ผลของยาเอมิโอดาโรนต่อการทำงานของหัวใจในสุนัขที่มีภาวะหัวใจเต้นไม่เป็นจังหวะ

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EFFECTS OF AMIODARONE ON CARDIAC PERFORMANCE IN ARRHYTHMIC DOGS

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ภาวะหัวใจเต้นไม่เป็นจังหวะเป็นปัญหาที่สำคัญมากในโรคของระบบหัวใจและหลอดเลือด ซึ่งนำไปสู่การเสียชีวิตแบบเฉียบพลัน ยาต้านภาวะหัวใจเต้นไม่เป็นจังหวะที่มีใช้ในทางสัตวแพทย์นั้นมีเพียงไม่กี่ชนิดที่มีประสิทธิภาพและปลอดภัย เอมิโอดาโรนเป็นยาต้านภาวะหัวใจเต้นไม่เป็นจังหวะ ซึ่งจัดอยู่ในกลุ่มที่สาม มีฤทธิ์ในการยับยั้งช่องไอออนโพแทสเซียม ซึ่งมีการใช้ในมนุษย์นานกว่า 50 ปี แต่อย่างไรก็ตาม รายงานการใช้ยาชนิดนี้ในทางสัตวแพทย์ยังมีน้อย และยายังออกฤทธิ์ยับยั้งตัวรับชนิดเบตาแอดรีเนอจิก และช่องไอออนแคลเซียม ซึ่งปัจจัยเหล่านี้ส่งผลต่อการทำงานของหัวใจ ทั้งการหดตัว การคลายตัว และยังส่งผลต่อการทำงานของไทรอยด์ฮอร์โมน การศึกษาครั้งนี้จึงมุ่งหาผลของการให้ยาเอมิโอดาโรนต่อการควบคุมภาวะหัวใจเต้นไม่เป็นจังหวะ การทำงานของหัวใจ และระดับไทรอยด์ฮอร์โมน สุนัข ป่วยเป็นโรคหัวใจที่เข้ารับการรักษาที่หน่วยโรคหัวใจ โรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย จำนวน 8 ตัว ซึ่งสุนัขทั้งหมดมีภาวะหัวใจโตและได้รับการยืนยันชนิดของภาวะหัวใจเต้นไม่เป็นจังหวะจากการตรวจคลื่นไฟฟ้าหัวใจในโรงพยาบาล และการบันทึกติดตามคลื่นไฟฟ้าหัวใจต่อเนื่อง 24 ชั่วโมง สุนัขที่เข้าร่วมการศึกษาจะได้รับยาเอมิโอดาโรนเริ่มต้นในขนาด 10 มก./กก. วันละสองครั้ง เป็นเวลา 7 วัน จากนั้นจึงให้ขนาดยา 5 มก./กก. วันละครั้ง ทุกวัน เพื่อควบคุมภาวะหัวใจเต้นไม่เป็นจังหวะ ผลการศึกษาพบว่าหลังจากให้ยาเอมิโอดาโรนนาน 15 วัน ค่าเฉลี่ยของอัตราการเต้นของหัวใจ ($p < 0.05$) และจำนวนคลื่นไฟฟ้าหัวใจที่ผิดปกติลดลงอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) การประเมินความเสี่ยงในการเกิดภาวะหัวใจเต้นไม่เป็นจังหวะอันเนื่องมาจากการเหนี่ยวนำของยาพบว่า ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ทั้งความแปรปรวนของการเกิดรีโพลาไรเซชันในหัวใจห้องล่าง (transmural of ventricular repolarization) และความแปรปรวนในระยะสั้นของช่วงเวลา QT (short term variability of QT interval) แม้ว่าช่วงเวลา QT มีแนวโน้มที่จะนานขึ้นหลังได้รับยา ($p = 0.06$) ผลการตรวจหัวใจด้วยคลื่นเสียงสะท้อนพบว่าค่า PEP และ IVCT นานขึ้นอย่างมีนัยสำคัญทางสถิติ (ทั้งคู่ $p < 0.05$) และค่า Tei index มีแนวโน้มเพิ่มขึ้น ($p = 0.07$) หลังได้รับยาเอมิโอดาโรนนาน 60 วัน ระดับ total T_3 และ T_4 ในพลาสมาเปลี่ยนแปลงอย่างไม่มีนัยสำคัญทางสถิติ ($p = 0.44$ และ 0.37 ตามลำดับ) จากการศึกษาครั้งนี้ชี้ให้เห็นว่าขนาดยาเอมิโอดาโรนมีประสิทธิภาพในการควบคุมภาวะหัวใจเต้นไม่เป็นจังหวะ มีผลเหนี่ยวนำให้เกิดภาวะหัวใจเต้นไม่เป็นจังหวะจากยาเองต่ำ มีผลกระทบต่อการทำงานของหัวใจน้อย ไม่พบผลข้างเคียงที่ชัดเจน และไม่ส่งผลเสียต่อสภาพสัตว์โดยรวมในทางคลินิก และการใช้ยาเอมิโอดาโรนเพื่อควบคุมภาวะหัวใจเต้นไม่เป็นจังหวะมีความปลอดภัยเพียงพอที่จะใช้รักษาสุนัขที่ป่วยเป็นโรคหัวใจ

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KEYWORDS: AMIODARONE / ARRHYTHMIAS / CARDIAC CONTRACTILITY / DOGS / THYROID

PAKIT BOONPALA: EFFECTS OF AMIODARONE ON CARDIAC PERFORMANCE IN ARRHYTHMIC DOGS. ADVISOR: ASST. PROF. SUWANAKIET SAWANGKOON, D.V.M., Ph.D., 103 pp.

Arrhythmia is one of a serious cardiovascular problem that can lead to sudden cardiac death. In veterinary medicine, there are a few effective and safe anti-arrhythmic agents clinically available. Amiodarone is a class III potassium channel blocking agent that has prescribed more than 50 years in humans; however, there are few reports in the veterinary medicine. The drug also acts as beta-adrenergic and calcium channel antagonist which may produce adverse effects on cardiac contractility, relaxation, and thyroid functions. This study aimed to investigate the effects of amiodarone on arrhythmic control, cardiac performance, and thyroid hormone levels in dogs. Eight client-owned dogs with heart diseases were recruited from the cardiology unit, small animal teaching hospital, Chulalongkorn University. All dogs were had dilated Hearts and confirmed types of arrhythmia by Lead II ECGs and 24-hour Holter monitoring. Amiodarone were administrated at a loading dose of 10 mg/kg twice a day for 7 days, and followed by a maintenance dose of 5 mg/kg once a day. After treatment with amiodarone for 15 days, the results showed significant decreases in heart rate and total arrhythmic count ($p < 0.05$). Proarrhythmic assessment were found insignificantly changes both transmural dispersion of ventricle repolarization and beat-to-beat variability while QT interval was tended to lengthen ($p = 0.06$). Echocardiograms were displayed significantly prolongation of the pre - ejection period and isovolumic contraction time ($p < 0.05$). Moreover, Tei index tended to increase ($p = 0.07$). After 60 days of amiodarone treatment, both total plasma tri-iodothyronine (T_3) and tetra-iodothyronine (T_4) showed insignificant changes when compared with the baseline ($p = 0.44$ and 0.37, respectively). We conclude that the dosage of amiodarone treated in this study is effective, low risk of proarrhythmia, less negative inotropy, and fewer side effects which do not have any impact on clinical conditions. Therefore, amiodarone is safe for treated cardiac arrhythmias in dogs with organic heart diseases.

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LIST OF ABBREVIATIONS

APD	Action potential duration
ALT	Alanine amiotransaminase
ALP	Alkaline phosphatase
AIH	Amiodarone induced-hypothyroidism
AIT	Amiodarone induced-thyrotoxicosis
DCM	Dilated cardiomyopathy
EF (%)	Ejection fraction (%)
ECGs	Electrocardiogram
EDV	End diastolic volume
ESV	End systolic volume
FS (%)	Fractional shortening (%)
IVCT	Isovolumic contraction time
IVRT	Isovolumic relaxation time
LV	Left ventricle
LVIDd	Left ventricular internal dimension, diastole
LVIDs	Left ventricular internal dimension, systole
MAP	Monophasic action potential
MI	Myocardial infarction
PEP	Pre-ejection period
PEP/ET ratio	Pre-ejection period/Ejection time ratio
TpTe	QT dispersion of ventricular repolarization
QTc (VdW)	QT interval with Van de water formula Correction
SERCA	Sarcoplasmic reticulum released calcium ATPase
STV	Short term variability of beat-to-beat repolarization

NCX	Sodium-calcium exchanger
SV	Stroke volume
SPCs	Supraventricular premature complex
T_4	Tetra-iodothyronine
TgAb	Thyroglobulin autoantibodies
TSH	Thyroid stimulating hormone
TdP	Torsade de pointes
TDR	Transmural dispersion of repolarization
T_3	Tri-iodothyronine
VPC	Ventricular premature complex
VT	Ventricular tachycardia
VHS	Vertebral heart scale

CHAPTER I
INTRODUCTION

Arrhythmia is an anomaly cardiac electrophysiology (Dean and Lab, 1989). It could be established as a complication of several structural heart diseases, cardiac fibrosis, or atrial enlargement (Brundel et al., 2005). In addition, it could be found in a non-structural heart disease that often occurred in several large breed dogs, for example, the arrhythmogenic right ventricular cardiomyopathy (ARVC) in Boxer (Harpster, 1991). Arrhythmias can be classified from the origin of abnormal impulse formation as supraventricular or ventricular arrhythmias. Their mechanisms are varies among re-entry, abnormal automaticity, and triggered activity (Wit and Cranefield, 1978). Malignant arrhythmias affect both cardiac functions and hemodynamics such as decreased cardiac output decreased contractility, impaired ventricular relaxation, increased electrical instability, and increased risk of sudden cardiac death (Bayes de Luna et al., 1989). Dilated cardiomyopathy (DCM) is a structural heart disease that often complicated by arrhythmias. When DCM was complicated by arrhythmias, cardiac output was more decreased than lonely DCM. At present, there are a few effective anti-arrhythmic drugs clinically available due to their proarrhythmic properties and serious side effects (IMPACT Research Group, 1984; CAST Investigators, 1989; Coplen et al., 1990; Moosvi et al., 1990; CAST II Investigators, 1992; Flaker et al., 1992; Nattel et al., 1994; Waldo et al., 1996). In veterinary medicine, the famous antiarrhythmic drugs are including, quinidine, procainamide, atenolol, propranolol, sotalol, amiodarone and diltiazem, which each drug has unique antiarrhythmic properties, efficacies and side effects. Antiarrhythmic drugs must be carefully selected because some antiarrhythmic drugs have negative inotropic effects and cause deteriorate cardiac output. Some drugs may have less side effects, however, less efficacy had been reported.

Amiodarone has been classified as a class III antiarrhythmic drug by Singh and Vaughan Williams (Singh and Vaughan Williams, 1970). It has been used to

treat various types of arrhythmias (Connolly, 1999; Fuster et al., 2011; Pedro et al., 2012). Amiodarone acts by inhibiting β-adrenergic receptors and several ion channels including inward rectifier potassium channels (I_{K1}), slow component of the delayed rectifier potassium channels (I_{Ks}), rapid component of the delayed rectifier potassium channels (I_{Kr}), voltage-gated sodium channel (I_{Na}), and voltage-gated calcium channels (I_{Ca}) (Polster and Broekhuysen, 1976; Mason et al., 1983; Lubic et al., 1994; Watanabe and Kimura, 2000). Eventhough pharmacodynamics and pharmacokinetics complication of amiodarone and prior studies of drug toxicity in humans (Jafari-Fesharaki and Scheinman, 1998) and dogs had been reported such as increase levels of hepatic enzymes, keratopathy and thyroid dysfunction (Pasquali et al., 1990; Jacobs et al., 2000; Bicer et al., 2002a). However, many studies indicated that amiodarone is the most potential antiarrhythmic drug available (Lewis et al., 1990; Pasquali et al., 1990; Kodama et al., 1999; Merot et al., 1999; Jacobs et al., 2000; Pachucki et al., 2001; Bicer et al., 2002a; Singh, 2006; Van Herendael and Dorian, 2010).

As mentioned above, amiodarone plays a crucial role as the functional inhibition i.e., blocking the ion channels, attrition of beta adrenergic receptors, and lowering the concentration of plasma tri-iodothyronine (T_3) in humans. These properties may directly affect cardiac contractility and relaxation. However, some studies reported that amiodarone had no obviously effect on hemodynamic and contractility (Pritchard et al., 1975; Polster and Broekhuysen, 1976; Buch and Andersen, 1984; Gagnol et al., 1985; Perret et al., 1992). Moreover, previous studies in healthy beagle dogs did not provide any specific information regarding to the evaluation of cardiac functions (Bicer et al., 2002b; Zhou et al., 2012).

At present, echocardiography is a useful technology, non-invasive, and practical for clinical studies to evaluate cardiac functions. However, there is no studies report using this tool on evaluation of cardiac performance after amiodarone treatment. Therefore, the present study aimed to evaluate the effects of amiodarone on cardiac function and to follow the thyroid hormone levels in arrhythmic dogs presented with structural heart diseases.

Objectives of the study

1. To evaluate effects of amiodarone in term of controlling arrhythmias in dogs
2. To evaluate effects of amiodarone on cardiac contractility and relaxation in arrhythmic dogs
3. To evaluate the impacts of amiodarone on thyroid hormone levels in arrhythmic dogs

CHAPTER II
LITERATURE REVIEWS

A. Antiarrhythmic and problems

Antiarrhythmic drugs can reduce risk of sudden cardiac death. However, some drugs have proarrhythmia and negative inotroph effects which results may be more deteriorate animal condition than benefits. Right now the antiarrhythmic drugs available in veterinary medicine are including;

1. Quinidine

In class I antiarrhythmic drug, quinidine is subcategories as class IA that decreases dV/dt in phase 0 of myocardial action potential by fast sodium channel inhibitory effect (Chen et al., 1975; Hondeghem and Matsubara, 1988). Moreover, quinidine has mild class III effects that increase the APD (Roden et al., 1988). Proarrhythmic effects were reported in using of quinidine that cause from the prolong QT interval, depress conduction of myocardial tissue, and may lead to generate torsades de pointes (TdP) (Walsh and Horwitz, 1979; Davidenko et al., 1989). Quinidine is also possessed muscarinic receptor inhibition, and causes increasing of sympathetic tone via vagolytic effects which facilitated atrioventricular conduction and lead to increment of sinus firing rate (Josephson et al., 1974; Juul-Moller et al., 1990). For this reason, quinidine dose not suite for treatment of supraventricular arrhythmia (Watanabe and Chiba, 1982). Furthermore, in previous human study showed the dreadful results of using quinidine with sinus rhythm was at the expensed of shorten survival time (Roden et al., 1986). Briefly, quinidine is far away from ideal antiarrhythmic drugs.

2. Procainamide

Procainamide is one of a potent antiarrhythmic drug with low side effect that widely use in equine medicine for primary indication of ventricular tachycardia (Dembek et al., 2014). Supraventricular and ventricular arrhythmia can be treated with procainamide (Pearle et al., 1983). However, procainamide has a short half-life in the blood stream, therefore, oral administration must be given three times per day and may not practical. Furthermore, previous study in the veterinary field showed ineffective of procainamide in treatment of ventricular arrhythmia in boxers (Meurs et al., 2002).

3. Beta-adrenergic blocker

Sympathetic activation and catecholamine releasing play roles in ischemic processes. In human, beta-blocking agents are effective drugs for treatment in post myocardial infarction patients via decrease sympathetic tone which cause reduction in myocardial oxygen demand, decrease heart rate, and acting as a vasodilator. However, in veterinary medicine, beta-blocking agents have an infamous, no clinical trial showed obviously beneficial results in treatment of arrhythmia. Furthermore, nature of DCM has anomaly of myocardial fibers (Tidholm et al., 1998), which impaired cardiac contraction and relaxation. Giving potent beta-blocking agent in DCM dogs may be more negatively impacted on cardiac function than survival benefit. A non-selective type of beta-adrenergic antagonist drug; propranolol, has causes bronchoconstriction. Meurs and colleagues (2002) were compared efficacy of antiarrhythmic drugs in Boxer with VPCs. The result showed atenolol was not effective. The advantage of this drug group is less proarrhythmic effects, and serve as a first line of antiarrhythmic drug in cases whose has risk of sudden cardiac death without congestive heart failure.

4. Sotalol

Sotalol is a combination of class II and III antiarrhythmic drugs. The drug has efficacy and quite safety (Meurs et al., 2002). However, sotalol has narrow therapeutic dosage and non-selective beta-blocking properties. There are few reports of sotalol in canine species. Thomsen and colleagues (2004) reported increase in short term variability of beat-to-beat repolarization (STV) which represented to increase risk of TdP. Sotalol has active metabolites and excreted by kidneys. Sotalol is suited for loan arrhythymia without systolic dysfunction. Further investigation and clinical trials in canine species are required.

5. Diltiazem

Calcium ion is one of the key factors in cardiac contractility and firing rate of the cardiac pacemaker. Diltazem is a class IV antiarrhythmic drug that was widely used in supraventricular arrhythmia, however, it is rarely used in ventricular arrhythmias. Diltiazem is used in advance heart disease cases with atrial fibrillation (Gelzer et al., 2009). The drug has effects on sinus firing rate, slow AV conduction time and ventricular response rate (Kawai et al., 1981). However, administration of diltiazem should be avoided in case that has impaired systolic function.

B. Amiodarone

1. Structure / class / synthesis

Amiodarone is 2-butyl, 3-(4,diethylaminoethoxy, 3,5-diiodo, benzoyl) benzofuran hydrochloride (figure 1) which was synthesized by Labaz Inc. (Belgium) in 1962 as a vasodilator agent (Singh, 1983) and used for anti-anginal treatment. Anti-arrhythmic properties were discovered years later. Amiodarone is classified as a class III antiarrhythmic drug by Willium-Vaughan classification (Singh and Vaughan Williams, 1970). Many studies have shown that amiodarone is the most potent antiarrhythmic agent (Kodama et al., 1999; Merot et al., 1999; Singh, 2006). In 1985, the U.S. Food and Drug Administration (U.S. FDA) approved the use of amiodarone as the last resort

treatment for recurrent life threatening ventricular arrhythmias (U.S. Food and Drug Administration, 2011). Eventhough, amiodarone was classified in class III antiarrhythmic drug, the drug has inhibitory effects on various types of ion channels, including some types of receptors. Amiodarone inhibits sodium ion channel activity, beta-adrenergic function, activity of sodium-calcium exchanger (NCX), and calcium ion channel activity (Lubic et al., 1994); (Mason et al., 1983; Watanabe and Kimura, 2000). Therefore, amiodarone has been named as a multichannel blocker with low proarrhythmic profiles (Singh, 2006). These essential properties decrease the risk of TdP as well as others (Sicouri et al., 1997; Drouin et al., 1998; van Opstal et al., 2001).

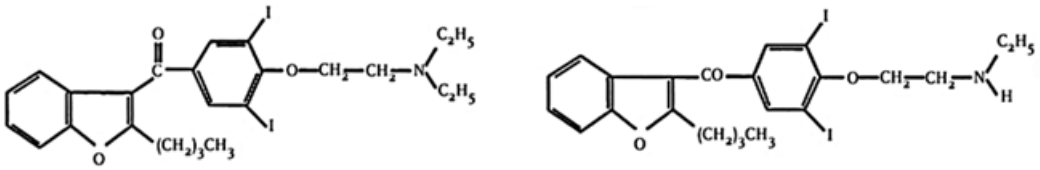

Figure 1. Structure of amiodarone and its metabolite.

2. Pharmacokinetic and pharmacodynamics of amiodarone

The structure of amiodarone is a benzofluran derivative containing iodine in the molecular structure (Rao et al., 1986). Mainly, amiodarone was metabolized by cytochrome P450 3A4 in the liver (Trivier et al., 1993). After first-pass metabolism, amiodarone is dealkylated to form desethylamiodarone, the active metabolite (Holt et al., 1983).

Amiodarone is a lipophilic compound that distributes and deposits mainly in adipose tissues; however, desethylamiodarone possesses lower lipophilic properties than the parent compound (Plomp et al., 1985). Therefore, in adipose tissues, desethylamiodarone concentration is lower than amiodarone concentration.

Conversely, desethylamiodarone concentration is higher than amiodarone 10-50 times in myocardial tissues (Nattel et al., 1992).

Since amiodarone takes times to reach the peak plasma concentration, therefore, in order to rush the electrophysiological effects, the loading dose is necessary for stabilizing the level of plasma drug concentration which Opie and Gersh (2009) were recommended in human medicine. The administration route is influenced with the half-life of drug elimination (Latini et al., 1984). In general, the drug is eliminated via bile and excreted in feces. Some portion of desethylamiodarone might be absorbed via enterohepatic circulation and dealkylated again to produce inactive metabolite which may excrete in feces (Bicer et al., 2002b). The small portion of both amiodarone and desethylamiodarone was excreted by the kidneys (Latini et al., 1984).

Phamacokinetics of amiodarone in dogs differ from humans. Per oral administration, the half-life of amiodarone is 3 days in dogs (Brien et al., 1990) compared to humans that have variation between 25 to 110 days (Opie and Gersh, 2009). Brien and colleagues (1990) reported high ratio of amiodarone and desethylamiodarone, however, this ratio is individually altered in humans (Plomp et al., 1984).

3. Antiarrhythmic properties of amiodarone

According to several publications in both humans and dogs, amiodarone can be used for treatment of various types of arrhythmias. Amiodarone has multi-channel blocking properties; therefore, the drug can be used to treat both supraventricular and ventricular arrhythmias (Connolly, 1999; Fuster et al., 2011; Pedro et al., 2012). Chronic amiodarone administration increases atrial effective refractory period and slows conduction velocity via increasing action potential duration (APD) and inhibiting sodium ion channel, respectively (Burashnikov et al., 2008). Several researchers conducted the studies in canine ventricular wedge preparations and found that amiodarone decreased transmural dispersion of repolarization, one of the causations of TdP (Sicouri et al., 1997; Drouin et al., 1998;

van Opstal et al., 2001). Moreover, Sicouri et al. (2010) reported that the combined administration between amiodarone and ranolazine caused post repolarization refractoriness by inhibiting sodium channel with difference in timing of gating operation. Interestingly, the increasing of APD by amiodarone does not enhance proarrhythmias. This may be due to the multichannel properties that balance ions across the cell membrane with more atrial specific than ventricle. In addition, amiodarone has use-dependent property that is the more heart rate, the more effective of the drug (Sicouri et al., 2009). These properties result from the unique structure and pharmacokinetics of amiodarone, which is beneficial for the treatment of tachyarrhythmias.

In 2002, Bicer and colleagues had conducted research in healthy beagles. The dogs were given oral amiodarone at a dose of 25 mg/kg twice a day. Assessment of cardiac function was done by echocardiography. The results showed that amiodarone had no effect on cardiac contractility, interventricular septum, and left ventricular posterior wall thickness (Bicer et al., 2002b). In addition, Zhou et al. (2012) conducted the research in dogs with congestive heart failure induced by rapid ventricular pacing. The results indicated that amiodarone had no significant changes in blood pressure when compared with congestive heart failure dogs that were not received amiodarone. However, the contractility and relaxation of the heart were not interrogated in the study.

4. Dosage of amiodarone in canine species

Based on previous research and recommendations, amiodarone has various trial dosages in dogs. Jacobs et al., (2000) suggested that dosage does not exceed 5 mg/kg twice a day, hepatic lesion was found in a higher dose. Kraus and coworkers suggested the loading dose of amiodarone administration in Doberman pinscher is 9.0-12.0 mg/kg twice a day for 7 days, and the maintenance dose is 4.3-6.3 mg/kg once a day (Kraus et al., 2009). The British Small Animal Veterinary Association (BSAVA) has guided amiodarone administration protocol in 3 phases. The loading dose is 10 mg/kg twice a day for 7 days. Next, the dog should receive 5

mg/kg twice a day for 15 days. Finally, the maintenance dose of the drug should be administrated 5 mg/kg once a day (BSAVA, 2011).

5. Effects of amiodarone on thyroid function

Since amiodarone contains iodine atom in its molecular structure (figure 2), various toxic effects of amiodarone on thyroid function have been reported (Wolff, 1969). These adverse effects were including cytotoxic effect, free radical formation, and change in metabolites of serum thyroid hormones such as decreasing conversion of T_4 to T_3, increasing conversion of T_4 to rT_3 (Chiovato et al., 1994; Di Matola et al., 2000).

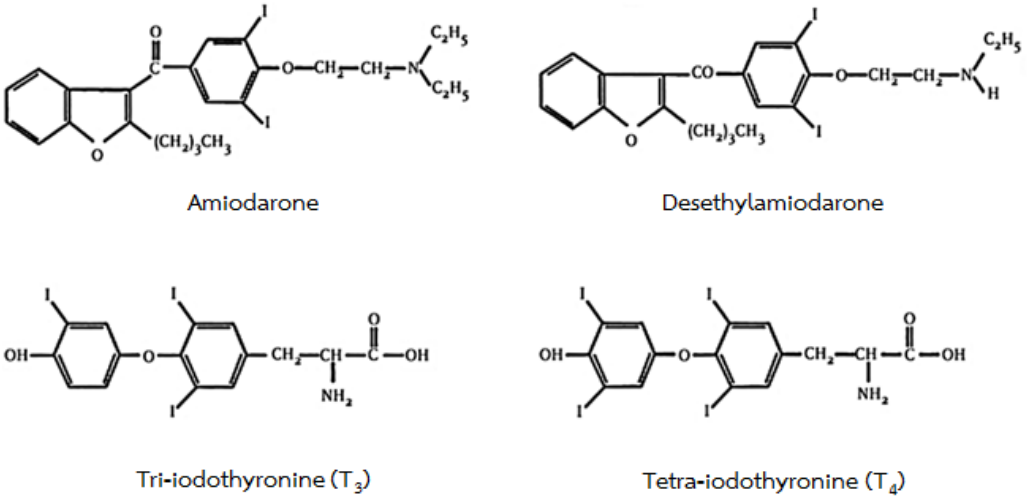

Figure 2. Molecular structure of amiodarone and thyroid hormone.

Since only T_3, but not T_4 was found in cardiomyocyte, T_3 is a major hormone in which it controls the cardiac function (Pachucki et al., 2001). Additionally, T_3 has direct effect on peripheral blood vessels (Park et al., 1997). When T_3 enters the cell membrane, T_3 may bind with the intracellular thyroid receptor and works as transcription factor that affects the gene expression (figure 3). Moreover, T_3 plays an importance role on controlling of key structure synthesis and regulatory proteins

such as SERCA, Na^{+}/K^{+}ATPase, beta-adrenergic receptor, alpha- and beta-myosin heavy chain in myocardial tissue. These genomic changings alter the structure, intracellular Ca^{2+} and K^{+} concentrations of the heart that is directly affected cardiac contractility, relaxation, and heart rate (Kahaly and Dillmann, 2005). T_3 also affects vascular and blood volumes via smooth muscle and renin-angiotensin-aldosterone system, respectively. The net effects showed as an increasing of cardiac output (Resnick and Laragh, 1982).

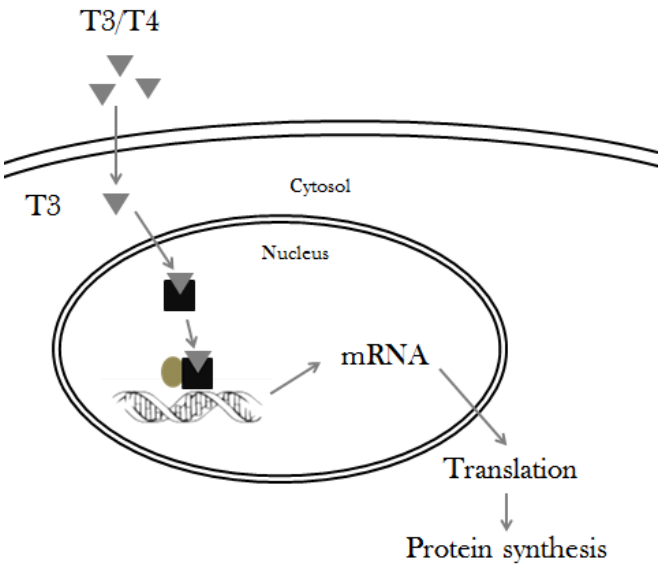

Figure 3. A diagram of T_3 and its mechanism of action on thyroid hormone receptor (Modified from Klein and Danzi, 2007).

C. Arrhythmias

1. Supraventricular arrhythmias in dogs

Arrhythmia is a cardiac electrophysiological anomaly (Dean and Lab, 1989). Mechanism of cardiac arrhythmia has various, depending on types of arrhythmias (Lilly, 2011). In dogs, atrial fibrillation is the most commonly found. Brundel and coworkers (Brundel et al., 2005) showed that atrial fibrillation was related to the atrial size. Basically, arrhythmias degrade both cardiac function and cardiac output, reduce blood supply to organs and myocardial tissue. Furthermore, arrhythmias increase cardiac work overload via compensatory mechanism. In term of electrophysiology, arrhythmias cause electrical instability and increase the risk of sudden cardiac death (Bayes de Luna et al., 1989). Moreover, Wiffels et al. (1995) had studied inducing atrial fibrillation in goats. They showed that atrial fibrillation can induce more severe atrial fibrillation known as AF begets AF. This may be due to ion channel remodeling.

2. Grading of ventricular arrhythmias

Lown's criteria have been used for classified ventricular arrhythmias. There are 4 grades accompany with 2 subgrades of grade 3 (Lown et al., 1975). Modified Lown's criteria have been extensively used in veterinary medicine. In this modification, ventricular arrhythmias are classified according to their severity and patterns into 4 grades (figure 4). Grade 1 is the singlet type ventricular premature complex that frequency less than 30 VPCs/hour. Grade 2 is composed of ventricular bigeminy and ventricular trigeminy. Grade 3 is composed of couplet, triplet, and salvo. The Last, R on T phenomenon, ventricular tachycardia and ventricular fibrillation are classified as the grade 4 (Meurs et al., 2001).

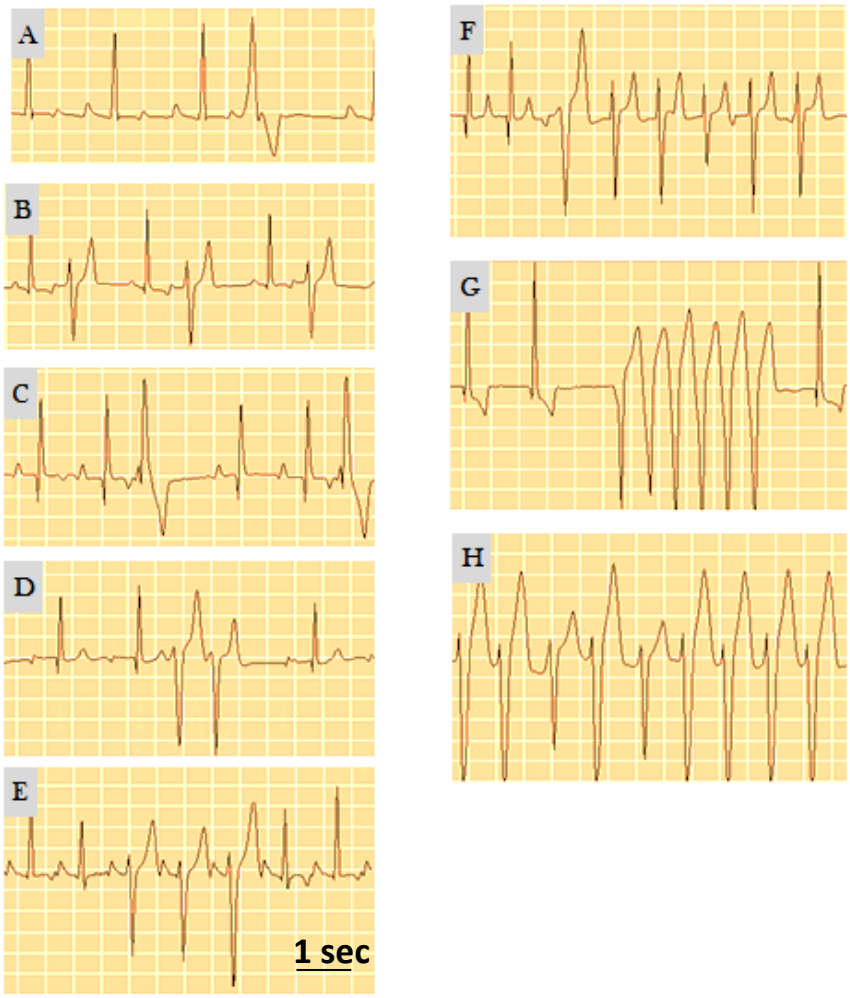

Figure 4. Schematic classification of ventricular arrhythmia. Paper speed = 25 mm/s. A) Singlet type VPCs B) Ventricular bigeminy C) Ventricular trigeminy D) Couplet E) Triplet F) Salvo G) RonT phenomenon H) Ventricular tachycardia.

3. Mechanisms of arrhythmias

Mechanisms of arrhythmia can be classified in various types. Lilly (2011) explained the mechanisms of arrhythmia can be caused by disorders of impulse generation, impulse conduction, or both of them. Abnormal impulse formation is classified into increasing or decreasing cardiac automaticity. This could be occurred in pacemaker cells, latent pacemaker cells, or a myocardial cell that is usually lacking automaticity properties. In some conditions such as cardiac ischemia

or myocardial cell membrane damaging, myocardial cells may generate impulses. Disorders of impulse conduction are described as reentry circuits and conduction blocks. Some factors are necessary to produce the reentry pathways such as unidirectional block, retrograde conduction, appropriate circuits length, and shortage of absolute refractory period (figure 5).

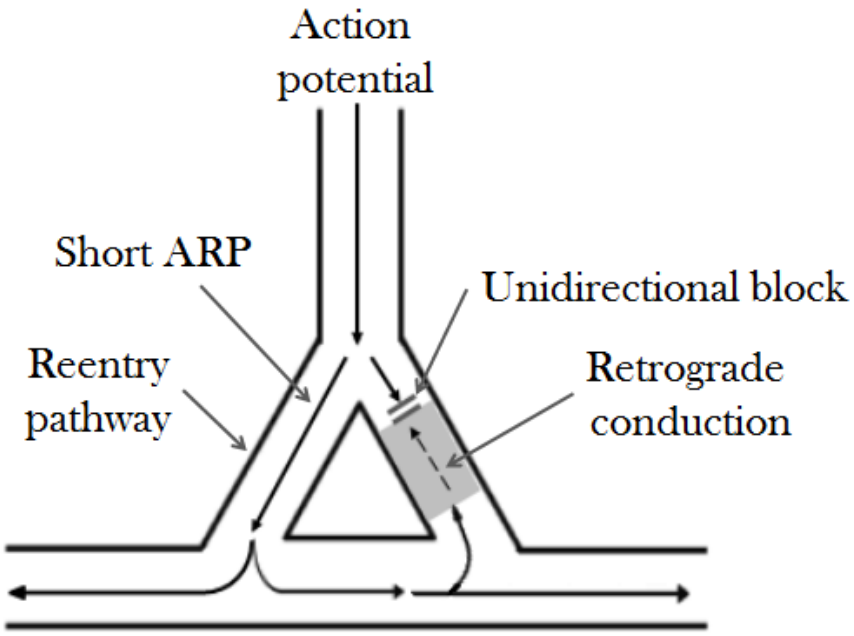

Figure 5. Reentry (Modified from Lilly, 2011).

Triggered activity is another mechanism of arrhythmias that can be classified into early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) (figure 6). EADs are the oscillations of the membrane potential during phase 2 or 3 of the action potential. Once the EADs reach the threshold potential, the abnormal electrocardiogram may present. Ca^{2+} and Na^{+} are the ions responsible for the formation of EADs. According to previous publications, EADs are the trigger of torsades de pointes arrhythmias (el-Sherif et al., 1989). On the other hands, DADs are

the oscillations of the membrane potential during phase 4 of the action potential. DADs are usually caused by an intracellular diastolic Ca^{2+} overload.

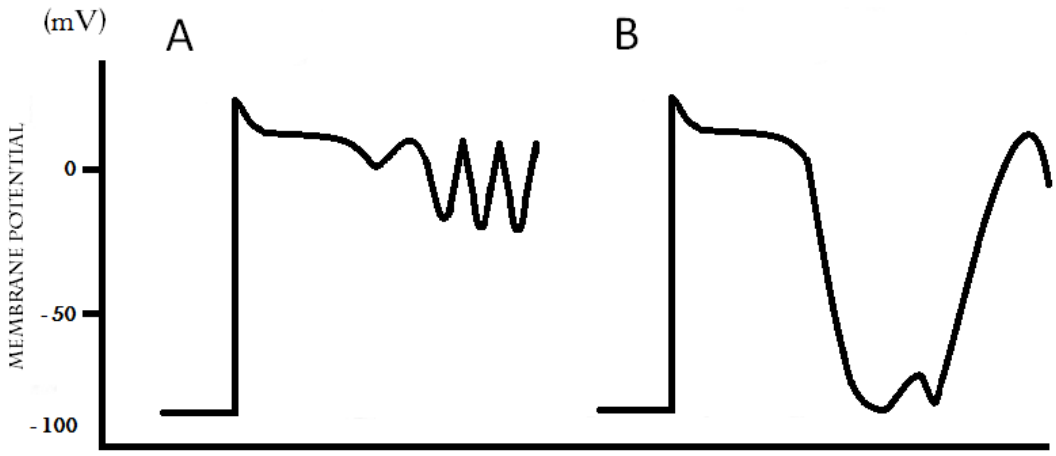

Figure 6. Triggered activity A) Early afterdepolarization (EADs) B) Delayed afterdepolarization (DADs) (Modified from Lilly, 2011).

D. Dilated cardiomyopathy

1. DCM, heartworm, failing heart and arrhythmias

DCM is one of the acquired heart diseases which cause by myocardial anomaly that showed heart enlargement and impaired cardiac functions. The disease increase sympathetic activity, myocardial wall stress, leads to alter gene expression which changes components of intracellular structures, sarcomeric proteins and contractile elements. The results of the gene alteration showed remodeling both structure and electrophysiology of cardiac tissue. Moreover, alteration of sarcomeric proteins caused loss integrity and elasticity of the heart; diastolic dysfunction, which can be early detection in occult DCM (O'Sullivan et al., 2007). Dogs which affect DCM have high morbidity and mortality rates. Average survival time is about 180-240 days, depending on breeds (Petric et al., 2002). Arrhythmia can be commonly found in dog with DCM. The reasons were not obvious and may be

multi-factors and breed predisposing. Importantly, DCM characterized malfunction of chronotropy, inotopy and lusitropy which more deteriorate cardiac output when complicated by arrhythmias.

Reviews of arrhythmogenicity causing by genetic mutation in the molecular level of humans showed left ventricular structure remodelling by gene alteration of myosin heavy chain (Antzelevitch, 2003). Moreover, Wu et al. (2002) showed changing of the titin isoform in rapid pacing- induced failing hearts resulting in increasing LV stiffness. In term of electrophysiological remodeling, the recent studies showed down regulation of connexion-43 (Cx43), hypophosphorelation of Cx43 together with L-type calcium channel; LV gap junction remodelling, LV repolarization abnormalily and E-C coupling proteins malfunction which these anomalies can be responsible for arrhythmogenesis in the failing heart (Ebinger et al., 2005; Jin et al., 2008).

Various canine DCM models can be found in a field of research. Mainly, the Doberman breed, rapid heart pacing and coronary microembolism models are well known and accepted.

Doberman breed model

Doberman is the most common breeds for DCM which Meurs and colleagues (2007) suggested as a familial disease inherited as autosomal dominant trait, however, gene that caused DCM still obscured in familial DCM. Doberman with DCM had high sympathetic tone causing high concentrations of norepinephrine and epinephrine in blood stream (Sisson, 2004).

Rapid pacing model

Wu et al. (2002) induced heart failure by four weeks of rapid ventricular pacing in a canine model. The results of rapid pacing caused alteration of titin isoform expression causing left ventricular more stiffness and lead to diastolic heart failure. Moreover, Akar et al. (2004) reported reduction of Cx43 expression in canine failing heart model and hypophosphorylation activity on Cx43 which may cause slow conduction via remodelled gap junction and lead to arrhythmias. Pathophysiology of this model is similar in human DCM, however, not actual nature in the dog.

Microembolism

Lavine et al. (1991) had performed experimental myocardial dysfunction in canine species which induced by injected microembolism into the left coronary vessel. Chronic coronary embolism-induced myocardial ischemia had showed left ventricular enlargement, systolic and diastolic dysfunction with restrictive filling pattern.

2. Diastolic dysfunction

Cardiac relaxation is one of the main parts of cardiac function. Diastolic phases divided into 2 continuous phases. After maximal ejection phase of systole, the heart starts to reuptake Ca^{2+} back into sarcoplasmic reticulum (SR) and mitochondria but mainly into SR by Sarcoplasmic reticulum calcium ATPase (SERCA) in which phospholamban controls the reuptake process (Bers, 2000). When the intracellular calcium concentration was reduced, cross-bridge cycles have released and left ventricular pressure starts to fall; however, slow LV ejection is still continuously flowing by momentum of the blood. Next, LV pressure continually decreases until the LV pressure is lower than the aortic pressure, the aortic valve is closed and the aortic flow is stopped. The LV pressure has continually reducing until the pressure is lower than the left atrium pressure so that the mitral inflow begins. When the passive filling has completed, atrial contraction pumps approximately 20 to 30 percentage of blood into the left ventricle in which it depends on ventricular compliance.

When the heart develops diastolic disorder, the ability of relaxation is malfunction and causes anomaly left ventricular filling pattern demonstrating both velocity and time. Diastolic dysfunction is categorized into 3 grades, impaired relaxation, pseudonormal, and restrictive filling.

In the first grade, impaired relaxation (figure 7B), cardiac chamber is more difficult to relax than normal process. Consequently, the rate of ventricular relaxation is delayed. The decreasing of left ventricular pressure is slower than

normal process resulting in lengthening of passive filling time and isovolumic relaxation time (Nagueh et al., 2009).

The second grade, pseudonormal (figure 7C), the heart has adjusted to the change and the remodeling of the heart occurs. This process results in an increasing of LV compliance and left atrial remodeling. The LA remodeling causes LA enlargement and increases LA filling pressure. The results of remodeling are increased IVRT or minimally decreased, and increased E/A ratio. The passive filling time is shortening than the first grade.

Last, the third grade, severe, restrictive filling (figure 7D), the IVRT is severely reduced because the LV chamber has loosed its compliance. The process of the mitral valve opening is faster than normal process. The Doppler flow patterns show very high E peak and very low A peak, and passive filling time is obviously deteriorating.

Diastolic dysfunction in DCM dogs has been reported in previous studies, In Doberman which can be found DCM more than 50 percent, pseudonormal and restrictive filling transmitral flow patterns were reported in occult and overt canine DCM, respectively. However, IVRT between these groups was insignificantly different (O'Sullivan et al., 2007). Rapid heart pacing in canine species model was reported by Neumann et al. (1999) which described by malformation arrangement of collagen fiber in heart tissues. Coronary microembolism in dogs has distinquishly reported. In this model, Gill and colleagues (2006) were reported increasing of left ventricular stiffness, prolonged IVRT, and increased collagen fiber types I and III.

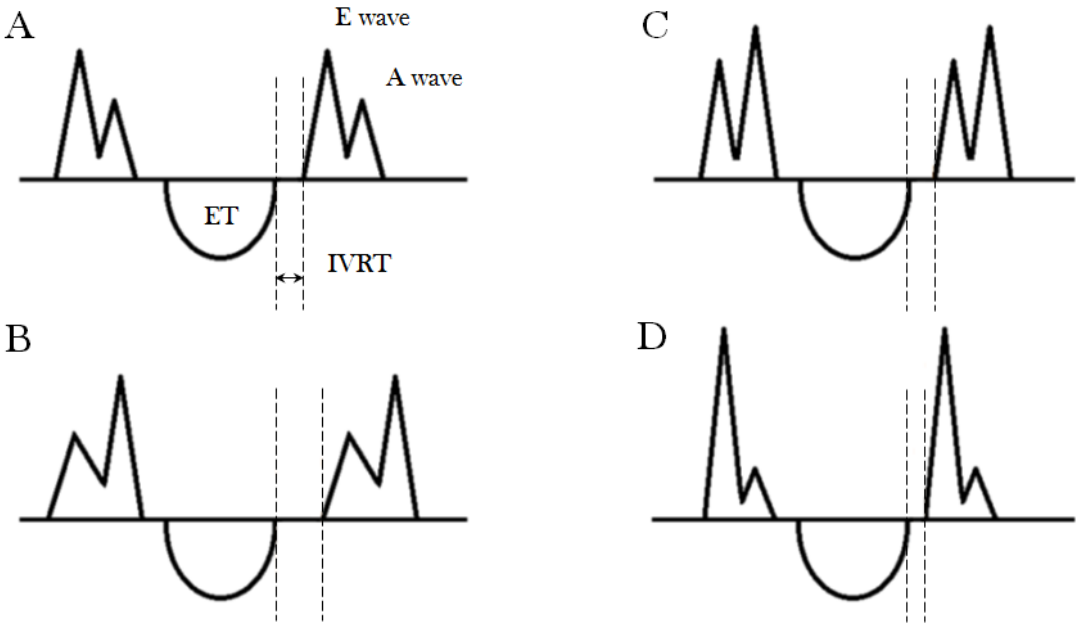

Figure 7. Doppler schematic patterns for assess diastolic function which obtain from Doppler mode echocardiogram, the left parasternal long axis 5-chamber view. The Isovolumic relaxation period starts after the end of aortic flow continuously until passive filling has begun. E wave = passive filling; A wave = atrial contraction; IVRT = isovolumic relaxation time; ET = ejection time. A) Normal pattern B) Impair relaxation C) Pseudonormal pattern D) Restrictive pattern, between dash line is IVRT (Modified from Boon, 2011).

E. Ultrasound techonology and Tei index

1. Ultrasound technology

Ultrasound machine is very useful medical diagnostic equipment which is effective in early detection of anomaly. However, computer and software are limitation of ultrasound technology. Moreover, dogs have a higher heart rate than humans, therefore, the time interval in each phase of the cardiac cycle in dogs is shorter than humans. For this reason, display resolution should have high resolution for accurate measurement, and the computer must have a powerful processor to obtain adequate sampling frequency. At present, computer, software and technology were greatly developed compared to the previous days and unlocked information that old technology did not discovered (O'Brien and Holmes, 2007). Ultrasound is a non-invasive technique which is very useful in veterinary medicine. It can be used to obtain organ structures, blood flow and functions. This non-invasive technique is not required general anesthetic procedure, better penetration into the body and less animal preparation procedure which is advantage for weak animals and practical in clinical use.

2. Tei index

Tei index or myocardial performance index is a global heart assessment echocardiographic parameter coming from the concept that the heart has two phases of functions, not only systole. Researchers were proved that Tei index was reliable parameter and has minor effects on heart rate, preload, afterload and other factors in different models (Tei et al., 1995; Sousa et al., 2007; Teshima et al., 2007; Sousa et al., 2014), however, the results still require to elucidate. Lavine reported that preload and afterload play role on Tei index in diseased hearts (Lavine, 2005a; Lavine, 2005b). Effect of contractility on Tei index was uncleared. Lavine (2006) found that positive inotropic agents increased left ventricular filling time. However, these effect might influenced by preload.

CHAPTER III
MATERIALS AND METHODS

A. Animals and criteria

This study was performed in clinical cases at the Cardiology clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The study was approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Science, Chulalongkorn University (Protocol number 13310009). Eight dogs with arrhythmic problems originated from organic heart diseases were recruited. The inclusion criteria were cardiac murmur, abnormal cardiac structure from chest radiography, presented with clinical signs of heart diseases, and cardiac arrhythmias assessed by electrocardiograms. Functional evaluation of cardiac health (FETCH) score was used to evaluated clinical conditon followed questionaries by Freeman et al. (2005). Furthermore, all dogs had neither clinical signs of acute heart failure, such as dyspnea at rest, severe pulmonary edema, low blood pressure (< 60 mmHg), and ascites nor received drugs that effect on the cytochrome P450 3A4 enzyme. However, they might receive the drugs that use for controlling heart failure, which were included furosemide (< 4 mg/kg per day), enalapril (< 1 mg/kg per day), and pimobendan (< 0.4 mg/kg per day). These three drugs might use as a single drug or in combination. Dogs were excluded if their treatment cannot be followed up.

B. Treatment and experimental procedures

After physical examination and body condition score assessment (0-5), animals enrolled in the study were subjected to measure blood pressure, electrocardiography, thoracic radiography, echocardiography, Holter monitoring, and blood collection for complete blood count and serum chemistry profiles. After completion of the evaluation, amiodarone was given to each dog at a loading dose of 10 mg/kg, oral, daily for 7 days. After that, a maintenance dose was given at 5

mg/kg, SID (figure 8). The dose was selected according to previous publication in dogs (Kraus et al., 2009). All parameters were re-evaluated after 15 days after receiving amiodarone. In addition, blood collection for measurements of thyroid hormone assay (tT_3 and tT_4) was also obtained at 60 days after receiving amiodarone.

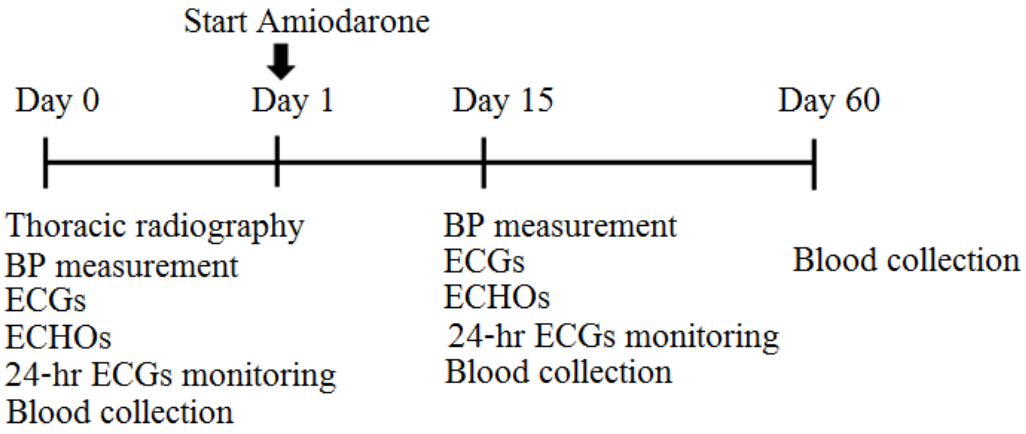

Figure 8. Treatment and experimental procedure in arrhythmic dogs.

C. Methods

1. Blood pressure measurement

Indirect blood pressure measurement was obtained by Doppler technique. The animal was restrained on the lateral recumbency position and the left plantar was shaved. Blood pressure measurement was performed during the dog had calm down. Cuff size was chosen 30 to 40 percentage of the limb circumference (Chalifoux et al., 1985). An arterial marker of the cuff was placed at the ventral area of the limb (metatarsal artery) and laid at the same plane of the heart (Haberman et al., 2006). Doppler transducer was placed on the plantar area above the median artery (figure 9). Ultrasonic gel was applied for sound conduction and then inflated cuff with 200 mmHg pressure. The systolic pressure was obtained from the first pulse sound returning after reduced pressure inside the cuff. Measurement was repeated for 5 times, but the 1st value was discarded. Three

values were averaged (Acierno and Labato, 2005). In case of animal movement or large errors of value, the re-measurement was performed.

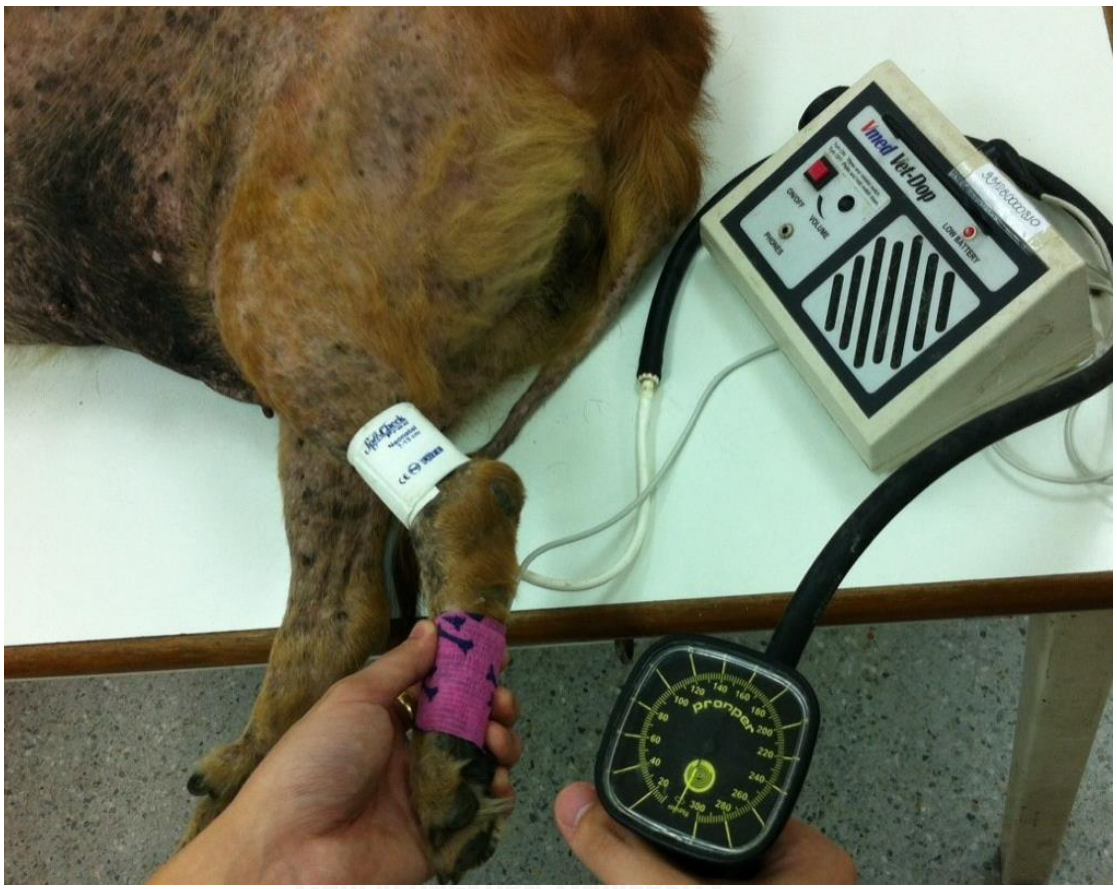

Figure 9. Example of indirect blood pressure measurement from dog No.7 when the dog was restrained on the examination table.

2. Electrocardiogram (ECGs)

Measurement of surface electrocardiogram was obtained from an ECGs machine. Dogs were restrained in right lateral recumbency position, forelimbs and hind limbs were pendicular to the long axis of the body (figure 10). Electrode clips were attached on the skin area under elbow or stifle. Six leads ECGs (lead I, II, III, aVR, aVL, and aVF) were obtained at least 30 seconds. These ECG tracings were used only for defining the types of arrhythmias for example the atrial flutter, atrial fibrillation, supraventricular premature complex (SPCs), ventricular premature complex (VPCs), and ventricular tachycardia (VT).

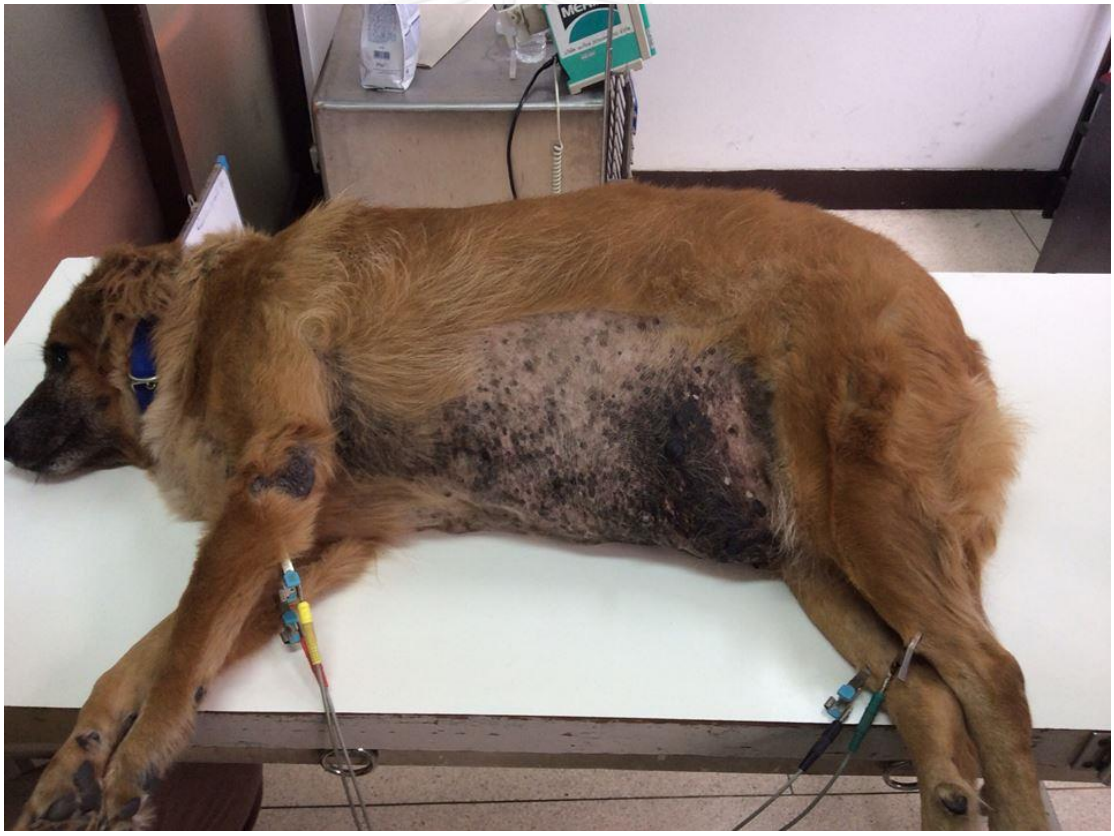

Figure 10. An example of surface electrocardiogram measurement from dog No.7 when the dog was restrained on the examination table.

3. Thoracic radiography

Assessment of the lungs and the heart were performed by thoracic radiography in both lateral and dorso-ventral views. Vertebral heart size (VHS) was calculated by sum of long axis and short axis of the heart in term of thoracic vertebrae starting at the edge of the 4th thoracic vertebra (figure 11). The long axis was measured from the main stem of bronchi to the left ventricular apex. The length of short axis was started at the lower edge of the caudal vena cava perpendicular to the long axis (Buchanan and Bucheler, 1995).

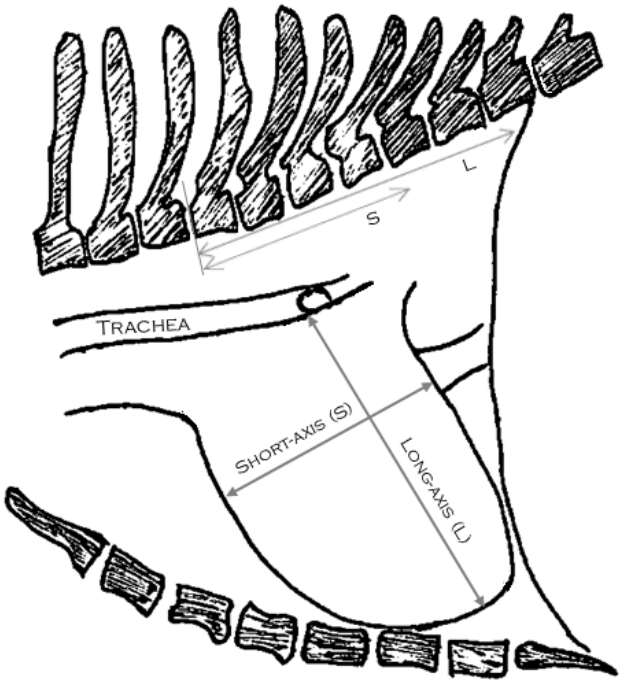

Figure 11. Example of vertebral heart size measurement (Modified from Buchanan and Bucheler, 1995).

4. Holter monitoring procedure and analysis

To obtain 24-hr Holter recording, attachment of the ECG electrode locations was modified from Petrie (2005). Before placement of the electrodes, the hair of the dogs over the chest was clipped and shaped. Skin was cleaned with rubbing alcohol. Seven electrodes were attached; left side at the area of apex of the heart; right side at the area of the heart base, 3 electrodes per side (figure 11). The ground electrode was attached at the right side near the ridge of the last rib. The ECG cables were connected and secured with adhesive tape. Tree channels ambulatory ECG (FM-180) was located on the dorsal area between the scapulae. The Holter unit was placed inside the plastic cage secured with adhesive tape. Then, the equipments were covered with the coverall. A new battery cells 1.5 volts (AAA) were used each time. Data were stored in the computer. The analytical process was performed by Holter analysis program (SCM-510w software, FUKUDA DENSHI, Co. Ltd., Japan).

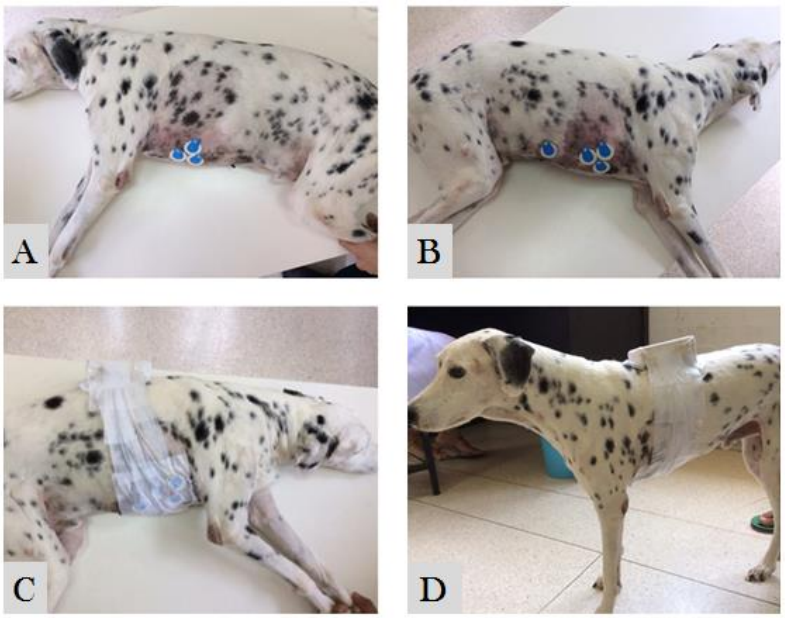

Figure 12. Example of Holter's unit installation from dog No.8. A) Position of electrodes attachment on left side B) Position of electrodes attachment on right side C) Adhesive tape attachment after applying all electrodes D) Complete installation of Holter's unit.

Holter parameters were including heart rate and time durations. Moreover, the time between the peak to the end of T wave (TpTe) (Antzelevitch et al., 1999) was measured and normalized by QT (Gupta et al., 2008) and QTc (Kilicaslan et al., 2012). QT interval was defined as the time from the beginning of the Q wave to the end of the T wave (Antzelevitch, 2001). The end of repolarization was measured by considering end of T waves all 3 channels of ECG from Holter tracings and selected the point obviously seen. In case of unidentification end of T wave, the tangential approach of the maximal amplitude of T wave to intersection point of baseline was used (figure 12). The baseline was determined as the same line of PR interval line at the most stable isoelectricity. If the line from PR interval is not stable, the other lines that show the most stable electric event was selected. In cases of biphasic T wave, peak of T wave was selected as the second peak and the measurement was performed to end of T wave. QTc was calculated according to Van de Water's method as shown in the equation 1 (Van de Water et al., 1989).

Short term of beat-to-beat repolarization (STV) was calculated from 31 consecutive normal beats of QT intervals before VPCs occured. The STV refers to mean of distance from the points of the Poincaré plots to identity line. This parameter was proposed a variation of ventricular repolarization in short period and can be used to predict risk of arrhythmia and proarrhythmia (Thomsen et al., 2004; Oosterhoff et al., 2010). The calculated equation was demonstrated in the equation 5.

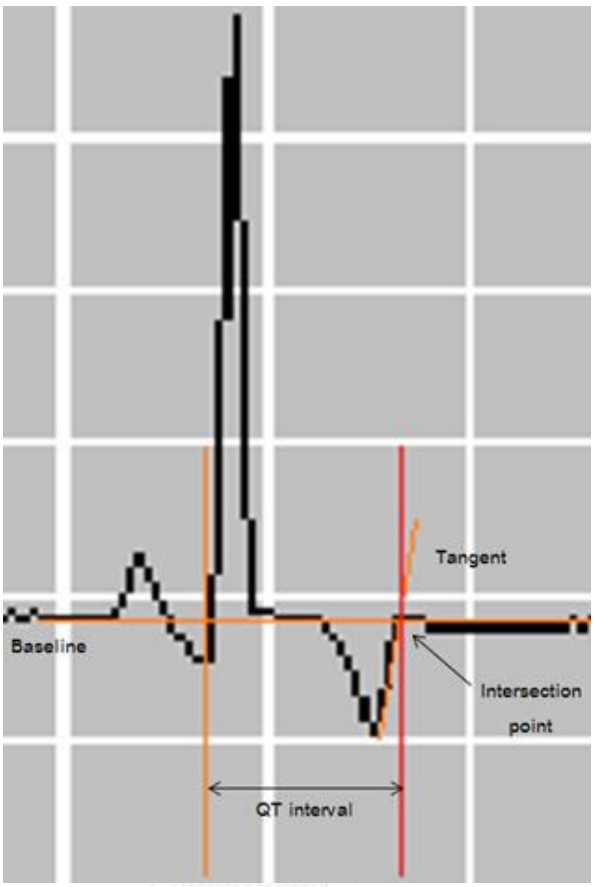

Figure 13. ECG from Holter's monitoring and QT measurement method.

Equation 1. Correction of QT interval by Van de Water formula (Van de Water et al., 1989).

$$QTc\ (VdW) = QT - 0.087(\frac{60}{HR} - 1)$$

When

QTc (VdW) = QT interval with Van de Water formula correction (ms)

QT = QT interval (ms)

HR = Heart rate (bpm)

Equation 2. Transmural dispersion of repolarization (Antzelevitch, 2001).

$$TDR = |Tp - Te|$$

When

TDR = Transmural dispersion of repolarization (ms)

Tp = Druation of start QRS complex until peak of T wave (ms)

Te = Duration of start QRS complex until end of T wawe (ms)

Equation 3. Ratio of TpTe and QT intervals (Gupta et al., 2008)

$$Ratio\ of\ TpTe\ and\ QT = \frac{|Tp - Te|}{QT}$$

When

Tp = Duration of start QRS complex until peak of T wave (ms)

Te = Duration of start QRS complex until end of T wawe (ms)

QT = QT interval (ms)

Equation 4. Ratio of TpTe and QTc intervals (Kilicaslan et al., 2012).

$$Ratio\ of\ TpTe\ and\ QTc = \frac{|Tp - Te|}{QTc}$$

When

Tp = Druation of start QRS complex until peak of T wave (ms)

Te = Duration of start QRS complex until end of T wawe (ms)

QTc = QT interval with Van de Water formula correction (ms)

Equation 5. Short term beat-to-beat variation of repolarization (Thomsen et al., 2004).

$$STV = \sum_{n=1}^{n=30} \frac{|QT(n+1) - QTn|}{30x\sqrt{2}}$$

When

STV = Short term variability of repolarization (ms)

QT_{n} = QT interval of any beat (ms)

$QT_{(n+1)}$ = QT interval of n+1 beat (ms)

5. Echocardiography

Cardiac functions were assessed by transthoracic echocardiography (EKO 7, SAMSUNG MEDISON, Korea) which all dogs were in conscious stage during the examination. Parameters were categorized into 4 groups for assessments of systolic function, diastolic function, cardiac geometry, and overall performance (Boon, 2011). Each echocardiographic parameter was measured from 5 consecutive beats. All dogs were evaluated from two echocardiographic positions, right parasternal and left parasternal positions.

Echocardiographic Parameters

5.1. Assessment of systolic function

5.1.1 Fractional shortening (%)

Fractional shortening (%) demonstrates the changing in percentage of left ventricular internal dimension during complete diastole and systole phases. The parameter was obtained from M-mode echocardiogram on right parasternal recumben position in long-axis view, and the M-mode cursor was placed cross over left ventricle between papillary muscle and the tip of mitral valve leaflets over chordae tendinae (figure 13). The equation was calculated as below.

Equation 6. Calculation of fractional shortening (Boon, 2011).

$$FS(\%) = \frac{LVIDd - LVIDs}{LVIDd} x100$$

When

FS (%) = Fractional Shortening (%)

LVIDd = Left Ventricular Internal Dimension at end-diastole (cm)

LVIDs = Left Ventricular Internal Dimension at end-systole (cm)

5.1.2 Ejection fraction (%) is the percentage change in left ventricular volume during systole and diastole. To archive the parameter, methods were performed similar as FS (%). However, Teicholz formula was used to calculate left ventricular volume based on the assumption that the left ventricular chamber in dog is elliptical shape. The Teicholz equation was showed below (Teichholz et al., 1976).

Equation 7. Calculation of ejection fraction from Teicholz formula (Teichholz et al., 1976).

$$LVEDV = \frac{7x(LVIDd)^3}{2.4 + LVIDd}$$

$$LVESV = \frac{7x(LVIDs)^3}{2.4 + LVIDs}$$

$$EF(\%) = \frac{LVEDV - LVESV}{LVEDV} x100$$

When

EF (%) = Ejection Fraction (%)

LVEDV = Left Ventricular Volume at end-diastole (cm)

LVESV = Left Ventricular Volume at end-systole (cm)

5.1.3 Pre-ejection period/Ejection time (PEP/ET) ratio

To obtain these parameters, echocardiogram was performed in the right parasternal long-axis 5-chamber view. The aortic valve should be seen obviously at the center of the aorta during diastole. Line of the M - mode cursor was placed through both aortic annulus and cut through the left atrium chamber at the center which separating the left atrium into 2 parts symmetrically (figure 14). (PEP/ET) ratio was calculated via parameters below.

Pre-ejection period (PEP) is the period of time since left ventricle has been electrical stimulated until the aortic valve was opened. This timing can be measured by simultaneously performed echocardiogram with electrocardiogram recording.

Left ventricular ejection time (LVET) is the time from the beginning of aortic flow until the end of the flow. The ET was measured from duration between opening of the aortic valve to closing of the aortic valve in M-mode view.

5.1.4 Isovolumetric contraction time (IVCT)

IVCT is the duration when the LV pressure is rapidly building up without changing in the LV volume. The end point of IVCT is occurred when the left ventricular pressure overcomes the aortic pressure. Images were taken from the left parasternal apical 5-chamber view by placing the pulse wave gate between the left ventricular outflow tract and the mitral valve (figure 15).

5.2 Assessment of diastolic function

5.2.2 Isovolumic relaxation time (IVRT)

IVRT is the duration between the end of aortic flow until starting of the inflow period. IVRT was measured from the end of aortic outflow to the beginning of the mitral inflow. The echocardiographic image was taken from left caudal parasternal 5-chamber view by placing the pulse wave gate between the left ventricular outflow tract and the septal leaflet of the mitral valve.

5.3 Assessment of cardiac geometry

Intra-structure of the heart was obtained from M-mode echocardiogram by right parasternal long axis 5-chamber view and M-mode cursor was placed cross over the left ventricle between papillary muscle and tip of mitral valve leaflets over the chordae tendinae. The technique to obtain image was similar to the method for obtaining FS (%). Interventricular septum, LV internal diameter, LV free

walls both systolic and diastolic phase were measured in the same measurement line. The left atrium and aortic diameter were obtained from the same view with the M-mode cursor line placing through aortic annulus and left atrium. Cursor line was perpendicular with both annulus and cut through center of the left atrium.

5.4 Overall cardiac function assessment

5.4.1 Tei index

Tei index is a whole heart assessment that used to determine both systolic and diastolic functions. Tei and co-workers calculated cardiac performance from both phases of isovolumic indices and aortic outflow period by adding isovolumic contraction time and isovolumic relaxation time, then divided by duration of aortic outflow (Tei et al., 1997). All parameters in Tei index were obtained from a Doppler mode echocardiography of the left apical 5-chamber view. Tei index is calculated as the following equation.

Equation 8. Calculation of Tei index (Tei et al., 1997).

$$IVCT + IVRT = a - ET$$

$$Tei\ index = \frac{IVCT + IVRT}{ET}$$

When

IVCT = Isovolumic Contraction Time (ms)

IVRT = Isovolumic Relaxation Time (ms)

ET= Ejection Time (ms)

a = Time between end of mitral inflow and start mitral inflow (ms)

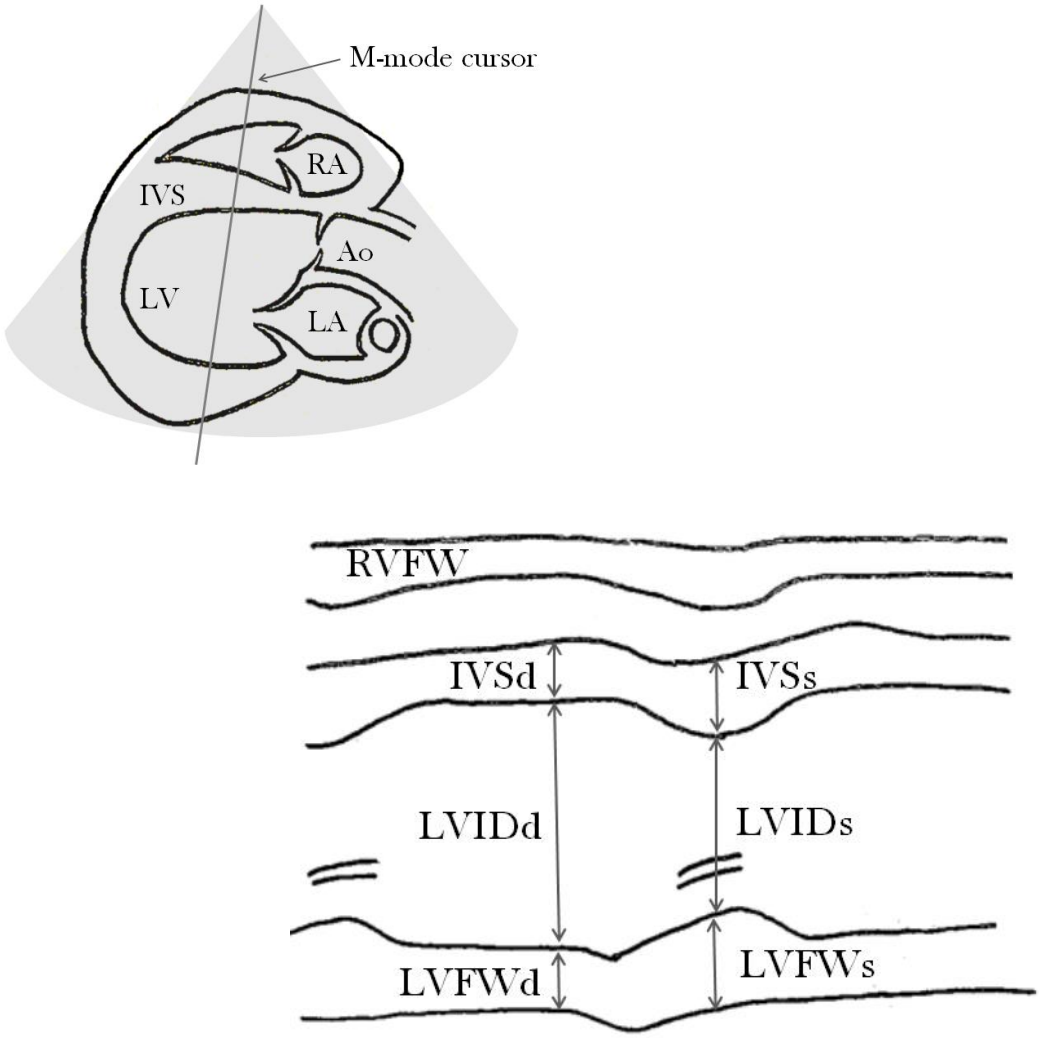

Figure 14. M-mode echocardiogram from the LV chordae tendinae level, right Parasternal 5-chamber view (Modified from Boon, 2011). RVFW = Right ventricular free wall; IVSd = Interventricular septum, diastole; IVSs = Interventricular septum, systole; LVIDd = Left ventricular internal dimension, diastole; LVIDs = Left ventricular internal dimension, systole; LVFWd = Left ventricular free wall, diastole; LVFEs = Left ventricular free wall, systole.

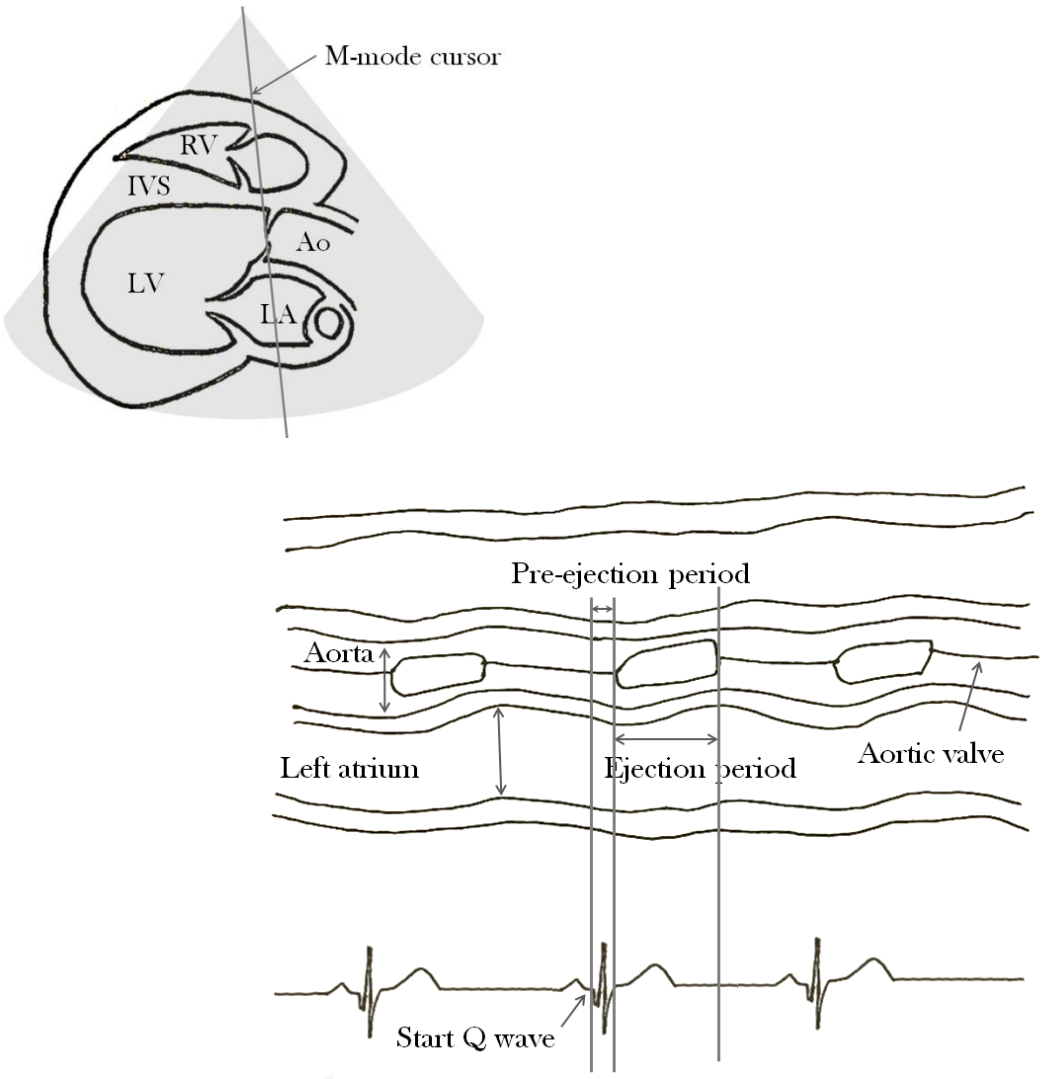

Figure 15. M-mode echocardiography from the Aorta-Left atrium level, right parasternal long-axis 5 chambers view (Modified from Boon, 2011).

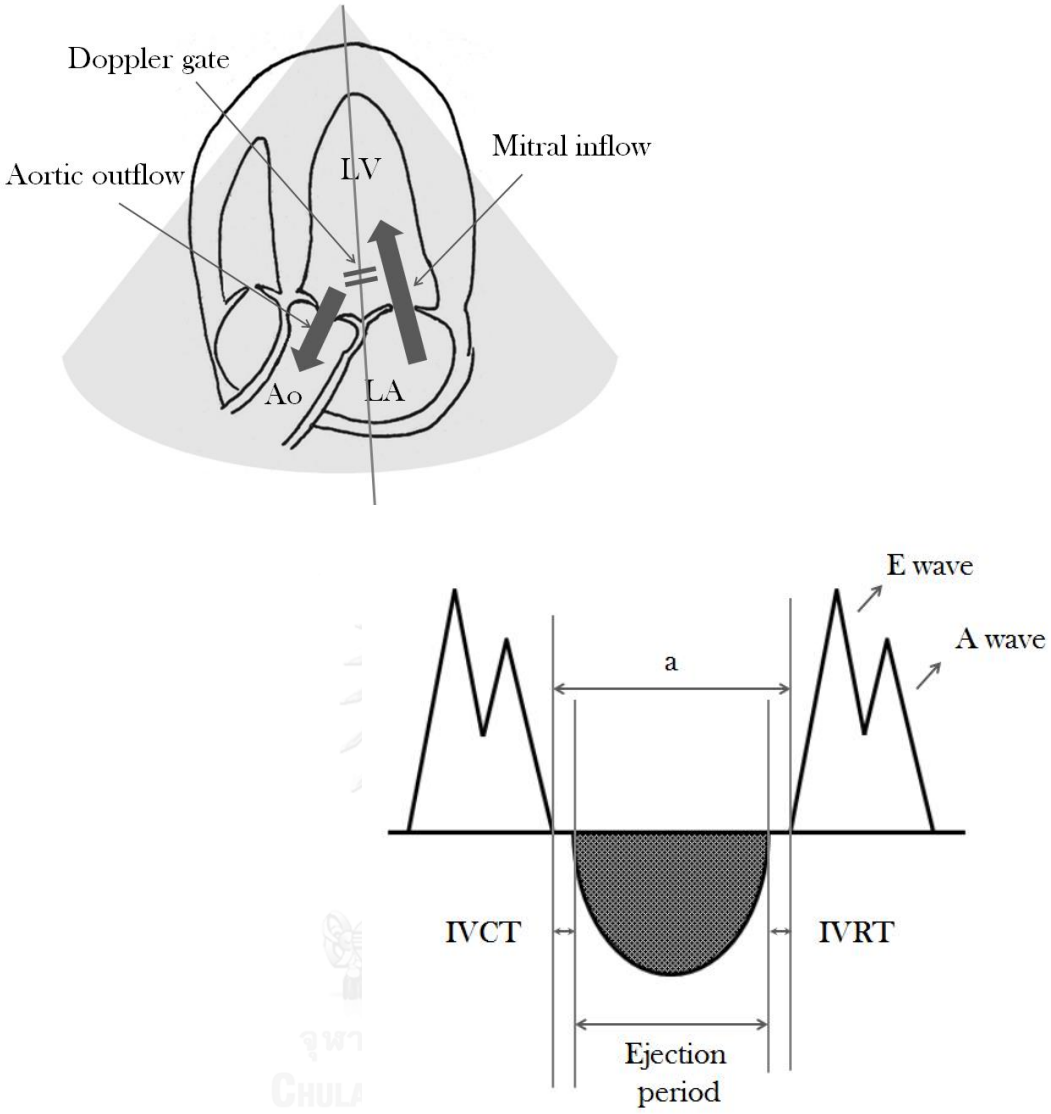

Figure 16. Left apical long-axis 5-chamber view for Doppler echocardiographic study (Modified from Boon, 2011). IVCT = Isovolumic contraction time; IVRT = Isovolumic relaxation time.

6. Blood collection and analytical procedures

Blood samples were withdrawn from the cephalic or saphenous vein by using the No.21 needle and divided into two micorcentrifuge tubes (2 ml each) containing ethylene diamine tetraacetic acid (EDTA) and heparin for determining complete blood count and serum chemistry profiles, respectively. Complete blood count was performed by using an automated hematology analyzer (The CELL-DYN 3700, Abbott Laboratory, USA). Blood chemistry profiles (alanine amiotransaminase, alkaline phosphatase, total serum protein, serum albumin, blood urea nitrogen, and creatinine) were analyzed by using an automate chemistry analyzer (The IL ILab 650 Chemistry Analyzer, Diamond diagnostic, MA, USA). Thyroid hormones, tri-iodothyronine (T_3) and tetra-iodothyronine (T_4), were anylyzed by using a chemiluminescence analyzer (The immulite one, DPC, USA).

7. Statistical analysis

Values were shown in mean ± standard error of means (SE). Statistical analysis was performed to compare the parameters between before and after amiodarone administration. Student paired-*t* test was used to demonstrate the difference between before and after treatment with normal distribution data. In case of abnormal distribution data, Wilcoxon-signed rank test was performed instead. The Pearson's correlation was done to determine the correlation in each parameter including correlation between total arrhythmic beats and cardiac contractility, total arrhythmic beats and cardiac relaxation, cardiac contractility and thyroid hormone level, and cardiac relaxation and thyroid hormone level. Spearman's rank was used if the data was not distributed normally. A statistical significance was considered when P value less than 0.05.

CHAPTER IV
RESULTS

A. General condition

The signalment of all enrolled dogs before initiating the study were shown in table 1. Eight dogs enrolled in this study consisted of four male castrated (50%), three male intract (37.5%) and one female sprayed dog (12.5%). The mean body weight was 25.0 kg, ranging from 15.5 kg to 34.0 kg. The average body condition score was 2.9 ± 0.29. The mean of age was 11.37 ± 0.26 years. Physical examinations of all eight dogs revealed murmur heart sound and pulse deficit. Systolic blood pressure was successfully measured in five of eight dogs and insignificantly changed after on amiodarone for 15 days (103 ± 6 VS 83 ± 17, p=0.48).

Both supraventricular and ventricular arrhythmias were found 3/8 (37.5%) and 5/8 (62.5%), respectively. The two of SVA dogs were atrial fibrillation and the others were supraventricular premature complex. Vertebral heart scores of all dogs were larger than normal value (9.7 ± 0.5) (Buchanan and Bucheler, 1995). All dogs were diagnosed as the dilated heart with mitral regurgitation. A dog with dilated heart was also had heartworm infestration. The average of functional evaluation of cardiac health (FETCH) score was 41.5 ± 9.9 before on amiodarone, and 30.8 ± 6.1 for after on amiodarone for 15 days.

Table 1. Signalment of enrolled dogs of the study.

Breed	BW	BCS	Sex	Age	VHS	FETCH Score	
						Pre	Post
Dalmatian	34.0	3.5/5	Mc	11	13.5	36	33
Mixed	23.5	2.0/5	M	12	12.8	62	36
English Cocker	15.5	2.5/5	M	12	14.4	41	33
Boxer	22.5	3.0/5	Mc	11	13.2	41	40
Golden R.	27.8	3.0/5	M	12	10.7	25	18
Mixed	19.9	2.0/5	Mc	12	13	46	29
Mixed	38.0	4.5/5	Fs	10	10.8	36	29
Dalmatian	28.4	3.0/5	Mc	11	11.4	40	25

BW = Body weight (kg); BCS = Body condition score; Mc = Male castrated; M = Male; Fs = Memale sprayed; AF = Atrial fibrillation; VPC = Ventricular premature complex; SVPC = Supraventricular premature complex; RonT = R wave is on the T wave; VPC = Ventricular premature complex; Hw = Heart worm disease; VHS = Vertebral heart scale; FETCH = functional evaluation of cardiac health

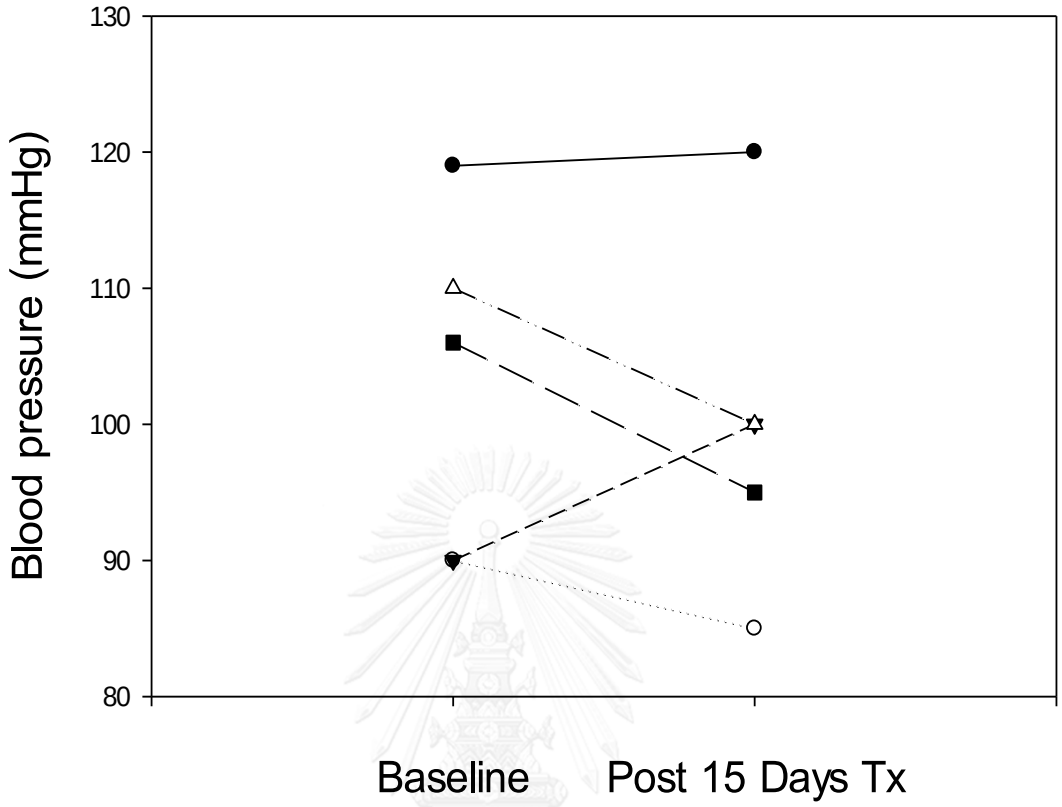

Figure 17. Blood pressure measurement by Doppler technique of five dogs.

The results of blood profile values were shown in table 2. Both complete blood count and serum chemistry profile values of all eight dogs were in normal range along the baseline and post 60 days of amiodarone treatment.

Table 2. Blood profile information at baseline and post 60 days of treatment.

	N	Pre	Post 60 days	*p* value
RBC ($x10^6$ per µL)	6	6.07 ± 0.33	5.69 ± 0.56	0.52
Hb (g/dL)	6	13.07 ± 0.88	12.05 ± 1.03	0.44
Hct (%)	6	40.83 ± 2.73	38.67 ± 2.56	0.52
Platelet ($x\ 10^3$ per µL)	6	341 ± 76	410 ± 55	0.43
WBC (per µL)	6	14,288 ± 1,462	17,647 ± 5,492	0.61
ALT (IU/L)	7	93 ± 18	119 ± 53	0.62
ALP (IU/L)	7	171 ± 37	123 ± 21	0.14
BUN (mg/dL)	6	27.15 ± 7.44	21.62 ± 3.49	0.47
Creatinine (mg/dL)	6	1.25 ± 0.20	1.23 ± 0.14	0.91

RBC = Red blood cell count; Hb = Hemoglobin; Hct = Haematocrit; WBC = White cell count; ALT = Alanine amino transferase; ALP = Alkaline phosphatase; BUN = Blood urea nitrogen.

The values were presented as means ± SEM.

B. Effect of amiodarone on Holter recording parameters

The result of 24-hour Holter monitoring was shown in table 3. The average 24-hour heart rate and total beats per day were significantly decreased after given amiodarone for 14 days (136 ± 8 VS 124 ± 8, p=0.02 and 184,137 ± 12,659 VS 168,073 ± 13,432, p=0.03, respectively). While the normal beats was not markedly changed, the total arrhythmic count per day was decreased significantly (15,482 ± 4,137 VS 8,424 ± 2,896, p=0.01).

We classified ventricular arrhythmia into V-run, V-couple, R on T type, bigeminy, trigeminy and V-single. After treated amiodarone for 15 days, the numbers of SVT were not significantly changed, however, VPCs was significantly decreased (14,663 ± 4,397 VS 8,392 ± 2,901, p=0.02). Ventricular trigeminy showed significantly altered (353 ± 125 VS 90 ± 48, p=0.04), and V-single tended to decrease in numbers (9,728 ± 3,187 VS 6,689 ± 2,612, p=0.06). (Percentage of ventricular arrhythmic types of each dog were shown in appendix tables ii, iv and v.)

The ECG from Holter monitoring was also used for measurement of ECG durations and proarrhythmic ratio (table 4). After 15 days of oral amiodarone administration, the QT, QTc, and TpTe tended to lengthen when compared to the baseline while |Tp-Te|/QT ratio, P duration, and PR intervals did not differ from the baseline. However, the QRS duration was lengthened significantly (67.41 ± 8.45 ms VS 77.50 ± 9.72 ms, p=0.005). Short term variability of repolarization (STV) did not alter by oral amiodarone (p=0.26). Sample of Poincaré plots of dog No. 6 was demonstrated in figure 18.

Table 3. Data from 24-hour ECGs recording.

	N	Pre	Post 15 days	*p* value
Max HR	8	214 ± 11	222 ± 16	0.522
Mean HR	8	136 ± 8	124 ± 8	0.016*
Min HR	8	85 ± 9	71 ± 7	0.037*
Total beats	7	184,137 ± 12,659	168,073 ± 13,432	0.030*
Normal beats	7	168,358 ± 14,823	158,712 ± 14,369	0.206
Total arrhythmic count	7	15,482 ± 4,137	8,424 ± 2,896	0.012*
Arrhythmic types				
VPCs	7	14,663 ± 4,397	8,392 ± 2,901	0.022*
SVPCs	7	484 ± 427	30 ± 18	0.313
V-run	7	105 ± 63	62 ± 32	0.422
V-couple	7	802 ± 419	279 ± 140	0.163
RonT (250 ms)	7	275 ± 112	116 ± 63	0.267
Bigeminy	7	240 ± 176	111 ± 77	0.436
Trigeminy	7	353 ± 125	90 ± 48	0.044*
V-single	7	9,728 ± 3,187	6,689 ± 2,612	0.061

HR = Heart rate; VPCs = Ventricular premature complex; SVPC = Supraventricular premature complex.

The values were presented as means ± SEM.

*$p<0.05$ when compared with pre amiodarone treatment.

Table 4. Proarrhythmic and electrophysiologic parameters of amiodarone.

	N	Pre	Post 15 days	*p* value
QT interval (ms)	8	215.32 ± 6.08	230.47 ± 8.03	0.069
QTc (VdW)	8	261.97 ± 6.00	276.77 ± 7.40	0.054
|Tp-Te|	8	36.94 ± 1.79	41.56 ± 3.02	0.085
|Tp-Te|/QT	8	0.17 ± 0.01	0.18 ± 0.01	0.285
|Tp-Te|/QTc	8	0.14 ± 0.01	0.15 ± 0.01	0.215
P duration (ms)	6	71.05 ± 21.93	63.65 ± 15.48	0.359
PR interval (ms)	6	205.69 ± 58.77	184.69 ± 55.16	0.205
QRS duration (ms)	8	67.41 ± 8.45	77.50 ± 9.72	0.005*
STV	6	5.72 ± 0.65	7.29 ± 1.34	0.257

QTc (VdW) = QT interval with Van de Water formula correction; |Tp-Te| = QT dispersion of ventricular repolarization; STV = Short-term variability of the QT interval.

The values were presented as means ± SEM.

*$p<0.05$ when compared with pre amiodarone treatment.

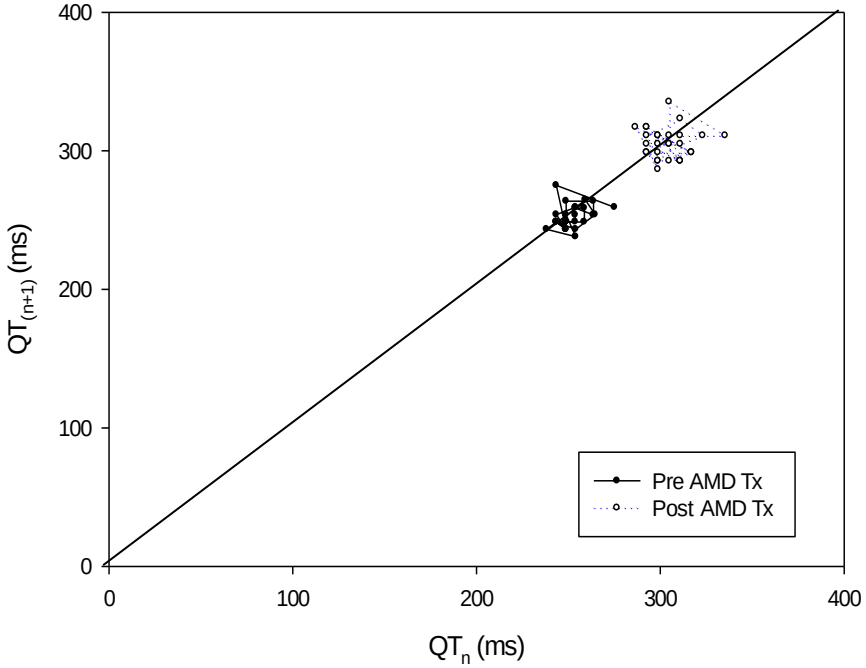

Figure 18. Poincaré plots from QT_n and $QT_{(n+1)}$ of dog No.6, before (close dot) and after amiodarone treatment (open dot). Perpendicular distance from dot plots to the diagonal line is a variation of beat-to-beat repolarization. The long distance represent high variation and high proarrhythmic risk.

C. Effect of amiodarone on echocardiographic parameters

Systolic function was assessed by echocardiograms with B-mode and M-mode. The results were shown in table 5. After oral amiodarone for 15 days, PEP was significantly increased (58.17 ± 3.64 VS 73.43 ± 5.44, p=0.02) when compared to baseline. In addition, IVCT was lengthened significantly after received amiodarone for 15 days (27.67 ± 2.79 VS 42.41 ± 5.86, p=0.04). Surprisingly, the stroke volume was significantly increased after treatment (69.72 ± 9.24 VS 88.81 ± 14.88, p=0.04). While there was no change in ET, the PEP/ET ratio and EF (%), FS (%) tended to increase when compared with the baseline.

The diastolic function was assessed spectral flows by the Doppler method. The isovolumetric relaxation time (IVRT) did not alter by oral amiodarone for 15 days. Tei index tended to increase after amiodarone treatment (0.28 ± 0.02 VS 0.36 ± 0.04, p= 0.07).

All atrial and ventricular geometric parameters were not significantly changed after received amiodarone (table 6).

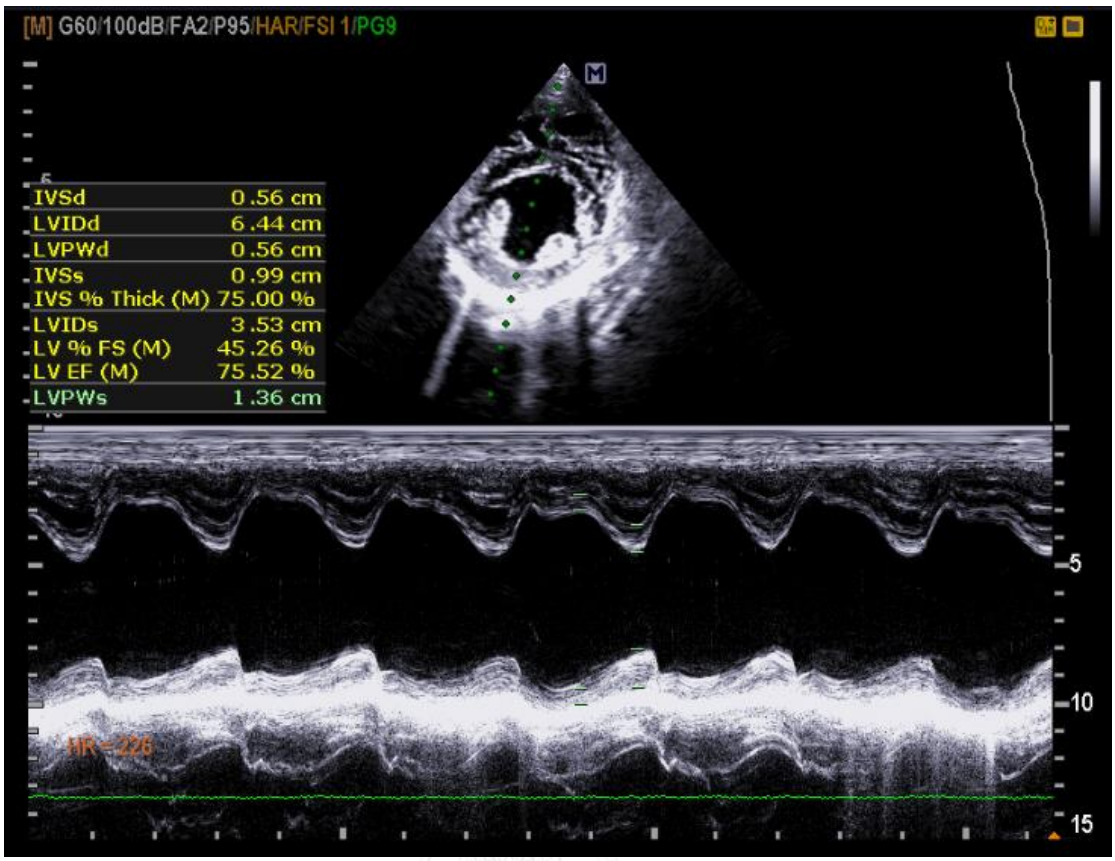

Figure 19. Echocaridography M-mode, right parasternal short-axis view, chordae tendinae level.

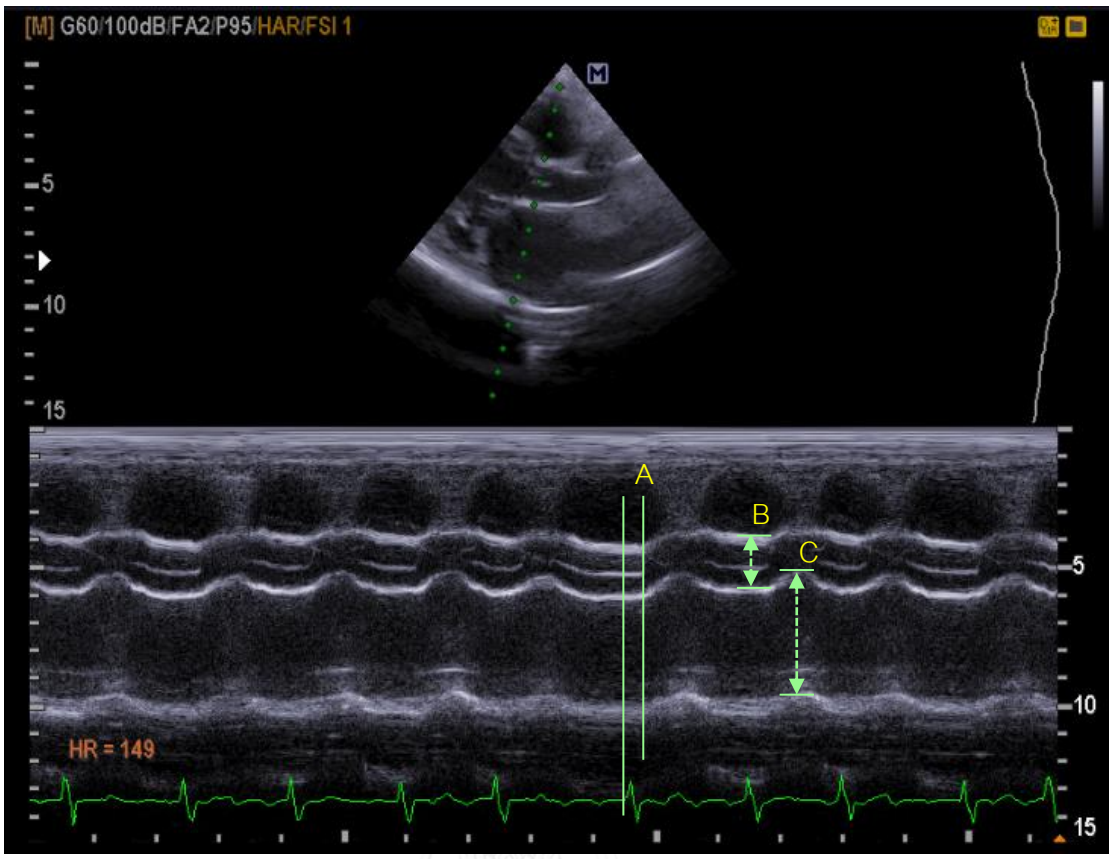

Figure 20. Echocaridography of the right parasternal long axis, 5-chamber view, A) Pre-ejection period B) Aortic diameter C) Left atrium diameter.

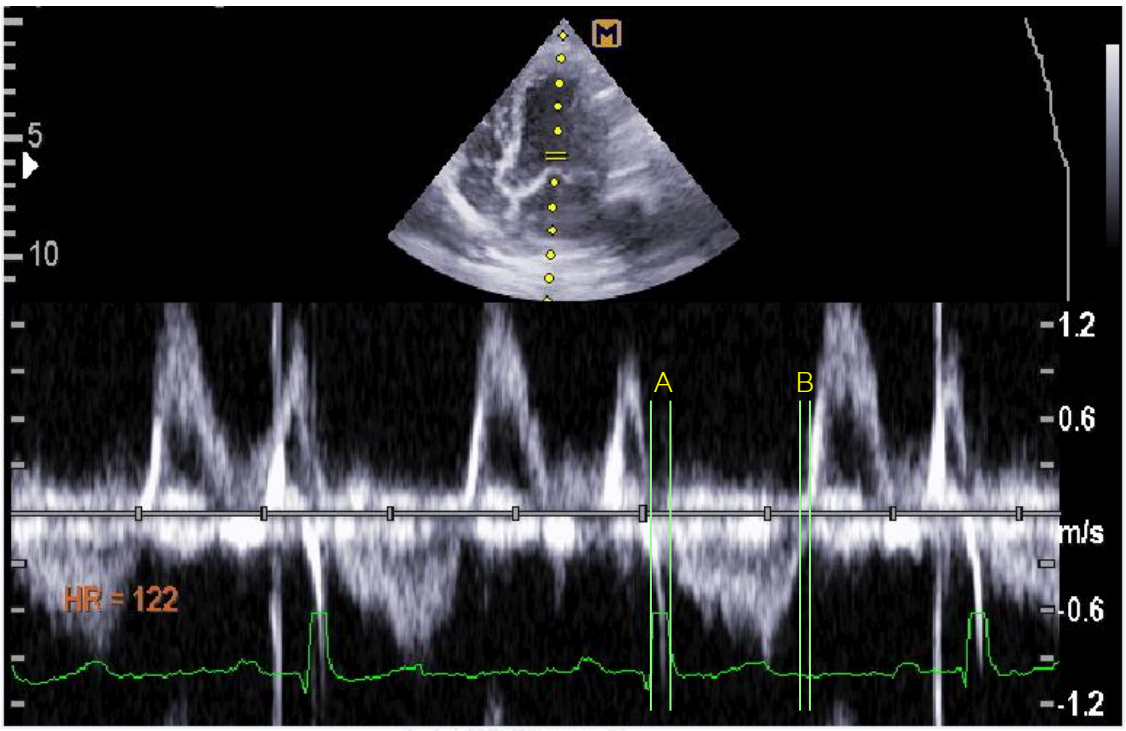

Figure 21. A Doppler mode echocardiogram, left parasternal long axis, 5-chambers view, the trans mitral flow is upward while the aortic flow is downward A) Isovolumic contraction time B) Isovolumic relaxation time.

Table 5. Results of echocardiograms which represented cardiac performance.

	N	Pre	Post 15 days	*p* value
Systolic function				
PEP (ms)	7	58.17 ± 3.64	73.43 ± 5.44	0.021*
ET (ms)	8	206.86 ± 22.14	185.82 ± 16.80	0.212
PEP/ET ratio	7	0.31 ± 0.03	0.44 ± 0.05	0.053
IVCT (ms)	8	27.67 ± 2.79	42.41 ± 5.86	0.042*
FS (%)	8	33.64 ± 2.80	37.72 ± 2.37	0.061
EF (%)	8	61.57 ± 3.78	67.03 ± 3.09	0.063
Diastolic function				
IVRT (ms)	8	25.59 ± 3.26	29.02 ± 2.78	0.409
Overall performance				
Tei index	8	0.28 ± 0.02	0.36 ± 0.04	0.071

PEP = Pre-ejetion period; ET = Ejection time; IVCT = Isovolumic contraction time; FS (%) = Fractional shortening (%); EF (%) = Ejection fraction (%); IVRT = Isovolumic relaxation time.

The values were presented as means ± SEM.

* $p<0.05$ when compared between pre and post amiodarone treatment.

Table 6. Results of echocardiograms which represented cardiac geometry.

	N	Pre	Post 15 days	*p* value
IVSd (cm)	8	0.84 ± 0.06	0.87 ± 0.70	0.732
LVIDd (cm)	8	4.85 ± 0.25	5.14 ± 0.39	0.176
LVPWd (cm)	8	0.81 ± 0.05	0.80 ± 0.05	0.862
IVSs (cm)	8	1.06 ± 0.11	1.31 ± 0.10	0.084
LVIDs (cm)	8	3.22 ± 0.21	3.20 ± 0.27	0.458
LVPWs (cm)	8	1.12 ± 0.05	1.19 ± 0.11	0.468
EDV (ml)	8	113.32 ± 13.59	133.07 ± 22.80	0.130
ESV (ml)	8	43.60 ± 6.32	44.26 ± 9.64	0.905
SV (ml)	8	69.72 ± 9.24	88.81 ± 14.88	0.040*
Ao (cm)	8	1.97 ± 0.13	1.97 ± 0.12	0.969
LA (cm)	8	3.48 ± 0.29	3.75 ± 0.38	0.355
La/Ao ratio	8	1.89 ± 0.25	1.99 ± 0.31	0.736

IVSd, IVDs = Interventricular septum during diastole and systole phases, respectively; LVIDd, LVIDs = Left ventricular internal dimension of diastole and systole phases, respectively; LVPWd, LVPWs = Left ventricular posterior wall during diastole and systole phases, respectively; EDV = End diastolic volume; ESV = End systolic volume; SV = Stroke volume; Ao = Aortic root diameter; LA = Left atrium diameter.

The values were presented as means ± SEM.

* $p<0.05$ when compared between pre and post amiodarone treatment.

D. Effect of amiodarone on plasma thyroid hormones

Total T_3 (tT_3) and Total T_4 (tT_4) were measured in this study as shown in figure 22 and figure 23, respectively. After amiodarone administration, both plasma tT_3 and plasma tT_4 were not significantly changed. One of the data was missing because the dog died before 60 days of treatment.

After amiodarone administration, both plasma tT_3 (40.2 ± 6.4 VS 55.9 ± 19.9 ng/dL, p=0.44, n=7) and plasma tT_4 (0.9 ± 0.3 VS 1.2 ± 0.3 µg/dL, p=0.37, n=7) were not significantly changed. In this study, four dogs had lower levels of tT_3 after given amiodarone. Two dogs had increased both tT_3 (46.1 VS 99.2 and 36.1 VS 151 ng/dL) and tT_4 (0.21 VS 0.71 and 0.01 VS 1.2 µg/dL) levels. Another two dogs had minimally changed tT_3 (43.6 VS 44.3 and 64.8 VS 53.8 ng/dL) and increased tT_4 (1.2 VS 2.1 and 1.7 VS 2.3 µg/dL). However, one of five dog has declined both tT_3 (52.0 VS 28.9 ng/dL) and plasma tT_4 (1.0 VS 0.54 µg/dL) after on amiodarone for 60 days. In contrast, two dogs with low circulatory tT_3 levels at the beginning of the study had decreased both plasma tT_3 (13.8 VS 0.00 and 24.9 VS 14.2 ng/dL) and plasma tT_4 (0.64 VS 0.02 and 1.8 VS and 1.5 µg/dL).

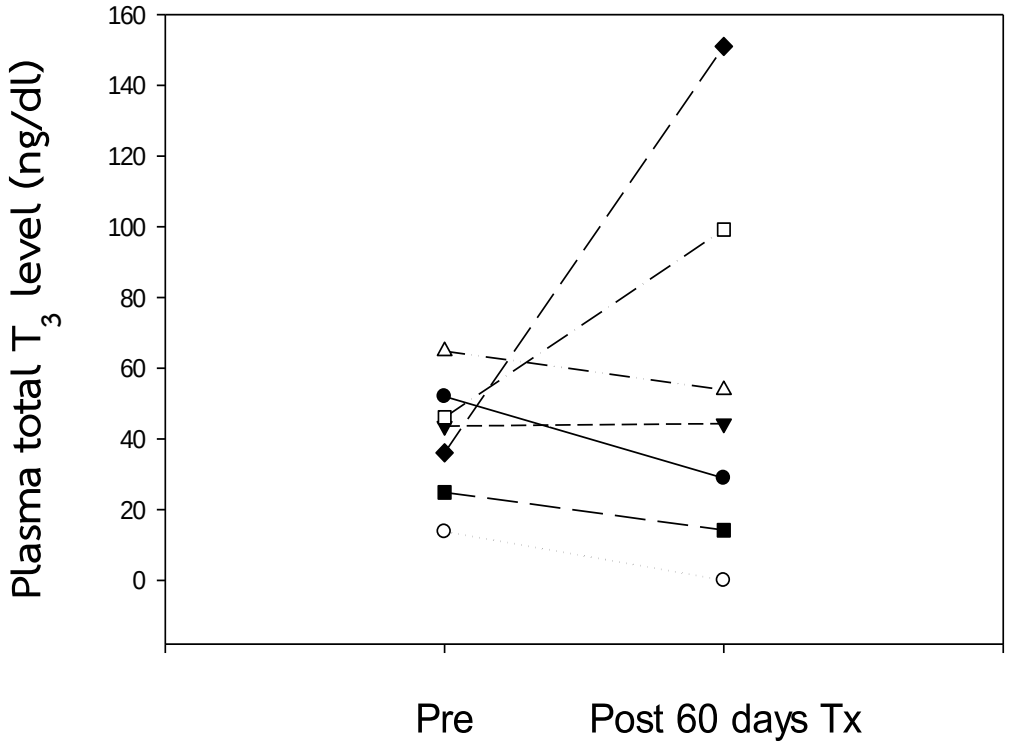

Figure 22. Plasma total tri-iodothyronine (T_3) of seven dogs after 60 days of amiodaorne treatment

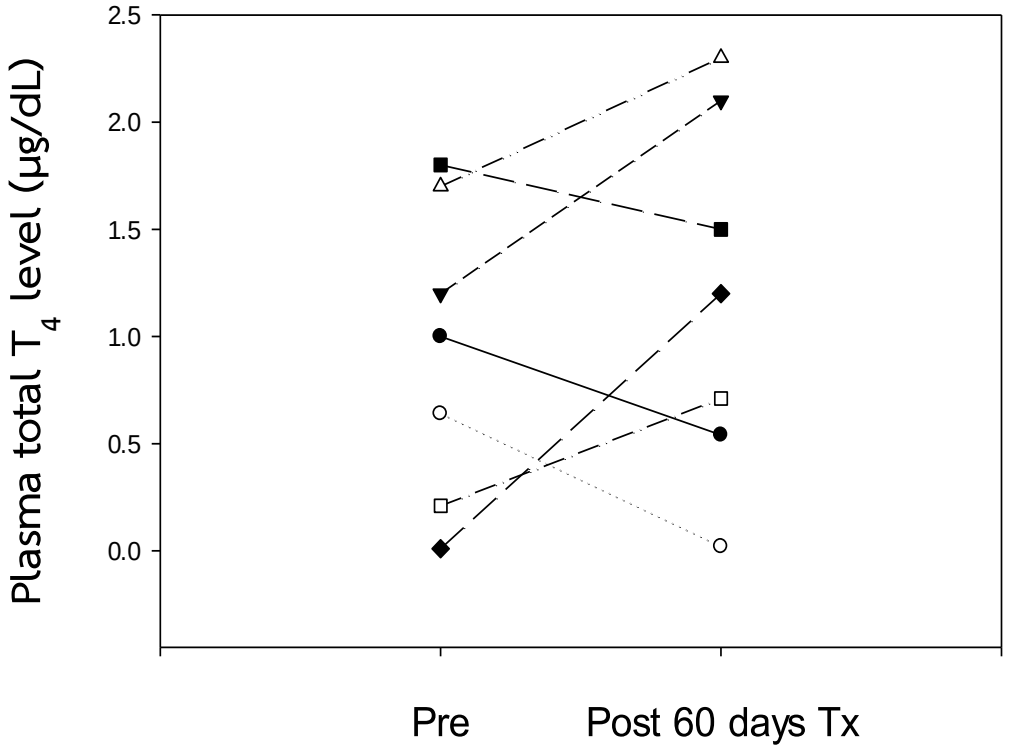

Figure 23. Plasma total tetra-iodothyronine (T_4) of seven dogs after 60 days of amiodaorne treatment

E. Correlation between Holter, echocardiographic parameters and thyroid hormones

Before amiodarone treatment, total beats had a high positive correlation with nomal beats (r^2=0.96, n=7, *p*=0.0005). VPCs had a positive relationship with total arrhythmic count (r^2=0.99, n=7, *p*<0.0001). Interestingly, QT interval had high correlation with IVCT (r^2=0.93, n=6, *p*=0.007) and Tei index (r^2=0.95, n=6, *p*=0.004). Tei index has positive correlation with IVCT (r^2=0.81, n=8, *p*=0.01).

After 15 days on amiodarone treatment, Correlation between normal beats and total beats still positively strong (r^2=0.98, n=7, *p*<0.0001), similar relationship between QT and IVCT (r^2= 0.83, n=6, *p*=0.04). Moreover, QT has correlation with stroke volume (r^2=0.82, n=6, *p*=0.046). Correlation between the Tei index and QT interval was diminished after treated with amiodarone (r^2=0.62, n=6, *p*=0.19). However, correlation between Tei index and IVCT was still persisted (r^2=0.89, n=8, *p*=0.003). Furthermore, we found total T_3 had relationship with PEP (r^2=-0.88, n=6, *p*=0.02) and IVRT (r^2=0.80, n=7, *p*=0.03).

CHAPTER V
DISCUSSION

From the present study, we hypothetized that; first, amiodarone has efficacy in controlling of arrhythmia. Second, negative inotropy and lusitropy are inferior by oral amiodarone. Third, plasma thyroid hormones are changed after 60 days of amiodarone treatment. However, some of our results were not followed our postulation. We will discuss in details later in this chapter.

A. Effect of amiodarone on animal clinical status

Dogs with dilated hearts may be affected by a common cardiac disease, dilated cardiomyopathy (DCM). Previous study had reported prevalence of DCM in Doberman pinschers about 58.2% (Wess et al., 2010). However, a study in Irish wolf hound dogs found only 25 percentage of 500 dogs, and 20 percent of them had AF (Vollmar, 2000). In a recent study conducted by Pedro et al. (2012), they found only 4 of 28 dogs affected by DCM and a mean age of these dogs was 6.75 ± 0.85 years. The major arrhythmia type in DCM dogs of that study was from the ventricular origin (3/4), another was AF which similar to our study. All of them were large breed dogs (one Great Dane, elses were Dobermans). They were two intacted male, a male castrated and a female, however, our study mainly had neutered or male dogs. From this point, supporting by previous studies, estrogen may have cardioprotective effects against to cardiac arrhythmia (McHugh et al., 1995; Tsai et al., 2002). A dog in our study had heartworm infestration. Heartworm may induce dilated heart from obstruction of pulmonary blood flow and produce congestive heart failure. In case of no any other complications, dilated heart chambers causing by heartworm is commonly found on the right side of the heart. The left ventricular systolic function had less affected on the early stage of heart worm disease when compared with DCM that had obviously systolic dysfunction.

After given a standard therapy with angiotensin converting enzyme inhibitor and furosemide, all dogs entered the stable heart failure stage, however, all dogs had shown obviously arrhythmias meeting criteria for our study. Systolic blood pressure did not change after oral amiodarone. After treatment for 15 days, almost dogs had improved clinical signs. There were no significantly change of complete blood count and serum chemistry profile value in this study, similarly to the study of Pedro and colleagues (2012). Using amiodarone may be relieved in arrhythmic dogs, but the bood check for liver enzymes should be monitors in cases that tend to have high liver enzymes to prevent the situation of hepatic failure.

B. Antiarrhythmic and proarrhythmic effects of amiodarone

The average loading dose of amiodarone in this study was 10.3 ± 0.7 mg/kg twice a day for 7 days, following by the maintenance dose at 5.8 ± 0.2 mg/kg daily. During loading period (amiodaronization), the decrease of VPC number on 24-hr ECGs monitoring was observed. However, one dog showed an increase in VPCs after changing to maintenance dose. This may due to the insufficient dosage of amiodarone. Therefore, An Increase of dosage may be necessary for unresponsive treatment. After 60 days of amiodarone treatment, recurrences of arrhythmias in some cases were observed. The higher dosage of amiodarone or adding other antiarrhythmic drugs may be considered.

It has been known that amiodarone possesses the potassium channel blocking property resulting in QT interval prolongation. In this study, QT interval tended to prolong after treat with amiodarone for 15 days (P=0.07). This is consistent with previous study in which amiodarone was given at a loading dose of 25 mg/kg twice a day, and following by a maintenance dose at 30 mg/kg once a day in healthy beagle dogs which had a minimal effect on QT interval prolongation (Bicer et al., 2002b). We also observed a prolongation of QRS complex after amiodarone administration in this study. However, previous studies in healthy dogs were not found significant changes of QRS complex duration (Merot et al., 1999; Bicer et al., 2001; van Opstal et al., 2001; Bicer et al., 2002b). On the other hand, Burashnikov et al. (2008) reported the significant QT interval prolongation in the left ventricular

tissue preparation after amiodarone administration, but less dominant than P wave. The results of lengthening QRS complex in DCM dogs may be explained by the sodium channel blocking property of amiodarone which increase the depolarization time.

Short term variability (STV) of QT intervals is one of arrhythmic risk prognostic parameters recently using in the pharmacological safety testing. Our study did not find any significant changes of STV after oral amiodarone for 15 days agreed with a previous study by Thomsen et al. (2004) in which dogs received amiodarone for 4 weeks. The dispersion of repolarization and the heterogeneity of tissue repolarization were measured in the present study. Due to multi-channel blocking properties of amiodarone, transmural dispersion ventricular repolarization (TDR) did not alter in this study. Merot and coworker (1999) studied the effects of chronic amiodarone on TDR in canine wedge preparation and found that TDR was insignificantly increased.

C. Effect of amiodarone on cardiac function and performance

From our study, 15 days of oral amiodarone administration had minimally effects on cardiac contractility indicating by slightly lengthening of the pre-ejection period (PEP) and isovolumic contraction time (IVCT) after 15 days of oral amiodarone administration but not the stroke volume nor ejection fraction. This result is consistent with Paulus et al. (1980). PEP is the duration between an electrical event to the opening of aortic valve while the IVCT is the time from the end of mitral inflow to the beginning of aortic outflow (figure 24). The events occurring under PEP are including ventricular depolarization, calcium-induced calcium release, cross-bridge cycle formation during isovolumic contraction before aortic valve opening. Lengthening of the two periods indicated a negative inotropy of the heart that may be caused by multichannel blocking properties of amiodarone, especially the calcium-inward inhibitory effect. Inhibition of calcium channel may interfere intracellular calcium homeostasis resulting from abnormal SERCA function, ryanodine receptor and troponin C function (Bers, 2000).

The present study also showed significantly increased stroke volume (SV) for 24% after treatment with amiodarone. The increased in SV is consistent with a previous study in humans in which the SV was increased by 19 % without changing the cardiac output (Trobaugh et al., 1984). It has been known that amiodarone possesses beta-adrenergic and calcium channel blocking properties which may decrease the heart rate and contractility as well. According to Frank-Starling law of the heart, decrease in heart rate may prolong the left ventricular filling time and causes increasing preload which finally increases the stroke volume.

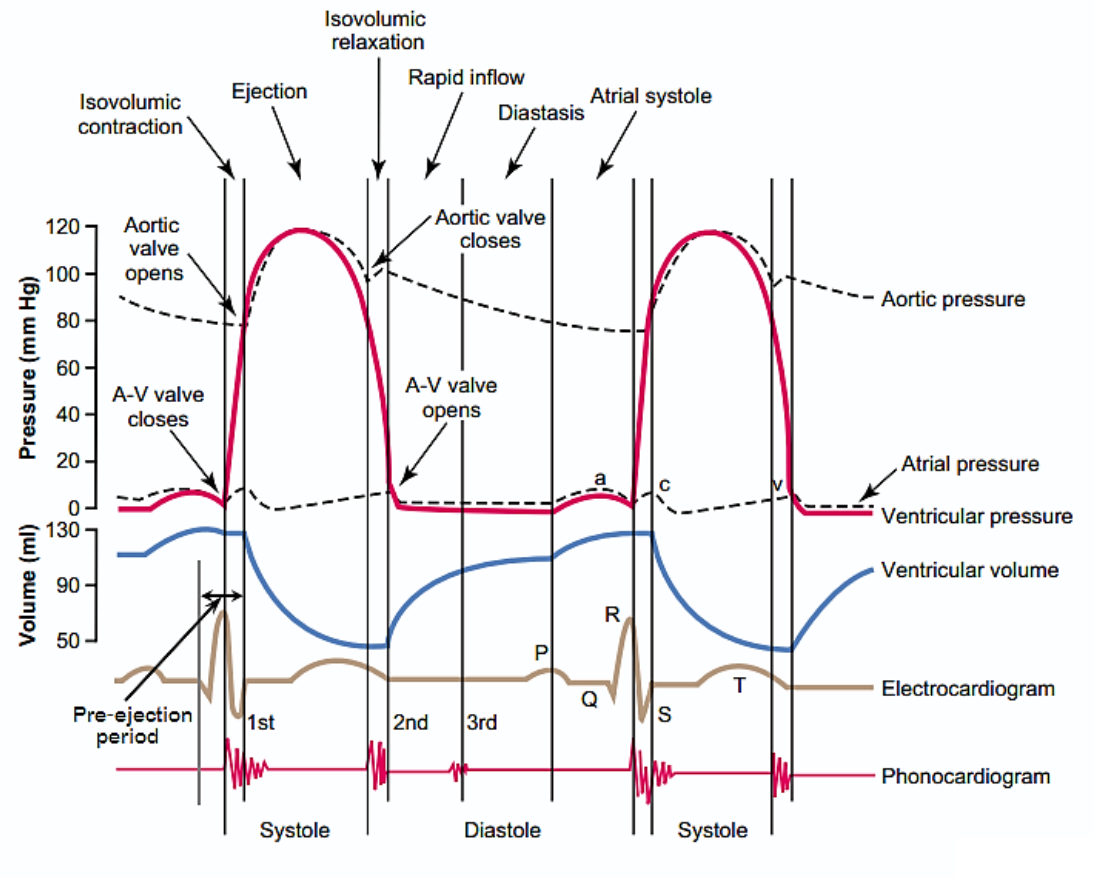

Figure 24. Left ventricular function and relative parameters of cardiac cycle (modified from Guyton and Hall, 2006).

We also performed the measurement of isovolumic relaxation time (IVRT) from a left apical parasternal 5-chamber view that showed insignificant changes in IVRT after 15-day of amiodarone administration. However, dogs with dilated hearts in this study had shorter IVRT than reference value (~70 ms) (Myreng and Smiseth, 1990). This could be due to a reduction of LV compliance (Jaber et al., 2008). Various factors affect the IVRT including preload, afterload, heart rate and aging (Myreng and Smiseth, 1990). Moreover, volume overload misguides the interpretation of the Doppler flow pattern in which the E peak seems to have higher velocity (Masutani et al., 2008). In this case, the measurement of both aortic pressure and left atrium have been suggested for estimating the diastolic function together with IVRT or performed a higher sensitivity parameter (Boon, 2011).

Tei index was tended to increase in our study. In humans, Tei index is a strong prognostic parameter in myocardial infarction patients, which worsen condition was founded on high increasing of the value (Harjai et al., 2002; Sasao et al., 2004; Larina et al., 2013). In the veterinary field, Tei index was adapted to assess right side and left side heart functions (Teshima et al., 2006; Teshima et al., 2007), even in occult DCM dogs which had high Tei index referred to a poor outcome the same as in humans (Lee et al., 2002). Therefore, Tei index may be a very sensitive parameter and can detect early anomaly that clinical signs are still normal.

D. Side effect of amiodarone on thyroid hormone levels

A previous publication on amiodarone-induced thyroid dysfunction had demonstrated side effects of amiodarone on both plasma tT_3 and tT_4 levels (Bogazzi et al., 2001). In our study, however, we have not found any obvious thyroid toxicity during 60 days of amiodarone therapy. T_3 is a key hormone that the body try to preserve at a steady level. Lower circulatory T_3 concentration may reflex to abnormal thyroid function. Moreover, many factors that may affect the thyroid functions such as overall animal health, concurrent diseases, iodine intake, daily caloric intake, and thyroid function itself (Wolff and Chaikoff, 1948; Wadden et al., 1990; Katzeff et al., 1997; McIver and Gorman, 1997). Several studies in humans (Bogazzi et al., 2001; Batcher et al., 2007; van Erven and Schalij, 2010) showed results

of amiodarone induced-thyroid dysfunction as amiodarone induced-hypothyroidism (AIH), amiodarone induced-thyrotoxicosis (AIT), and mixed form of thyroid dysfunction which each type has various specific pathophysiology (Martino et al., 2001). However, there have no any report this thyroid dysfunction in clinically canine-disease models before. Unfortunately, the physiology of iodine metabolism, kinetic of hormone binding proteins, secretion, and clearance of canine thyroid hormones are different from humans (Daminet and Ferguson, 2003). In dog, plasma iodine and intra-follicular iodide concentrations are higher than human thyroid glands about 10-20 times and 12.5 times, respectively (Kaptein et al., 1990). Thyroid binding proteins in canine species have high affinity than humans, however, dogs have binding protein levels about 15 percent compared to humans (Larsson et al., 1985). Iodide uptake rate, T_3 synthesis, and total secretion of T_4 in canine thyroid glands are higher than humans about 5, 3, and 2 times, respectively (Belshaw et al., 1974). In dog, plasma half-life of T_3 and T_4 are 5 to 6 hours and 8 to 16 hours, respectively, which theses clearance are more rapid when compared with plasma T_3 and T_4 clearances in humans (24 to 36 hours and ~7 days, respectively) (Kaptein et al., 1993). From these points, dogs may tolerate iodine overload situation more than humans. Even so, amiodarone induced-thyroid dysfunction has been reported eslewhere (Pasquali et al., 1990). That study was conducted in vitro and in acute phase, which may not be represent the whole compensatory processes. In long term, normal thyroid glands possibly could escape from iodine overload and show normal serum thyroid profiles.

Other side effects of amiodarone administration have been reported by previous studies such as keratopathy, increasing of liver enzyme, lung fibrosis and neuropathy (McGovern et al., 1983; Gittinger and Asdourian, 1988; Jacobs et al., 2000; Bicer et al., 2002a). However, in this study, amiodarone administration approximately in a loading dose of 10 mg/kg twice a day for 7 days, and followed by a maintenance dose of 5 mg/kg once a day had no significant adverse effect. The liver enzyme panels of all dogs showed insignificant changes after 15 days and 60 days of amiodarone administration. This is consistent with previous publication by Pedro et al. (2012). None of the dogs in this study had high hepatic enzymes or was dropped out of the study by the liver failure.

E. Relationships among heart rate, QT interval, PEP, and IVCT

QT and IVCT have a relationship because the duration of IVCT is overlapping with QT. Variation of both parameters may be due to the effect of amiodarone on heart rate. Tei index has correlation with QT and IVCT before and after amiodarone treatment. Since IVCT is a part of the calculation of Tei index; therefore, Tei index may be influenced by heart rate similar to IVCT. Dogs treated with amiodarone may have benefits from a reduction of heart rate and energy economy but not the cardiac performance.

F. Conclusion

In conclusion, the present study proved that amiodarone is a potent antiarrhythmic drug with has a low risk of proarrhythmic effect. The drug possesses the minor negative inotropy, which should be concerned in cases of impaired systolic function. However, we did not find a significant thyroid toxicity causing by amiodarone, and have no impact on clinical signs. Therefore, the dose of amiodarone recommended in this study is safe for treating arrhythmic dogs with organic heart diseases.

G. Limitations of the study

One of limitation is wash out period, especially digoxin. Digoxin is a well known antiarrhythmic drug that prolongs the conduction time on AV node. Effects of the drug may interfere the action of amiodarone in the study. However, drug withdrawal in cardiac patients may increase the fatal risk. For this reason, adding new drug may take time to reach maximal effects and may be influenced by other prescripted drugs. Moreover, multiple drugs interaction should be concerned. Diuretic drug enhances water and electrolyte excretion, which may lead to a significant change of the intracellular potassium ion and affect the electrical and mechanical events. Last, atrial fibrillation is a rapid supraventricular arrhythmia. QT interval of AF is highly variable and should be interpreted cautiously with ventricular arrhythmia.

REFERENCES

Acierno MJ and Labato MA 2005. Hypertension in renal disease: diagnosis and treatment. Clin Tech Small Anim Pract. 20(1): 23-30.

Akar FG, Spragg DD, Tunin RS, Kass DA and Tomaselli GF 2004. Mechanisms underlying conduction slowing and arrhythmogenesis in nonischemic dilated cardiomyopathy. Circ Res. 95(7): 717-725.

Antzelevitch C 2001. Transmural dispersion of repolarization and the T wave. Cardiovasc Res. 50(3): 426-431.

Antzelevitch C 2003. Molecular genetics of arrhythmias and cardiovascular conditions associated with arrhythmias. J Cardiovasc Electrophysiol. 14(11): 1259-1272.

Antzelevitch C, Shimizu W, Yan GX, Sicouri S, Weissenburger J, Nesterenko VV, Burashnikov A, Di Diego J, Saffitz J and Thomas GP 1999. The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart. J Cardiovasc Electrophysiol. 10(8): 1124-1152.

Batcher EL, Tang XC, Singh BN, Singh SN, Reda DJ, Hershman JM and Investigators S-T 2007. Thyroid function abnormalities during amiodarone therapy for persistent atrial fibrillation. Am J Med. 120(10): 880-885.

Bayes de Luna A, Coumel P and Leclercq JF 1989. Ambulatory sudden cardiac death: mechanisms of production of fatal arrhythmia on the basis of data from 157 cases. Am Heart J. 117(1): 151-159.

Belshaw B, BARANDES M, BECKER D and BERMAN M 1974. A Model of Iodine Kinetics in the Dog. Endocrinology. 95(4): 1078-1093.

Bers DM 2000. Calcium fluxes involved in control of cardiac myocyte contraction. Circ Res. 87(4): 275-281.

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 H, Nakayama T, Strauch SM and Hamlin RL 2001. Effects of chronic, oral amiodarone on left ventricular pressure, electrocardiograms, and

action potentials from myocardium in vivo and from Purkinje fibers in vitro. Vet Ther. 2(4): 325-333.

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.

Bogazzi F, Bartalena L, Gasperi M, Braverman LE and Martino E 2001. The various effects of amiodarone on thyroid function. Thyroid. 11(5): 511-519.

Boon JA 2011. Evaluation of size, function, and hemodynamics. 2 ed. In: Veterinary echocardiography. UK, Wiley-Blackwell. 195-246.

Brien JF, Jimmo S, Brennan FJ, Armstrong PW and Abdollah H 1990. Disposition of amiodarone and its proximate metabolite, desethylamiodarone, in the dog for oral administration of single-dose and short-term drug regimens. Drug Metab Dispos. 18(6): 846-851.

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

BSAVA 2011. Amiodarone. 7 ed. In: BSAVA Small Animal Formulary. Rhondda Wales, HSW Print. 16-17.

Buch J and Andersen ED 1984. Non-invasive evaluation of the haemodynamic effects of amiodarone. Acta Cardiol. 39(5): 317-328.

Buchanan JW and Bucheler J 1995. Vertebral scale system to measure canine heart size in radiographs. J Am Vet Med Assoc. 206(2): 194-199.

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.

CAST II Investigators 1992. Effect of the antiarrhythmic agent moricizine on survival after myocardial infarction. The Cardiac Arrhythmia Suppression Trial II Investigators. N Engl J Med. 327(4): 227-233.

CAST Investigators 1989. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. N Engl J Med. 321(6): 406-412.

Chalifoux A, Dallaire A, Blais D, Lariviere N and Pelletier N 1985. Evaluation of the arterial blood pressure of dogs by two noninvasive methods. Can J Comp Med. 49(4): 419-423.

Chen CM, Gettes LS and Katzung BG 1975. Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of (dV/dt)max in guinea pig ventricular myocardium. Circ Res. 37(1): 20-29.

Chiovato L, Martino E, Tonacchera M, Santini F, Lapi P, Mammoli C, Braverman LE and Pinchera A 1994. Studies on the in vitro cytotoxic effect of amiodarone. Endocrinology. 134(5): 2277-2282.

Connolly SJ 1999. Evidence-based analysis of amiodarone efficacy and safety. Circulation. 100(19): 2025-2034.

Coplen SE, Antman EM, Berlin JA, Hewitt P and Chalmers TC 1990. Efficacy and safety of quinidine therapy for maintenance of sinus rhythm after cardioversion. A meta-analysis of randomized control trials. Circulation. 82(4): 1106-1116.

Daminet S and Ferguson DC 2003. Influence of drugs on thyroid function in dogs. J Vet Intern Med. 17(4): 463-472.

Davidenko JM, Cohen L, Goodrow R and Antzelevitch C 1989. Quinidine-induced action potential prolongation, early afterdepolarizations, and triggered activity in canine Purkinje fibers. Effects of stimulation rate, potassium, and magnesium. Circulation. 79(3): 674-686.

Dean JW and Lab MJ 1989. Arrhythmia in heart failure: role of mechanically induced changes in electrophysiology. Lancet. 1(8650): 1309-1312.

Dembek KA, Hurcombe SD, Schober KE and Toribio RE 2014. Sudden death of a horse with supraventricular tachycardia following oral administration of flecainide acetate. J Vet Emerg Crit Care (San Antonio). 24(6): 759-763.

Di Matola T, D'Ascoli F, Fenzi G, Rossi G, Martino E, Bogazzi F and Vitale M 2000. Amiodarone induces cytochrome c release and apoptosis through an iodine-independent mechanism. J Clin Endocrinol Metab. 85(11): 4323-4330.

Drouin E, Lande G and Charpentier F 1998. Amiodarone reduces transmural heterogeneity of repolarization in the human heart. J Am Coll Cardiol. 32(4): 1063-1067.

Ebinger MW, Krishnan S and Schuger CD 2005. Mechanisms of ventricular arrhythmias in heart failure. Curr Heart Fail Rep. 2(3): 111-117.

el-Sherif N, Bekheit SS and Henkin R 1989. Quinidine-induced long QTU interval and torsade de pointes: role of bradycardia-dependent early afterdepolarizations. J Am Coll Cardiol. 14(1): 252-257.

Flaker GC, Blackshear JL, McBride R, Kronmal RA, Halperin JL and Hart RG 1992. Antiarrhythmic drug therapy and cardiac mortality in atrial fibrillation. The Stroke Prevention in Atrial Fibrillation Investigators. J Am Coll Cardiol. 20(3): 527-532.

Freeman LM, Rush JE, Farabaugh AE and Must A 2005. Development and evaluation of a questionnaire for assessing health-related quality of life in dogs with cardiac disease. J Am Vet Med Assoc. 226(11): 1864-1868.

Fuster V, Ryden LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA, Halperin JL, Kay GN, Le Huezey JY, Lowe JE, Olsson SB, Prystowsky EN, Tamargo JL and Wann LS 2011. 2011 ACCF/AHA/HRS focused updates incorporated into the ACC/AHA/ESC 2006 Guidelines for the management of patients with atrial fibrillation: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines developed in partnership with the European Society of Cardiology and in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. J Am Coll Cardiol. 57(11): e101-198.

Gagnol JP, Devos C, Clinet M and Nokin P 1985. Amiodarone. Biochemical aspects and haemodynamic effects. Drugs. 29 Suppl 3: 1-10.

Gelzer AR, Kraus MS, Rishniw M, Moise NS, Pariaut R, Jesty SA and Hemsley SA 2009. Combination therapy with digoxin and diltiazem controls ventricular rate in chronic atrial fibrillation in dogs better than digoxin or diltiazem monotherapy: a randomized crossover study in 18 dogs. J Vet Intern Med. 23(3): 499-508.

Gill RM, Jones BD, Corbly AK, Wang J, Braz JC, Sandusky GE, Wang J and Shen W 2006. Cardiac diastolic dysfunction in conscious dogs with heart failure induced by

chronic coronary microembolization. Am J Physiol Heart Circ Physiol. 291(6): H3154-3158.

Gittinger JW, Jr. and Asdourian GK 1988. Amiodarone-related optic neuropathy. Mayo Clin Proc. 63(2): 210.

Gupta P, Patel C, Patel H, Narayanaswamy S, Malhotra B, Green JT and Yan GX 2008. T(p-e)/QT ratio as an index of arrhythmogenesis. J Electrocardiol. 41(6): 567-574.

Guyton AC and Hall JE 2006. The Heart. 11th ed. In: Textbook of Medical Physiology. Elsevier Saunders, China. 107.

Haberman CE, Kang CW, Morgan JD and Brown SA 2006. Evaluation of oscillometric and Doppler ultrasonic methods of indirect blood pressure estimation in conscious dogs. Can J Vet Res. 70(3): 211-217.

Harjai KJ, Scott L, Vivekananthan K, Nunez E and Edupuganti R 2002. The Tei index: a new prognostic index for patients with symptomatic heart failure. J Am Soc Echocardiogr. 15(9): 864-868.

Harpster NK 1991. Boxer cardiomyopathy. A review of the long-term benefits of antiarrhythmic therapy. Vet Clin North Am Small Anim Pract. 21(5): 989-1004.

Holt DW, Tucker GT, Jackson PR and Storey GC 1983. Amiodarone pharmacokinetics. Am Heart J. 106(4 Pt 2): 840-847.

Hondeghem LM and Matsubara T 1988. Quinidine blocks cardiac sodium channels during opening and slow inactivation in guinea-pig papillary muscle. Br J Pharmacol. 93(2): 311-318.

IMPACT Research Group 1984. International mexiletine and placebo antiarrhythmic coronary trial: I. Report on arrhythmia and other findings. Impact Research Group. J Am Coll Cardiol. 4(6): 1148-1163.

Jaber WA, Maniu C, Krysiak J, Shapiro BP, Meyer DM, Linke WA and Redfield MM 2008. Titin isoforms, extracellular matrix, and global chamber remodeling in experimental dilated cardiomyopathy: functional implications and mechanistic insight. Circ Heart Fail. 1(3): 192-199.

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

Jafari-Fesharaki M and Scheinman MM 1998. Adverse effects of amiodarone. Pacing Clin Electrophysiol. 21(1 Pt 1): 108-120.

Jin H, Lyon AR and Akar FG 2008. Arrhythmia mechanisms in the failing heart. Pacing Clin Electrophysiol. 31(8): 1048-1056.

Josephson ME, Seides SF, Batsford WP, Weisfogel GM, Akhtar M, Caracta AR, Lau SH and Damato AN 1974. The electrophysiological effects of intramuscular guinidine on the atrioventricular conducting system in man. Am Heart J. 87(1): 55-64.

Juul-Moller S, Edvardsson N and Rehnqvist-Ahlberg N 1990. Sotalol versus quinidine for the maintenance of sinus rhythm after direct current conversion of atrial fibrillation. Circulation. 82(6): 1932-1939.

Kahaly GJ and Dillmann WH 2005. Thyroid hormone action in the heart. Endocr Rev. 26(5): 704-728.

Kaptein EM, Hoopes MT, Ferguson DC, Satyadi EC and Akmal M 1990. Comparison of reverse triiodothyronine distribution and metabolism in normal dogs and humans. Endocrinology. 126(4): 2003-2014.

Kaptein EM, Moore GE, Ferguson DC and Hoenig M 1993. Thyroxine and triiodothyronine distribution and metabolism in thyroxine-replaced athyreotic dogs and normal humans. Am J Physiol. 264(1 Pt 1): E90-100.

Katzeff HL, Powell SR and Ojamaa K 1997. Alterations in cardiac contractility and gene expression during low-T3 syndrome: prevention with T3. Am J Physiol. 273(5 Pt 1): E951-956.

Kawai C, Konishi T, Matsuyama E and Okazaki H 1981. Comparative effects of three calcium antagonists, diltiazem, verapamil and nifedipine, on the sinoatrial and atrioventricular nodes. Experimental and clinical studies. Circulation. 63(5): 1035-1042.

Kilicaslan F, Tokatli A, Ozdag F, Uzun M, Uz O, Isilak Z, Yiginer O, Yalcin M, Guney MS and Cebeci BS 2012. Tp-e interval, Tp-e/QT ratio, and Tp-e/QTc ratio are prolonged in patients with moderate and severe obstructive sleep apnea. Pacing Clin Electrophysiol. 35(8): 966-972.

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.

Kraus MS, Thomason JD, Fallaw TL and Calvert CA 2009. Toxicity in Doberman Pinchers with ventricular arrhythmias treated with amiodarone (1996-2005). J Vet Intern Med. 23(1): 1-6.

Larina VN, Bart B, Dergunova EN and Alekhin MN 2013. [Prognostic value of the myocardial performance (Tei) index in patients with chronic heart failure]. Kardiologiia. 53(11): 37-44.

Larsson M, Pettersson T and Carlstrom A 1985. Thyroid hormone binding in serum of 15 vertebrate species: isolation of thyroxine-binding globulin and prealbumin analogs. Gen Comp Endocrinol. 58(3): 360-375.

Latini R, Tognoni G and Kates RE 1984. Clinical pharmacokinetics of amiodarone. Clin Pharmacokinet. 9(2): 136-156.

Lavine SJ 2005a. Effect of heart rate and preload on index of myocardial performance in the normal and abnormal left ventricle. J Am Soc Echocardiogr. 18(2): 133-141.

Lavine SJ 2005b. Index of myocardial performance is afterload dependent in the normal and abnormal left ventricle. J Am Soc Echocardiogr. 18(4): 342-350.

Lavine SJ 2006. Effect of changes in contractility on the index of myocardial performance in the dysfunctional left ventricle. Cardiovasc Ultrasound. 4: 45.

Lavine SJ, Prcevski P, Held AC and Johnson V 1991. Experimental model of chronic global left ventricular dysfunction secondary to left coronary microembolization. J Am Coll Cardiol. 18(7): 1794-1803.

Lee BH, Dukes-McEwan J, French AT and Corcoran BM 2002. Evaluation of a novel doppler index of combined systolic and diastolic myocardial performance in Newfoundland dogs with familial prevalence of dilated cardiomyopathy. Vet Radiol Ultrasound. 43(2): 154-165.

Lewis JH, Mullick F, Ishak KG, Ranard RC, Ragsdale B, Perse RM, Rusnock EJ, Wolke A, Benjamin SB, Seeff LB and et al. 1990. Histopathologic analysis of suspected amiodarone hepatotoxicity. Hum Pathol. 21(1): 59-67.

Lilly LS 2011. Clinical aspects of cardiac arrhythmias. 5th ed. In: Pathophysiology of Heart Disease: A Collaborative Project of Medical Students and Faculty. Lippincott Williams & Wilkins, China. 261-277.

Lown B, Calvert AF, Armington R and Ryan M 1975. Monitoring for serious arrhythmias and high risk of sudden death. Circulation. 52(6 Suppl): III189-198.

Lubic SP, Nguyen KP, Dave B and Giacomini JC 1994. Antiarrhythmic agent amiodarone possesses calcium channel blocker properties. J Cardiovasc Pharmacol. 24(5): 707-714.

Martino E, Bartalena L, Bogazzi F and Braverman LE 2001. The effects of amiodarone on the thyroid. Endocr Rev. 22(2): 240-254.

Mason JW, Hondeghem LM and Katzung BG 1983. Amiodarone blocks inactivated cardiac sodium channels. Pflugers Arch. 396(1): 79-81.

Masutani S, Little WC, Hasegawa H, Cheng HJ and Cheng CP 2008. Restrictive left ventricular filling pattern does not result from increased left atrial pressure alone. Circulation. 117(12): 1550-1554.

McGovern B, Garan H, Kelly E and Ruskin JN 1983. Adverse reactions during treatment with amiodarone hydrochloride. Br Med J (Clin Res Ed). 287(6386): 175-180.

McHugh NA, Cook SM, Schairer JL, Bidgoli MM and Merrill GF 1995. Ischemia- and reperfusion-induced ventricular arrhythmias in dogs: effects of estrogen. Am J Physiol. 268(6 Pt 2): H2569-2573.

McIver B and Gorman CA 1997. Euthyroid sick syndrome: an overview. Thyroid. 7(1): 125-132.

Merot J, Charpentier F, Poirier JM, Coutris G and Weissenburger J 1999. Effects of chronic treatment by amiodarone on transmural heterogeneity of canine ventricular repolarization in vivo: interactions with acute sotalol. Cardiovasc Res. 44(2): 303-314.

Meurs KM, Fox PR, Norgard M, Spier AW, Lamb A, Koplitz SL and Baumwart RD 2007. A prospective genetic evaluation of familial dilated cardiomyopathy in the Doberman pinscher. J Vet Intern Med. 21(5): 1016-1020.

Meurs KM, Spier AW, Wright NA, Atkins CE, DeFrancesco TC, Gordon SG, Hamlin RL, Keene BW, Miller MW and Moise NS 2002. Comparison of the effects of four

antiarrhythmic treatments for familial ventricular arrhythmias in Boxers. J Am Vet Med Assoc. 221(4): 522-527.

Meurs KM, Spier AW, Wright NA and Hamlin RL 2001. Use of ambulatory electrocardiography for detection of ventricular premature complexes in healthy dogs. J Am Vet Med Assoc. 218(8): 1291-1292.

Moosvi AR, Goldstein S, VanderBrug Medendorp S, Landis JR, Wolfe RA, Leighton R, Ritter G, Vasu CM and Acheson A 1990. Effect of empiric antiarrhythmic therapy in resuscitated out-of-hospital cardiac arrest victims with coronary artery disease. Am J Cardiol. 65(18): 1192-1197.

Myreng Y and Smiseth OA 1990. Assessment of left ventricular relaxation by Doppler echocardiography. Comparison of isovolumic relaxation time and transmitral flow velocities with time constant of isovolumic relaxation. Circulation. 81(1): 260-266.

Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA and Evangelista A 2009. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr. 22(2): 107-133.

Nattel S, Hadjis T and Talajic M 1994. The treatment of atrial fibrillation. An evaluation of drug therapy, electrical modalities and therapeutic considerations. Drugs. 48(3): 345-371.

Nattel S, Talajic M, Fermini B and Roy D 1992. Amiodaone: Pharmacology,Clinical Actions, and Relationships Between Them. J Cardiovasc Electrophysiol. 3: 226-280.

Neumann T, Vollmer A, Schaffner T, Hess OM and Heusch G 1999. Diastolic dysfunction and collagen structure in canine pacing-induced heart failure. J Mol Cell Cardiol. 31(1): 179-192.

O'Brien RT and Holmes SP 2007. Recent advances in ultrasound technology. Clin Tech Small Anim Pract. 22(3): 93-103.

O'Sullivan ML, O'Grady MR and Minors SL 2007. Assessment of diastolic function by Doppler echocardiography in normal Doberman Pinschers and Doberman Pinschers with dilated cardiomyopathy. J Vet Intern Med. 21(1): 81-91.

Oosterhoff P, Thomsen MB, Maas JN, Atteveld NJ, Beekman JD, HV VANR, MA VDH and Vos MA 2010. High-rate pacing reduces variability of repolarization and prevents repolarization-dependent arrhythmias in dogs with chronic AV block. J Cardiovasc Electrophysiol. 21(12): 1384-1391.

Opie LH and Gersh BJ 2009. Antiarrhythmic Agents and strategies. 7 ed. In: Drugs for the heart. Philadelphia, Saunders Elsevier. 235-292.

Pachucki J, Hopkins J, Peeters R, Tu H, Carvalho SD, Kaulbach H, Abel ED, Wondisford FE, Ingwall JS and Larsen PR 2001. Type 2 iodothyronin deiodinase transgene expression in the mouse heart causes cardiac-specific thyrotoxicosis. Endocrinology. 142(1): 13-20.

Park KW, Dai HB, Ojamaa K, Lowenstein E, Klein I and Sellke FW 1997. The direct vasomotor effect of thyroid hormones on rat skeletal muscle resistance arteries. Anesth Analg. 85(4): 734-738.

Pasquali D, Tseng FY, Rani CS and Field JB 1990. Inhibition of intermediary metabolism by amiodarone in dog thyroid slices. Am J Physiol. 259(4 Pt 1): E529-533.

Paulus WJ, Ranquin R and Parizel G 1980. Systolic time intervals: a valuable parameter of thyroid function. Angiology. 31(2): 100-108.

Pearle DL, Souza JD and Gillis RA 1983. Comparative vagolytic effects of procainamide and N-acetylprocainamide in the dog. J Cardiovasc Pharmacol. 5(3): 450-453.

Pedro B, Lopez-Alvarez J, Fonfara S, Stephenson H and Dukes-McEwan J 2012. Retrospective evaluation of the use of amiodarone in dogs with arrhythmias (from 2003 to 2010). J Small Anim Pract. 53(1): 19-26.

Perret G, Yin YL, Nicolas P, Pussard E, Vassy R, Uzzan B and Berdeaux A 1992. Amiodarone decreases cardiac beta-adrenoceptors through an antagonistic effect on 3,5,3' triiodothyronine. J Cardiovasc Pharmacol. 19(4): 473-478.

Petric AD, Stabej P and Zemva A 2002. Dilated cardiomyopathy in Doberman Pinschers: Survival, Causes of Death and a Pedigree Review in a Related Line. J Vet Cardiol. 4(1): 17-24.

Petrie JP 2005. Practical application of holter monitoring in dogs and cats. Clin Tech Small Anim Pract. 20(3): 173-181.

Plomp TA, van Rossum JM, Robles de Medina EO, van Lier T and Maes RA 1984. Pharmacokinetics and body distribution of amiodarone in man. Arzneimittelforschung. 34(4): 513-520.

Plomp TA, Wiersinga WM and Maes RA 1985. Tissue distribution of amiodarone and desethylamiodarone in rats after repeated oral administration of various amiodarone dosages. Arzneimittelforschung. 35(12): 1805-1810.

Polster P and Broekhuysen J 1976. The adrenergic antagonism of amiodarone. Biochem Pharmacol. 25(2): 131-134.

Pritchard DA, Singh BN and Hurley PJ 1975. Effects of amiodarone on thyroid function in patients with ischaemic heart disease. Br Heart J. 37(8): 856-860.

Rao RH, McCready VR and Spathis GS 1986. Iodine kinetic studies during amiodarone treatment. J Clin Endocrinol Metab. 62(3): 563-568.

Resnick LM and Laragh JH 1982. PLasma renin activity in syndromes of thyroid hormone excess and deficiency. Life Sci. 30(7-8): 585-586.

Roden DM, Bennett PB, Snyders DJ, Balser JR and Hondeghem LM 1988. Quinidine delays IK activation in guinea pig ventricular myocytes. Circ Res. 62(5): 1055-1058.

Roden DM, Woosley RL and Primm RK 1986. Incidence and clinical features of the quinidine-associated long QT syndrome: implications for patient care. Am Heart J. 111(6): 1088-1093.

Sasao H, Noda R, Hasegawa T, Endo A, Oimatsu H and Takada T 2004. Prognostic value of the Tei index combining systolic and diastolic myocardial performance in patients with acute myocardial infarction treated by successful primary angioplasty. Heart Vessels. 19(2): 68-74.

Sicouri S, Belardinelli L, Carlsson L and Antzelevitch C 2009. Potent antiarrhythmic effects of chronic amiodarone in canine pulmonary vein sleeve preparations. J Cardiovasc Electrophysiol. 20(7): 803-810.

Sicouri S, Burashnikov A, Belardinelli L and Antzelevitch C 2010. Synergistic electrophysiologic and antiarrhythmic effects of the combination of

ranolazine and chronic amiodarone in canine atria. Circ Arrhythm Electrophysiol. 3(1): 88-95.

Sicouri S, Moro S, Litovsky S, Elizari MV and Antzelevitch C 1997. Chronic amiodarone reduces transmural dispersion of repolarization in the canine heart. J Cardiovasc Electrophysiol. 8(11): 1269-1279.

Singh BN 1983. Amiodarone: historical development and pharmacologic profile. Am Heart J. 106(4 Pt 2): 788-797.

Singh BN 2006. Amiodarone: a multifaceted antiarrhythmic drug. Curr Cardiol Rep. 8(5): 349-355.

Singh BN and Vaughan Williams EM 1970. The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. Br J Pharmacol. 39(4): 657-667.

Sisson DD 2004. Neuroendocrine evaluation of cardiac disease. Vet Clin North Am Small Anim Pract. 34(5): 1105-1126.

Sousa MG, Carareto R, De-Nardi AB, Brito FL, Nunes N and Camacho AA 2007. Effects of isoflurane on Tei-index of myocardial performance in healthy dogs. Can Vet J. 48(3): 277-282.

Sousa MG, Paulino D, Pascon JPE, Pereira-Neto GB, Carareto R and Camacho AA 2014. Assesment of the TEl index of myocardial performance in dogs with doxorubicin-induced cardiomiopathy. Archivos De Medicina Veterinaria. 46(1): 63-68.

Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, Tajik AJ and Seward JB 1995. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. J Cardiol. 26(6): 357-366.

Tei C, Nishimura RA, Seward JB and Tajik AJ 1997. Noninvasive Doppler-derived myocardial performance index: correlation with simultaneous measurements of cardiac catheterization measurements. J Am Soc Echocardiogr. 10(2): 169-178.

Teichholz LE, Kreulen T, Herman MV and Gorlin R 1976. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol. 37(1): 7-11.

Teshima K, Asano K, Iwanaga K, Koie H, Uechi M, Kato Y, Kutara K, Edamura K, Hasegawa A and Tanaka S 2006. Evaluation of right ventricular Tei index (index of myocardial performance) in healthy dogs and dogs with tricuspid regurgitation. J Vet Med Sci. 68(12): 1307-1313.

Teshima K, Asano K, Iwanaga K, Koie H, Uechi M, Kato Y, Kutara K, Kanno N, Seki M, Edamura K, Hasegawa A and Tanaka S 2007. Evaluation of left ventricular Tei index (index of myocardial performance) in healthy dogs and dogs with mitral regurgitation. J Vet Med Sci. 69(2): 117-123.

Thomsen MB, Verduyn SC, Stengl M, Beekman JD, de Pater G, van Opstal J, Volders PG and Vos MA 2004. Increased short-term variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs. Circulation. 110(16): 2453-2459.

Tidholm A, Haggstrom J and Jonsson L 1998. Prevalence of attenuated wavy fibers in myocardium of dogs with dilated cardiomyopathy. J Am Vet Med Assoc. 212(11): 1732-1734.

Trivier JM, Libersa C, Belloc C and Lhermitte M 1993. Amiodarone N-deethylation in human liver microsomes: involvement of cytochrome P450 3A enzymes (first report). Life Sci. 52(10): PL91-96.

Trobaugh GB, Kudenchuk PJ, Greene HL, Tutt RC, Kingston E, Gorham JR, Gross BW, Graham EL, Sears GK and Werner JA 1984. Effect of amiodarone on ventricular function as measured by gated radionuclide angiography. Am J Cardiol. 54(10): 1263-1266.

Tsai CH, Su SF, Chou TF and Lee TM 2002. Differential effects of sarcolemmal and mitochondrial K(ATP) channels activated by 17 beta-estradiol on reperfusion arrhythmias and infarct sizes in canine hearts. J Pharmacol Exp Ther. 301(1): 234-240.

U.S. Food and Drug Administration 2011. Amiodarone hydrochloride marketed as Cordarone and Pacerone) Information.

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 Erven L and Schalij MJ 2010. Amiodarone: an effective antiarrhythmic drug with unusual side effects. Heart. 96(19): 1593-1600.

Van Herendael H and Dorian P 2010. Amiodarone for the treatment and prevention of ventricular fibrillation and ventricular tachycardia. Vasc Health Risk Manag. 6: 465-472.

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.

Vollmar AC 2000. The prevalence of cardiomyopathy in the Irish wolfhound: a clinical study of 500 dogs. J Am Anim Hosp Assoc. 36(2): 125-132.

Wadden TA, Mason G, Foster GD, Stunkard AJ and Prange AJ 1990. Effects of a very low calorie diet on weight, thyroid hormones and mood. Int J Obes. 14(3): 249-258.

Waldo AL, Camm AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt B, Pratt CM, Schwartz PJ and Veltri EP 1996. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival With Oral d-Sotalol. Lancet. 348(9019): 7-12.

Walsh RA and Horwitz LD 1979. Adverse hemodynamic effects of intravenous disopyramide compared with quinidine in conscious dogs. Circulation. 60(5): 1053-1058.

Watanabe H and Chiba S 1982. Cardiovascular effects of quinidine and procainamide on intact dogs and isolated cross-perfused canine atria. J Cardiovasc Pharmacol. 4(2): 226-231.

Watanabe Y and Kimura J 2000. Inhibitory effect of amiodarone on Na(+)/Ca(2+) exchange current in guinea-pig cardiac myocytes. Br J Pharmacol. 131(1): 80-84.

Wess G, Schulze A, Butz V, Simak J, Killich M, Keller LJ, Maeurer J and Hartmann K 2010. Prevalence of dilated cardiomyopathy in Doberman Pinschers in various age groups. J Vet Intern Med. 24(3): 533-538.

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.

Wit AL and Cranefield PF 1978. Reentrant excitation as a cause of cardiac arrhythmias. Am J Physiol. 235(1): H1-17.

Wolff J 1969. Iodide goiter and the pharmacologic effects of excess iodide. Am J Med. 47(1): 101-124.

Wolff J and Chaikoff IL 1948. The inhibitory action of iodide upon organic binding of iodine by the normal thyroid gland. J Biol Chem. 172(2): 855.

Wu Y, Labeit S, Lewinter MM and Granzier H 2002. Titin: an endosarcomeric protein that modulates myocardial stiffness in DCM. J Card Fail. 8(6 Suppl): S276-286.

Zhou SX, Fang C, Zheng SX, Zhang YL, Lei J and Wang JF 2012. Effect of amiodarone on dispersion of ventricular repolarization in a canine congestive heart failure model. Clin Exp Pharmacol Physiol. 39(3): 241-246.

APPENDIX

Table i Drugs and dosage which dogs received before initiation of study. Loading and maintenance doseages of amiodarone were showed as below.

	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
Amiodarone (L)	5.9	6.4	12.9	11.1	10.8	10.1	10.5	10.6
Amiodarone (M)	5.9	6.4	6.5	6.7	5.4	5.0	5.3	5.3
Digoxin	0.003	-	0.004	0.002	-	-	-	-
Doxycycline	-	10.6		8.9	-	12.6	-	-
Enalapril	0.3	0.2	0.6	0.9	0.7	1.0	0.5	0.7
Fish oil	+	-	-	-	-	+	-	-
Furosemide	3.5	3.4	1.3	3.6	-	3.0	-	0.7
Moduratic	0.7	-	-	-	-	-	-	-
Pimobendan	0.4	-	0.4	-	0.2	-	0.3	-
Samarin	-	-	-	-	+	-	-	-
SAME	-	-	-	-	+	-	-	-
Ursolin	-	-	-	-	+	-	-	-

Dosages were showed in table as mg/kg/day.

(-) = The drug had not administration.

(+) = The drug had administration during the study.

Amiodarone (L) = loading dose of amiodarone, twice a day.

Amiodarone (M) = maintenance dose of amiodarone, once a day.

Table ii Overview results of 24-hour Holter monitoring.

No.	Total beats		Normal beats		Total arrhythmic count	
	Pre	Post	Pre	Post	Pre	Post
1	178154	179881	152166 (85.41%)	168989 (93.94%)	25924 (14.55%)	10885 (6.05%)
2	NA	NA	NA	NA	NA	NA
3	251051	239697	250021 (99.59%)	234431 (97.80%)	1030 (0.41%)	1655 (0.69%)
4	192865	166367	178129 (92.36%)	153774 (92.43%)	14731 (7.64%)	11202 (6.73%)
5	161322	140249	157711 (97.76%)	139863 (99.72%)	3603 (2.23%)	379 (0.27%)
6	192339	154448	163583 (85.05%)	134362 (86.99%)	28289 (14.71%)	19863 (12.86%)
7	152245	131587	126683 (83.21%)	115873 (88.06%)	24029 (15.78%)	14398 (10.94%)
8	160982	164281	150212 (93.31%)	163694 (99.64%)	10766 (6.69%)	584 (0.36%)
Means	184137	168073	168358	158712	15482	8424
SE	12659	13432	14823	14369	4137	2896

NA = Not available due to data dose not complete 24 hours.

Percentage of each value was compared the value with total beats.

Table iii Information of heart rate from calclualtion of SCM-510w program.

	Max HR		Mean HR		Min HR	
No.	Pre	Post	Pre	Post	Pre	Post
1	220	241	127	128	78	87
2	185	156	152	138	122	108
3	256	300	181	172	113	81
4	205	243	137	118	96	65
5	257	231	119	103	59	54
6	184	172	137	110	84	55
7	227	212	117	106	51	47
8	179	217	114	118	75	70
Means	214	222	136	124	85	71
SE	11	16	8	8	9	7

Table iv Types of arrhythmia.

No.	VPCs		SVPCs	
	Pre	Post	Pre	Post
1	25924 (14.5%)	10873 (6.0%)	0	0
2	NA	NA	NA	NA
3	1030 (0.4%)	1654 (0.7%)	0	0
4	14726 (7.6%)	11212 (6.7%)	0	0
5	1155 (0.7%)	259 (0.2%)	3039 (1.8%)	116 (0.08%)
6	28289 (14.7%)	19860 (12.8%)	0	0
7	23782 (15.6%)	14315 (10.8%)	247 (0.16%)	83 (0.06%)
8	7733 (4.8%)	571 (0.34%)	105 (0.06%)	13 (0.008%)
Means	14663	8392	484	30
SE	4397	2901	427	18

NA = Not available due to data does not complete 24 hours.

Percentage of each arrhythmia type was compared the value with total beats.

Table v Types of ventricular arrhythmia.

No.	V-run		V-couplet		RonT (250 ms)	
	Pre	Post	Pre	Post	Pre	Post
1	206 (0.12%)	232 (0.13%)	2042 (1.14%)	280 (0.15%)	607 (0.34 %)	195 (0.1%)
2	NA	NA	NA	NA	NA	NA
3	7 (0.003%)	40 (0.016%)	19 (0.007%)	47 (0.02%)	91 (0.04%)	466 (0.19%)
4	40 (0.02%)	51 (0.03%)	515 (0.27%)	687 (0.41%)	458 (0.24%)	50 (0.03%)
5	1 (0.0006%)	0	3 (0.002%)	0	686 (0.42%)	50 (0.04%)
6	14 (0.007%)	3 (0.002%)	250 (0.13%)	33 (0.21%)	34 (0.02%)	0
7	444 (0.29%)	109 (0.08%)	2714 (1.78%)	904 (0.68%)	43 (0.03%)	51 (0.04%)
8	20 (0.01%)	0	73 (0.04%)	3 (0.002%)	6 (0.004%)	3 (0.002%)
Means	105	62	802	279	275	116
SE	63	32	419	140	112	63

NA = Not available due to data does not complete 24 hours.

Percentage of each arrhythmia type was compared the value with total beats.

Table v Type of ventricular arrhythmia (continue).

No.	Bigeminy		Trigeminy		V-single	
	Pre	Post	Pre	Post	Pre	Post
1	1263 (0.71%)	230 (0.13%)	289 (0.16%)	122 (0.07%)	14864 (8.3%)	7016 (3.9%)
2	NA	NA	NA	NA	NA	NA
3	0	4 (0.002%)	4 (0.002%)	0	946 (0.37%)	1333 (0.56%)
4	31 (0.016%)	12 (0.007%)	609 (0.31%)	129 (0.08%)	11096 (5.7%)	8988 (5.4%)
5	2 (0.001%)	1 (0.0007%)	24 (0.015%)	0	1059 (0.65%)	256 (0.18%)
6	9 (0.005%)	2 (0.001%)	782 (0.41%)	33 (0.02%)	25080 (13.04%)	19658 (12.73%)
7	327 (0.2%)	530 (0.4%)	671 (0.44%)	347 (0.26%)	8098 (5.32%)	9005 (6.84%)
8	47 (0.29%)	0	89 (0.06%)	0	6955 (4.32%)	565 (0.34%)
Means	240	111	353	90	9728	6689
SE	176	77	125	48	3187	2612

NA = Not available due to data does not complete 24 hours.

Percentage of each arrhythmia type was compared the value with total beats.

Table vi Duration of P wave, PR interval and QRS complex of ECG from Holter.

	P duration (ms)		PR interval (ms)		QRS duration (ms)	
No.	Pre	Post	Pre	Post	Pre	Post
1	NA	NA	NA	NA	68.551	90.884
2	109.544	76.667	294.882	208.750	74.827	77.778
3	NA	NA	NA	NA	59.167	61.264
4	69.295	55.694	231.674	232.222	66.528	83.472
5	64.192	51.667	161.863	147.500	51.237	65.972
6	80.258	84.951	239.056	255.837	76.538	84.529
7	54.885	44.939	161.351	145.344	70.402	78.543
8	48.101	67.959	145.288	118.475	72.011	77.519
Means	71.046	63.646	205.686	184.688	67.408	77.495
SE	8.953	6.321	23.999	22.521	2.988	3.438

NA = Not available due to atrial fibrillation.

Table vii QT interval and corrtected QT by Van de Water formula.

	QT (ms)		QTc (VdW)	
No.	Pre	Post	Pre	Post
1	211.087	229.388	256.675	279.964
2	250.553	247.165	300.201	296.001
3	194.792	197.856	251.864	255.392
4	214.108	243.638	268.280	290.850
5	201.754	200.972	244.326	243.660
6	205.342	258.228	254.178	305.324
7	223.491	248.051	259.103	277.167
8	221.429	218.487	261.101	265.815
Means	215.319	230.473	261.966	276.772
SE	6.080	8.029	5.995	7.401

Table viii Information of transmural dispersion of ventricular repolarization and short term beat-to-beat variability of ventricular repolarization (STV), data were obtained from Holter monitoring.

	|Tp-Te|		|Tp-Te|/QT		|Tp-Te|/QTc		STV	
No.	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	35.266	47.951	0.167	0.209	NA	NA	0.138	0.172
2	41.262	38.690	0.165	0.157	4.686	5.407	0.138	0.131
3	35.417	37.082	0.182	0.187	NA	NA	0.140	0.145
4	26.997	30.278	0.126	0.125	4.549	9.198	0.101	0.104
5	33.941	32.813	0.168	0.164	3.726	4.219	0.139	0.135
6	40.595	48.900	0.198	0.190	7.465	4.476	0.160	0.160
7	40.374	55.108	0.180	0.222	7.458	12.664	0.156	0.199
8	41.637	41.637	0.188	0.190	6.418	7.779	0.159	0.157
Means	36.936	41.557	0.172	0.185	5.717	7.290	0.141	0.150
SE	1.787	3.019	0.007	0.011	0.658	1.337	0.007	0.010

NA = Not available due to atrial fibrillation.

Table ix Echocardiogram results, systolic functions.

	PEP (ms)		ET (ms)		PEP/ET	
No.	Pre	Post	Pre	Post	Pre	Post
1	NA	NA	249.40	207.60	NA	NA
2	59.60	97.40	231.49	198.60	0.26	0.49
3	51.20	74.40	170.20	157.60	0.30	0.54
4	59.20	75.20	200.00	134.40	0.30	0.56
5	65.20	77.40	156.20	147.60	0.44	0.52
6	74.20	72.60	197.60	252.20	0.38	0.30
7	52.60	68.60	325.80	247.80	0.16	0.28
8	45.20	48.40	124.20	140.80	0.36	0.34
Means	58.17	73.43	206.86	185.82	0.31	0.44
SE	3.64	5.44	22.135	16.80	0.03	0.05

NA = Not available due to lack of ECGs.

Table ix Echocardiogram results, systolic functions (continue).

No.	EF (%)		FS (%)	
	Pre	Post	Pre	Post
1	56.159	55.456	29.866	29.641
2	60.325	70.444	32.389	40.664
3	56.872	74.513	29.731	43.167
4	70.556	72.603	40.382	42.506
5	50.778	54.858	25.602	28.248
6	71.625	72.755	41.140	42.165
7	48.208	60.066	24.119	31.464
8	78.010	75.564	45.917	43.913
Means	61.567	67.032	33.643	37.721
SE	3.780	3.091	2.799	2.366

Table x Doppler echocardiogram results, IVCT, IVRT and overall assessment.

	IVCT (ms)		IVRT (ms)		Tei index	
No.	Pre	Post	Pre	Post	Pre	Post
1	26.72	31.89	27.58	24.14	0.23	0.27
2	31.03	30.17	18.97	22.41	0.29	0.24
3	14.80	58.80	17.20	22.40	0.21	0.46
4	34.00	63.40	29.80	24.20	0.36	0.43
5	17.20	29.40	23.40	27.20	0.19	0.24
6	37.20	64.00	17.80	34.20	0.31	0.57
7	28.80	26.00	45.40	32.60	0.35	0.31
8	31.60	35.60	24.60	45.00	0.28	0.39
Means	27.67	42.41	25.59	29.02	0.28	0.36
SE	2.79	5.86	3.26	2.78	0.02	0.04

Table xi Echocardiogram results, left ventricular geometry.

	IVSd (cm)		LVIDd (cm)		LVPWd (cm)	
No.	Pre	Post	Pre	Post	Pre	Post
1	0.838	1.008	5.980	6.680	1.036	0.804
2	0.804	0.736	5.156	6.202	0.868	1.010
3	0.914	0.610	4.608	4.420	0.656	0.716
4	0.830	0.724	5.448	6.192	0.670	0.916
5	0.448	1.080	4.234	4.326	0.968	0.736
6	0.968	0.792	5.192	5.284	0.752	0.686
7	0.920	1.182	4.428	3.852	0.884	0.918
8	0.954	0.858	3.772	4.140	0.640	0.602
Means	0.835	0.874	4.852	5.137	0.809	0.799
SE	0.059	0.070	0.254	0.389	0.054	0.049

Table xi Echocardiogram results, left ventricular geometry (continue).

	IVSs (cm)		LVIDs (cm)		LVPWs (cm)	
No.	Pre	Post	Pre	Post	Pre	Post
1	1.460	1.400	4.194	4.700	1.184	0.840
2	0.942	1.342	3.486	3.680	1.278	1.738
3	0.864	1.090	3.238	2.512	1.006	1.018
4	0.980	0.896	3.248	3.560	1.170	1.430
5	0.598	1.344	3.150	3.104	1.116	0.976
6	1.298	1.222	3.056	3.056	1.108	0.996
7	0.924	1.876	3.360	2.640	1.242	1.398
8	1.402	1.330	2.040	2.322	0.868	1.152
Means	1.059	1.313	3.222	3.197	1.122	1.194
SE	0.106	0.100	0.210	0.274	0.047	0.107

Table xii Echocardiogram results, cardiac geometry.

	Ao (cm)		LA (cm)		La/Ao	
No.	Pre	Post	Pre	Post	Pre	Post
1	2.054	1.960	4.168	4.012	2.048	2.062
2	1.404	2.140	4.290	3.512	3.071	1.646
3	1.468	1.464	3.776	4.200	2.582	2.871
4	2.112	1.504	4.160	5.404	1.984	3.618
5	2.386	2.126	2.000	2.688	0.838	1.269
6	2.118	2.342	3.654	4.816	1.782	2.059
7	2.314	2.400	2.964	2.334	1.285	0.973
8	1.872	1.836	2.832	3.006	1.516	1.452
Means	1.966	1.972	3.481	3.747	1.888	1.994
SE	0.128	0.124	0.286	0.375	0.251	0.310

Table xiii Data of end diastolic volume, end systolic volume and stroke volume.

	EDV (ml)		ESV (ml)		SV (ml)	
No.	Pre	Post	Pre	Post	Pre	Post
1	178.631	229.796	78.313	102.361	100.318	127.435
2	126.983	194.131	50.380	57.377	76.603	136.754
3	97.733	88.630	42.151	22.589	55.582	66.041
4	144.228	193.418	42.467	52.991	101.761	140.427
5	80.089	84.256	39.422	38.035	40.668	46.221
6	129.046	134.400	36.617	36.617	92.429	97.783
7	89.008	63.994	46.099	25.555	42.908	38.439
8	60.868	75.949	13.385	18.559	47.483	57.390
Means	113.323	133.072	43.604	44.261	69.719	88.811
SE	13.591	22.803	6.324	9.637	9.242	14.876

Table xiv Thyroid profiles of dogs, before and after 60 days amiodarone treatment.

	tT3 (ng/dL)		tT4 (µg/dL)	
No.	Pre	Post	Pre	Post
1	52.00	28.90	1.00	0.54
2	13.80	0.00	0.64	0.02
3	NA	NA	NA	NA
4	43.60	44.30	1.20	2.10
5	64.80	53.80	1.70	2.30
6	24.90	14.20	1.80	1.50
7	46.10	99.20	0.21	0.71
8	36.10	151.00	0.01	1.20
Means	40.19	55.91	1.20	1.20
SE	6.43	19.91	0.32	0.32

NA = Not available due to not enough sample.

Table xv Correlation between Holter, echocardiographic parameters and thyroid hormones, before amiodarone administration.

	TB	NB	TAC	VPCs	P dur	PR int	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
Mean HR	0.990	0.959	-0.399	-0.306	0.943	0.998	-0.104	-0.146	0.217	-0.253	-0.002	-0.349	0.182	-0.053	-0.081	-0.570	-0.240	-0.700	0.299	0.397
	1.76E-05	0.0006	0.376	0.504	0.005	0.000004	0.806	0.730	0.605	0.629	0.997	0.396	0.666	0.902	0.849	0.140	0.568	0.080	0.515	0.436
	7	7	7	7	6	6	8	8	8	6	7	8	8	8	8	8	8	7	7	6
TB		0.964	-0.384	-0.295	0.826	0.955	-0.189	-0.732	-0.092	-0.073	-0.004	-0.362	0.243	0.074	0.049	-0.606	-0.270	-0.447	0.612	0.556
		0.0005	0.395	0.521	0.085	0.011	0.685	0.061	0.845	0.907	0.995	0.425	0.600	0.875	0.917	0.150	0.558	0.375	0.197	0.330
		7	7	7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
NB			-0.615	-0.537	0.629	0.700	-0.388	-0.767	-0.179	-0.542	-0.107	-0.520	0.058	0.043	0.016	-0.635	-0.393	-0.138	0.639	0.164
			0.142	0.214	0.255	0.188	0.389	0.044	0.701	0.345	0.839	0.232	0.901	0.927	0.973	0.126	0.383	0.794	0.172	0.792
			7	7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
TAC				0.994	0.413	0.517	0.785	0.476	0.343	0.842	0.418	0.739	0.545	0.085	0.107	0.383	0.548	-0.536	0.009	0.730
				5.26E-06	0.490	0.372	0.037	0.280	0.452	0.073	0.410	0.058	0.206	0.857	0.819	0.397	0.203	0.273	0.987	0.162
				7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
VPCs					0.444	0.563	0.778	0.429	0.362	0.801	0.420	0.717	0.587	0.063	0.084	0.373	0.559	-0.519	0.032	0.757
					0.454	0.323	0.040	0.337	0.425	0.103	0.407	0.070	0.166	0.893	0.858	0.410	0.192	0.292	0.952	0.138
					5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
P dur						0.946	0.326	0.567	0.749	-0.295	0.492	0.225	0.530	-0.044	-0.065	-0.555	-0.004	-0.685	0.287	0.137
						0.004	0.529	0.240	0.087	0.570	0.321	0.669	0.279	0.934	0.903	0.253	0.995	0.133	0.581	0.796
						6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
PR int							0.460	0.514	0.756	-0.203	0.480	0.472	0.762	0.149	0.132	-0.491	0.271	-0.741	0.287	0.417
							0.359	0.297	0.082	0.699	0.335	0.345	0.078	0.778	0.803	0.322	0.603	0.092	0.581	0.411
							6	6	6	6	6	6	6	6	6	6	6	6	6	6
QRS								0.597	0.555	0.675	0.044	0.862	0.432	0.502	0.511	0.069	0.646	-0.845	-0.381	0.785
								0.118	0.153	0.141	0.925	0.006	0.285	0.205	0.196	0.871	0.084	0.017	0.400	0.064
								8	8	6	7	8	8	8	8	8	8	7	7	6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

Table xv Correlation between Holter, echocardiographic parameters and thyroid hormones, before amiodarone administration (continue).

	TB	NB	TAC	VPCs	P dur	PR int	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
QT									0.910	-0.049	-0.176	0.484	0.056	0.079	0.079	0.170	0.436	-0.687	-0.616	0.242
									0.002	0.927	0.706	0.224	0.895	0.852	0.852	0.687	0.280	0.088	0.141	0.644
									8	6	7	8	8	8	8	8	8	7	7	6
QTcW										-0.200	-0.086	0.435	0.283	0.174	0.164	-0.100	0.396	-0.759	-0.370	0.361
										0.704	0.855	0.281	0.497	0.680	0.698	0.815	0.331	0.048	0.414	0.482
										6	7	8	8	8	8	8	8	7	7	6
STV											-0.092	0.566	-0.043	0.186	0.202	0.324	0.473	-0.315	-0.316	0.521
											0.862	0.242	0.936	0.724	0.701	0.531	0.343	0.543	0.542	0.290
											6	6	6	6	6	6	6	6	6	6
PEP												0.250	0.514	-0.020	-0.021	-0.322	0.026	-0.082	0.928	-0.052
												0.589	0.238	0.966	0.965	0.482	0.955	0.878	0.008	0.922
												7	7	7	7	7	7	6	6	6
IVCT													0.542	0.638	0.653	0.153	0.810	-0.751	-0.136	0.929
													0.165	0.089	0.079	0.718	0.015	0.052	0.771	0.007
													8	8	8	8	8	7	7	6
SV														0.365	0.368	-0.210	0.330	-0.299	0.401	0.697
														0.373	0.370	0.618	0.425	0.515	0.373	0.124
														8	8	8	8	7	7	6
EF (%)															0.999	-0.378	0.361	-0.488	-0.057	0.485
															4.92E-10	0.355	0.380	0.267	0.903	0.330
															8	8	8	7	7	6
FS (%)																-0.352	0.377	-0.476	-0.0636	0.493
																0.393	0.357	0.280	0.892	0.321
																8	8	7	7	6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

Table xv Correlation between Holter, echocardiographic parameters and thyroid hormones, before amiodarone administration (continue).

	TB	NB	TAC	VPCs	P dur	PR int	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
IVRT																	0.516	0.422	-0.486	0.193
																	0.191	0.346	0.269	0.714
																	8	7	7	6
Tei																		-0.434	-0.285	0.947
																		0.330	0.535	0.004
																		7	7	6
tT3																			0.206	-0.575
																			0.658	0.233
																			7	6
tT4																				-0.209
																				0.691
																				6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

Table xvi Correlation between Holter, echocardiographic parameters and thyroid hormones, after amiodarone administration.

	TB	NB	TAC	VPCs	P dur	PR	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
Mean HR	0.986	0.965	-0.354	-0.350	0.506	0.271	-0.413	-0.399	-0.144	-0.162	0.256	0.289	0.194	0.419	0.430	-0.480	0.138	-0.392	-0.694	0.328
	4.77E-05	0.0004	0.436	0.441	0.306	0.604	0.309	0.327	0.734	0.758	0.579	0.488	0.645	0.302	0.287	0.228	0.745	0.384	0.084	0.526
	7	7	7	7	6	6	8	8	8	6	7	8	8	8	8	8	8	7	7	6
TB		0.981	-0.355	-0.351	0.529	0.348	-0.370	-0.505	-0.214	-0.216	0.093	0.469	0.204	0.439	0.458	-0.431	0.326	-0.208	-0.194	0.553
		9.28E-05	0.434	0.440	0.359	0.566	0.414	0.247	0.645	0.727	0.861	0.289	0.660	0.325	0.302	0.334	0.476	0.693	0.712	0.333
		7	7	7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
NB			-0.528	-0.524	0.297	-0.073	-0.467	-0.651	-0.376	-0.303	0.023	0.364	0.109	0.400	0.416	-0.380	0.212	0.032	-0.053	0.119
			0.223	0.227	0.627	0.907	0.290	0.113	0.406	0.620	0.965	0.422	0.816	0.374	0.353	0.400	0.648	0.953	0.921	0.849
			7	7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
TAC				1.000	0.388	0.747	0.701	0.937	0.905	0.202	0.257	0.291	0.422	-0.020	-0.007	-0.029	0.404	-0.562	-0.316	0.673
				2.93E-12	0.578	0.147	0.079	0.002	0.005	0.744	0.623	0.526	0.345	0.966	0.989	0.951	0.369	0.245	0.542	0.213
				7	5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
VPCs					0.341	0.749	0.703	0.937	0.907	0.202	0.255	0.295	0.426	-0.016	-0.002	-0.029	0.407	-0.562	-0.317	0.676
					0.574	0.146	0.078	0.002	0.005	0.745	0.625	0.520	0.341	0.973	0.996	0.951	0.364	0.246	0.541	0.210
					5	5	7	7	7	5	6	7	7	7	7	7	7	6	6	5
P dur						0.550	0.439	0.403	0.623	-0.636	0.176	0.418	0.489	0.679	0.690	0.135	0.493	-0.436	-0.303	0.326
						0.259	0.383	0.429	0.186	0.175	0.738	0.409	0.325	0.138	0.129	0.799	0.320	0.387	0.559	0.529
						6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
PR							0.647	0.702	0.820	-0.315	0.529	0.790	0.789	0.401	0.441	-0.504	0.573	-0.825	0.060	0.855
							0.165	0.120	0.046	0.543	0.280	0.061	0.062	0.430	0.381	0.308	0.234	0.043	0.911	0.030
							6	6	6	6	6	6	6	6	6	6	6	6	6	6
QRS								0.745	0.745	0.297	-0.074	-0.0004	0.597	-0.115	-0.073	0.136	0.050	-0.273	-0.381	0.850
								0.034	0.034	0.568	0.875	0.999	0.118	0.785	0.864	0.748	0.906	0.554	0.399	0.032
								8	8	6	7	8	8	8	8	8	8	7	7	6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

Table xvi Correlation between Holter, echocardiographic parameters and thyroid hormones, after amiodarone administration (continue).

	TB	NB	TAC	VPCs	P dur	PR	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
QT									0.934	0.267	0.220	0.123	0.475	0.138	0.152	0.093	0.210	-0.401	-0.397	0.743
									0.0007	0.610	0.635	0.771	0.234	0.744	0.719	0.827	0.617	0.373	0.378	0.091
									8	6	7	8	8	8	8	8	8	7	7	6
QTcW										0.024	0.299	0.326	0.689	0.326	0.359	-0.030	0.343	-0.533	-0.389	0.831
										0.964	0.515	0.431	0.059	0.431	0.383	0.944	0.406	0.218	0.389	0.041
										6	7	8	8	8	8	8	8	7	7	6
STV											-0.348	-0.171	-0.231	-0.057	-0.097	0.157	-0.061	0.528	-0.208	0.111
											0.500	0.745	0.659	0.914	0.856	0.766	0.909	0.282	0.693	0.834
											6	6	6	6	6	6	6	6	6	6
PEP												-0.040	0.568	-0.194	-0.150	-0.847	-0.353	-0.884	-0.297	0.256
												0.932	0.183	0.676	0.748	0.016	0.437	0.019	0.567	0.624
												7	7	7	7	7	7	6	6	6
IVCT													0.309	0.649	0.668	-0.120	0.890	-0.291	0.492	0.833
													0.457	0.082	0.070	0.777	0.003	0.527	0.262	0.039
													8	8	8	8	8	7	7	6
SV														0.189	0.262	-0.502	0.028	-0.697	-0.248	0.819
														0.654	0.531	0.205	0.947	0.082	0.592	0.046
														8	8	8	8	7	7	6
EF (%)															0.997	0.251	0.688	0.126	0.008	0.604
															8.94E-08	0.549	0.059	0.787	0.987	0.204
															8	8	8	7	7	6
FS (%)																0.211	0.683	0.070	0.004	0.631
																0.616	0.062	0.882	0.994	0.179
																8	8	7	7	6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

Table xvi Correlation between Holter, echocardiographic parameters and thyroid hormones, after amiodarone administration (continue).

	TB	NB	TAC	VPCs	P dur	PR	QRS	QT	QTcW	STV	PEP	IVCT	SV	EF (%)	FS (%)	IVRT	Tei	tT3	tT4	QTn
IVRT																	0.273	0.800	0.113	-0.357
																	0.513	0.031	0.810	0.487
																	8	7	7	6
Tei																		0.002	0.363	0.619
																		0.996	0.424	0.190
																		7	7	6
tT3																			0.141	-0.492
																			0.762	0.322
																			7	6
tT4																				0.049
																				0.927
																				6

TB = Total beats, NB = Normal beat, TAC= Total arrhythmic beat, VPCs = Ventricular premature complex.

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