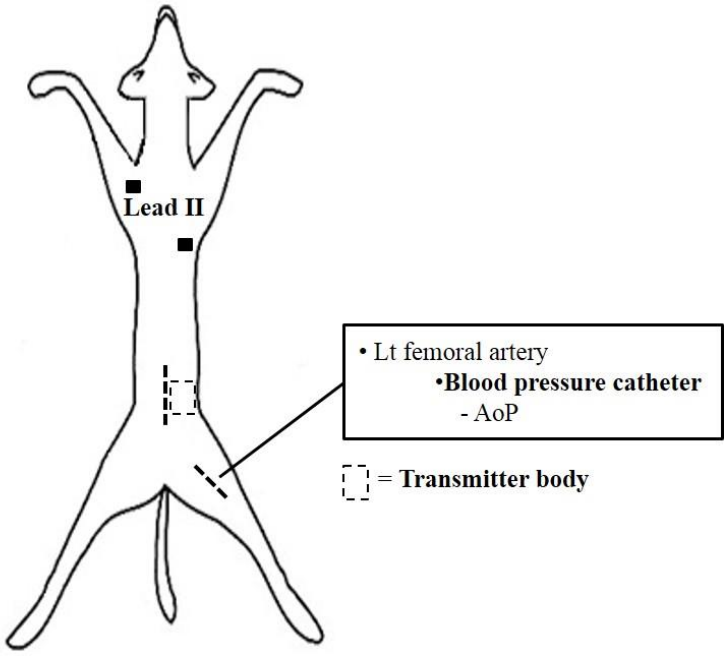


**Figure 12.** A representative of anesthetized dog instrumented with radiotelemetry (TL11M2-D70-PCT, Data Sciences International, St. Paul, MN, USA) for studying the effects of single oral dronedarone administration on blood pressures and electrocardiograms. AoP = aortic pressure, Lead II = transthoracic ECG representation of the lead II electrocardiograms

To determine chronic effects of dronedarone on ECG, BP, and LV mechanics, four dogs were implanted with radiotelemetry unit, sono-micrometry crystals, a solid-state pressure transducer, venous occluder, and fluid-filled pressure catheters (figure 13-14). A left-thoracotomy was performed and the animal was chronically instrumented with a radio telemetry unit (TL11M3-D70-PCTP, Data Sciences International, St. Paul, MN, USA). The body of transmitter was sutured on the left chest wall behind the scapula. The first pressure catheter was placed into the descending aorta to obtain systemic arterial pressure while the second pressure catheter was placed into the left ventricular chamber via left apex to obtained left ventricular pressure (LVP). The sono-micrometry crystals (Sonometrics, London, ON, Canada) providing left-ventricular (LV) dimensions/volume were implanted inside the LV muscle at lateral and posterior LV free walls. Additionally, a solid-state pressure transducer (Konigsberg P22, Konigsberg Instruments Inc., Pasadena, CA, USA) was placed into the LV chamber for pressure monitoring. A hydraulic occluder (OC2, In Vivo Metric, Healdsburg, CA, USA) was placed/secured around the caudal vena cava, in order to allow its controlled constriction for the generation of LV pressure-volume curves during heterometric auto-regulation. All catheters/wires were aseptically tunneled and externalized next to the incision line. The two ECG electrodes were tunneled subcutaneously and secured to the superficial muscles of the chest wall (modified lead II configuration). Prior to closure, a chest tube was placed for drainage of any fluid and/or gas that accumulates from the surgical procedure. The tube was aspirated twice daily until the amount of fluid removed is less than 35 ml per aspiration in an approximate 24 hr period. Prophylactic antibiotic, pain medication, and post-operative care were performed as described previously. If necessary, an additional analgesic may also be administered which may include a fentanyl patch (25-50 μg/hr). All surgical incisions were closed in layers; the underlying musculature was closed with absorbable sutures, and the skin was closed with staples. Throughout the recovery phase, the animals were observed daily for routine signs of recovery, and the wound sites were observed for any signs of potential infections. Animals experiencing pain, distress and/or infections were brought to the attention of the attending veterinarian.

The skin incision staples were removed at 7 days after surgery. All dogs were allowed to recover for 2 weeks and transmitter signals were verified before the beginning of the study.

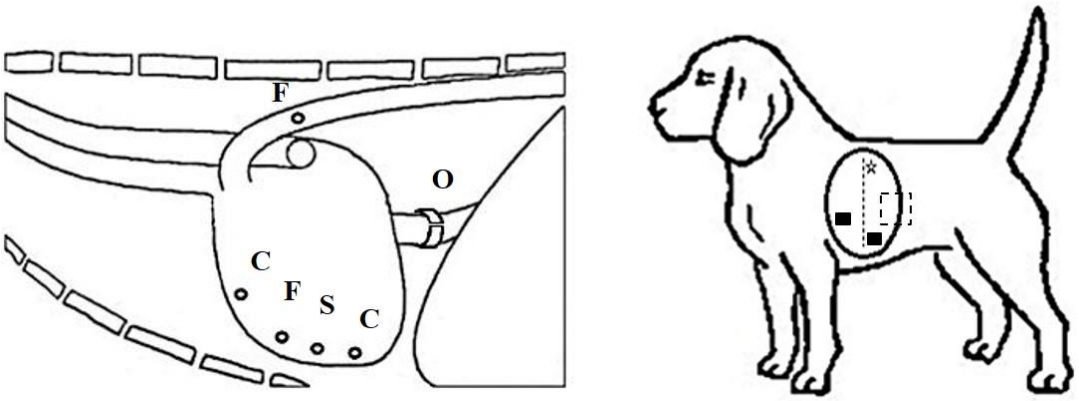


**Figure 13.** Diagrams of instrumentation for the studying of chronic effects of oral dronedarone administration on cardiac functions, blood pressures and electrocardiograms in dogs. F = fluid-filled pressure catheters, C = sono-micrometry crystals, S = solid-state pressure transducer, O= vascular occluder, black square = ECG electrodes (lead II), dashed line square = radiotelemetry unit, dashed line = incision line, star = all catheters/wires externalization

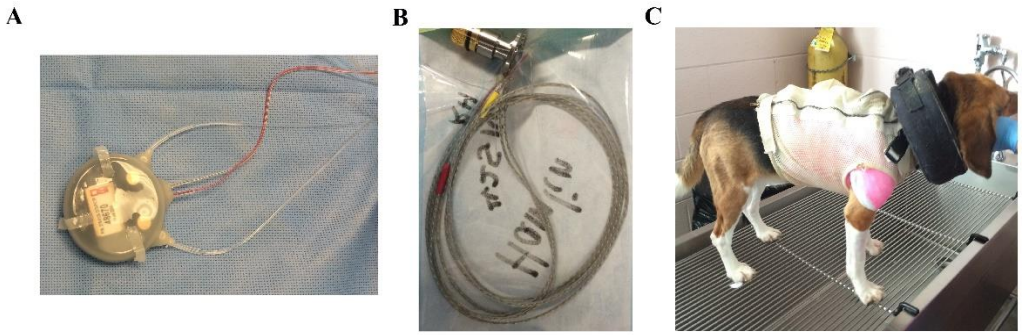


**Figure 14.** Illustration of instrumentation in dogs for the studying of chronic effects of oral dronedarone administration on cardiac functions, blood pressures and electrocardiograms. Body of radiotelemetry unit (TL11M3-D70-PCTP, Data Sciences International, St. Paul, MN, USA) (A), sono-micrometry crystals (Konigsberg P22, Konigsberg Instruments Inc., Pasadena, CA, USA) (B), dog at recovery from anesthesia with completed instrumentation (C)

**4. Experimental procedures and drug administration**

To determine oral dose of dronedarone, two dogs instrumented with TL11M2-D70-PCT were used. Dogs were randomized to receive single oral dose of either placebo or dronedarone (10, 20, or 40 mg/kg). The range of oral dose was chosen based on our preliminary experiment in anesthetized dogs and previous publications in dogs (Verduyn et al., 1999; Djandjighian et al., 2000). ECG, BP, and temperature were recorded before dosing and continue to monitor up to 36 hr after dosing. All parameters were obtained hourly at 1-6 hr and at 12-, 24-, and 36-hr after dosing. Due to its short elimination half-life, the washout period between each treatment was at least 14 days, which yield approximately 11-24 times of the half-life (figure 15) (Naccarelli et al., 2011).

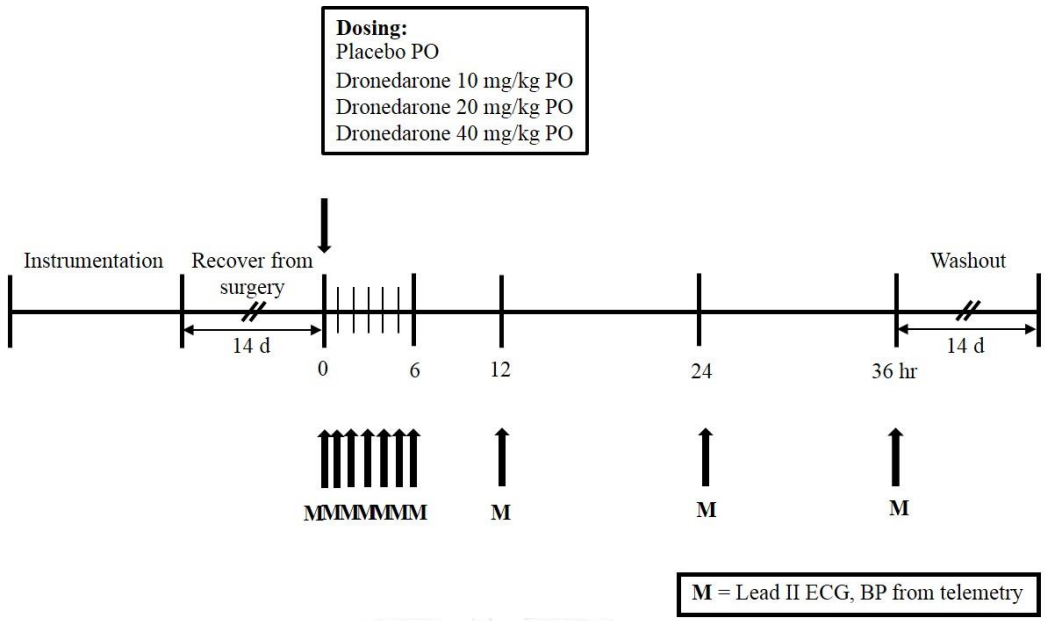


**Figure 15.** Experimental procedures for studying effects of single oral dronedarone administration on blood pressures and electrocardiograms in conscious telemetry dogs. HR = heart rate, BP = blood pressure

To determine chronic effects of dronedarone on ECG, BP, and LV mechanics, the rest of the dogs (n = 4) were used. Base on the results of single oral dose of dronedarone in our study, each dog was randomized to receive either placebo or dronedarone at a dose of 20 mg/kg, BID, for 7 Days. ECG, BP, LVP, and temperature were recorded before dosing and continue to monitor up to 7 days after dosing. All parameters including pressure-volume loops were obtained hourly at 4-8 hr after the first dose and at 12-, 96- (day 4), and 168-hr (day 7) after dosing (figure 16). The detail of generating of pressure-volume loops was previously described (Kijtawornrat et al., 2014). Briefly, at a given time point after dosing, left ventricular preload was acutely reduced by means of brief (approximately 8-10 beats) caudal vena cava occlusions in order to generate a family of pressure-volume curves; approximately three occlusions were performed at each time point, allowing for hemodynamic recovery between occlusions.

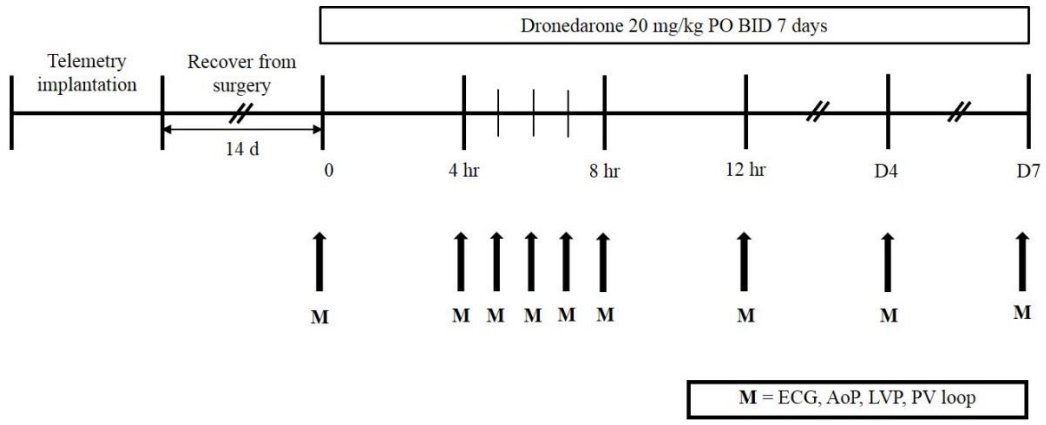


**Figure 16.** Experimental procedures to study chronic effects of oral dronedarone administration on cardiac functions, blood pressures, and electrocardiograms in conscious telemetry dogs. ECGs = electrocardiograms, AoP = aortic pressure, LVP = left ventricular pressure, PVL = pressure-volume loop

**5. Data analysis**

Telemetric device was programmed using the Dataquest ART 3.1 software to record electrocardiograms, blood pressure, and body temperature. Standard ECG intervals (RR, PQ, QRS, and QT) were manually measured by using ECG auto software (EMKA Technologies, Falls Church, VA, USA). A mean of the averaged 60 sec per time-point was reported. The QT interval was corrected for heart rate by using van de Water formula (QTc(V)) (Van de Water et al., 1989).

Statistical analyses were performed with commercially available software. Data are presented as mean ± standard error of the mean. Comparisons were made for each parameter in each time point versus the baseline. Differences among time points were determined using one-way ANOVA with repeated measures design. When indicated by a significant F-statistic, specific means were compared by Dunnett's test for multiple comparisons with the baseline. Statistical significance was considered at $P < 0.05$ for all analyses.

**Study part 3: Dronedarone attenuates the duration of atrial fibrillation in dog model of sustained atrial fibrillation**

**1. Approvals**

This study was approved by the Institutional Animal Care and Use Committee of QTest Labs, LLC, Columbus, OH, USA (SPD14-022). All experimental animal procedures were performed in compliance with QTest IACUC regulation, and followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (2011).

**2. Animals**

Six healthy mature Beagles of either gender were purchased from Marshall BioResources (North Rose, NY, USA). They were housed individually from the time of arrival to the end of study in a dog run maintained at a temperature of 21 ± 2 °C, a relative humidity of 50 ± 20 %, and a 12 hr:12 hr light:dark cycle. All animals were received commercial chow twice daily, and water was provided ad libitum in stainless steel containers. Physical examination, routine lead II ECG recording, complete blood count, and blood chemistry analysis were performed to evaluate healthy status in all dogs before beginning of the experiment. Complete blood count and blood chemistry analysis were performed again after dogs received dronedarone for 7 days. Surgical procedures were started after at least 6 hr period of fasting.

**3. Surgical procedures for measurement of atrial effective refractory period, action potential duration of atrium, and induction of sustained atrial fibrillation**

All dogs were given butorphanol (0.1 mg/kg, IV) 10 min before receiving propofol (4-6 mg/kg, IV, Abbott Laboratories, North Chicago, IL, USA, to effect). Orotracheal intubation was performed and ventilated mechanically with ascending-bellows, volume-cycled, pressure-regulated ventilator. The ventilator was set to deliver a tidal volume of 12-15 ml/kg (maximum allowed pressure, 20 $cmH_2O$) at a rate of 8 to 12 breaths per minute, sustaining the end-tidal partial pressure of $CO_2$ between 35 and 45 mmHg and that of $O_2$ greater than 80 mmHg. The endotracheal tube was

connected to a circle anesthetic rebreathing circuit, and anesthesia was maintained with isoflurane in oxygen delivered by a use of vaporizer. The end-tidal inhalant concentration was maintained between 1.4-1.6 %. Body temperature was maintained at 36.5-37 °C by a warm water heating pump. Each animal was shaved and scrubbed at the surgical areas and prepared as aseptic technique (left femoral triangle and left jugular area).

A mikro-tip® catheter pressure transducer (5Fr, Millar® instrument, Houston, TX, USA) was inserted into the left femoral artery and advanced to the aortic arch for measuring systemic arterial blood pressure. With the help of fluoroscope guidance, monophasic action potential catheter was inserted through the left jugular vein and push against the endocardium of the right atrium for obtaining action potential duration (APD) (figure 17). Atrial effective refractory period (AERP) was obtained by using program electrical stimulator. Extrastimuli were introduced at premature coupling interval ($S_1$-$S_2$) during constant atrial pacing with an interval $S_1$-$S_1$ of 300 ms by progressively shortening $S_1$-$S_2$ for 10 ms pacing cycle length until atria fails to depolarize (figure 18). Then, increasing by 5 ms pacing cycle length was introduced until causes atria depolarization. AERP is the longest $S_1$-$S_2$ interval that fails to cause atrial depolarization.

Lead II Electrocardiograms was monitored for rhythm and rate. After obtaining APD and ERP of the right atrium, a bipolar pacing catheter was positioned at the right atrial appendage through the left jugular vein for induction of atrial fibrillation (AF) (figure 17). Sustained AF was induced according to previous publication (Kijtawornrat et al., 2008). Briefly, the right atrial appendage was pacing at a rate of 40 Hz with square waves of 20 V and 2 ms duration. Simultaneously, phenylephrine (PE, 2 µg/kg/min) (Baxter Healthcare Corporation, Deerfield, IL, USA) was infused constantly through peripheral intravenous catheter placed inside right cephalic vein until the end of session. After 20 min of rapid atrial pacing (RAP), the pacing was stopped and the duration of AF was observed. AF was identified by (1) a presence of irregularly irregular rapid ventricular response, (2) an absence of P wave, (3) a presence of low-frequency irregular oscillations (f waves), and (4) a presence of irregular systemic arterial pressure

pulses and variable in amplitude with a pulse deficit. At the end of experiment, all vessels were sutured with 8-0 prolene. Tissues and muscles were sutured with absorbable 3-0 suture materials. Skin was closed with sterile staples. Butorphanol (0.05-0.4 mg/kg, once a day, subcutaneously; Abbott Laboratories, North Chicago, IL, USA), acepromazine (0.05-0.2 mg/kg, once a day, subcutaneously; Butler Animal Health Supply, Dublin, OH, USA), and cephazolin (15 mg/kg, once a day, subcutaneously; Butler Animal Health Supply, Dublin, OH, USA) were administered for 7 days.

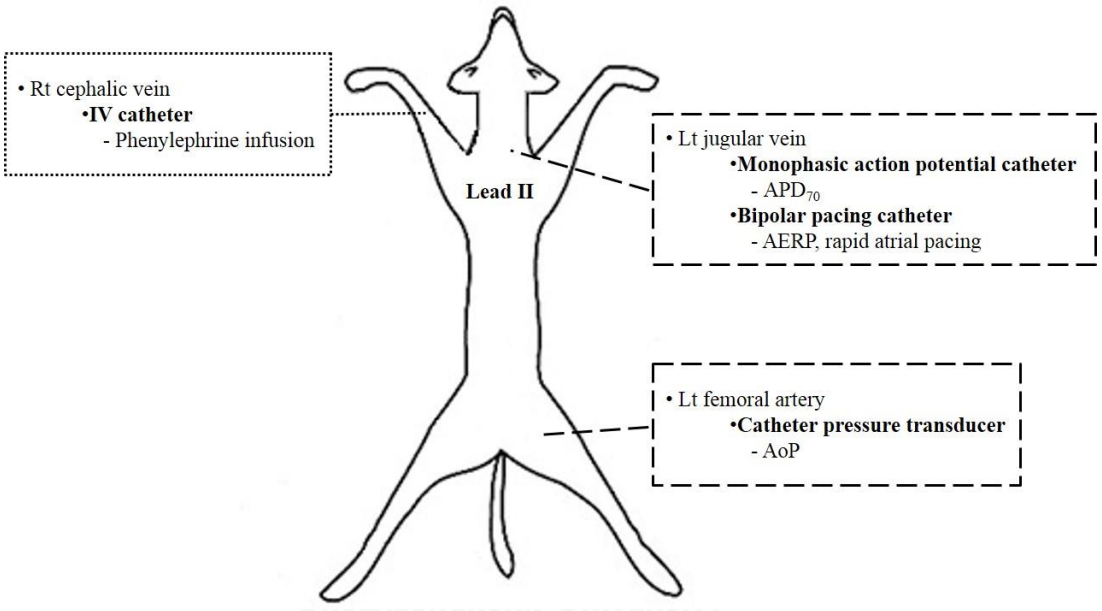


**Figure 17.** Drawing of instrumentation in dogs model of sustained atrial fibrillation for the studying of effects of dronedarone on the duration of atrial fibrillation. $APD_{70}$ = action potential duration at 70 % repolarization of atrial action potential, AERP = atrial effective refractory period, AoP = aortic pressure

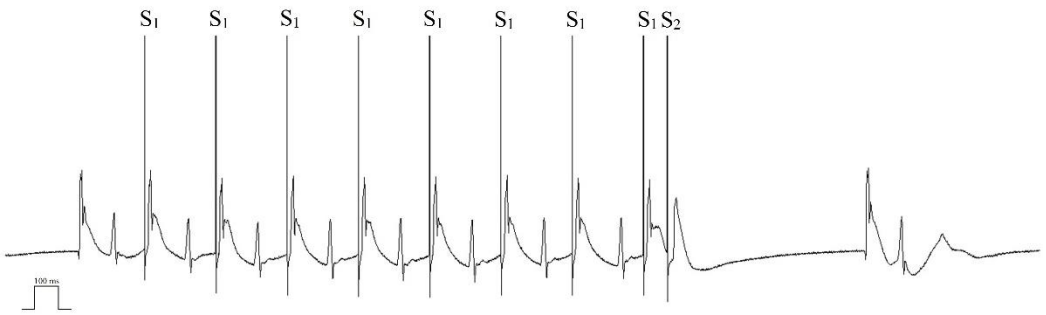


**Figure 18.** Representative of atrial action potential tracing for obtaining atrial effective refractory period (AERP) by using program electrical stimulator. Constant atrial pacing ($S_1$-$S_1$) = 300 ms, premature coupling interval ($S_1$-$S_2$) = 100 ms (ECG-Auto v3.3.0.15).

**4. Experimental procedures**

After instrumentation, dogs were allowed to stabilize for at least 20 min. Then ECG, BP, MAP, and AERP were obtained. After all data were collected, sustained AF was induced. The incidence and duration of AF were recorded after stop pacing. Dogs were allowed to recover from anesthesia and dronedarone (20 mg/kg, BID, PO) was given beginning on the next day for 7 days. The dose of dronedarone was chosen based on our previous experiment in conscious dogs instrumented with telemetry unit. On day 7 after drug was given, dogs were anesthetized and instrumented as described earlier for obtaining ECG, BP, MAP, and AERP. Each dog was infused with phenylephrine at dose of 2 µg/kg/min. Simultaneously, RAP was initiated as described earlier. After 20 min of pacing, pacing was stopped and the cardiac rhythm was monitored. Then, the concentration of PE was increased to 4 µg/kg/min to confirm efficacy of dronedarone on attenuation AF duration and the atrium was paced again for 20 min. At the end of the experiment, all animals were allowed to recover from anesthesia and post-operative care was performed for 7 days. Then all dogs were transferred back to the in-house colony (figure 19).

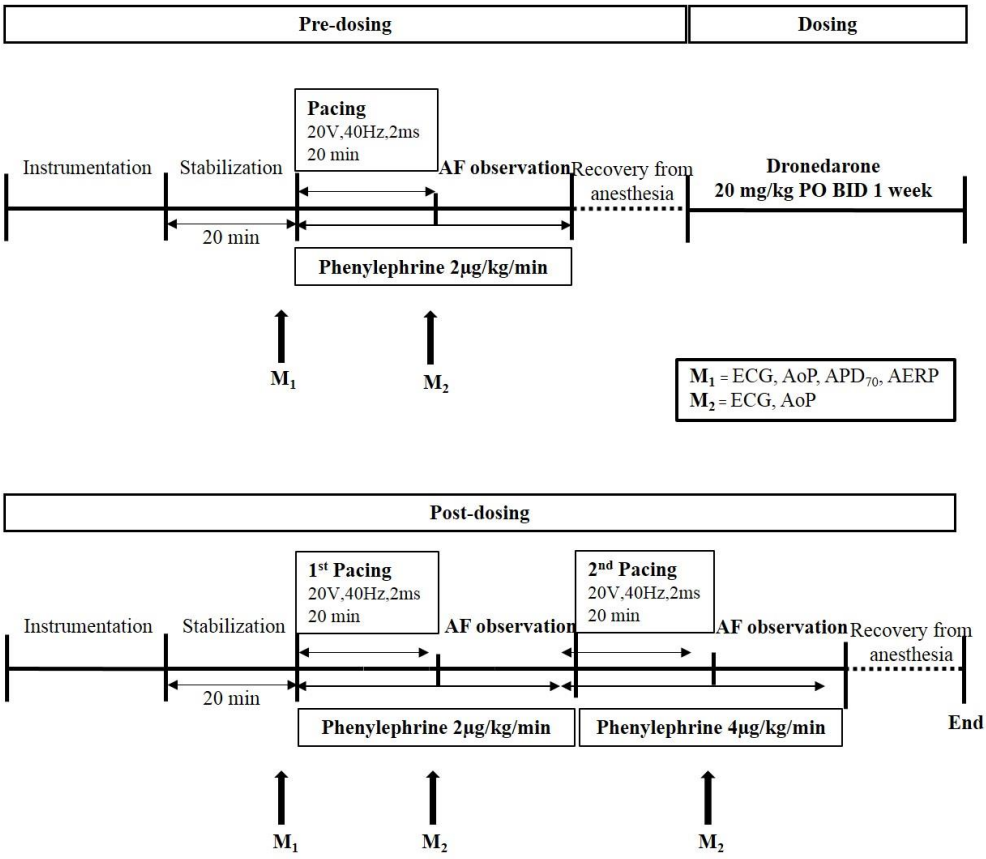


**Figure 19.** Experimental procedures for investigation of dronedarone effects on the duration of atrial fibrillation in dogs model of sustained atrial fibrillation. AoP = aortic pressure, $APD_{70}$ = action potential duration at 70 % repolarization obtained from atrium, AERP = atrial effective refractory period

## 5. Data analysis

Electrocardiograms and blood pressure were analyzed for heart rate or ventricular response rate and mean blood pressure at pre-dosing (at baseline before PE infusion with RAP and at 20 min during PE infusion with RAP) and 7 days post-dosing [at baseline, 20 min of the first trial during PE infusion (2 µg/kg/min) with RAP, and 20 min of second trial of PE infusion (4 µg/kg/min) with RAP] by using ECG Auto software (EMKA Technologies, Falls Church, VA, USA). $APD_{70}$ was defined as action potential duration at 70 % repolarization of atrial action potential (figure 20A). $APD_{70}$ and AERP were obtained at pre-dosing and 7 day post-dosing. Post-repolarization refractoriness

(PRR) (figure 20B) was calculated as the difference between AERP and atrial $APD_{70}$. In atria, AERP usually corresponded with $APD_{70}$ to $APD_{75}$ (Burashnikov et al., 2010b). The duration of sustained AF at post-dosing was averaged between the duration of AF of the first and second trial.

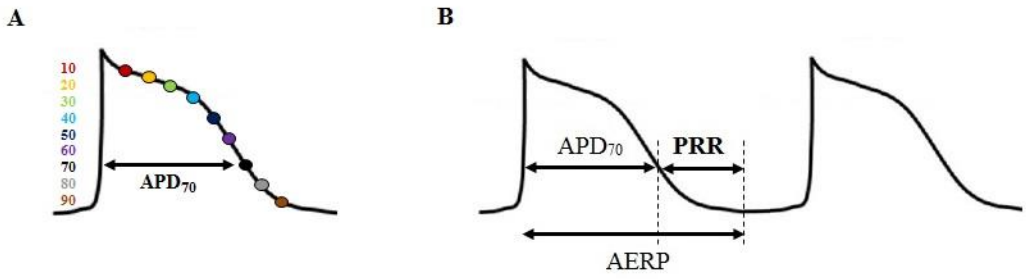


**Figure 20.** A representative action potential duration at 70 % repolarization ($APD_{70}$) (A) and post-repolarization refractoriness (PRR) (B). AERP = atrial effective refractory period; Post-Repolarization Refractoriness (PRR) is defined as the difference of the AERP and $APD_{70}$.

Statistical analyses were performed with commercially available software. Data were presented as mean ± standard error of mean. All data points were averaged from 60 sec of recording. Differences between pre-dosing and post-dosing were determined using paired t-test. Fisher's exact tests were used to compare the incidence of reduction of AF duration between pre- and post-dosing. A probability value of $P < 0.05$ was considered to be significant.

# CHAPTER IV
# RESULTS

**The results of this study were organized into 3 study parts as follow:**

## Study part 1: Acute effects of intravenous dronedarone on electrocardiograms, hemodynamics, and cardiac functions in anesthetized dogs

In general, all parameters at each time-point were measurable from all dogs anesthetized with isoflurane. There was no substantial change among parameters of ECG, hemodynamics, and cardiac functions in vehicle-treated dogs at each time-point for at least 150 min after stabilization period. From beginning to the end of experiment in vehicle-treated dogs, the heart rate was maintained between 102-114 beats per minute (bpm) while mean aortic pressure was maintained between 83-91 mmHg.

### A. Acute effects of dronedarone on electrocardiograms

At the highest dose of dronedarone (2.5 mg/kg), PQ interval was significantly prolonged by 18.3 % when compared with baseline ($P < 0.05$; figure 21A). In both vehicle group and dronedarone group, all 3 cumulative doses did not alter QRS complex, QT and QTc intervals, and heart rate (figure 21B-21D). It could be noticed that all ECG parameters of dogs receiving only vehicle were unaltered.

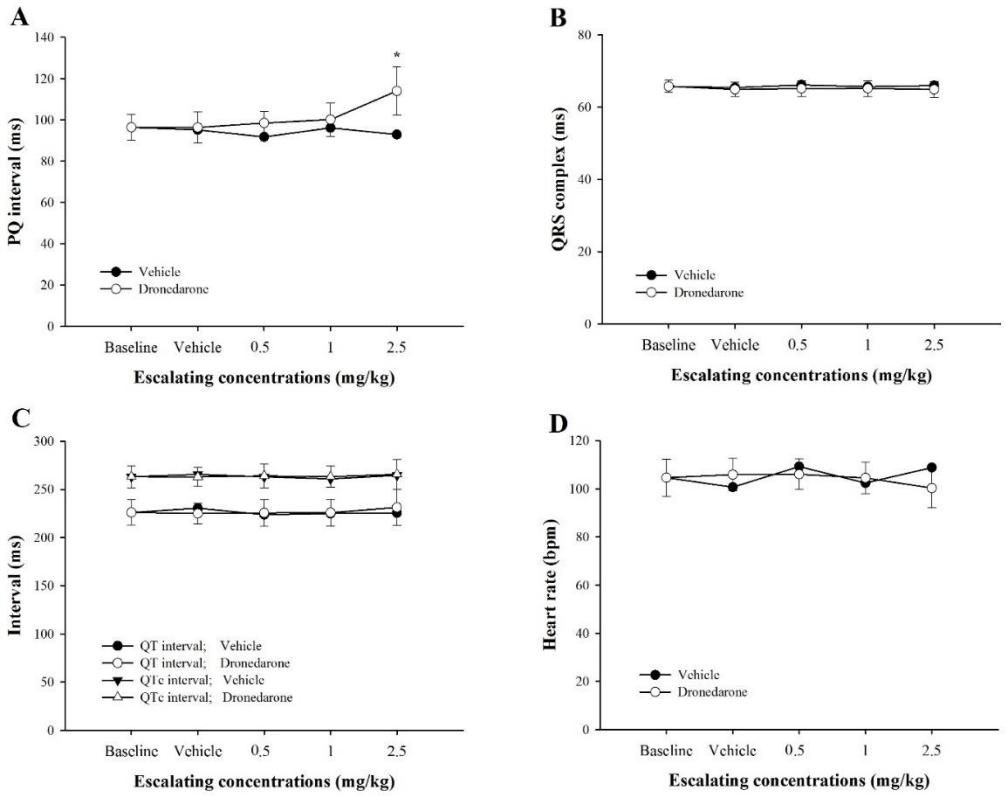


**Figure 21.** Effects of escalating doses of dronedarone (0.5, 1.0, and 2.5 mg/kg) versus vehicle-treated dogs measured at the same time-point on baseline adjusted PQ interval (A), QRS complex (B), QT and QTc intervals (C), and heart rate (D). Values were presented as mean ± standard error of means (SEM) in dronedarone-treated dogs (n = 5) while those values in the vehicle-treated dogs were presented as an average of 2 dogs. *indicates $P < 0.05$ versus baseline by one-way repeated measure ANOVA.

**B. Acute effects of dronedarone on His-bundle electrogram**

The AH interval at baseline was 79.0 ± 0.2 ms. It can be noticed that dronedarone tended to lengthen AH interval in a dose-dependent manner and peaked at 2.5 mg/kg with a 19.8 % increased from baseline (figure 22A). The HV interval at baseline was 50.5 ± 1.5 ms. All 3 cumulative doses of dronedarone had no effect on HV interval (figure 22B).

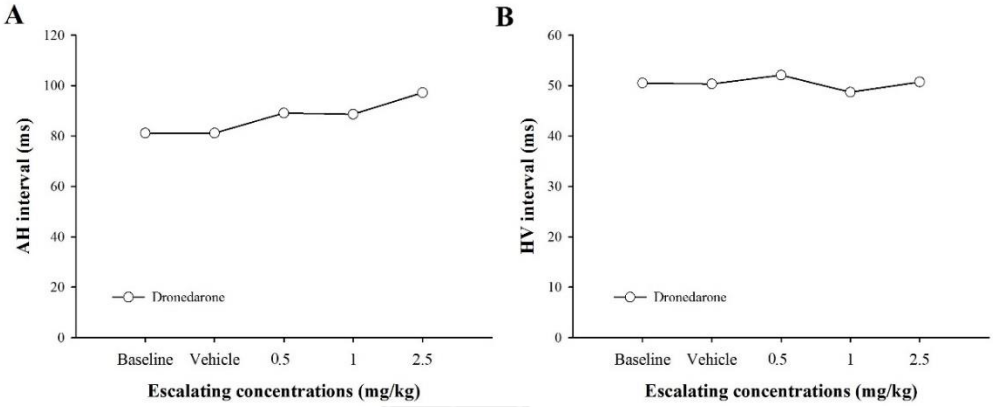


**Figure 22.** Effects of escalating doses of dronedarone (0.5, 1.0, 2.5 mg/kg) on AH interval (A) and HV interval (B). Values were presented as an average of two dogs treated with vehicle followed by dronedarone.

### C. Acute effects of dronedarone on hemodynamics

Figure 23 reveals plots of baseline adjusted cardiac output (A), mean aortic pressure (B), pulmonary vascular resistance (C), and systemic vascular resistance (D) versus cumulative doses obtained during incremental dosing in dronedarone-treated dogs and in vehicle-treated dogs. When dogs were given dronedarone, cardiac output tended to decrease at 1.0 mg/kg and significantly decreased at 2.5 mg/kg when compared with baseline ($P < 0.01$) whereas mean AoP did not change. In response to graded doses of dronedarone, systemic vascular resistance was sharply increased at 2.5 mg/kg when compared with baseline ($P < 0.01$), while PVR did not change. Mean pulmonary arterial pressure (mPAP) and mean RAP at baseline were 14.2 ± 0.82 mmHg and 4.79 ± 0.8 mmHg, respectively. While the mPAP was remained unchanged in dogs receiving either escalating dronedarone or vehicle from baseline to the end of experiment, the mRAP was significantly increased in dogs receiving dronedarone at 2.5 mg/kg (6.65 ± 1.28, $P < 0.05$) when compared with baseline.

Figure 24 reveals plots of baseline adjusted stroke volume, end-diastolic volume, and end-systolic volume versus escalating doses of dronedarone or vehicle. In response to incremental doses of dronedarone, SV and ESV were trivially changed at 0.5 mg/kg and 1.0 mg/kg. Then the SV was significantly decreased at 2.5 mg/kg ($P < 0.05$, -42.48 %) when compared to baseline whereas the ESV was increased at 1.0 mg/kg and significantly increased at 2.5 mg/kg ($P < 0.05$) when compared to baseline (15.57 % and 24.40 %, respectively). The EDV did not change in response to either dronedarone or vehicle. The SV, EDV, and ESV were unchanged in dogs receiving vehicle.

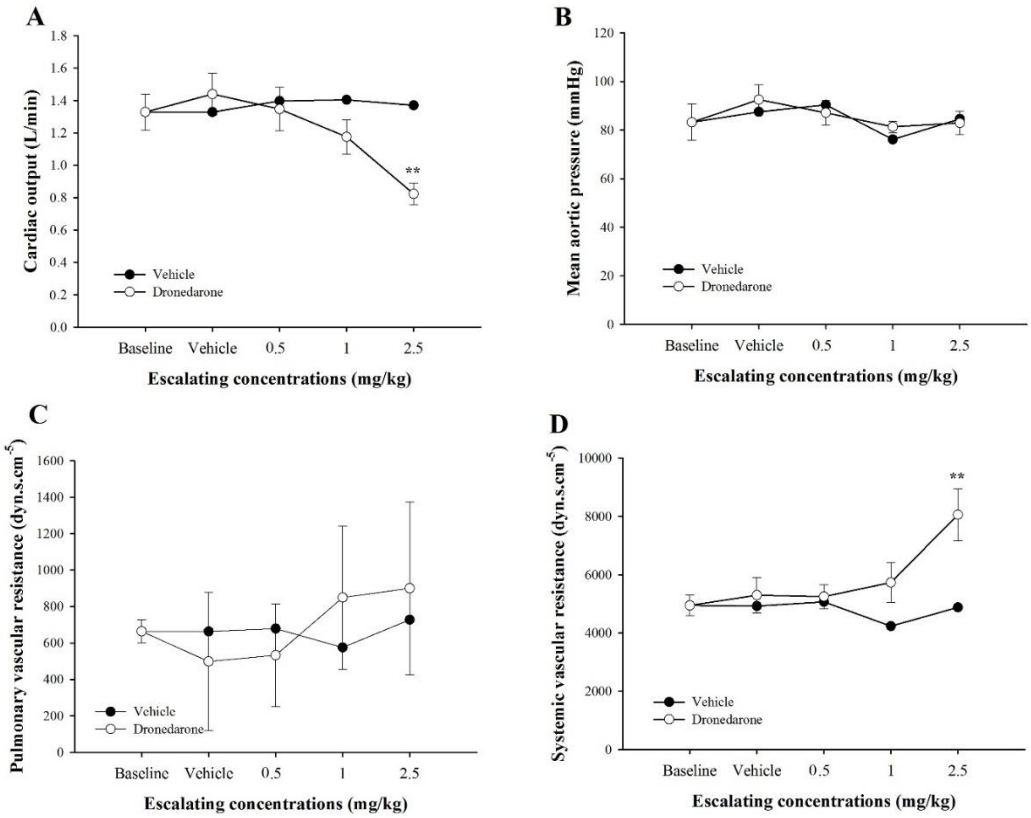


**Figure 23.** Effects of escalating doses of dronedarone (0.5, 1.0, and 2.5 mg/kg) versus vehicle-treated dogs measured at the same time-point on baseline adjusted cardiac output (A), mean aortic pressure (B), pulmonary vascular resistance (PVR, C), and systemic vascular resistance (SVR, D). Values were presented as mean ± standard error of means (SEM) in dronedarone-treated dogs (n = 5) while those values in the vehicle-treated dogs were presented as an average of 2 dogs. ** indicates $P < 0.01$ versus baseline by one-way repeated measure ANOVA.

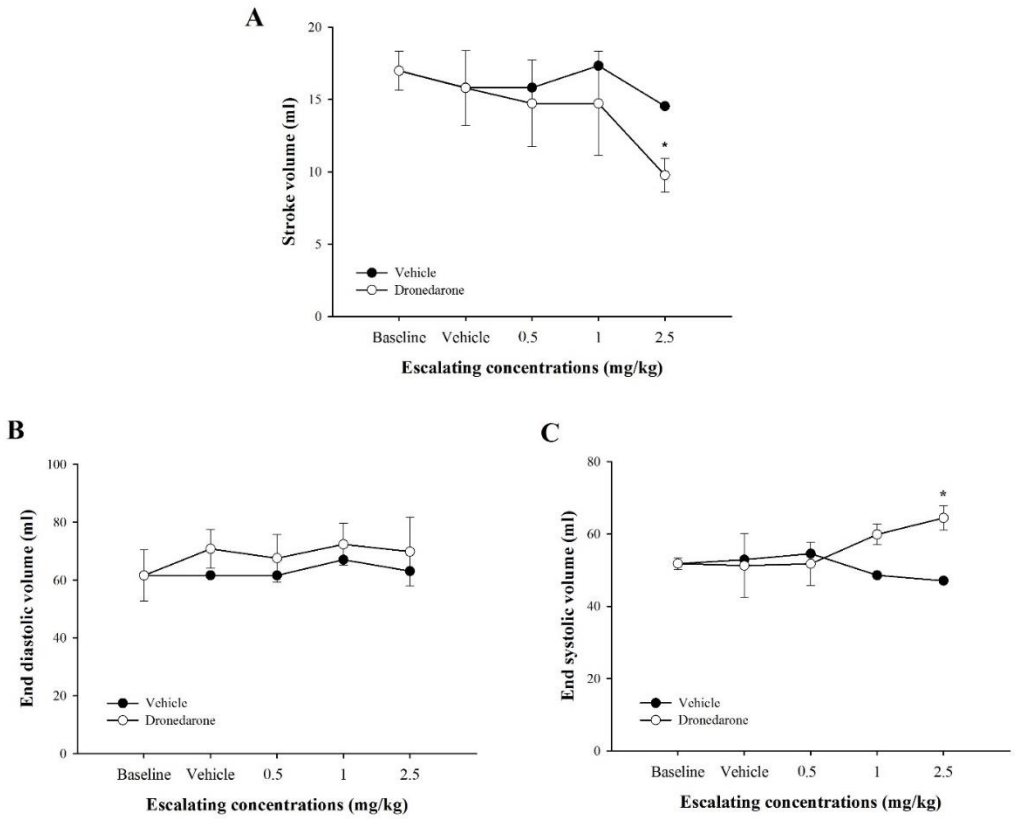


**Figure 24.** Effects of cumulative doses of dronedarone (0.5, 1.5, and 4 mg/kg) versus vehicle-treated dogs measured at the same time-point on baseline adjusted stroke volume (SV, A), end-diastolic volume (EDV, B), and end-systolic volume (ESV, C). Values were presented as mean ± standard error of means (SEM) in dronedarone-treated dogs (n = 5) while those values in the vehicle-treated dogs were presented as an average of 2 dogs. It can be noticed that acute dronedarone administration significantly reduced stroke volume whereas the end-systolic volume was significantly increased. There is no significant change in end-diastolic volume. *indicates $P < 0.05$ versus baseline by one-way repeated measure ANOVA.

**D. Acute effects of dronedarone on left ventricular functions**

Inotropic and lusitropic properties of left ventricle were assessed by parameters obtained from both pressure-volume loop and left ventricular pressure (figure 25-26). In response to escalating doses of dronedarone ESPVR, PRSW, CI, and $dP/dt_{max}$ were decreased in a dose-dependent manner. The end-systolic pressure volume relationship (figure 25A) was significantly decreased at 1.0 and 2.5 mg/kg when compared to baseline ($P < 0.05$; -49.30 % and -44.37 %, respectively). The representative family of pressure-volume loops in 1 dog receiving vehicle and escalating doses of dronedarone (0.5 and 1.0 mg/kg) was also plot in figure 26. The PRSW (figure 25B) and CI (figure 25C) were decreased continuously from baseline and became significantly decreased at 2.5 mg/kg when compared with baseline ($P < 0.05$; -22.29 % and -19.02 %, respectively). The $dP/dt_{max}$ (figure 25D) was continuously decreased from a dose of 0.5 mg/kg and the change became significant at 1.0 and 2.5 mg/kg when compared to baseline ($P<0.05$, -22.62 % and $P < 0.01$, -30.26 %, respectively). All parameters of contractility were unchanged in dogs receiving vehicle.

In response to escalating doses of dronedarone, end-diastolic pressure volume relationship (EDPVR), tau, and $dP/dt_{min}$ were increased in a dose-dependent manner (figure 27). The EDPVR (figure 27A) was unchanged at 0.5 mg/kg but it significantly increased at 1.0 and 2.5 mg/kg when compared with baseline ($P < 0.05$; 47.55 % and 54.33 %, respectively). The tau (figure 27B) and $dP/dt_{min}$ (figure 27C) were significantly increased only at 2.5 mg/kg when compared with baseline ($P < 0.01$, 50.85 %; $P < 0.05$, 25.61 %, respectively). All parameters of relaxation were unchanged in dogs receiving vehicle.

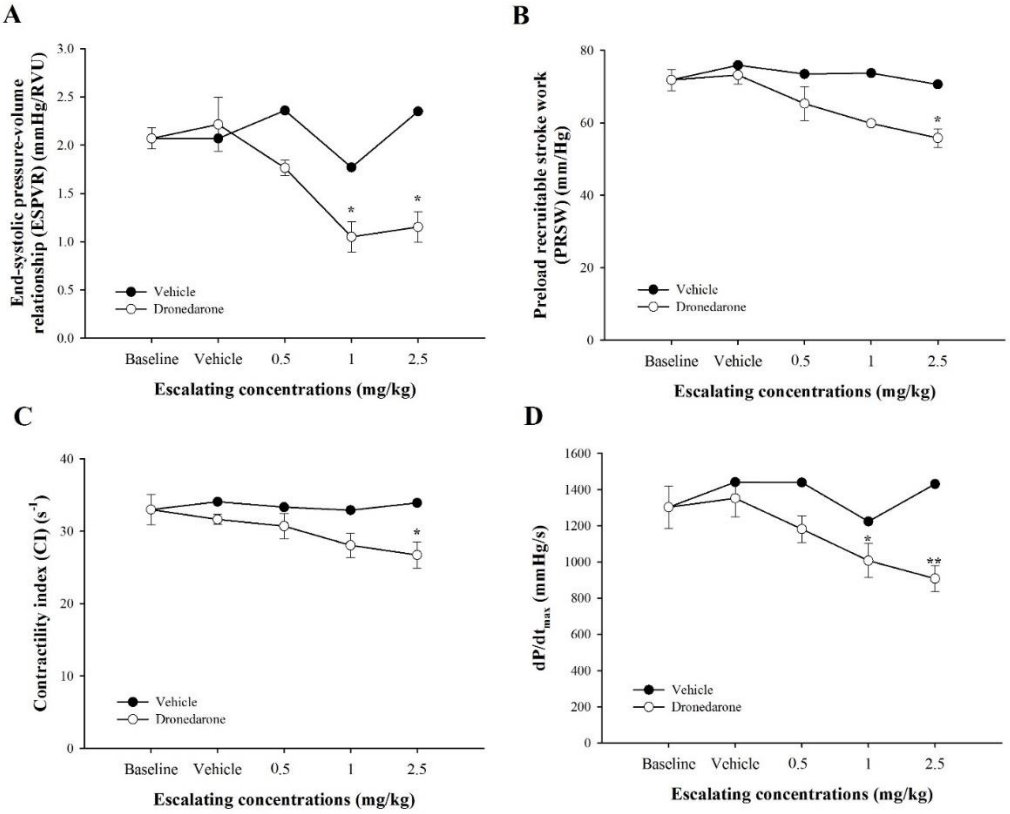


**Figure 25.** Effects of escalating doses of dronedarone (0.5, 1.0, and 2.5 mg/kg) versus vehicle-treated dogs measured at the same time-point on baseline adjusted introtropic indices, end-systolic pressure-volume relationship (ESPVR, A), preload recruitable stroke work (PRSW, B), contractility index (CI, C), and $dP/dt_{max}$ (D). Values were presented as mean ± standard error of means (SEM) in dronedarone-treated dogs (n = 5) while those values in the vehicle-treated dogs were presented as an average of 2 dogs. *indicates $P < 0.05$ and **indicates $P < 0.01$ versus baseline by one-way repeated measure ANOVA.

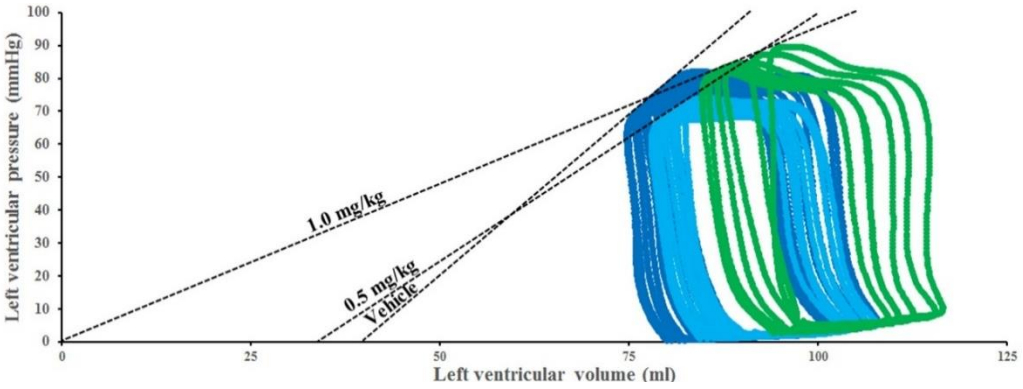


**Figure 26.** Representative left ventricular pressure-volume relationship in a single dog receiving vehicle and after administration of dronedarone (0.5 and 1.0 mg/kg) in isoflurane anesthetized dog. The slopes of the end-systolic pressure-volume relationship were fitted by linear.

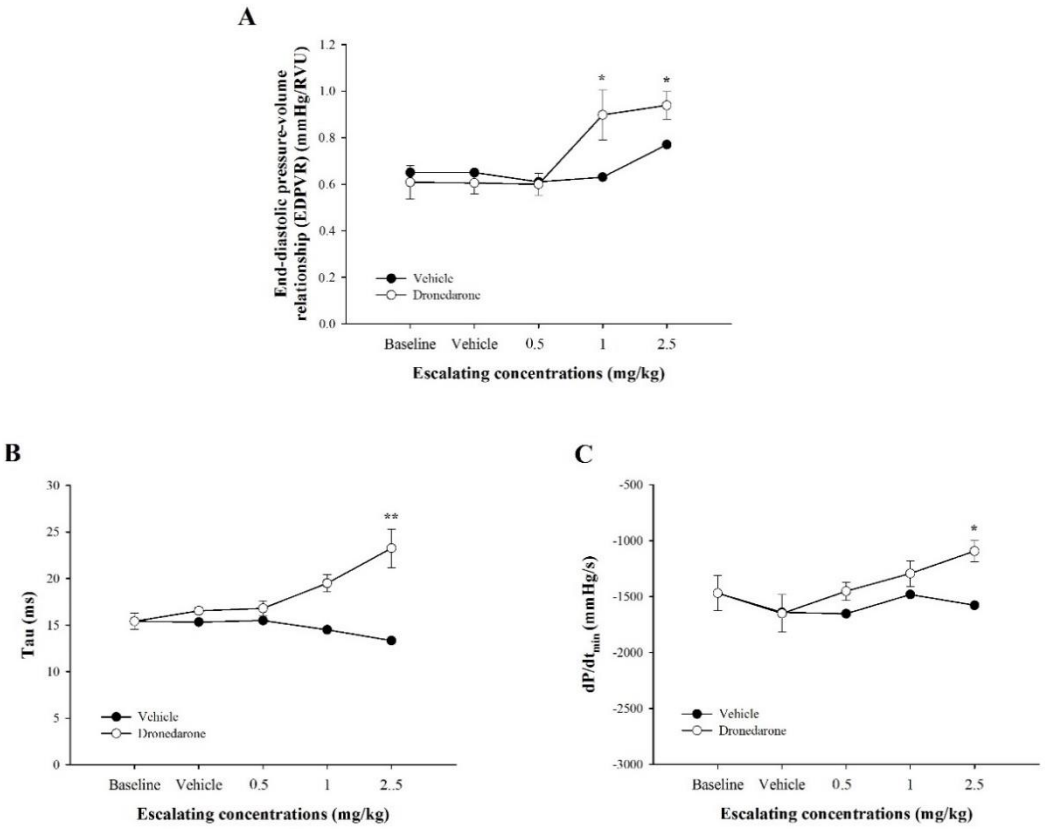


**Figure 27.** Effects of escalating doses of dronedarone (0.5, 1.0, 2.5 mg/kg) versus vehicle-treated dogs measured at the same time-point on baseline adjusted lusitropic indices, end-diastolic pressure-volume relationship (EDPVR, A), tau (B), and $dP/dt_{min}$ (C). Values were presented as mean ± standard error of means (SEM) in dronedarone-treated dogs (n = 5) while those values in the vehicle-treated dogs were presented as an average of 2 dogs. *indicates $p < 0.05$ and **indicates $P < 0.01$ versus baseline by one-way repeated measure ANOVA.

**Study part 2: Effects of single and short-term oral dose of dronedarone administration on cardiac functions, blood pressures, and electrocardiograms in conscious telemetry dogs**

**A. Effects of a single oral dose of dronedarone on heart rate, ECG parameters, and blood pressure**

In the first set of experiment, two dogs were randomly received 3 doses of dronedarone (10, 20, or 40 mg/kg, PO) and placebo. At baseline, the averaged heart rate of both dogs was 86.88 ± 5.59 bpm and the mean blood pressure was 93.84 ± 8.0 mmHg. The means of PQ, QRS, QT and QTc were 91.15 ± 3.23 ms, 35.44 ± 1.46 ms, 201.9 ± 5.68 ms, and 228.54 ± 1.8 ms respectively. All of single oral doses of dronedarone had no effect on those parameters throughout the monitoring period (36 hr) except for the PQ interval when compared with placebo (figure 28A-28F). Single oral dose of dronedarone at 40 mg/kg seems to increase the PQ interval clearly from effects of other doses. It can be noticed that heart rate, QRS complex and mean blood pressure were highly variable during the monitoring period.

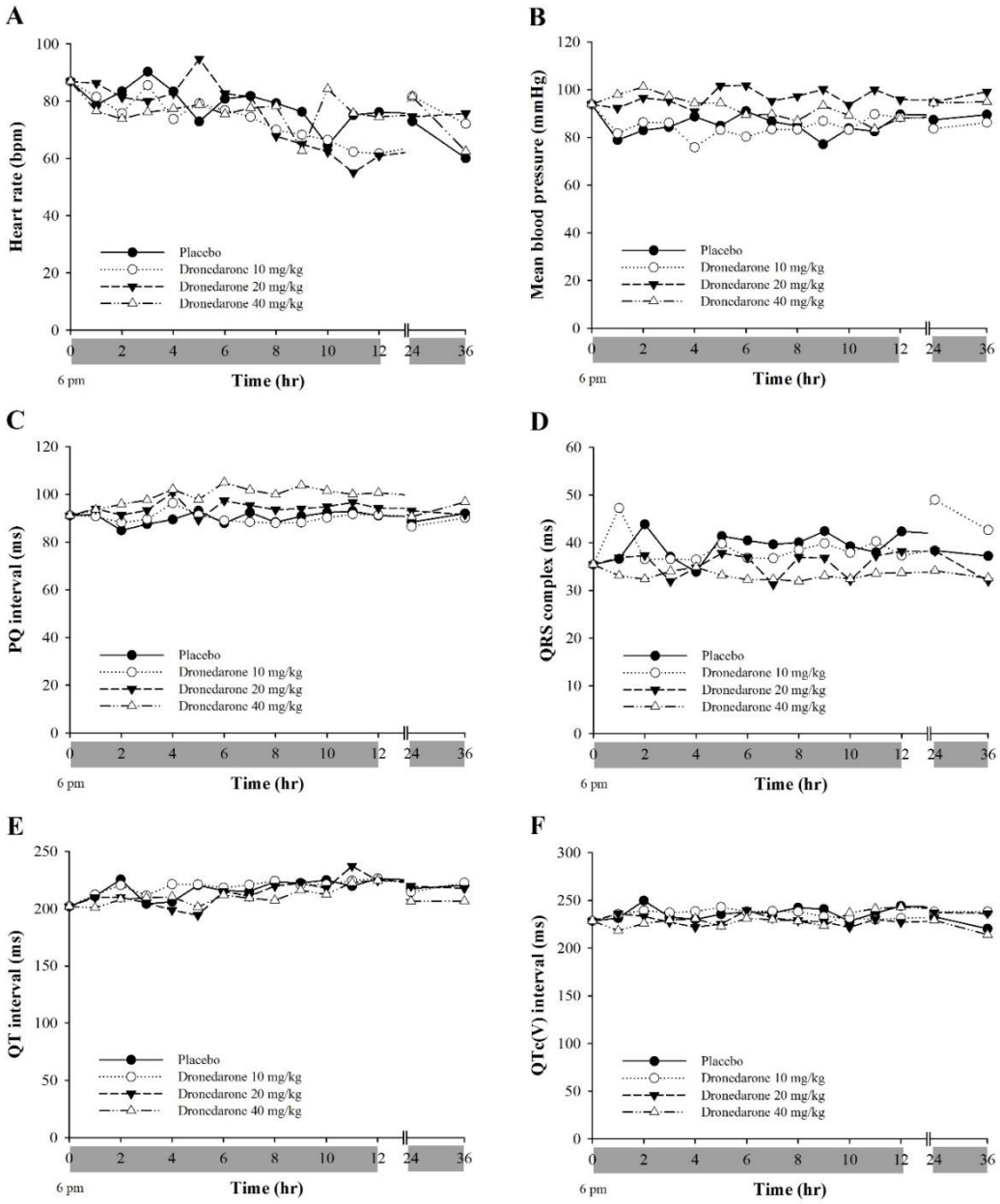


**Figure 28.** Effects of single oral dose of placebo or dronedarone (10, 20, and 40 mg/kg) on heart rate (A), mean blood pressure (B), PQ interval (C), QRS complex (D), QT interval (E), and QTc(V) (F) in two conscious dogs instrumented with telemetered unit for obtaining electrocardiograms and blood pressure. Values were obtained from an average of two dogs acquired at baseline, hourly at 1-12 hr, 24 and 36 hr after dosing. Data of each time-pointed was averaged from 1 min.

**B. Effects of chronic oral dronedarone on cardiac contractility**

In this study, cardiac contractility was assessed by end-systolic pressure-volume relationship (ESPVR), preload recruitable stroke work (PRSW), contractility index (CI), and $dp/dt_{max}$. Figure 29 shows a family of pressure-volume loops generated after a brief period of posterior vena cava occlusion. Notice that the slopes of those loops were similar when measured at baseline (A), 4 hr after the first dose (B), day 4 (C), and day 7 (D). Overall, the indices of cardiac contractility did not change significantly after chronic dronedarone treatment for 7 days (20 mg/kg, BID, PO) when compared with baseline. It could be noticed that at 4 hr after the first dose, the PRSW, CI, and $dP/dt_{max}$ were decreased when compared with baseline (15.01 %, 10.30 %, and 12.81 %, respectively) but those values did not reach the statistical significance. PRSW and $dP/dt_{max}$ seems to be constant after 12 hr of the first dose until day 7 except for the CI which seems to be increase (13.11 %, P = 0.063) at day 7 (figure 30).

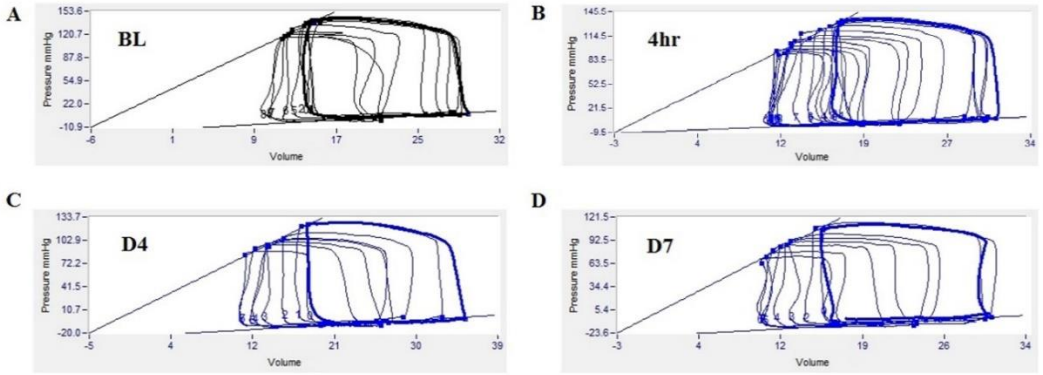


**Figure 29.** Representative left ventricular pressure-volume relationship at baseline (A) and after oral dronedarone administration (20 mg/kg, twice per day) at 4 hr after the first dose (B), at day 4 (D4, C), and at day 7 (D7, D) in conscious dogs. The slopes of the end-systolic pressure-volume relationship (ESPVR) were fitted by linear.

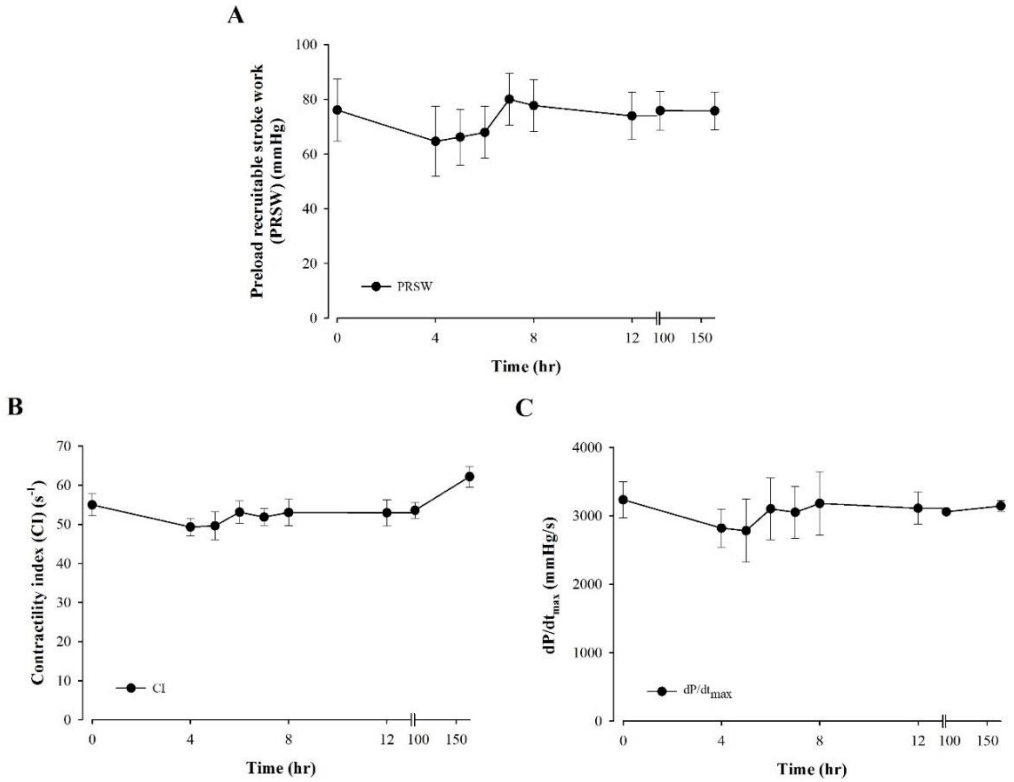


**Figure 30.** Effects of oral dronedarone administration (20 mg/kg, BID) for 7 days on preload recruitable stroke work (PRSW, A), contractility index (CI, B), and the maximal rate of rise of the left ventricular pressure ($dP/dt_{max}$, C) in conscious dogs (n = 4). Values were obtained at baseline (0 hr), 4-8 and 12 hr after the first dose, 96 hr (day 4) and 168 hr (day 7) after dosing.

### C. Effects of chronic oral dronedarone on cardiac relaxation

Cardiac relaxation was evaluated by end-diastolic pressure-volume relationship (EDPVR), tau, and $dP/dt_{min}$ (figure 31A-31C). All of relaxation indices measured in this study were highly variable and did not change significantly when compared with baseline. Similar with the inotropic indices, EDPVR and tau seem to be constant at 12 hr after the first dose until day 7.

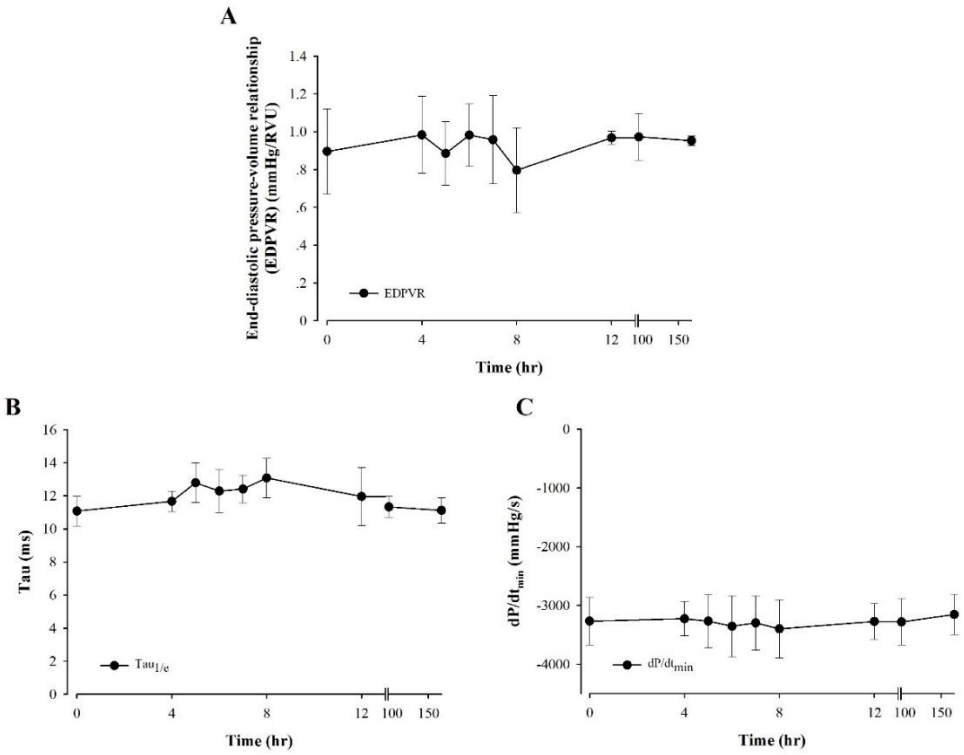


**Figure 31.** Effects of oral dronedarone administration (20 mg/kg, BID) for 7 days on end-diastolic pressure-volume relationship (EDPVR, A), tau (B), and the maximal rate of fall of the left ventricular pressure ($dP/dt_{min}$, C) in conscious dogs (n = 4). Values were obtained at baseline (0 hr), 4-8 and 12 hr after the first dose, 96 hr (day 4) and 168 hr (day 7) after dosing.

**D. Effects of chronic oral dronedarone on cardiac output and blood pressure**

Chronic administration of dronedarone tended to decrease cardiac output (figure 32A) beginning at 4 hr after the first dose (-22.58 %, P = 0.273) until the last time-point of measurement (day 7, -33.83 %, P = 0.273); however, these decreases did not achieve statistical significance when compared with baseline. The end-systolic volume, end-diastolic volume, and stroke volume were unaltered (figure 32B). Figure 32C illustrates the mean blood pressures (MBP) in response to chronic dronedarone administration. MBP seems to be unaltered from the beginning to 8 hr after the first dose. After 8 hr, it started to decline to the level lower than baseline. At day 4-7, the decreases of MBP became constant and that values were significantly lower when compared with baseline (day 4, -20.18 %, $P < 0.018$; day 7, - 17.76 %, $P < 0.044$, respectively).

**E. Effect of chronic oral dronedarone on heart rate and ECG parameters**

In response to chronic dronedarone administration, the heart rate (HR) tended to decrease and it was almost significantly reduction at day 7 (-25.43 %, P = 0.057) when compared with baseline (figure 33A). While QRS complex was not change (data not show), the PQ interval (figure 33B) was constant from the beginning until 12 hr after the first dose. After that it was significantly lengthened at day 4 and day 7 (21.7 %, $P < 0.001$ and 18.01 %, $P < 0.001$, respectively). The QT interval (figure 33C) was gradually lengthened and almost significantly prolonged at day 4 (9.63 %, P = 0.067) when compared with baseline. However, when the QT was corrected for heart rate by van de Water formula, it was not significantly prolonged (figure 33D).

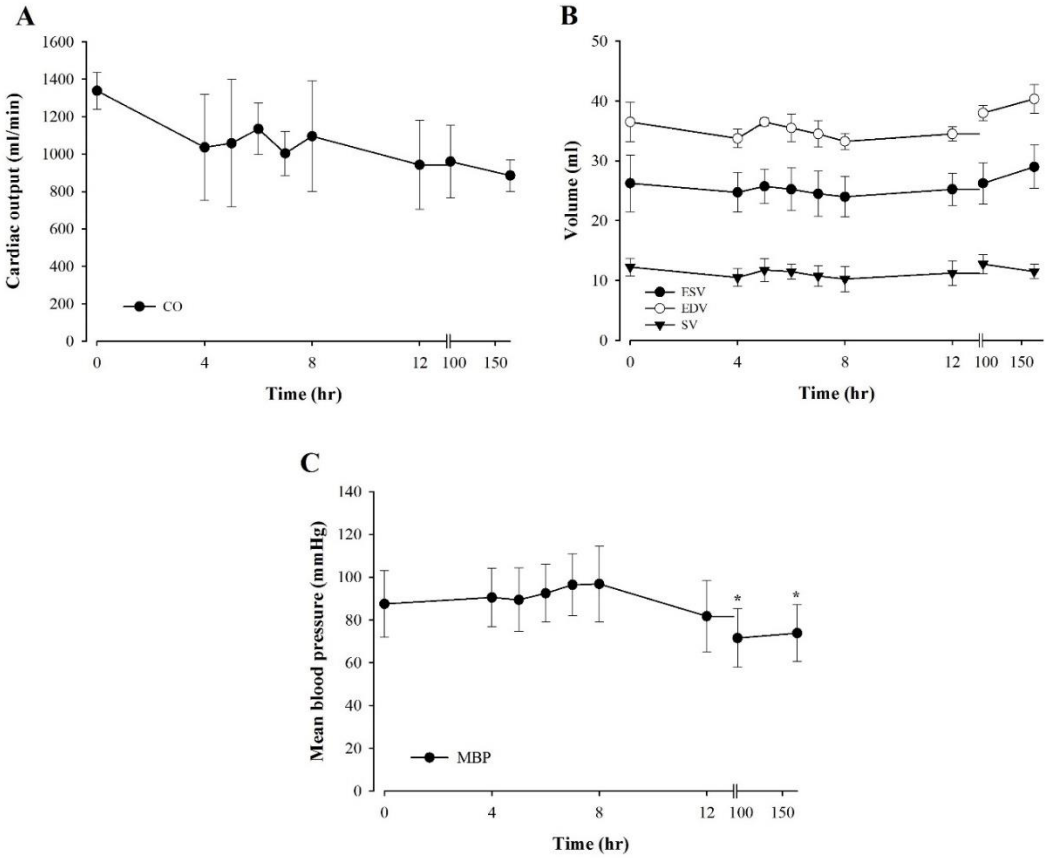


**Figure 32.** Effects of oral dronedarone administration (20 mg/kg, BID) for 7 days on cardiac output (CO, A), end-systolic volume (ESV), end-diastolic volume (EDV), and stroke volume (B), and mean blood pressure (MBP, C) in conscious dogs (n = 4). Values were obtained at baseline (0 hr), 4-8 and 12 hr after the first dose, 96 hr (day 4) and 168 hr (day 7) after dosing. *indicates $P < 0.05$ versus baseline by one-way repeated measure ANOVA

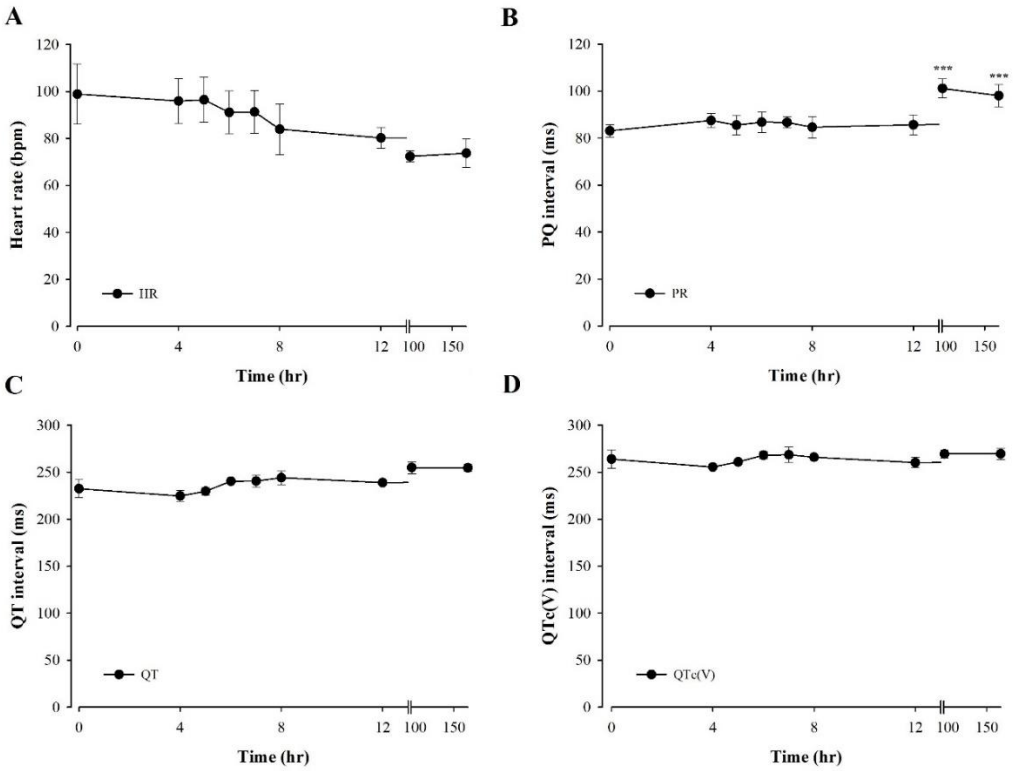


**Figure 33.** Effects of oral dronedarone administration (20 mg/kg, BID) for 7 days on heart rate (HR, A), PQ interval (B), QT interval (C), and QTc(V) interval (D) in conscious dogs (n = 4). Values were obtained at baseline (0 hr), 4-8 and 12 hr after the first dose, 96 hr (day 4) and 168 hr (day 7) after dosing. ***P < 0.001 versus baseline by one-way repeated measure ANOVA

**Study part 3: Dronedarone attenuates the duration of atrial fibrillation in dog model of sustained atrial fibrillation**

All dogs used in this study were healthy. Their complete blood count (CBC), blood chemistry analysis (i.e. blood urea nitrogen, creatinine, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), alanine transaminase (ALT)) and electrocardiograms were within normal limit. After receiving dronedarone for 7 day, their CBC and blood chemistry analysis were still within normal limit.

Before the onset of phenylephrine (PE) infusion (baseline) while the dog was anesthetized, heart rate (HR) and mean blood pressure (MBP) at pre-dosing were 106 ± 1.0 bpm and 69.7 ± 1.7 mmHg, respectively (figure 34-35). After oral dronedarone administration (20 mg/kg, twice a day) for 7 days, the HR was significantly reduced 15.6 % ($P < 0.05$) whereas the MBP was declined significantly (14.0 %, $P < 0.05$) when compared with pre-dosing. During RAP simultaneously with PE infusion at pre-dosing, VR was 163 ± 31 bpm. At post-dosing, the VR during RAP and PE infusion (2 µg/kg/min) was declined significantly (21.6 %, $P < 0.05$) while the VR during RAP and PE infusion (4 µg/kg/min) did not change when compared with pre-dosing (figure 34). The MBP at pre-dosing was 137.2 ± 15.4 mmHg. At post-dosing, neither MBP during RAP and PE infusion (2 µg/kg/min) nor MBP during RAP and PE infusion (4 µg/kg/min) were different from MBP during RAP and PE infusion at pre-dosing (figure 35).

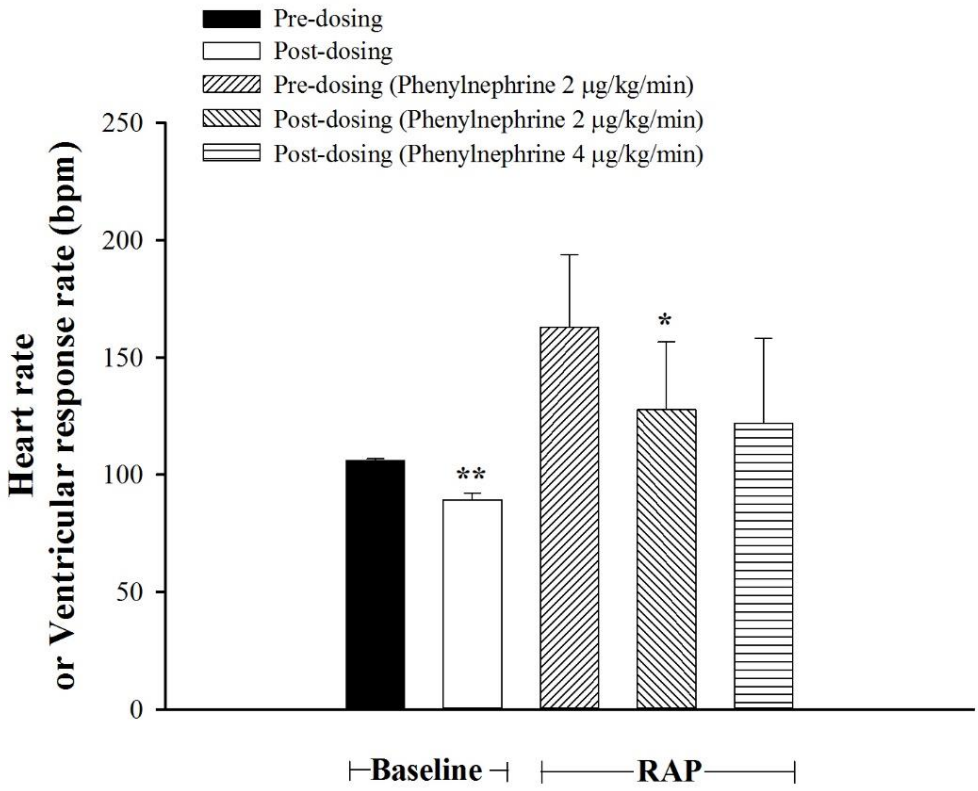


**Figure 34.** Plots of heart rate (during baseline before the beginning of phenylephrine infusion) and ventricular response rate (during rapid atrial pacing, RAP) for pre- and post-dosing with oral dronedarone administration (20 mg/kg, BID) for 7 days in dogs while they were anesthetized with isoflurane. $^{*}$indicates $P < 0.05$, $^{**}$indicates $P < 0.01$ versus pre-dosing by paired t-test

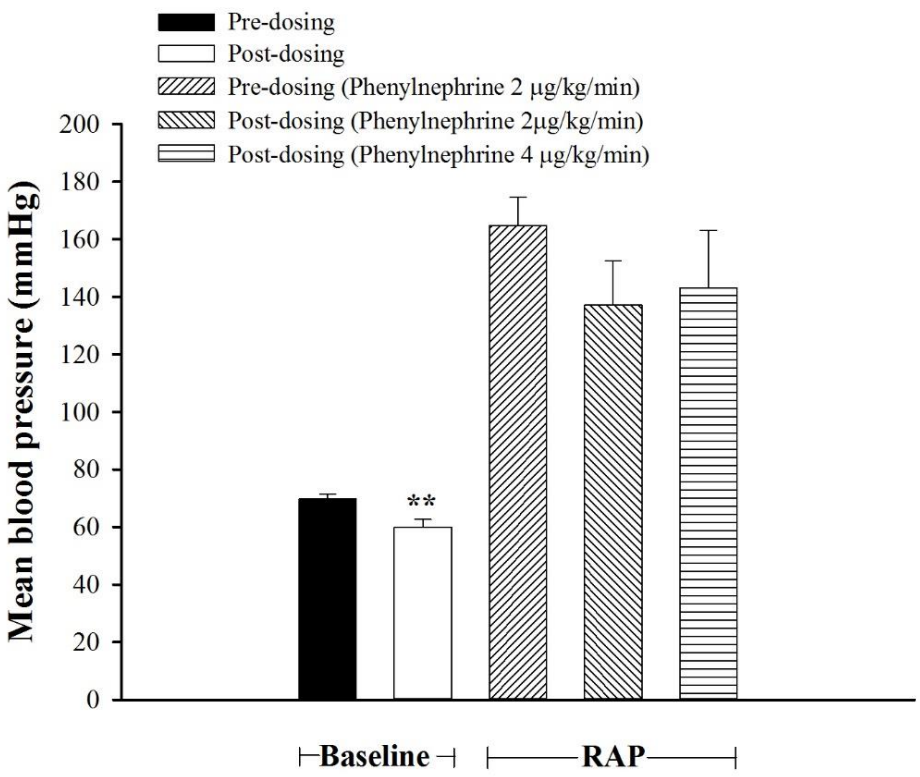


**Figure 35.** Plots of mean blood pressure during baseline (before the beginning of phenylephrine infusion) and rapid atrial pacing (RAP) for pre- and post-dosing with oral dronedarone administration (20 mg/kg, BID) for 7 days in dogs while they were anesthetized with isoflurane. **indicates $P < 0.01$ versus pre-dosing by paired t-test

In response to dronedarone, $APD_{70}$ (figure 36-37) of atrium was significantly increased 19.3 % compared with pre-dosing ($P < 0.001$). The AERP was also significantly increased 23.1 % compared with pre-dosing ($P < 0.01$). Hence PRR significantly increased 36.7 % compared with pre-dosing (from 21.1 ± 4.6 ms to 28.8 ± 6.4 ms, $P < 0.01$).

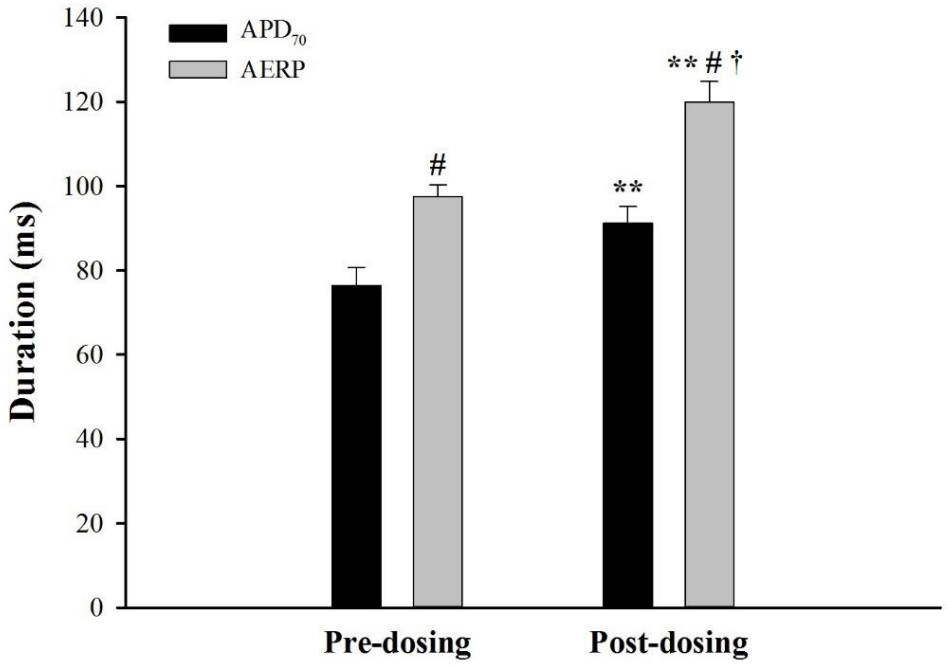


**Figure 36.** Plots of atrial action potential duration at 70 % or repolarization ($APD_{70}$) and atrial effective refractory period (AERP) obtained at pre-dosing and post-dosing with oral dronedarone administration (20 mg/kg, BID) for 7 days in dogs while they were anesthetized with isoflurane. Post-repolarization refractoriness (PRR) was defined as the difference between the AERP and the $APD_{70}$. $^{\#}$indicates $P < 0.05$ when compared between $APD_{70}$ and AERP at the same time-point, $^{**}$indicates $P < 0.01$ when compared between the same parameter with pre-dosing, $^{\dagger}$indicates $P < 0.05$ when compared PRR at post-dosing with pre-dosing versus pre-dosing by paired t-test

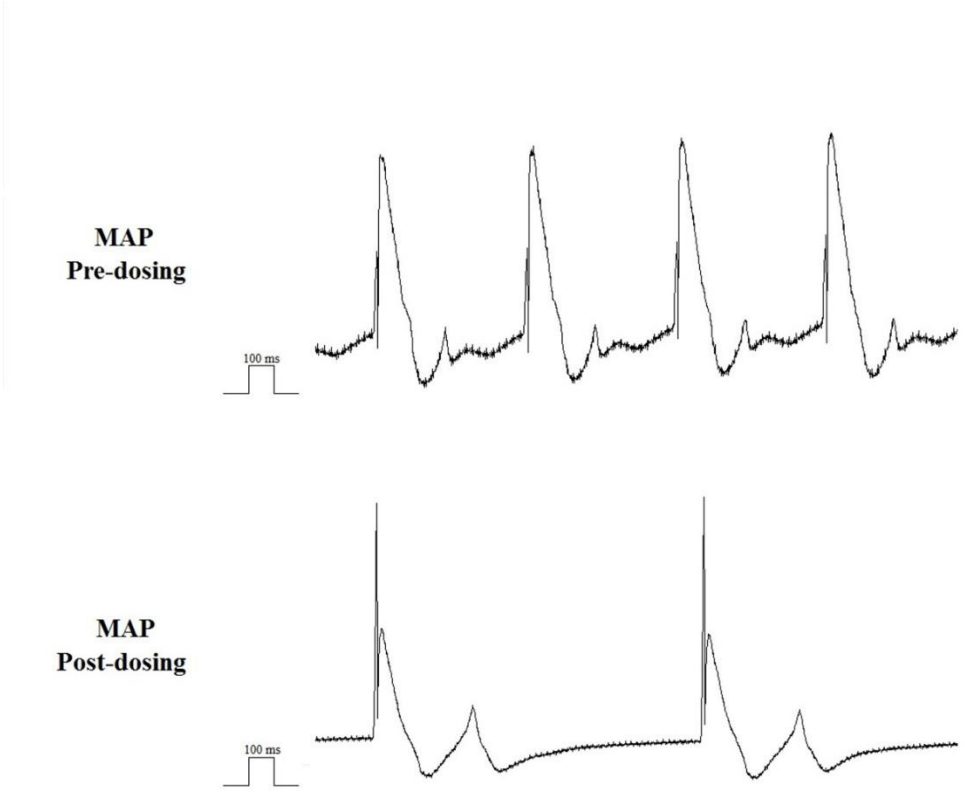


**Figure 37.** A representative monophasic action potential (MAP) of the right atrium in one of the dog during baseline (anesthetized but no pacing) at pre-dosing and at 7 day post-dosing with dronedarone (20 mg/kg, BID, PO) (ECG-Auto v3.3.0.15).



At pre-dosing, after PE infusion and RAP, atrial fibrillation persisted in all dogs after cessation of pacing with an average of 88.8 sec (ranging from 441.7 to 1.89 sec) (figure 38). After 7 days of dronedarone administration, sustained AF was induced in 5 dogs in which the average duration of sustained AF was reduced to 5.7 sec (ranging from 4.3 to 10.7 sec). While the sustained AF was unable to induce in one dog (figure 39), the duration of sustained AF was increased in one dog (from 1.9 to 5.8 sec). Therefore the overall percentage of dronedarone which attenuates the duration of AF in dogs model of sustained AF was 83.3 % (5/6 dogs, $P < 0.05$).

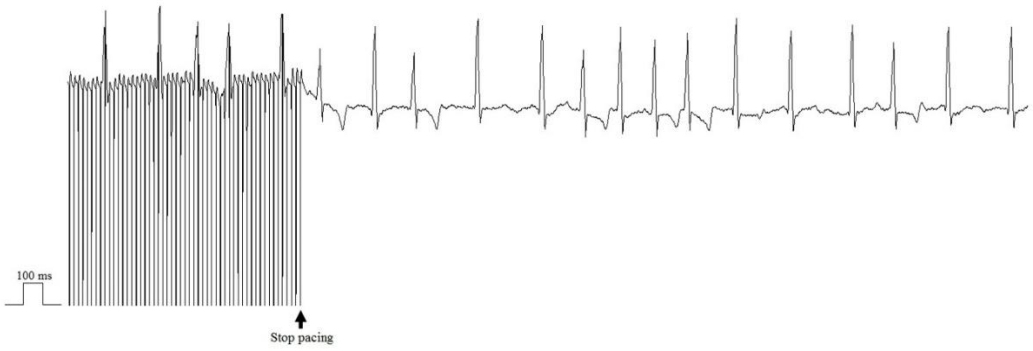


**Figure 38.** Lead II electrocardiograms in the anesthetized dog with rapid atrial pacing (40 Hz, 20 V, and 2 ms) and phenylephrine infusion (2 μg/kg/min). Atrial fibrillation characterized by fibrillatory waves that varied in amplitude, shape, and timing occurred after cessation of rapid atrial pacing.

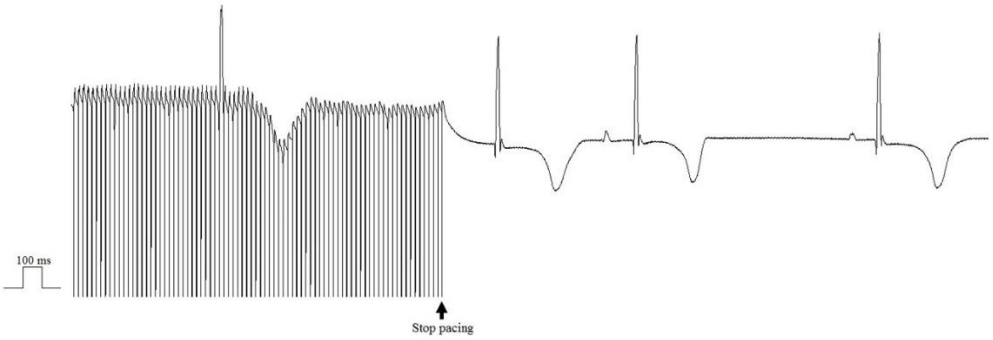




**Figure 39.** Lead II electrocardiograms in the anesthetized dog treated with dronedarone (20 mg/kg, BID, PO) for 7 days. Atrial fibrillation was induced by rapid atrial pacing (40 Hz, 20 V, and 2 ms) and phenylephrine infusion (2 μg/kg/min) and it was converted to normal sinus rhythm soon after rapid atrial pacing was stopped.

# CHAPTER V
# DISCUSSION

## Study part 1: Acute effects of intravenous dronedarone on electrocardiograms, hemodynamics, and cardiac functions in anesthetized dogs

Dronedarone has been used widely for management of AF in humans. While the acute effects of dronedarone on ECG parameters and his bundle electrograms were studied previously (Manning et al., 1995), there are no reference to hemodynamics and cardiac mechanics in dogs. This study was conducted to determine acute effects of dronedarone on electrocardiograms, hemodynamics, and cardiovascular functions especially the left ventricular mechanics in intact dogs anesthetized with isoflurane.

In this study, an escalating dose of dronedarone 2.5 mg/kg caused PQ interval to lengthen to values greater than obtained during baseline and vehicle. In addition, dronedarone doses, used in the present study, tended to prolong AH interval in a dose-dependent manner. The prolongation of PQ interval as well as AH interval can be explained by dronedarone binding to its binding site in cardiac calcium channel, which reduces calcium conductance through voltage-gated L-type calcium channel ($I_{Ca,L}$). Previous *in vitro* whole cell patch clamp study showed that dronedarone blocks $I_{Ca,L}$ with $IC_{50}$ of 0.18 $\mu$M at a stimulation frequency of 0.033 Hz in a use-and frequency-dependent manner (Gautier et al., 2003). Similarly, acute dronedarone application in dog's papillary muscle demonstrated strong $I_{Ca,L}$ inhibitory effect (Varro et al., 2001). In alpha–chloralose anesthetized dogs, intravenous administration of dronedarone (5 mg/kg) has been demonstrated to markedly lengthen PQ interval (Manning et al., 1995). The lengthening of PQ interval may be attributed to blocking of the $I_{Ca,L}$ or the fast sodium channel ($I_{Na}$) by dronedarone. However, it has been showed both in our study and a previous study (Manning et al., 1995) that dronedarone did not affect the HV interval together with a lack of any effect on QRS interval in this study suggesting that acute effect of dronedarone at an escalating dose of 2.5 mg/kg did not significantly alter sodium channel. Our study confirmed the findings of previous studies in that

acute effect of intravenous dronedarone caused PQ interval prolongation (Manning et al., 1995; Verrier et al., 2013). It has been suggested that the effect of dronedarone on APD and QT/QTc intervals is depending on species and the duration of drug administration partly due to its protein-binding property that may interfere with its electrical properties (Patel et al., 2009). In this study the QT and QTc intervals were unchanged. The result of QTc interval was agreed with previous studies (Manning et al., 1995; Varro et al., 2001). In alpha–chloralose anesthetized dogs in which a cumulative dose of dronedarone at 4.5 mg/kg did not affect QTc interval. In conscious normal dogs, chronic dronedarone administration orally (25 mg/kg, twice per day, 4 weeks) did not show any effect on QT interval. In contrast to our results, intravenous dronedarone in dogs with complete atrioventricular block produced a QTc shortening effect and suppressed EAD-induced torsades de pointes (Verduyn et al., 1999). Varro and colleagues (Varro et al., 2001) suggested that it might be because dronedarone has multiple sites of action involving $I_{Ca,L}$, $I_{Na}$, and rapid component of delayed rectifier potassium channel ($I_{Kr}$). These multiple blocking effects may balance depolarizing and repolarizing currents resulted in unaltered QTc intervals. Therefore the possible explanation for unchanged QT/QTc intervals in our study would be the duration of drug administration (i.e. acute vs sustained administration) together with the multichannel blocking effects. It has been known that dronedarone reduces heart rate by blockade of both $I_{Ca,L}$ and beta-adrenergic receptors. In addition, recent studies have showed that dronedarone also inhibits funny channel ($I_f$) in pacemaker cells (Bogdan et al., 2011; Sobrado et al., 2013; Verrier et al., 2013). The heart rate measured in this study was minimally changed in response to escalating doses of dronedarone. Similar results were observed in the study of Hodeige and colleagues in which intravenous dronedarone administration at either 1 mg/kg or 5 mg/kg failed to attenuate isoprenaline-induced increases in HR in anesthetized dog but oral dose of dronedarone (12.5 mg/kg) significantly reduced the elevation of HR induced by isoprenaline in conscious dogs (Hodeige et al., 1995). In contrast to our result, a cumulative dose of 4.5 mg/kg demonstrated a reduction of heart rate 10-20 % in dogs anesthetized with alpha–chloralose, an anesthetic known to produce minimal cardiac and respiratory depression. The differences between previous studies and our study

are the anesthetic regimens and the experimental conditions which may be responsible for the different outcome (Manning et al., 1995).

A previous study in anesthetized pigs showed that cumulative doses of dronedarone (5 mg/kg, IV) had no effect on mean arterial pressure and contractility as evaluated by left ventricular $dP/dt_{max}$ (Sobrado et al., 2013). In our study, left ventricular contractility was decreased significantly at an escalating dose of 2.5 mg/kg as assessed by end-systolic pressure-volume relationship (ESPVR), preload recruitable stroke work (PRSW), and $dP/dt_{max}$. The first two parameters were derived from a family of pressure-volume loops, a gold standard for measurements of cardiac contractility, since they are load-independent indices (Suga et al., 1973). On the other hand, $dP/dt_{max}$, a measure of baroinometry, is determined by loading conditions (i.e. preload, afterload), heart rate (Bowditch effect), and myocardial contractility (Hamlin and del Rio, 2012). Since the mean arterial blood pressure, end-diastolic volume, and heart rate remained unchanged in this experiment, the decrease in $dP/dt_{max}$ could be a consequence of the negative inotropy of the dronedarone. Therefore, the reduction in LV contractility in our study could be explained by multichannel blocking properties of dronedarone mainly $I_{Ca,L}$ and non-competitive binding to beta-adrenergic receptors (Chatelain et al., 1995). The poor LV contraction resulted in increased end-systolic volume. Since the end-diastolic volume did not change, the stroke volume was markedly reduced. As a result, a cardiac output (CO) in this study was markedly reduced (-38.02 %) from baseline.

Interestingly, while cardiac output markedly decreased, the blood pressure was remained unchanged. Since blood pressure is a product of CO and total peripheral resistance (TPR), the reduction in CO must be counteract by an elevation of TPR. In our study, systemic vascular resistance (SVR) was noticeably increased at an escalating dose of 2.5 mg/kg while the pulmonary vascular resistance (PVR) was trivially changed. The SVR was mainly determined by the diameter of the blood vessels. However, dronedarone has been known to possess both alpha- and beta-adrenergic blocking effects which may dilate the resistant vessels. This discrepancy could be explained by the fact that when test article with beta-blockade property was acutely administered to the dog, the compensatory rise in SVR was usually observed because a fall in CO

activates baroreceptor reflex (van den Meiracker et al., 1988; Lund-Johansen and Omvik, 1991). However, the compensatory increase in heart rate was not detected since the blocking effects of dronedarone on adrenergic receptors and $I_f$ channels prevent the compensatory reflex.

In this study, effect of dronedarone 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 (Garcia et al., 2000). The left ventricular $dP/dt_{min}$ is determined by lusitrope, reduction in heart rate, diastolic systemic arterial pressure, structural properties of myocardium, and constriction of the pericardium or pericardial effusion (McConnell et al., 2009). Tau is determined by both heart rate and beta-blocking effect of dronedarone. In response to escalating doses of dronedarone, $dP/dt_{min}$ and tau changed in dose-dependent manner. Since the heart rate remained unchanged throughout the experiment, the observed change in tau (i.e. negative lusitrope) may result from beta-blocking effect of dronedarone prevents phosphorylation of phospholamban; therefore, less calcium is resequestrated through the $SERCa^{2+}$ channel resulted in slow relaxation (Bristow, 2011). This result is also in accordance with our previous study in which $dP/dt_{min}$ and tau were lengthened when metoprolol was given to anesthetized guinea pigs (Kijtawornrat et al., 2014). The end-diastolic pressure-volume relationship (EDPVR) has been used as an index of lusitrope since it measures the relationship between pressure and volume at the end-diastole (Burkhoff, 2013). Similar to the results of tau and $dP/dt_{min}$, EDPVR was elevated at an escalating dose of 2.5 mg/kg compared to baseline suggesting diastolic dysfunction due to acute effect of beta-blockade. The similar result was observed when escalating doses of metoprolol were given intravenously in anesthetized guinea pigs (Kijtawornrat et al., 2014).

It is well known that dronedarone and other amiodarone-like agents had a slow onset of action (Naccarelli and Jalal, 1995). It is also known that these compounds had profound effects on hemodynamics when given intravenously (Cushing et al., 2010). Therefore, the parameters of ECG, hemodynamics, and cardiac mechanics obtained in this study were measured 15 min after the end of infusion of each dose

to allow the recovery of hemodynamics during infusion period and permit the drug to stabilize.

In humans, dronedarone is used for either maintaining sinus rhythm or reducing ventricular rate in atrial fibrillation (Singh et al., 2007). Base on successful clinical trials in humans, dronedarone might be useful in veterinary medicine as well. This study was conducted on healthy beagle dogs anesthetized with isoflurane. The typical clinical patients with arrhythmias especially AF are large breed dogs or small breed dogs with heart diseases (Guglielmini et al., 2000; Westling et al., 2008). Data from this study must be interpreted cautiously in clinical patients. Since dronedarone exerts its negative inotropy and lusitropy, caution should be exercised when using dronedarone in dogs with supra- or ventricular arrhythmias with ventricular compromise especially when the arrhythmias comorbidity with unstable heart failure. Furthermore, it is difficult for veterinarian practitioners to extrapolate therapeutic dosage from the present study since the cumulative doses were investigated intravenously. Therefore, further study should be performed to explore therapeutic oral dose in conscious dogs or dogs with atrial fibrillation.

**Limitations of study part 1:**

We did not measure plasma concentrations of dronedarone and its metabolite, N-debutyl metabolite. In humans, the steady state plasma concentration of oral dronedarone (a therapeutic dose of 400 mg twice daily) is 84-167 ng/ml (Patel et al., 2009). As far as we are aware, there is only one study measured the plasma and tissue levels of dronedarone in dogs after chronic oral administration (25 mg/kg, twice per day, 4 weeks) (Varro et al., 2001). After 4 weeks, the plasma concentration of dronedarone was 1.01 ± 0.32 $\mu$g/m*l* and the plasma concentration of N-debutyl metabolite was 0.09 ± 0.03 $\mu$g/m*l*. That plasma concentration does not produce any change on APD and QT/QTc intervals except a strong use-dependent $V_{max}$ depression. The pharmacokinetics of dronedarone have been reported in dogs previously (Product Monograph, 2014). After an oral dose, the time to maximum plasma concentration ($t_{max}$) is between 1-4 hr and the steady state plasma concentrations were achieved

between 7-14 days (Patel et al., 2009; Product Monograph, 2014). The absolute bioavailability of dronedarone was between 14-22 %. Dronedarone was greatly bound to dog's plasma proteins (>99.5 %) without concentration dependent. It is rapidly and extensively distributed to several organs (i.e. kidneys, spleen, lung, and liver). After absorption, dronedarone was extensively metabolized to metabolites (i.e. N-debutyl metabolite). It is eliminated by metabolic clearance and excreted mainly by biliary excretion. The escalating doses of dronedarone used in our study are within the range of previous study (1-17 mg/kg, IV) that have been demonstrated to produce physiological effects in anesthetized dogs (Manning et al., 1995). Based on the pharmacokinetics of dronedarone in dogs, the lowest and highest cumulative dose dronedarone in our study (0.5 and 4 mg/kg) would yield a plasma concentration approximately 10 and 80 times higher than the study of Varro and colleagues (Varro et al., 2001). Since that study did not report the percentage of recovery of dronedarone's extraction process, it is possible that the cumulative doses of dronedarone in our study are lower than those calculations.

**Study part 2: Effects of single and short-term oral dose of dronedarone administration on cardiac functions, blood pressures, and electrocardiograms in conscious telemetry dogs**

This study aimed to evaluate the cardiac function, blood pressure, and ECG of chronic oral dose of dronedarone (20 mg/kg, twice per day) in conscious dogs instrumented with telemetry units. Although electrocardiographic effects on dronedarone were assessed previously in both anesthetized and conscious dogs (Manning et al., 1995; Verduyn et al., 1999; van Opstal et al., 2001; Varro et al., 2001), its inotropic and lusitropic properties had not been evaluated in conscious dogs. Since dogs with atrial fibrillation almost always possess underlying cardiac diseases, drugs for management of AF in veterinary medicine should be given to the patients with caution. Because drugs that alter inotropy and lusitropy might worsen the cardiac function of the patients. We provided the first evidence that chronic dronedarone administration (20 mg/kg, twice per day, orally) for 7 day did not alter cardiac contraction and relaxation while its electrophysiology is still preserved (i.e. lengthened PQ interval). We have used the pressure-volume loop technique in this study because it has been accepted as a gold standard to assess the cardiac inotropy and lusitropy (Suga et al., 1973).

The dose of dronedarone was selected based on our preliminary study and previous publications (Verduyn et al., 1999; Djandjighian et al., 2000; van Opstal et al., 2001; Varro et al., 2001). In literatures, oral doses of dronedarone used in dogs were between 10-30 mg/kg (Verduyn et al., 1999; Djandjighian et al., 2000; van Opstal et al., 2001; Varro et al., 2001). In our previous report, a cumulative dose of intravenous dronedarone at 1.5 mg/kg can prolong PQ interval without adverse effects on cardiac function. In dogs, the absorption is 64-95% and the oral bioavailability is 14-22% (Product Monograph, 2014). If a dog weighing 10 kg was given oral dronedarone at a dose of 20 mg/kg, the expected dose of dronedarone when given by injection would be between 1.79 to 4.18 mg/kg. Therefore, we decided to vary doses of dronedarone from 10, 20, and 40 mg/kg and administered randomly to our two pilot dogs instrumented with telemetry unit for obtaining ECG and blood pressure. As shown in

figure 24, we decided to use a dose of 20 mg/kg for the main experiment since it did not show any dramatically effect on ECG parameters and did not cause hypotension after an acute dose.

The present study was designed to evaluate the effects repeated doses of dronedarone for only 7 days. Although the steady state of drug when given to humans is 7-14 day, we think that this duration should be enough to assess short-term effect of drug since the results of both electrophysiology and cardiac function demonstrated stable responses after day 4 (figure 30-31).

In our main experiment (n = 4), all dogs were given oral dronedarone for 7 days and cardiac contractility and relaxation were evaluated. The results showed that dronedarone did not alter the left ventricular inotropy. The slope of PRSW was used as a gold standard since it is relatively constant among conscious animals (Glower et al., 1985; Feneley et al., 1992; Kijtawornrat et al., 2014). The slope of ESPVR could also be used as a gold standard for measurement of cardiac contractility. However, it has been showed to be curvilinear at higher or lower contractile states (Kass et al., 1989). The CI and $dP/dt_{max}$ were also demonstrated consistent with the results of ESPVR and PRSW even though these two parameters are affected by loading condition (Hamlin and Del Rio, 2010; Kijtawornrat, 2013). In this study both active (tau and $dP/dt_{min}$) and passive (EDPVR) relaxation indices were evaluated and the results showed that dronedarone did not change neither active nor passive indices of left ventricular relaxation. Similar findings were observed in our previous anesthetized dogs study in which dronedarone had no effect on lusitropy until it was given at a high dose.

In this study, cardiac output tended to decrease from the beginning of the study and continue to decline until the end of observation time-point. This could be due to heart rate reduction effects of dronedarone since the stroke volume did not change. It has been demonstrated previously that dronedarone lowers heart rate in both humans and dogs (Verduyn et al., 1999; Djandjighian et al., 2000; van Opstal et al., 2001; Varro et al., 2001; Singh et al., 2007; Davy et al., 2008). The primary mechanism responsible for the bradycardia effects has been proved to be the inhibition of the funny channel (Sobrado et al., 2013). Mean blood pressure was

demonstrated to be significantly decreased after chronic dronedarone administration. This is in consistent with previous data in humans (Christiansen et al., 2010b; Naccarelli et al., 2011). The hypotension effect of dronedarone may be partly due to a decline in cardiac output or mainly due to its alpha-adrenergic blocking effect (Naccarelli et al., 2011). In both conscious and anesthetized dogs, dronedarone at 10 and 30 mg/kg attenuates alpha-adrenoceptor stimulation demonstrated by a reduction of adrenaline-induced increases in blood pressure (Hodeige et al., 1995). This hypotension effect alerts the veterinary practitioners to be caution when prescribed dronedarone to their patients.

It has been known previously that dronedarone affects PQ interval which may partly be due to decrease firing rate from the sinoatrial node (SAN) or slow conduction velocity from SAN to the head of atrioventricular node (Naccarelli et al., 2011). The results of this study confirm the previous findings both in animal experiment and in clinical trials (Varro et al., 2001; Touboul et al., 2003). In this study, the effect of dronedarone on QT and QTc liability confirmed the result of a previous study in conscious normal dogs in which chronic dronedarone administration orally (25 mg/kg, BID, 4 weeks) did not show any effect on QT interval (Varro et al., 2001). Our previous data in anesthetized dogs was also demonstrated the similar effects on QT and QTc intervals.

The current dog model were instrumented with telemetry units for obtaining ECG, blood pressure and left ventricular pressure together with sono-micrometry crystals and a vascular occluder to obtain pressure-volume loop relationship in conscious state. From our results, the model demonstrates that it is feasible to obtain cardiovascular effects of drugs through the incorporation of contractility and relaxation using sono-micrometry crystal and occluder while the dog was trained to collect data in the sling. Although the model requires open-chest surgery, all dogs recovered and showed no adverse effects of surgery.

**Limitations of study part 2:**

We performed experiment in normal healthy dogs treated with dronedarone (20 mg/kg, BID) orally for 7 days. We do not know whether or not this dose is effective for management of AF in dogs. Further study must be investigated the effectiveness of dronedarone (20 mg/kg, BID, PO) in AF models.

**Study part 3: Dronedarone attenuates the duration of atrial fibrillation in dog model of sustained atrial fibrillation**

The present study demonstrated that dronedarone was effective against experimentally induced sustained AF. The sustained AF used in this study was induced by RAP simultaneously with PE infusion. According to previous publication, PE was used to elevate systemic arterial pressure which will increase stimulation of vagal efferents via baroreceptor reflex (Kijtawornrat et al., 2008). Since vagal fiber in atria distributes heterogeneously, the stimulation of vagus nerve creates heterogeneity of repolarization which contributed to AF.

Dronedarone has been reported to prevent and terminate AF previously in *in vitro* models (Burashnikov et al., 2010a; Burashnikov et al., 2010b). In canine isolated arterially perfused right atria, acute dronedarone (10 μM) prevents acetylcholine-mediated AF and terminates persistent AF (Burashnikov et al., 2010a). Furthermore, a combination of dronedarone (10 μmol/l) and ranolazine (5 μmol/l) has been shown to prevent the induction of AF in canine isolated coronary-perfused right and left atrium (Burashnikov et al., 2010b). In this study, AF was induced in all dogs before receiving dronedarone. After treatment, drondarone has been showed to reduce the duration of AF in 5 of 6 dogs in which one of those 5 dogs was unable to induce AF at all. Thus, our data on the management of AF are generally consistent with those reported previously. The mechanisms by which dronedarone reduced the duration of sustained AF in our study may be due to its effects on APD of atria and its effects on AERP. In this study, dronedarone increased atrial APD 19.3 % which less than its effect on AERP (increased 23.1 %) so that the PRR was developed. The development of PRR was also consistent with a previous study (Burashnikov et al., 2010b). In canine right atrial preparation, dronedarone has been shown to alter APD minimally while its effect on AERP was markedly lengthened leading to development of PRR (Burashnikov et al., 2010b). It has been known that atrial cells have intrinsically a more depolarized resting membrane potential (RMP) than ventricular's RMP (Burashnikov et al., 2007; Bogdan et al., 2009). Bogdan and colleagues (2011) suggested that dronedarone exhibits PRR by the state-dependent block of fast $Na^+$ channels in which a marked inhibition happened

at more depolarized holding potential. This effect could imply that dronedarone possesses atrial-selective effect. Furthermore, when compared the shape of atrial APD with the shape of ventricular APD, atrial APD has a slow phase 3 repolarization; therefore, atrial $Na^+$ channels will rest in the inactivated state for longer time than ventricular $Na^+$ channels (Bogdan et al., 2011). Dronedarone as well as amiodarone, a structurally related compound, have been demonstrated to preferentially block $Na^+$ channels during inactivation state (Ehrlich and Dobrev, 2011).

The present study also showed that dronedarone produces heart rate reduction in anesthetized dogs both at baseline (before the onset of RAP) and after RAP with PE infusion (2 μg/kg/min). This effect was consistent with our previous study in isoflurane anesthetized dogs and in conscious dogs instrumented with telemetry units. The efficacy of dronedarone on a reduction of ventricular rate was also observed in patients with permanent AF both at rest and during exercise (Davy et al., 2008). It has been shown previously that dronedarone exerts bradycardia effect by combinations of inhibitions of pacemaker current and blockade of beta-adrenergic receptor and calcium channels (Chatelain et al., 1995; Rocchetti et al., 1998; Gautier et al., 2003; Bogdan et al., 2011).

**Limitations of study part 3:**

All investigations of the present study were performed in anesthetized healthy dogs. In clinical practice, however, dogs with AF almost always have underlying heart diseases. Therefore, extrapolations of results obtained from this study to the clinic should be done with caution.

# CHAPTER VI

# SUMMARY

Atrial fibrillation (AF) is a supraventricular arrhythmia led to decrease cardiac output and impair mechanical function of the heart and quality of life. Dronedarone has an atrial-selective property and has been used for management of atrial fibrillation in humans but limited information was observed in dogs. The overall objective of the present study was to investigate potential for dronedarone to control atrial fibrillation in dogs. Firstly, the acute effects of escalating concentrations of dronedarone on electrocardiograms (ECG), hemodynamics and cardiac mechanics in anesthetized healthy dogs were determined. A total of 7 beagle dogs were anesthetized with isoflurane and instrumented to obtain lead II ECG, pressures at ascending aorta, right atrium, pulmonary artery, and left ventricle, and left ventricular pressure-volume relationship, a gold standard for measuring cardiac inotropy and lusitropy. Five dogs were given vehicle and followed by escalating doses of dronedarone (0.5, 1.0, and 2.5 mg/kg, 15 min for each dose) and two dogs were used as vehicle-treated control. All parameters were measured at 15 min after the end of each dose. The results showed that all parameters in vehicle-treated dogs were unaltered. Dronedarone at 2.5 mg/kg significantly lengthened PQ interval ($P<0.01$), reduced cardiac output ($P<0.01$), and increased systemic vascular resistance ($P<0.01$). Dronedarone produced negative inotropy assessed by significantly lowered end-systolic pressure-volume relationship (ESPVR), preload recruitable stroke work (PRSW), contractility index (CI), and the maximal rate of rise of the left ventricular (LV) pressure ($dP/dt_{max}$). It also impaired diastolic function by significantly increased end-diastolic pressure-volume relationship (EDPVR), tau, and the maximal rate of fall of the LV pressure ($dP/dt_{min}$). These results suggested that acute effects of dronedarone produced negative dromotropy, inotropy, and lusitropy in anesthetized dogs. Since the pharmacokinetics of dronedarone in dogs have been established earlier, the cumulative doses of intravenous dronedarone were then be extrapolate to oral doses vary from 10 to 40 mg/kg, BID. Those doses were used in the experiment part II.

Next, the study was designed to determine the chronic effects of oral dronedarone on cardiac inotropy and lusitropy, blood pressure, ECG in healthy dogs. A total of 6 beagle dogs were instrumented with telemetry units and sono-micrometry crystals to obtain left ventricular pressure-volume relationship, mean blood pressure (MBP), and ECG. Dogs were given orally dronedarone (20 mg/kg, twice per day) for 7 days. All parameters were obtained hourly at 4-8 hr after the first dose and at 12-, 96-(day 4), and 168-hr (day 7) after dosing. The results showed that dronedarone had no effect on inotropy and lusitropy while it significantly lengthened PQ interval ($P<0.001$) and lowered MBP ($P<0.05$). Dronedarone also tended to reduce cardiac output ($P=0.237$) and heart rate ($P=0.057$). These results suggested that chronic effects of oral dronedarone administration at a dose of 20 mg/kg, twice per day, produced negative dromotropy and induced hypotension in conscious dogs. The dose of dronedarone was used for the next study for studying its efficacy and mechanism to attenuate atrial fibrillation.

Finally, the last study was designed to evaluate efficacy of dronedarone to attenuate the duration of AF in dog model of sustained atrial fibrillation. Six beagle dogs were anesthetized with isoflurane and instrumented to measure atrial action potential duration (APD) by monophasic action potential catheter and atrial effective refractory period (AERP) by program electrical stimulation. Then AF was induced by rapid right atrial pacing (20 V, 40 Hz) simultaneously with infusion of phenylephrine (2μg/kg/min, IV) for 20 min. The duration of sustained AF was recorded and the animals were allowed to recover. Dronedarone was given at a dose of 20 mg/kg, BID, PO for 7 days. On the last day, dogs were anesthetized again to record APD and AERP and the AF was induced with the similar procedure. The results showed that after dronedarone administration the APD was lengthened significantly from 76.4±4.2 ms to 91.2±3.9 ms ($p<0.05$) and AERP was prolonged significantly from 97.5±2.8 ms to 120±4.8 ms ($p<0.05$). The duration of sustained AF was also significantly attenuated after receiving dronedarone ($p<0.05$). These results indicated that oral dronedarone (20 mg/kg, BID) attenuates duration of sustained AF in dog model of AF by extended the AERP more than APD suggesting post-repolarization refractoriness.

**Beneficial to veterinary practitioners**

The crucial and novel findings in all of these studies are that oral dronedarone at a dose of 20 mg/kg, when given BID, can attenuate the duration of atrial fibrillation in a dog model of sustained AF. The possible mechanism responsible for this result is at least in part by developing a post-repolarization refractoriness suggesting that dronedarone blocks fast sodium channels in a state-dependent manner. This study is also the first to demonstrate that dronedarone may develop PRR *in vivo* experiment. Furthermore, oral dronedarone at a dose of 20 mg/kg (BID) had no effect on cardiac inotropy and lusitropy. However, it lowers blood pressure. In most cases, dogs with atrial fibrillation almost always have underlying heart diseases. Therefore, care should be taken when prescribed dronedarone to dogs especially when the dogs have impaired cardiac function.

When compared with amiodarone, dronedarone has been proved in human medicine to possess an excellent safety profile with no pulmonary or thyroid toxicity. However, there is no chronic test in veterinary medicine. In case of the price, dronedarone is more expensive than amiodarone approximately 2.5 times. In case of efficacy, dronedarone has been demonstrated to be 100 times more potent than amiodarone for inhibition of $I_{K.Ach}$. However, some investigators have shown that efficacy of dronedarone for antiarrhythmic is inferior to amiodarone when tested in vivo. All in all, with consideration of safety profile and its efficacy dronedarone should be first-line prescribed to patients instead of amiodarone especially atrial fibrillation patient with minimal heart disease.

# REFERENCES


Aliot E, Capucci A, Crijns HJ, Goette A and Tamargo J 2011. Twenty-five years in the making: flecainide is safe and effective for the management of atrial fibrillation. Europace. 13(2): 161-173.

Allessie M, Ausma J and Schotten U 2002. Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovasc Res. 54(2): 230-246.

Allessie MA, Bonke FI and Schopman FJ 1973. Circus movement in rabbit atrial muscle as a mechanism of trachycardia. Circ Res. 33(1): 54-62.

Allessie MA, Bonke FI and Schopman FJ 1977. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. Circ Res. 41(1): 9-18.

Armengol J, Man GC, Balsys AJ and Wells AL 1981. Effects of the respiratory cycle on cardiac output measurements: reproducibility of data enhanced by timing the thermodilution injections in dogs. Crit Care Med. 9(12): 852-854.

Artwhol J, Carbone L, Flecknell P, Friedman DP and Pritchett-Corning K 2013. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. In: the American Veterinary Medical Association, Schaumburg.

Australian Government 2010. "Subject: Australian Public Assessment Report for Dronedarone Hydrochloride" (online). Available: https://www.tga.gov.au/auspar/auspar-dronedarone-hydrochloride.

Bicer S, Fuller GA, Wilkie DA, Yamaguchi M and Hamlin RL 2002a. Amiodarone-induced keratopathy in healthy dogs. Vet Ophthalmol. 5(1): 35-38.

Bicer S, Nakayama T and Hamlin RL 2002b. Effects of chronic oral amiodarone on left ventricular function, ECGs, serum chemistries, and exercise tolerance in healthy dogs. J Vet Intern Med. 16(3): 247-254.

Bicer S, Patchell JS, Hamlin DM and Hamlin RL 2002c. Acute effects of escalating doses of amiodarone in isolated guinea pig hearts. J Vet Pharmacol Ther. 25(3): 221-226.

Bogdan R, Goegelein H and Ruetten H 2009. Viable heart slices: action potential recordings from atrial and ventricular tissues. Acta Physiol. 195(suppl 669): 64.

Bogdan R, Goegelein H and Ruetten H 2011. Effect of dronedarone on Na+, Ca2+ and HCN channels. Naunyn Schmiedebergs Arch Pharmacol. 383(4): 347-356.

Bolderman RW, Hermans JJ and Maessen JG 2009. Determination of the class III antiarrhythmic drugs dronedarone and amiodarone, and their principal metabolites in plasma and myocardium by high-performance liquid chromatography and UV-detection. J Chromatogr B Analyt Technol Biomed Life Sci. 877(18-19): 1727-1731.

Bright JM, Martin JM and Mama K 2005. A retrospective evaluation of transthoracic biphasic electrical cardioversion for atrial fibrillation in dogs. J Vet Cardiol. 7(2): 85-96.

Bright JM and zumBrunnen J 2008. Chronicity of atrial fibrillation affects duration of sinus rhythm after transthoracic cardioversion of dogs with naturally occurring atrial fibrillation. J Vet Intern Med. 22(1): 114-119.

Bristow MR 2011. Treatment of chronic heart failure with beta-adrenergic receptor antagonists: a convergence of receptor pharmacology and clinical cardiology. Circ Res. 109(10): 1176-1194.

Brundel BJ, Henning RH, Kampinga HH, Van Gelder IC and Crijns HJ 2002. Molecular mechanisms of remodeling in human atrial fibrillation. Cardiovasc Res. 54(2): 315-324.

Brundel BJ, Melnyk P, Rivard L and Nattel S 2005. The pathology of atrial fibrillation in dogs. J Vet Cardiol. 7(2): 121-129.

Burashnikov A, Belardinelli L and Antzelevitch C 2010a. Acute dronedarone is inferior to amiodarone in terminating and preventing atrial fibrillation in canine atria. Heart Rhythm. 7(9): 1273-1279.

Burashnikov A, Di Diego JM, Sicouri S, Ferreiro M, Carlsson L and Antzelevitch C 2008. Atrial-selective effects of chronic amiodarone in the management of atrial fibrillation. Heart Rhythm. 5(12): 1735-1742.

Burashnikov A, Di Diego JM, Zygmunt AC, Belardinelli L and Antzelevitch C 2007. Atrium-selective sodium channel block as a strategy for suppression of atrial fibrillation:

differences in sodium channel inactivation between atria and ventricles and the role of ranolazine. Circulation. 116(13): 1449-1457.

Burashnikov A, Sicouri S, Di Diego JM, Belardinelli L and Antzelevitch C 2010b. Synergistic effect of the combination of ranolazine and dronedarone to suppress atrial fibrillation. J Am Coll Cardiol. 56(15): 1216-1224.

Burkhoff D 2013. Pressure-volume loops in clinical research: a contemporary view. J Am Coll Cardiol. 62(13): 1173-1176.

Calvert CA, Sammarco C and Pickus C 2000. Positive Coombs' test results in two dogs treated with amiodarone. J Am Vet Med Assoc. 216(12): 1933-1936, 1926.

Chatelain P, Meysmans L, Matteazzi JR, Beaufort P and Clinet M 1995. Interaction of the antiarrhythmic agents SR 33589 and amiodarone with the beta-adrenoceptor and adenylate cyclase in rat heart. Br J Pharmacol. 116(3): 1949-1956.

Chaves AA, Zingaro GJ, Yordy MA, Bustard KA, O'Sullivan S, Galijatovic-Idrizbegovic A, Schuck H, Christian DB, Hoe CM and Briscoe RJ 2007. A highly sensitive canine telemetry model for detection of QT interval prolongation: studies with moxifloxacin, haloperidol and MK-499. J Pharmacol Toxicol Methods. 56(2): 103-114.

Christiansen CB, Torp-Pedersen C and Kober L 2010a. Efficacy and safety of dronedarone: a review of randomized trials. Expert Opin Drug Saf. 9(1): 189-199.

Christiansen CB, Torp-Pedersen C and Kober L 2010b. Impact of dronedarone in atrial fibrillation and flutter on stroke reduction. Clin Interv Aging. 5: 63-69.

Costard A, Liem LB and Franz MR 1989. Rate dependent effect of quinidine, mexiletine, and their combination on postrepolarization refractoriness in vivo. J Cardiovasc Pharm. 14: 810-817.

Crijns HJ 2005. Rate versus rhythm control in patients with atrial fibrillation: what the trials really say. Drugs. 65(12): 1651-1667.

Cushing DJ, Cooper WD, Gralinski MR and Lipicky RJ 2010. The hypotensive effect of intravenous amiodarone is sustained throughout the maintenance infusion period. Clin Exp Pharmacol Physiol. 37(3): 358-361.

Damiano RJ, Jr., Gaynor SL, Bailey M, Prasad S, Cox JL, Boineau JP and Schuessler RP 2003. The long-term outcome of patients with coronary disease and atrial fibrillation undergoing the Cox maze procedure. J Thorac Cardiovasc Surg. 126(6): 2016-2021.

Davy JM, Herold M, Hoglund C, Timmermans A, Alings A, Radzik D, Van Kempen L and Investigators ES 2008. Dronedarone for the control of ventricular rate in permanent atrial fibrillation: the Efficacy and safety of dRonedArone for the cOntrol of ventricular rate during atrial fibrillation (ERATO) study. Am Heart J. 156(3): 527 e521-529.

Djandjighian L, Planchenault J, Finance O, Pastor G, Gautier P and Nisato D 2000. Hemodynamic and antiadrenergic effects of dronedarone and amiodarone in animals with a healed myocardial infarction. J Cardiovasc Pharmacol. 36(3): 376-383.

Dobrev D and Nattel S 2010. New antiarrhythmic drugs for treatment of atrial fibrillation. Lancet. 375(9721): 1212-1223.

Doshi RN, Wu TJ, Yashima M, Kim YH, Ong JJ, Cao JM, Hwang C, Yashar P, Fishbein MC, Karagueuzian HS and Chen PS 1999. Relation between ligament of Marshall and adrenergic atrial tachyarrhythmia. Circulation. 100(8): 876-883.

Dukes-Mcewan J 2002. Atrial fibrillation: onset and perpetuation. Vet J. 164(2): 87-89.

Ehrlich JR and Dobrev D 2011. Atrial-selective sodium channel block by dronedarone: sufficient to terminate atrial fibrillation? Naunyn Schmiedebergs Arch Pharmacol. 384(2): 109-114.

Ehrlich JR, Nattel S and Hohnloser SH 2002. Atrial fibrillation and congestive heart failure: specific considerations at the intersection of two common and important cardiac disease sets. J Cardiovasc Electrophysiol. 13(4): 399-405.

European Heart Rhythm A, European Association for Cardio-Thoracic S, Camm AJ, Kirchhof P, Lip GY, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, Hindricks G, Prendergast B, Heidbuchel H, Alfieri O, Angelini A, Atar D, Colonna P, De Caterina R, De Sutter J, Goette A, Gorenek B, Heldal M, Hohloser SH, Kolh P, Le Heuzey JY, Ponikowski P and Rutten FH 2010. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial

Fibrillation of the European Society of Cardiology (ESC). Eur Heart J. 31(19): 2369-2429.

Fedida D, Orth PM, Chen JY, Lin S, Plouvier B, Jung G, Ezrin AM and Beatch GN 2005. The mechanism of atrial antiarrhythmic action of RSD1235. J Cardiovasc Electrophysiol. 16(11): 1227-1238.

Feneley MP, Skelton TN, Kisslo KB, Davis JW, Bashore TM and Rankin JS 1992. Comparison of preload recruitable stroke work, end-systolic pressure-volume and dP/dtmax-end-diastolic volume relations as indexes of left ventricular contractile performance in patients undergoing routine cardiac catheterization. J Am Coll Cardiol. 19(7): 1522-1530.

Franz MR and Costard A 1988. Frequency-dependent effects of quinidine on the relationship between action potential duration and refractoriness in the canine heart in situ. Circulation. 77(5): 1177-1184.

Garcia MJ, Smedira NG, Greenberg NL, Main M, Firstenberg MS, Odabashian J and Thomas JD 2000. Color M-mode Doppler flow propagation velocity is a preload insensitive index of left ventricular relaxation: animal and human validation. J Am Coll Cardiol. 35(1): 201-208.

Garrey W 1914. The nature of fibrillary contraction of the heart: its relation to tissue mass and form. Am J Physiol. 33: 397-414.

Gaspo R, Bosch RF, Talajic M and Nattel S 1997. Functional mechanisms underlying tachycardia-induced sustained atrial fibrillation in a chronic dog model. Circulation. 96(11): 4027-4035.

Gautier P, Guillemare E, Marion A, Bertrand JP, Tourneur Y and Nisato D 2003. Electrophysiologic characterization of dronedarone in guinea pig ventricular cells. J Cardiovasc Pharmacol. 41(2): 191-202.

Gillinov AM and McCarthy PM 2004. Advances in the surgical treatment of atrial fibrillation. Cardiol Clin. 22(1): 147-157.

Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, Tyson GS, Sabiston DC, Jr. and Rankin JS 1985. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation. 71(5): 994-1009.

Goldberger AL and Pavelec RS 1986. Vagally-mediated atrial fibrillation in dogs: conversion with bretylium tosylate. Int J Cardiol. 13(1): 47-55.

Guglielmini C, Chetboul V, Pietra M, Pouchelon JL, Capucci A and Cipone M 2000. Influence of left atrial enlargement and body weight on the development of atrial fibrillation: retrospective study on 205 dogs. Vet J. 160(3): 235-241.

Haissaguerre M, Jais P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Metayer P and Clementy J 1998. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med. 339(10): 659-666.

Hamlin RL and Del Rio C 2010. An approach to the assessment of drug-induced changes in non-electrophysiological properties of cardiovascular function. J Pharmacol Toxicol Methods. 62(1): 20-29.

Hamlin RL and del Rio C 2012. dP/dt(max)--a measure of 'baroinometry'. J Pharmacol Toxicol Methods. 66(2): 63-65.

Hodeige D, Heyndrickx JP, Chatelain P and Manning A 1995. SR 33589, a new amiodarone-like antiarrhythmic agent: anti-adrenoceptor activity in anaesthetized and conscious dogs. Eur J Pharmacol. 279(1): 25-32.

Hohnloser SH, Crijns HJ, van Eickels M, Gaudin C, Page RL, Torp-Pedersen C, Connolly SJ and Investigators A 2009. Effect of dronedarone on cardiovascular events in atrial fibrillation. N Engl J Med. 360(7): 668-678.

Jacobs G, Calvert C and Kraus M 2000. Hepatopathy in 4 dogs treated with amiodarone. J Vet Intern Med. 14(1): 96-99.

Kass DA, Beyar R, Lankford E, Heard M, Maughan WL and Sagawa K 1989. Influence of contractile state on curvilinearity of in situ end-systolic pressure-volume relations. Circulation. 79(1): 167-178.

Kijtawornrat A 2013. Indices of myocardial contractility. Thai J Vet Med. 43(2): 167-168.

Kijtawornrat A, Roche BM and Hamlin RL 2008. A canine model of sustained atrial fibrillation induced by rapid atrial pacing and phenylephrine. Comp Med. 58(5): 490-493.

Kijtawornrat A, Ueyama Y, del Rio C, Sawangkoon S, Buranakarl C, Chaiyabutr N and Hamlin RL 2014. Test of the usefulness of a paradigm to identify potential

cardiovascular liabilities of four test articles with varying pharmacological properties in anesthetized guinea pigs. Toxicol Sci. 137(2): 458-468.

Kober L, Torp-Pedersen C, McMurray JJ, Gotzsche O, Levy S, Crijns H, Amlie J and Carlsen J 2008. Increased mortality after dronedarone therapy for severe heart failure. N Engl J Med. 358(25): 2678-2687.

Kodama I, Kamiya K and Toyama J 1999. Amiodarone: ionic and cellular mechanisms of action of the most promising class III agent. Am J Cardiol. 84(9A): 20R-28R.

Kopecky SL, Gersh BJ, McGoon MD, Whisnant JP, Holmes DR, Jr., Ilstrup DM and Frye RL 1987. The natural history of lone atrial fibrillation. A population-based study over three decades. N Engl J Med. 317(11): 669-674.

Lee RJ, Liem LB, Cohen TJ and Franz MR 1989. Use dependent effect of class I antiarrhythmic drugs on the relationship between action potential duration and refractoriness in the human heart (abstract). Circulation. 80(Suppl II): II-327.

Lewis T, Fell HS and Stroud WD 1920. Observations upon flutter and fibrillation. II. The nature of auricular flutter. Heart J. 7: 191–233.

Li D, Fareh S, Leung TK and Nattel S 1999. Promotion of atrial fibrillation by heart failure in dogs: atrial remodeling of a different sort. Circulation. 100(1): 87-95.

Liu L and Nattel S 1997. Differing sympathetic and vagal effects on atrial fibrillation in dogs: role of refractoriness heterogeneity. Am J Physiol. 273(2 Pt 2): H805-816.

Lund-Johansen P and Omvik P 1991. Acute and chronic hemodynamic effects of drugs with different actions on adrenergic receptors: a comparison between alpha blockers and different types of beta blockers with and without vasodilating effect. Cardiovasc Drugs Ther. 5(3): 605-615.

Manning A, Thisse V, Hodeige D, Richard J, Heyndrickx JP and Chatelain P 1995. SR 33589, a new amiodarone-like antiarrhythmic agent: electrophysiological effects in anesthetized dogs. J Cardiovasc Pharmacol. 25(2): 252-261.

Mason PK and DiMarco JP 2009. New pharmacological agents for arrhythmias. Circ Arrhythm Electrophysiol. 2(5): 588-597.

Mayer AG 1906. Should Our Colleges Establish Summer Schools? Science. 23(592): 703-704.

McConnell BK, Popovic Z, Mal N, Lee K, Bautista J, Forudi F, Schwartzman R, Jin JP, Penn M and Bond M 2009. Disruption of protein kinase A interaction with A-kinase-anchoring proteins in the heart in vivo: effects on cardiac contractility, protein kinase A phosphorylation, and troponin I proteolysis. J Biol Chem. 284(3): 1583-1592.

Menaut P, Belanger MC, Beauchamp G, Ponzio NM and Moise NS 2005. Atrial fibrillation in dogs with and without structural or functional cardiac disease: A retrospective study of 109 cases. J Vet Cardiol. 7(2): 75-83.

Mines GR 1913. On dynamic equilibrium in the heart. J Physiol. 46(4-5): 349-383.

Mines GR 1914. Further experiments on the action of the vagus on the electrogram of the frog's heart. J Physiol. 47(6): 419-430.

Moe G 1920. On the multiple wavelet hypothesis of atrial fibrillation. Arch Int Pharmacodyn Ther. 140: 183 – 188.

Moe GK, Rheinboldt WC and Abildskov JA 1964. A Computer Model of Atrial Fibrillation. Am Heart J. 67: 200-220.

Morillo CA, Klein GJ, Jones DL and Guiraudon CM 1995. Chronic rapid atrial pacing. Structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. Circulation. 91(5): 1588-1595.

Moro S, Ferreiro M, Celestino D, Medei E, Elizari MV and Sicouri S 2007. In vitro effects of acute amiodarone and dronedarone on epicardial, endocardial, and M cells of the canine ventricle. J Cardiovasc Pharmacol Ther. 12(4): 314-321.

Naccarelli GV and Jalal S 1995. Intravenous amiodarone. Another option in the acute management of sustained ventricular tachyarrhythmias. Circulation. 92(11): 3154-3155.

Naccarelli GV, Wolbrette DL, Levin V, Samii S, Banchs JE, Penny-Peterson E and Gonzalez MD 2011. Safety and efficacy of dronedarone in the treatment of atrial fibrillation/flutter. Clin Med Insights Cardiol. 5: 103-119.

National Research Council 2011. Guide for the care and use of laboratory animals. 8th ed. In: National Academies Press, Washington D.C.

Nattel S 2002. New ideas about atrial fibrillation 50 years on. Nature. 415(6868): 219-226.

Nattel S, Khairy P, Roy D, Thibault B, Guerra P, Talajic M and Dubuc M 2002. New approaches to atrial fibrillation management: a critical review of a rapidly evolving field. Drugs. 62(16): 2377-2397.

Nishida K, Michael G, Dobrev D and Nattel S 2010. Animal models for atrial fibrillation: clinical insights and scientific opportunities. Europace. 12(2): 160-172.

Oyama MA and Prosek R 2006. Acute conversion of atrial fibrillation in two dogs by intravenous amiodarone administration. J Vet Intern Med. 20(5): 1224-1227.

Patel C, Yan GX and Kowey PR 2009. Dronedarone. Circulation. 120(7): 636-644.

Piccini JP, Hasselblad V, Peterson ED, Washam JB, Califf RM and Kong DF 2009. Comparative efficacy of dronedarone and amiodarone for the maintenance of sinus rhythm in patients with atrial fibrillation. J Am Coll Cardiol. 54(12): 1089-1095.

Product Monograph 2014. "Subject: $^{Pr}$MULTAQ® Dronedarone Tablets 400 mg dronedarone (as dronedarone hydrochloride) Antiarrhythmic Agent" (online). Available: http://products.sanofi.ca/en/multaq.pdf.

Raff GL and Glantz SA 1981. Volume loading slows left ventricular isovolumic relaxation rate. Evidence of load-dependent relaxation in the intact dog heart. Circ Res. 48(6 Pt 1): 813-824.

Rocchetti M, Bertrand JP, Nisato D and Guatier P 1998. Cellular electrophysiological study of dronedarone, a new amiodarone-like agent, in Guinea pig sinoatrial node. Nauyn Schmiedebergs Arch Pharmacol. 358(1 Suppl 2): R617.

Rothberger CJ and Winterberg H 1909. Vorhofflimmern und Arhythmia perpetua. Wiener Klinische Wochenschrift. 22: 839–844.

Roy D, Pratt CM, Torp-Pedersen C, Wyse DG, Toft E, Juul-Moller S, Nielsen T, Rasmussen SL, Stiell IG, Coutu B, Ip JH, Pritchett EL, Camm AJ and Atrial Arrhythmia Conversion Trial I 2008. Vernakalant hydrochloride for rapid conversion of atrial fibrillation: a phase 3, randomized, placebo-controlled trial. Circulation. 117(12): 1518-1525.

Roy D, Rowe BH, Stiell IG, Coutu B, Ip JH, Phaneuf D, Lee J, Vidaillet H, Dickinson G, Grant S, Ezrin AM, Beatch GN and Investigators C 2004. A randomized, controlled

trial of RSD1235, a novel anti-arrhythmic agent, in the treatment of recent onset atrial fibrillation. J Am Coll Cardiol. 44(12): 2355-2361.

Sarazan RD 2014. Cardiovascular pressure measurement in safety assessment studies: technology requirements and potential errors. J Pharmacol Toxicol Methods. 70(3): 210-223.

Sarazan RD, Mittelstadt S, Guth B, Koerner J, Zhang J and Pettit S 2011. Cardiovascular function in nonclinical drug safety assessment: current issues and opportunities. Int J Toxicol. 30(3): 272-286.

Satoh T and Zipes DP 1996. Unequal atrial stretch in dogs increases dispersion of refractoriness conducive to developing atrial fibrillation. J Cardiovasc Electrophysiol. 7(9): 833-842.

Saunders A, Gordon S and Miller M 2009. Canine atrial fibrillation. Compend Contin Educ Vet. 31(11): 1-9.

Saunders AB, Miller MW, Gordon SG and Van De Wiele CM 2006. Oral amiodarone therapy in dogs with atrial fibrillation. J Vet Intern Med. 20(4): 921-926.

Scherf D and Terranova R 1949. Mechanism of auricular flutter and fibrillation. Am J Physiol. 159(1): 137-142.

Singh BN and Cingolani E 2010. A new agent for atrial fibrillation: electrophysiological properties of dronedarone. J Cardiovasc Pharmacol Ther. 15(4 Suppl): 6S-14S.

Singh BN, Connolly SJ, Crijns HJ, Roy D, Kowey PR, Capucci A, Radzik D, Aliot EM, Hohnloser SH, Euridis and Investigators A 2007. Dronedarone for maintenance of sinus rhythm in atrial fibrillation or flutter. N Engl J Med. 357(10): 987-999.

Sinno H, Derakhchan K, Libersan D, Merhi Y, Leung TK and Nattel S 2003. Atrial ischemia promotes atrial fibrillation in dogs. Circulation. 107(14): 1930-1936.

Sobrado LF, Varone BB, Machado AD, Nearing BD, Zeng D, Belardinelli L and Verrier RL 2013. Dronedarone's inhibition of If current is the primary mechanism responsible for its bradycardic effect. J Cardiovasc Electrophysiol. 24(8): 914-918.

Soloviev MV, Hamlin RL, Shellhammer LJ, Barrett RM, Wally RA, Birchmeier PA and Schaefer GJ 2006. Variations in hemodynamic parameters and ECG in healthy,

conscious, freely moving telemetrized beagle dogs. Cardiovasc Toxicol. 6(1): 51-62.

Spach MS and Boineau JP 1997. Microfibrosis produces electrical load variations due to loss of side-to-side cell connections: a major mechanism of structural heart disease arrhythmias. Pacing Clin Electrophysiol. 20(2 Pt 2): 397-413.

Suga H, Sagawa K and Shoukas AA 1973. Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. Circ Res. 32(3): 314-322.

Takeuchi S, Akita T, Takagishi Y, Watanabe E, Sasano C, Honjo H and Kodama I 2006. Disorganization of gap junction distribution in dilated atria of patients with chronic atrial fibrillation. Circ J. 70(5): 575-582.

Tamariz LJ and Bass EB 2004. Pharmacological rate control of atrial fibrillation. Cardiol Clin. 22(1): 35-45.

Torp-Pedersen C, Crijns HJ, Gaudin C, Page RL, Connolly SJ, Hohnloser SH and Investigators A 2011. Impact of dronedarone on hospitalization burden in patients with atrial fibrillation: results from the ATHENA study. Europace. 13(8): 1118-1126.

Touboul P, Brugada J, Capucci A, Crijns HJ, Edvardsson N and Hohnloser SH 2003. Dronedarone for prevention of atrial fibrillation: a dose-ranging study. Eur Heart J. 24(16): 1481-1487.

Tsai CF, Tai CT, Hsieh MH, Lin WS, Yu WC, Ueng KC, Ding YA, Chang MS and Chen SA 2000. Initiation of atrial fibrillation by ectopic beats originating from the superior vena cava: electrophysiological characteristics and results of radiofrequency ablation. Circulation. 102(1): 67-74.

Van de Water A, Verheyen J, Xhonneux R and Reneman RS 1989. An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. J Pharmacol Methods. 22(3): 207-217.

van den Meiracker AH, Man in 't Veld AJ, van Eck HJ, Boomsma F and Schalekamp MA 1988. Hemodynamic and hormonal adaptations to beta-adrenoceptor blockade. A 24-hour study of acebutolol, atenolol, pindolol, and propranolol in hypertensive patients. Circulation. 78(4): 957-968.

van der Velden HM, van Kempen MJ, Wijffels MC, van Zijverden M, Groenewegen WA, Allessie MA and Jongsma HJ 1998. Altered pattern of connexin40 distribution in persistent atrial fibrillation in the goat. J Cardiovasc Electrophysiol. 9(6): 596-607.

van Opstal JM, Schoenmakers M, Verduyn SC, de Groot SH, Leunissen JD, van Der Hulst FF, Molenschot MM, Wellens HJ and Vos MA 2001. Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome. Circulation. 104(22): 2722-2727.

Varro A, Takacs J, Nemeth M, Hala O, Virag L, Iost N, Balati B, Agoston M, Vereckei A, Pastor G, Delbruyere M, Gautier P, Nisato D and Papp JG 2001. Electrophysiological effects of dronedarone (SR 33589), a noniodinated amiodarone derivative in the canine heart: comparison with amiodarone. Br J Pharmacol. 133(5): 625-634.

Vaughan Williams EM 1984. A classification of antiarrhythmic actions reassessed after a decade of new drugs. J Clin Pharmacol. 24(4): 129-147.

Veenhuyzen GD, Simpson CS and Abdollah H 2004. Atrial fibrillation. CMAJ. 171(7): 755-760.

Verduyn SC, Vos MA, Leunissen HD, van Opstal JM and Wellens HJ 1999. Evaluation of the acute electrophysiologic effects of intravenous dronedarone, an amiodarone-like agent, with special emphasis on ventricular repolarization and acquired torsade de pointes arrhythmias. J Cardiovasc Pharmacol. 33(2): 212-222.

Verheule S, Wilson E, Banthia S, Everett THt, Shanbhag S, Sih HJ and Olgin J 2004. Direction-dependent conduction abnormalities in a canine model of atrial fibrillation due to chronic atrial dilatation. Am J Physiol Heart Circ Physiol. 287(2): H634-644.

Verrier RL, Sobrado MF, Pagotto VP, Kanas AF, Machado AD, Varone BB, Sobrado LF, Nearing BD, Zeng D and Belardinelli L 2013. Inhibition of I(f) in the atrioventricular node as a mechanism for dronedarone's reduction in ventricular rate during atrial fibrillation. Heart Rhythm. 10(11): 1692-1697.

Wann LS, Curtis AB, Ellenbogen KA, Estes NA, 3rd, Ezekowitz MD, Jackman WM, January CT, Lowe JE, Page RL, Slotwiner DJ, Stevenson WG, Tracy CM, Fuster V, Ryden LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Kay GN, Le Heuzey JY, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL, Wann LS, Jacobs AK, Anderson JL, Albert N, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Kushner FG, Ohman EM, Stevenson WG, Yancy CW and American College of Cardiology Foundation/American Heart Association Task F 2011. 2011 ACCF/AHA/HRS focused update on the management of patients with atrial fibrillation (update on Dabigatran): a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation. 123(10): 1144-1150.

Wegener FT, Ehrlich JR and Hohnloser SH 2006. Dronedarone: an emerging agent with rhythm- and rate-controlling effects. J Cardiovasc Electrophysiol. 17 Suppl 2: S17-20.

Westling J, Westling W and Pyle RL 2008. Epidemiology of atrial fibrillation in the dog. Intern J Appl Res Vet Med. 6(3): 151-154.

Wijffels MC, Kirchhof CJ, Dorland R and Allessie MA 1995. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. Circulation. 92(7): 1954-1968.

Zimetbaum PJ 2009. Dronedarone for atrial fibrillation--an odyssey. N Engl J Med. 360(18): 1811-1813.

# APPENDIX

**Table I** Average of PQ, QRS, QT, QTc(V) and HR in isoflurane anesthetized dogs treated with vehicle infusion (n = 2)

| Vehicle group (n = 2) | PQ (ms) | QRS (ms) | QT (ms) | QTc(V) (ms) | HR (bpm) |
|---|---|---|---|---|---|
| **Baseline** | 96.4 | 65.8 | 226.0 | 263.1 | 104.7 |
| **Vehicle 1 IV** | 95.2 | 65.4 | 230.5 | 265.7 | 100.7 |
| **Vehicle 2 IV** | 91.8 | 66.2 | 223.8 | 263.0 | 109.3 |
| **Vehicle 3 IV** | 96.2 | 65.7 | 224.9 | 260.9 | 102.5 |
| **Vehicle 4 IV** | 93.0 | 66.0 | 225.6 | 264.5 | 108.9 |

PQ = PQ interval, QRS = QRS complex, QT = QT interval, QTc(V) = corrected QT interval calculated by van de Water equation, HR = heart rate, IV = intravenous

**Table II** Average of SBP, DBP, MBP, CO, ESV, EDV, SV, SVR and PVR in isoflurane anesthetized dogs treated with vehicle (n = 2)

| Vehicle group (n = 2) | SBP (mmHg) | DBP (mmHg) | MBP (mmHg) | CO (mmHg) | ESV (ml) | EDV (ml) | SV (ml) | SVR ($dyn.s.cm^{-5}$) | PVR ($dyn.s.cm^{-5}$) |
|---|---|---|---|---|---|---|---|---|---|
| **Baseline** | 112.8 | 66.8 | 83.3 | 1.3 | 51.8 | 61.6 | 17.0 | 4946.5 | 663.8 |
| **Vehicle 1 IV** | 120.1 | 68.5 | 87.5 | 1.4 | 52.9 | 61.7 | 15.8 | 4922.8 | 662.9 |
| **Vehicle 2 IV** | 121.2 | 72.1 | 90.4 | 1.4 | 54.6 | 61.6 | 15.8 | 5068.8 | 678.5 |
| **Vehicle 3 IV** | 105.6 | 60.0 | 76.2 | 1.4 | 48.6 | 67.1 | 17.3 | 4232.2 | 575.1 |
| **Vehicle 4 IV** | 114.8 | 66.9 | 84.6 | 1.4 | 47.1 | 63.1 | 14.5 | 4879.3 | 727.5 |

SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, CO = cardiac output, ESV = end systolic volume, EDV = diastolic volume, SV = stroke volume, SVR systemic vascular resistance, PVR = pulmonary vascular resistance, IV = intravenous

**Table III** Average of $dP/dt_{max}$, CI, ESPVR, PRSW, $dP/dt_{min}$, tau and EDPVR in isoflurane anesthetized dogs treated with vehicle (n = 2)

| | Inotropic parameters | | | | Lusitropic parameters | | |
|---|---|---|---|---|---|---|---|
| **Vehicle group (n = 2)** | **$dP/dt_{max}$ (mmHg/s)** | **CI ($s^{-1}$)** | **ESPVR (mmHg/RVU)** | **PRSW (mmHg)** | **$dP/dt_{min}$ (mmHg/s)** | **Tau (ms)** | **EDPVR (mmHg/RVU)** |
| **Baseline** | 1302.5 | 33.0 | 2.1 | 71.9 | -1468.2 | 15.4 | 0.7 |
| **Vehicle 1 IV** | 1441.6 | 34.1 | 2.1 | 75.9 | -1639.6 | 15.3 | 0.7 |
| **Vehicle 2 IV** | 1439.5 | 33.3 | 2.4 | 73.5 | -1652.0 | 15.5 | 0.6 |
| **Vehicle 3 IV** | 1307.5 | 32.9 | 1.8 | 73.7 | -1480.3 | 14.5 | 0.6 |
| **Vehicle 4 IV** | 1431.1 | 33.9 | 2.4 | 70.6 | -1576.3 | 13.3 | 0.8 |

$dP/dt_{max}$ = the maximal rate of rise of the left ventricular pressure, CI = contractility index, ESPVR = end-systolic pressure-volume relationship, PRSW = preload recruitable stroke work, $dP/dt_{min}$ = the maximal rate of fall of the left ventricular pressure, tau = isovolumic relaxation time constant, EDPVR = end-diastolic pressure-volume relationship, IV = intravenous

**Table IV** Mean±SEM of PQ, QRS, QT, QTc(V), HR, AH and HV in isoflurane anesthetized dogs treated with escalating doses of dronedarone (n = 5)

| DR group (n = 5) | PQ (ms) | QRS (ms) | QT (ms) | QTc(V) (ms) | HR (bpm) | AH (ms) | HV (ms) |
|---|---|---|---|---|---|---|---|
| **Baseline** | 104.0±6.2 | 66.1±1.7 | 256.1±13.0 | 283.5±11.5 | 90.5±7.7 | 81.2±2.0 | 50.7±1.0 |
| **Vehicle IV** | 104.0±7.7 | 65.3±2.0 | 254.9±10.8 | 283.4±9.6 | 91.7±6.7 | 81.2±0.2 | 50.5±1.5 |
| **DR 0.5 mg/kg IV** | 106.3±5.7 | 65.5±2.2 | 255.6±14.0 | 284.4±12.3 | 91.8±6.4 | 89.1±0.8 | 52.3±0.9 |
| **DR 1.0 mg/kg IV** | 108.1±8.1 | 65.5±2.2 | 255.9±13.9 | 283.6±11.0 | 90.5±6.7 | 88.7±10.0 | 48.0±0.7 |
| **DR 2.5 mg/kg IV** | 123.1±11.8 | 65.3±2.2 | 262.0±18.6 | 286.2±15.5 | 86.8±8.2 | 97.2±17.6 | 50.9±2.1 |

SEM = standard error of mean, PQ = PQ interval, QRS = QRS complex, QT = QT interval, QTc(V) = corrected QT interval calculated by van de Water equation, HR = heart rate, AH = AH interval, HV = HV interval, IV = intravenous

**Table V** Mean±SEM of SBP, DBP, MBP, CO, ESV, EDV, SV, SVR and PVR in isoflurane anesthetized dogs treated with escalating doses of dronedarone (n = 5)

| DR group (n = 5) | SBP (mmHg) | DBP (mmHg) | MBP (mmHg) | CO (l/mim) | ESV (ml) | EDV (ml) | SV (ml) | SVR ($dyn.s.cm^{-5}$) | PVR ($dyn.s.cm^{-5}$) |
|---|---|---|---|---|---|---|---|---|---|
| **Baseline** | 95.7±10.7 | 59.5±6.1 | 73.7±7.5 | 1.1±0.1 | 77.9±1.5 | 96.4±8.8 | 29.2±1.4 | 5098.9±425.2 | 685.6±82.0 |
| **Vehicle IV** | 103.7±9.8 | 66.2±4.9 | 82.0±6.1 | 1.2±0.1 | 77.1±8.9 | 110.8±6.7 | 27.2±2.6 | 5478.3±626.1 | 759.5±117.4 |
| **DR 0.5 mg/kg IV** | 97.7±7.9 | 62.6±4.1 | 77.2±5.0 | 1.1±0.1 | 77.8±6.0 | 105.7±8.2 | 25.3±3.0 | 5449.6±489.9 | 822.1±87.6 |
| **DR 1.0 mg/kg IV** | 91.0±5.6 | 59.8±2.7 | 72.1±2.5 | 1.0±0.1 | 90.1±2.9 | 113.3±7.3 | 25.3±3.6 | 5767.6±761.0 | 943.4±98.7 |
| **DR 2.5 mg/kg IV** | 94.7±7.8 | 59.9±4.1 | 73.5±4.8 | 0.7±0.1 | 96.9±3.4 | 109.3±11.9 | 16.8±1.2 | 8633.1±942.2 | 1366.1±232.2 |

SEM = standard error of mean, SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, CO = cardiac output, ESV = end systolic volume, EDV = diastolic volume, SV = stroke volume, SVR systemic vascular resistance, PVR = pulmonary vascular resistance, IV = intravenous

**Table VI** Mean±SEM of $dP/dt_{max}$, CI, ESPVR, PRSW, $dP/dt_{min}$, tau and EDPVR in isoflurane anesthetized dogs treated with escalating doses of dronedarone (n = 5)

| | Inotropic parameters | | | | Lusitroic parameters | | |
|---|---|---|---|---|---|---|---|
| DR group (n = 5) | $dP/dt_{max}$ (mmHg/s) | CI ($s^{-1}$) | ESPVR (mmHg/RVU) | PRSW (mmHg) | $dP/dt_{min}$ (mmHg/s) | Tau (ms) | EDPVR (mmHg/RVU) |
| **Baseline** | 1387.8±117.2 | 37.0±2.1 | 1.4±0.1 | 45.1±2.9 | -1354.7±156.5 | 13.7±0.9 | 0.4±0.1 |
| **Vehicle IV** | 1440.9±102.5 | 35.4±0.7 | 1.5±0.3 | 46.0±2.5 | -1522.1±168.1 | 14.7±0.4 | 0.4±0.05 |
| **DR 0.5 mg/kg IV** | 1259.2±74.2 | 34.4±1.7 | 1.2±0.1 | 41.0±4.7 | -1338.7±82.7 | 15.0±0.8 | 0.4±0.05 |
| **DR 1.0 mg/kg IV** | 1073.9±94.1 | 31.4±1.7 | 0.7±0.2 | 37.6±0.7 | -1192.0±114.7 | 17.4±0.9 | 0.7±0.1 |
| **DR 2.5 mg/kg IV** | 967.8±71.3 | 29.9±1.8 | 0.8±0.2 | 35.1±2.6 | -1007.7±93.1 | 20.7±2.1 | 0.7±0.1 |

SEM = standard error of mean, $dP/dt_{max}$ = the maximal rate of rise of the left ventricular pressure, CI = contractility index, ESPVR = end-systolic pressure-volume relationship, PRSW = preload recruitable stroke work, $dP/dt_{min}$ = the maximal rate of fall of the left ventricular pressure, tau = isovolumic relaxation time constant, EDPVR = end-diastolic pressure-volume relationship, IV = intravenous

**Table VII** Average of HR over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| HR (bpm) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vehicle** | 86.9 | 78.7 | 83.5 | 90.3 | 83.4 | 72.9 | 80.8 | 81.8 | 79.3 | 76.3 | 64.6 | 75.2 | 76.2 | 73.0 | 60.1 |
| **DR 10 mg/kg** | 93.3 | 87.5 | 81.4 | 91.9 | 79.2 | 85.1 | 82.5 | 80.1 | 75.2 | 73.3 | 71.4 | 67.0 | 66.3 | 87.7 | 77.5 |
| **DR 20 mg/kg** | 84.3 | 83.7 | 78.9 | 77.7 | 80.3 | 91.9 | 80.2 | 79.1 | 65.8 | 63.1 | 60.4 | 53.4 | 59.1 | 72.4 | 73.4 |
| **DR 40 mg/kg** | 95.6 | 84.3 | 81.3 | 83.9 | 85.1 | 86.7 | 83.1 | 85.5 | 86.2 | 69.1 | 92.8 | 83.4 | 82.1 | 89.9 | 68.8 |

HR = heart rate, DR = dronedarone

**Table VIII** Average of PQ over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| PQ (ms) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 91.2 | 91.5 | 84.9 | 87.5 | 89.5 | 93.1 | 87.9 | 92.4 | 88.3 | 90.8 | 92.4 | 92.9 | 91.0 | 88.4 | 92.0 |
| DR 10 mg/kg | 92.0 | 91.7 | 89.0 | 90.4 | 97.2 | 92.2 | 89.8 | 89.2 | 88.8 | 89.0 | 91.1 | 92.5 | 92.2 | 87.3 | 91.0 |
| DR 20 mg/kg | 88.5 | 91.3 | 88.7 | 90.6 | 97.8 | 86.6 | 94.5 | 92.6 | 90.9 | 91.3 | 92.1 | 93.8 | 91.5 | 90.4 | 88.8 |
| DR 40 mg/kg | 90.5 | 92.8 | 95.2 | 96.9 | 101.3 | 97.2 | 104.2 | 101.1 | 99.1 | 103.0 | 100.7 | 99.3 | 99.9 | 90.0 | 96.1 |

PQ = PQ interval, DR = dronedarone

**Table IX** Average of QRS over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| QRS (ms) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 35.4 | 36.6 | 43.9 | 37.1 | 33.9 | 41.4 | 40.5 | 39.7 | 40.1 | 42.4 | 39.3 | 38.0 | 42.4 | 38.4 | 37.2 |
| DR 10 mg/kg | 35.4 | 47.3 | 36.6 | 36.6 | 36.5 | 39.8 | 36.9 | 36.8 | 38.5 | 39.9 | 37.9 | 40.3 | 37.3 | 49.0 | 42.7 |
| DR 20 mg/kg | 40.6 | 42.2 | 42.8 | 36.6 | 39.9 | 43.3 | 42.3 | 35.8 | 42.3 | 42.2 | 36.8 | 42.7 | 43.8 | 43.9 | 36.6 |
| DR 40 mg/kg | 43.4 | 40.6 | 39.7 | 41.8 | 42.8 | 40.7 | 39.6 | 39.7 | 39.1 | 40.5 | 39.7 | 41.2 | 41.4 | 41.8 | 39.9 |

QRS = QRS complex, DR = dronedarone



**Table X** Average of QT over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| QT (ms) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | 201.9 | 210.7 | 225.4 | 204.2 | 206.0 | 220.3 | 215.6 | 215.1 | 223.3 | 222.3 | 224.9 | 219.6 | 226.0 | 217.5 | 220.4 |
| DR 10 mg/kg | 209.6 | 220.4 | 228.8 | 219.5 | 229.8 | 229.5 | 226.6 | 229.1 | 232.7 | 229.1 | 229.5 | 232.9 | 234.9 | 222.7 | 231.1 |
| DR 20 mg/kg | 217.6 | 226.3 | 226.4 | 221.0 | 214.2 | 209.4 | 232.0 | 227.3 | 236.9 | 239.5 | 234.9 | 255.7 | 242.1 | 236.7 | 234.7 |
| DR 40 mg/kg | 220.7 | 219.4 | 227.4 | 229.0 | 229.9 | 220.1 | 231.7 | 228.6 | 226.5 | 236.1 | 232.3 | 243.5 | 245.6 | 225.8 | 225.9 |

QT = QT interval, DR = dronedarone

**Table XI** Average of QTc(V) over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| QTc(V) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vehicle** | 228.5 | 231.4 | 249.7 | 232.7 | 230.1 | 235.5 | 238.0 | 236.8 | 242.3 | 240.9 | 228.1 | 236.5 | 244.2 | 232.9 | 220.4 |
| **DR 10 mg/kg** | 240.1 | 247.5 | 251.5 | 249.3 | 250.6 | 255.2 | 250.3 | 250.9 | 250.2 | 244.9 | 243.4 | 241.5 | 243.1 | 250.1 | 250.2 |
| **DR 20 mg/kg** | 242.1 | 250.0 | 247.2 | 240.7 | 235.3 | 238.6 | 253.8 | 245.3 | 242.4 | 241.1 | 234.9 | 244.0 | 240.6 | 251.3 | 250.6 |
| **DR 40 mg/kg** | 253.0 | 241.7 | 250.2 | 253.8 | 255.5 | 246.8 | 255.9 | 254.5 | 253.0 | 247.4 | 262.2 | 267.3 | 269.0 | 253.8 | 237.1 |

QTc(V) = corrected QT interval calculated by van de Water equation, DR = dronedarone

**Table XII** Average of MBP over time in 2 conscious telemetry dogs treated with vehicle, oral dronedarone 10-, 20- or 40 mg/kg

| MBP (mmHg) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 7 hr | 8 hr | 9 hr | 10 hr | 11 hr | 12 hr | 24 hr | 36 hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vehicle** | 93.8 | 78.9 | 83.0 | 84.3 | 88.7 | 84.9 | 91.0 | 86.6 | 84.8 | 77.1 | 83.8 | 82.5 | 89.6 | 87.4 | 89.5 |
| **DR 10 mg/kg** | 91.3 | 79.5 | 84.0 | 83.9 | 73.8 | 75.1 | 78.2 | 81.1 | 81.1 | 84.6 | 80.9 | 87.3 | 86.1 | 81.4 | 83.9 |
| **DR 20 mg/kg** | 84.8 | 83.4 | 87.2 | 86.2 | 82.1 | 89.0 | 91.9 | 86.1 | 87.9 | 90.5 | 84.6 | 90.4 | 86.6 | 85.7 | 89.5 |
| **DR 40 mg/kg** | 82.2 | 85.8 | 88.6 | 85.2 | 82.7 | 82.7 | 78.3 | 78.6 | 76.2 | 81.8 | 78.2 | 73.1 | 77.1 | 82.8 | 83.1 |

MBP = mean blood pressure, DR = dronedarone

**Table XIII** Mean±SEM of PQ, QRS, QT, QTc(V) and HR over time in 4 conscious telemetry dogs treated with oral dronedarone 20 mg/kg twice a day for 7 days

| | PQ (ms) | QRS (ms) | QT (ms) | QTc(V) (ms) | HR (bpm) |
|---|---|---|---|---|---|
| **Baseline** | 83.1±2.7 | 48.4±1.7 | 232.5±9.6 | 263.9±10.0 | 98.9±12.8 |
| **4 hr** | 87.5±3.0 | 47.3±1.2 | 224.7±5.6 | 255.5±2.2 | 95.9±9.6 |
| **5 hr** | 85.5±4.0 | 49.3±1.7 | 229.8±3.9 | 261.0±2.2 | 96.5±9.5 |
| **6 hr** | 86.8±4.3 | 48.9±1.1 | 240.4±2.6 | 268.1±4.2 | 91.1±9.1 |
| **7 hr** | 86.7±2.5 | 48.7±1.3 | 240.6±6.3 | 268.6±8.3 | 91.30±9.1 |
| **8 hr** | 84.7±4.6 | 50.7±1.4 | 244.1±7.3 | 266.0±1.8 | 83.9±10.8 |
| **12 hr** | 85.7±4.2 | 50.2±2.0 | 238.9±2.7 | 260.3±5.6 | 80.2±4.4 |
| **Day 4** | 101.1±4.0 | 43.7±3.6 | 254.9±6.4 | 269.5±4.4 | 72.4±2.4 |
| **Day 7** | 98.1±4.7 | 42.1±3.9 | 254.6±4.2 | 269.6±6.2 | 73.7±5.9 |

SEM = standard error of mean, PQ = PQ interval, QRS = QRS complex, QT = QT interval, QTc(V) = corrected QT interval calculated by van de Water equation, HR = heart rate

**Table XIV** Mean±SEM of SBP, DBP, MBP, CO, ESV, EDV and SV over time in 4 conscious telemetry dogs treated with oral dronedarone 20 mg/kg twice a day for 7 days

| | SBP (mmHg) | DBP (mmHg) | MBP (mmHg) | (CO ml/min) | ESV (ml) | EDV (ml) | SV (ml) |
|---|---|---|---|---|---|---|---|
| **Baseline** | 98.7±18.9 | 75.8±12.8 | 87.5±15.5 | 1338.5±98.0 | 26.3±4.8 | 36.5±3.3 | 12.3±1.5 |
| **4 hr** | 102.2±17.8 | 77.3±9.4 | 90.5±13.7 | 1036.3±283.9 | 24.8±3.3 | 33.8±1.5 | 10.5±1.5 |
| **5 hr** | 102.1±19.3 | 76.8±10.6 | 89.5±14.9 | 1058.5±339.7 | 25.8±2.9 | 36.5±0.6 | 11.8±1.9 |
| **6 hr** | 105.6±17.7 | 79.9±9.5 | 92.5±13.5 | 1134.5±137.4 | 25.3±3.6 | 35.5±2.3 | 11.5±1.3 |
| **7 hr** | 110.2±19.2 | 83.6±10.3 | 96.5±14.5 | 1004.0±116.9 | 24.5±3.8 | 34.5±2.2 | 10.8±1.8 |
| **8 hr** | 110.9±23.6 | 84.5±13.4 | 96.9±17.8 | 1096.0±297.0 | 24.0±3.4 | 33.3±1.3 | 10.3±2.1 |
| **12 hr** | 93.9±21.0 | 70.1±12.8 | 81.8±16.6 | 942.8±237.0 | 25.3±2.7 | 34.5±1.2 | 11.3±2.1 |
| **Day 4** | 84.2±17.5 | 60.5±11.4 | 71.6±13.8 | 960.3±193.6 | 26.3±3.4 | 38.0±1.3 | 12.8±1.5 |
| **Day 7** | 86.8±17.7 | 62.3±10.6 | 73.9±13.3 | 885.8±83.5 | 29.0±3.6 | 40.3±2.4 | 11.5±1.2 |

SEM = standard error of mean, SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, CO = cardiac output, ESV = end systolic volume, EDV = diastolic volume, SV = stroke volume

**Table XV** Mean±SEM of $dP/dt_{max}$, CI, ESPVR, PRSW, $dP/dt_{min}$, tau and EDPVR over time in 4 conscious telemetry dogs treated with oral dronedarone 20 mg/kg twice a day for 7 days

| | Inotropic parameters | | | | Lusitropic parameters | | |
|---|---|---|---|---|---|---|---|
| | $dP/dt_{max}$ (mmHg/s) | CI ($s^{-1}$) | ESPVR (mmHg/RVU) | PRSW (mmHg) | $dP/dt_{min}$ (mmHg/s) | Tau (ms) | EDPVR (mmHg/RVU) |
| **Baseline** | 3234.6±263.2 | 55.0±2.8 | 8.6±1.1 | 76.1±11.3 | -3267.6±405.6 | 11.1±0.9 | 0.9±0.2 |
| **4 hr** | 2820.4±279.2 | 49.3±2.2 | 7.3±0.8 | 64.6±12.8 | -3225.1±292.8 | 11.7±0.6 | 1.0±0.2 |
| **5 hr** | 2783.6±461.2 | 49.6±3.5 | 7.3±0.8 | 66.2±10.2 | -3267.9±446.9 | 12.8±1.2 | 0.9±0.2 |
| **6 hr** | 3103.3±451.7 | 53.1±2.9 | 7.5±0.8 | 67.9±9.4 | -3353.5±518.5 | 12.3±1.3 | 1.0±0.2 |
| **7 hr** | 3051.9±379.6 | 51.9±2.2 | 9.1±0.3 | 80.0±9.4 | -3297.0±462.0 | 12.4±0.8 | 1.0±0.2 |
| **8 hr** | 3182.1±460.8 | 53.0±3.4 | 8.9±0.8 | 77.7±9.5 | -3398.4±494.1 | 13.1±1.2 | 0.8±0.2 |
| **12 hr** | 3110.5±232.8 | 53.0±3.4 | 10.6±1.9 | 73.9±8.6 | -3272.4±305.7 | 12.0±1.7 | 1.0±0.03 |
| **Day 4** | 3060.1±32.4 | 53.6±2.0 | 6.8±0.6 | 75.8±7.1 | -3279.6±390.8 | 11.3±0.6 | 0.9±0.1 |
| **Day 7** | 3144.4±78.0 | 62.2±2.6 | 11.2±1.2 | 75.8±6.9 | -3154.5±345.2 | 11.1±0.8 | 0.9±0.05 |

SEM = standard error of mean, $dP/dt_{max}$ = the maximal rate of rise of the left ventricular pressure, CI = contractility index, ESPVR = end-systolic pressure-volume relationship, PRSW = preload recruitable stroke work, $dP/dt_{min}$ = the maximal rate of fall of the left ventricular pressure, tau = isovolumic relaxation time constant, EDPVR = end-diastolic pressure-volume relationship

**Table XVI** AF duration, $APD_{70}$, AERR and PRR at pre-dosing and post-dosing (oral dronedarone 20 mg/kg twice a day for 7 days) in canine AF models (n = 4)

| | AF duration (ms) | | $APD_{70}$ (ms) | | AERP (ms) | | PRR (ms) | |
|---|---|---|---|---|---|---|---|---|
| Dog no. | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing |
| **2469911** | 441.7 | 4.3 | 85.4 | 104.0 | 110 | 120 | 24.6 | 16 |
| **2485193** | 22.6 | 5.7 | 69.2 | 87.0 | 100 | 135 | 30.8 | 48 |
| **2487838** | 3.3 | 0 | 82.5 | 88.0 | 90 | 100 | 7.5 | 12 |
| **2490553** | 35.0 | 7.5 | 58.8 | 76.8 | 95 | 125 | 36.2 | 48.2 |
| **2185416** | 1.9 | 5.8 | 79.5 | 99.4 | 95 | 125 | 15.5 | 25.6 |
| **2484642** | 28.1 | 10.7 | 83.2 | 92.0 | 95 | 115 | 11.8 | 23 |

AF duration = AF starts until AF converts to sinus rhythm, $APD_{70}$ = action potential duration at 70% repolarization, AERR = atrial effective refractory period, PRR = post-repolarization refractoriness AF duration; pre-dosing measured after RAP with PE 2 µg/kg/min; post-dosing averaged after 1st RAP with PE 2 µg/kg/min and 2nd RAP with PE 4 µg/kg/min

$APD_{70}$, AERR, PRR; pre-dosing measured before RAP with PE 2 µg/kg/min; post-dosing measured before 1st RAP with PE 2 µg/kg/min

**Table XVII** Mean±SEM of HR, SBP, DBP and MBP at baseline, 20 min of 1st pacing and 20 min of 2nd pacing in canine AF models (n = 4)

| | HR (bpm) | | SBP (mmHg) | | DBP (mmHg) | | MBP (mmHg) | |
|---|---|---|---|---|---|---|---|---|
| | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing | Pre-dosing | Post-dosing |
| **Baseline** | 79.8±1.5 | 75.0±3.9 | 59.7±2.0 | 46.7±2.3 | 69.7±1.7 | 60.0±2.7 | 105.9±1.0 | 89.3±2.9 |
| **20 min of 1st pacing** | 184.8±9.2 | 166.1±19.6 | 149.0±10.7 | 117.0±13.1 | 164.8±9.7 | 137.2±15.4 | 162.9±30.9 | 127.7±28.8 |
| **20 min of 2nd pacing** | 200.4±13.0 | 173.8±26.6 | 148.4±18.9 | 122.2±15.6 | 170.4±15.3 | 143.1±20.0 | 118.3±39.1 | 121.9±36.1 |

SEM = standard error of mean, HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure

# VITA

NAME Miss Nakkawee Saengklub

DATE OF BIRTH November 09, 1986

EDUCATION

| | | |
|---|---|---|
| Ph.D. | 2011-present | Chulalongkorn University<br>Bangkok, Thailand |
| D.V.M. (Honor) | 2005-2010 | Chulalongkorn University<br>Bangkok, Thailand |

HONOR AND OTHER DISTINCTIONS

1. Graduated Second Class of Honors in D.V.M. 2010

2. Research Excellent Award, topic “A Comparison effects of intravenous flecainide, diltiazem, and dofetilide on rhythm control in a canine model of atrial fibrillation: a preliminary study”. VPAT Regional Veterinary Congress 2012, 13-16 May 2012, Bangkok, Thailand.

3. Jr. Investigator Travel Award and Poster Competition 1st Place, topic “Comparison of indices of left ventricular inotrope and lusitrope in isoflurane-anesthetized, closed chest, dogs”. The 13th Annual Safety Pharmacology Society Meeting, 16-19 Sep 2013, Rotterdam, Netherlands.

4. Excellent poster award (1st rank), research article, topic “Chronic effect of oral dronedarone administration on electrocardiography, hemodynamic, and cardiac function in conscious, telemetry dogs”. The 14th Chulalongkorn University Veterinary Conference, 20-22 April 2015, Bangkok, Thailand.

PUBLICATIONS

1. Saengklub, N., Limprasutr, V., Sawangkoon, S., Buranakarl, C., Chaiyabutr, N. and Kijtawornrat, A. (2015) Comparison of two locations for recording intracardiac conductivity in anesthetized dogs. Thai J Vet Med. 45(1): 35-41.

2. Saengklub, N., Limprasutr, V., Sawangkoon, S., Buranakarl, C., Hamlin, R. L. and Kijtawornrat, A. (2015) Acute effects of intravenous dronedarone on electrocardiograms, hemodynamics and cardiac functions in anesthetized dogs. J Vet Med Sci. (in press)