Studies on Myocardial Funny Channels and the Funny Current Inhibitor Ivabradine in Healthy Cats and Cats with Hypertrophic Cardiomyopathy

DISSERTATION

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Abstract

Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats and is associated with high morbidity and mortality. Evidence suggests that tachycardic events may trigger syncope, decompensation, or sudden death in otherwise well compensated human patients with HCM. Bradycardic agents used in feline HCM include beta-receptor antagonists or calcium channel blockers; hoverer, their clinical use may be limited by potential adverse effects. A drug that selectively and specifically lowers heart rate (HR), without intrinsic effects on cardiac function, may therefore be superior in the long-term treatment of feline HCM. Ivabradine is a highly selective funny current (I_f) inhibitor that acts directly on the sinoatrial node to induce a use- and dose-dependent reduction of HR. Ivabradine has been shown to have favorable effects on cardiac function in experimental animals but its use has not yet been studied in cats.

The first study describes the pharmacokinetics of ivabradine and its major metabolite S-18982 after single and repeated oral administration of ivabradine (Procorolan®, Les Laboratoires Servier, France) in healthy cats. Two-compartmental and one-compartmental models with first-order input and elimination provided the best fit to the data for both ivabradine and S-18982.The two models were combined to produce a single 4-compartment model characterizing pharmacokinetics of ivabradine and S-18982.

The second study describes the use of immunoblot analysis to evaluate myocardial expression of hyperpolarization-activated, cyclic nucleotide-gated (HCN) proteins in cats. In cats with HCM, HCN4 was significantly upregulated in left ventricular (LV) myocardial tissue whereas in right ventricular tissue only a trend was found. We also observed that maturation of ventricular cardiomyocytes toward the adult phenotype regarding HCN4 expression is completed prior to the age of 2-3 months.

The results of the third catheter-based study demonstrate that intravenous administration of ivabradine consistently reduces HR and the rate-pressure product in anesthetized cats with HCM. LV systolic and diastolic function as well as left atrial (LA) performance were either unchanged or only minimally affected by ivabradine. Elevation of HR induced by catecholamines was significantly blunted by ivabradine in healthy cats as well as in cats with HCM.

In the last two experiments, the non-inferiority of four weeks of oral ivabradine compared to atenolol and the effects of both drugs on reproducibility of echocardiographically-derived indices are described. Ivabradine demonstrated more favorable effects on several echocardiographic variables, including indices of LV systolic wall stress, LV relaxation, and LA function. Overall, the majority of echocardiographic variables obtained had excellent to good reproducibility justifying their use in the clinical setting. However, although unexpected, both drugs failed to increase reproducibility of the majority of echocardiographically-derived indices. Ivabradine was specifically useful in the separation of diastolic filling waves, making assessment of LV diastolic function clinically feasible.

Based on these studies, oral ivabradine appears to be clinically well tolerated, safe and useful for effective HR control in cats. This may make ivabradine attractive in the treatment of feline HCM, although its long-term effects in cats with HCM and in particular in cats with dynamic LV outflow obstruction require further investigation.

Dedication

This document is dedicated to my family and friends.

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Fields of Study

Major Field: Veterinary Clinical Science

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INTRODUCTION

Feline Cardiomyopathy: Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats and is associated with significant mortality.[1-3] The development of HCM in most cats is likely due to a genetic mutation of one or more sarcomeric proteins that results in symmetric or regional concentric hypertrophy of the left ventricle (LV) and rarely, the right ventricle, or the papillary muscles only.[4-7] Histomorphologic hallmarks of the disease include cardiomyocyte hypertrophy and myofiber disarray; myocardial ischemia and necrosis; and interstitial and replacement fibrosis.[2, 8] Left ventricular hypertrophy also results in a reduction of the LV chamber size, impaired ventricular relaxation, and increased wall stiffness all of which impede diastolic filling. As a consequence, LV filling pressures increase contributing to left atrial (LA) enlargement, arrhythmogenesis, congestion, blood stasis, and finally congestive heart failure (CHF), sudden death, or arterial thromboembolism. The majority of the cats with HCM develop dynamic outflow tract obstruction from hypertrophy of the interventricular septum and systolic anterior motion (SAM) of the mitral valve.[8] Presence of SAM further increases systolic LV wall stress and thus myocardial oxygen demand and may worsen LV hypertrophy, diastolic dysfunction, and LA enlargement.

Potential risk factors: The progression of HCM is variable with most cats having a long asymptomatic period that may end with the abrupt development of congestive

heart failure, arterial thromboembolism (ATE), syncope, or sudden cardiac death. Several risk factors of poor outcome have been identified including the degree of LV diastolic dysfunction, LA enlargement, and elevated heart rate (HR).[1, 3, 9, 10]

Effects of tachycardia on coronary perfusion and myocardial oxygen consumption: Studies in people indicate that tachycardia leads to chronic "stress" of the walls of the coronary arteries, which, in the long run may facilitate the development of artherosclerotic lesions.[11-14] Fast HR increases the magnitude of stretch (tensile stress) on the arterial wall, and by shortening of the diastolic phase of the cardiac cycle exposures the endothelium to oscillatory shear stress.[15, 16] In addition, high HR intensifies the pulsatile motion of the heart and, consequently, the frequency of the periodically changing geometry of the coronary vessels.[15, 16] The increase in total time spent in systole at high HR may also cause an increase in mean blood pressure within the coronary vessels since the shorter the duration of diastole, the more mean arterial pressure approaches systolic pressure.[15, 16] The blood pressure load and the hemodynamic stress are thus both proportionally greater in individuals with fast HR.[17] This will result in increased cardiac work and higher tensile stress on the arterial wall promoting vascular smooth muscle cell growth and collagen deposition. These mechanisms may facilitate the development of atherosclerosis and vascular stiffening and may result in myocardial ischemia.[18, 19]

The energy expended by the heart is used mainly to achieve isovolumetric ventricular contraction and relaxation.[20] When HR accelerates and exceeds the physiologic optimum of the force-frequency releationship, cardiac work becomes

uneconomical.[20] As a consequence, high HR may increase oxygen demand even when the external work performed by the heart is kept constant.[21] By lowering HR, myocardial oxygen demand is decreased and myocardial oxygen supply is increased, both of which may prevent the development of regional or global myocardial ischemia.

In summary, elevated HR may facilitate development of atherosclerosis and vascular stiffening, increasing oxygen consumption, and worsening coronary perfusion. Thus, tachycardia may facilitate the development of ventricular arrhythmias, progression of myocardial disease, and sudden cardiac death, an association that has been found in many epidemiologic studies in people.[22-24]

Pathogenesis of decompensation in cats with HCM: The pathogenesis of acute decompensation in clinically stable cats with HCM is not yet fully understood. However, evidence exists that stressful events such as routine examinations at a veterinary hospital, changes in the cat's home environment, pain, anesthesia, or elective surgery can trigger sudden development of CHF in previously asymptomatic animals.[3, 25] In people, physical or emotional stress may lead to unwanted tachyarrhythmias that are known triggers for acute decompensation of recently stable ischemic heart disease.[26] Thus, pharmacologic modulation of HR aimed at lowering resting HR and blunting the positive chronotropic effects induced by catecholamines may prevent sudden periods of uncontrolled tachycardia and thereby reduce the risk of acute decompensation, dynamic outflow obstruction, life-threatening arrhythmias during ischemic periods, and sudden cardiac death.[20]

Clinical management of cats with HCM: Treatment of feline HCM should be aimed at resolving all the underlying pathogenetic mechanism of the disease, such as diastolic and systolic dysfunction, dynamic outflow obstruction, ischemia, arrhythmias, neurohormonal activation, and hypercoagulability status. Beta receptor antagonists and calcium channel blockers are the most frequently used drugs in preclinical feline HCM although their general use remains controversial since administration of either drug has no proven efficacy on disease progression or survival.[27-32] The use of both drugs is based on their theoretical ability to improve ventricular diastolic function, to lower HR, or to reduce outflow tract obstruction. However, adverse effects including weakness, lethargy, salivation, weight loss, and reduced LA function may limit their clinical use.[27-32]

Role of funny channels in the heart: Under physiological conditions, HR is controlled by the sinoatrial node, the origin of pacemaker activity.[33-37] Sinoatrial myocytes have the unique capacity to generate slow diastolic depolarization, spontaneously driving the membrane voltage away from the hyperpolarized level towards the threshold level for initiating the following action potential.[33-37] The rhythmic action potentials generated in this way propagate through the conducting system of the heart and trigger myocardial contraction. Pacemaker activity involves the interplay between several ionic currents that influence spontaneous diastolic depolarization of the sinoatrial node, including the I_f-current. Heart rate in general and I_f channels in particular, are directly modulated by sympathetic activity.[33-37] The I_f-current is directly activated by intercellular cyclic adenosine monophosphate (cAMP) and is carried by the hyperpolarization-activated cyclic nucleotide-gated family (HCN) of ion channels.[33-37] The effect of cAMP

is to shift the I_f activation curve to more positive voltages and to accelerate activation and to slow deactivation kinetics.[33-37]

In ventricular myocytes, I_f is abundantly expressed during fetal and neonatal life.[38-40] At some stage of their electrophysiological maturation toward adult ventricular phenotype, these cells lose their capacity to generate spontaneous activity.[33, 38, 41] Both in mouse and rat hearts (and possibly other species), this is accompanied by a progressive decrease in I_f expression.[33, 38, 40] However, ventricular myocytes can re-express I_f during adult life under particular circumstances. A striking upregulation of I_f expression has been observed in a variety of animal models of cardiac hypertrophy and failure.[42-46] The degree of hypertrophy is correlated to the increase in I_f density,[43] and changes in expression levels are most pronounced in those cardiac regions with highest pressure load indicating that development of hypertrophy directly effects the magnitude of channel expression.[46, 47] In addition, changes in I_f density are correlated with the etiology of the disease with I_f overexpression being greater in ischemic than in idiopathic dilated cardiomyopathy.[48, 49] Moreover, voltage-dependence of I_f may also change as evident by comparing the I_f activation curve in ischemic cardiomyopathy and dilated cardiomyopathy to control hearts.[48, 49]

Expression of fetal phenotype and overexpression of I_f channels are a consequence of electrophysiological remodeling and, from a clinical point of view, may represent an arrhythmogenic substrate in heart failure, a condition associated with a high risk for sudden cardiac death.[40, 50] Thus, besides heart rate control, selective I_f -current inhibitors such as ivabradine may exert beneficial effects under those circumstances

where I_f is re-expressed in ventricular myocardium, possibly reducing the risk of sudden death.

Funny current inhibition: Ivabradine is a highly selective I_f -current inhibitor. It acts directly on the sinoatrial node to induce a rapid, sustained, use- and dose-dependent reduction of HR by reducing the slope of slow diastolic depolarization of cardiac pacemaker cells.[51-56] Ivabradine has been shown to preserve cardiac output, increase coronary blood flow, increase myocardial oxygen supply and decrease consumption, and prevent the progressive loss of coronary capillaries in failing myocardium in people and experimental animals.[57-62] Auxiliary effects may include modifications of the extracellular matrix including decreased myocardial collagen accumulation and increased LV capillary density as demonstrated in a rat model of congestive heart failure.[60] Ivabradine has also been shown in people to be non-inferior to atenolol and amlodipine for anti-anginal and anti-ischemic effects.[62-64] Due to ivabradine's unique selective heart rate reducing properties it may become a new therapeutic option for use in cats with HCM. It should prolong LV diastolic filling and coronary perfusion time, decrease myocardial oxygen consumption, and reduce myocardial ischemia and its chronic consequence, replacement fibrosis. It may also attenuate or relieve LV outflow tract obstruction due to improved LV filling making SAM less likely, and most importantly reduce tachycardia-induced crisis events.

The overall goal of this project was to evaluate the effects of ivabradine on cardiac function and HR in cats. In our first study (**Chapter 1**), we investigated the pharmacokinetic characteristics of ivabradine in healthy cats. Subsequently, we evaluated the myocardial expression of hyperpolarization-activated, cyclic nucleotide-gated (HCN) proteins, the molecular correlates of I_f channels, in healthy cats and cats with HCM (**Chapter 2**). The third study (**Chapter 3**) was designed to investigate the effects of ivabradine on HR, LV function, and LA performance by use of catheter-based and echocardiographic techniques in healthy cats and cats with HCM. Finally, our last two studies (**Chapter 4** and **5**) were devoted to the clinical use (short-term effects of oral administration) of ivabradine and its impact on the reproducibility of echocardiographic indices of left heart function in healthy cats.

We hypothesized that 1) single and repeated oral doses of ivabradine result in a plasma half-life and elimination kinetics suitable for 12 hour dosing intervals; 2) expression of myocardial HCN2 and HCN4 is significantly higher in ventricular myocardium of cats with HCM and HCN4 channel density is higher in healthy kittens as compared to adult healthy cats; 3) ivabradine exhibits only minimal effects on LV and LA function and significantly blunts the positive chronotropic response induced by catecholamine administration; 4) ivabradine is non-inferior to atenolol with regard to clinical tolerability and echocardiographic variables of LV and LA function; and 5) ivabradine and atenolol reduce observer variability and improve reproducibility of echocardiographic data in healthy cats.

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CHAPTER 1

PHARMACOKINETICS OF ORAL IVABRADINE IN HEALTHY CATS

Ivabradine belongs to a new class of heart rate-lowering agents called 'selective and specific I_f current inhibitors' that act on the cardiac pacemaker cells of the sinoatrial node[1, 2] without affecting other cardiac ion currents.[3] Ivabradine was developed for treatment of ischemic heart disease in human patients and may be of potential use in cats with hypertrophic cardiomyopathy.

The metabolism and overall disposition of ivabradine has been studied in a variety of species, including dog, rat, and human.[4, 5] Separation and detection of various ivabradine metabolites by liquid chromatography-tandem mass spectrometry (LC-MS-MS) methods were developed using human plasma, including the unsaturated compound S-33170 and O-demethylated derivatives (S-33171, S-33172, S-33173, S-33174).[6] The area under the plasma concentration–time curve (AUC) in humans was about 27% that of ivabradine, while AUCs of other metabolites were less than 5%. O-demethylated derivatives were not observed in human plasma.[6] Additionally, S-18982 is the only metabolite with observed activity.[7] Therefore, analyses were limited to ivabradine and its major metabolite (S-18982) in the present study.

Our group previously completed a study on the pharmacodynamic effects of oral ivabradine in healthy cats.[a] Findings revealed that a single oral dose of ivabradine at 0.3 mg/kg and 0.5 mg/kg predictably lowered heart rate for at least 12 hours. In order to characterize the pharmacokinetic profile of ivabradine in healthy cats, this study had the following objectives: (a) to cross-validate an analytical method for the determination of ivabradine and its major N-desmethylivabradine metabolite, S-18982, in feline plasma over a wide concentration range and (b) to establish the blood concentration-time profile of ivabradine and S-18982 after single and multiple oral administrations of ivabradine. Herein, we describe the cross-validation of an analytical assay for ivabradine quantification in feline plasma and its application in a pharmacokinetic study of oral ivabradine in cats. The data obtained was used to develop a pharmacokinetic (PK) model characterizing the combined pharmacokinetics of ivabradine and S-18982.

Material and Methods

Animals and husbandry: Eight experimental healthy adult domestic shorthair cats (all female spayed), aged 2-7 years and weighing 3.1-6.0 kg were included in the present study. Complete blood counts, serum biochemistry panels, systolic blood pressure, electrocardiogram, and echocardiography were unremarkable. Cats were housed in single cages in an American Association for the Accreditation of Laboratory Animal Care approved facility with non-restricted access to water and food during the entire study period. The study protocol was approved by the Institutional Animal Care and Use

Committee of The Ohio State University prior to initiation of the study (Protocol number 2008 A 0154).

Experimental conditions: Following a 12-hour fast, each cat was sedated with intramuscular ketamine (10 mg/kg; Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa) and midazolam (0.2 mg/kg; Midazolam, Bedford Laboratories, Bedford, OH). The ventral neck area was clipped, prepared in a sterile fashion, and a jugular vein catheter was placed (ARROW, double lumen, 4F, 8 cm length, ARROW International, Reading, PA). The first dose of ivabradine was administered after 12-hours of catheter placement. Patency of the catheter was maintained by repeated flushing (every 8 hours and following each sample collection) with 2 mL of heparinized saline.

Each cat received a single oral dose of 0.3 mg/kg ivabradine (corresponds to 1/5 to 1/3 of a 5 mg tablet of Procoralan®; Les Laboratoires Servier, Neuilly-sur-Seine, France) at baseline (time 0, Day 1), followed by twice daily dosing on Days 2 through 4. To ensure proper drug administration, the article was manually placed in the oropharynx at the base of the tongue in a consistent fashion by the principal investigator.

Sample collection: Samples were collected from the previously placed jugular vein catheters. Two and one half milliliters of whole blood was sampled into a tube containing 143 U.S.P. units of sodium heparin (MONOJECT, Tyco Healthcare Group LP, Mansfield, MA), followed by thorough mixing. Plasma samples were collected at the following time points on Day 1: baseline (time 0), 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after administration of ivabradine. Additional plasma samples were collected on Day 4 immediately before and 4, 8, 12, 24, and 36 hours after administration of ivabradine.

Samples were centrifuged at 20° C at 2000 x *g* for 10 min, and plasma was separated from the packed cells. Plasma was immediately stored at - 80° C (duration of storage 4-8 weeks) until shipment to the analyzing laboratory (AAIPharma, Neu-Ulm, Germany) on dry ice. Good shipping conditions of all samples were confirmed and documented by AAIPharma at arrival. In one cat, patency of the jugular vein catheter was lost prior to completion of the study. Therefore, the last 4 samples were collected by jugular venipuncture.

Sample preparation for analysis of ivabradine and its metabolite: Method cross-validation and sample analysis was performed as previously described in plasma from mouse, rat, rabbit, dog, and pig.[8] Cross-validation consisted of inter- and intra-day assessments of quality control samples (inter-day, 12 samples; intra-day lower limit of quantification [LLOQ], 4 samples; and inter-day LLOQ, 10 samples), and inter-day assessments of calibration standard samples (12 standard curves)[4] All compounds used for validation were of analytical quality and obtained from Technologie Servier (Servier, Neuilly-sur-Seine, France). Stock solution of ivabradine, S18982, and internal standard (IS, S-16070) were prepared separately by dissolving compounds in purified water (ivabradine and S-18982) or methanol (IS) at 1 mg/mL (expressed as free base). These stock solutions were stored at 4°C in the absence of light and were stable for at least 4 weeks. Further dilutions in methanol of these stock solutions were used to prepare working solutions for calibration standards and quality control (QC) samples in spiked matrix. Calibration standards in spiked plasma were prepared at nominal concentrations

of 0.250, 1.00, 2.50, 10.0, 25.0, 100, and 250 ng/mL and QC samples at nominal concentrations of 0.250, 0.750, 20.0, 200, and 1 000 ng/mL.

Extraction method and quantification by HPLC-MS: Plasma samples were thawed at room temperature for 10 to 15 min, vortexed for 15 sec, then centrifuged at 5°C ± 3°C for 15 min at 3 000 rpm. Each aliquot of plasma (50 µL) was transferred to a glass tube, and 25 µL of a 50 ng/mL solution of IS and 200 µL of 0.1N HCl were added. The sample was vortex-mixed for 1 min and slowly applied to Oasis MCX cartridges (1 cc, 30 mg; Waters GmbH, Eschborn, Germany) previously solvated with 0.5 mL of methanol and conditioned with 0.5 mL of 0.01N HCl, with centrifugation at 500 rpm for 1 min. After washing with 0.5 mL of 0.01N HCl and 0.5 mL MeOH, the analytes were eluted with 2 x 0.5 mL of methanol/ammonium hydroxide (25%) 25/1.3 (v/v) during additional centrifugation steps. The eluate was then evaporated to dryness under a stream of nitrogen at 40°C, and the residue reconstituted with 100 µL of mobile phase. The extract was then transferred to an appropriate autosampler vial for analysis.

Reconstituted samples were analyzed on an LC-10Advp 1100 HPLC system (Shimadzu GmbH, Dusiburg, Germany) connected to an API 365 mass spectrometer (SpectralLab Scientific Inc., Toronto, Canada) operated by Analyst software Version 1.4.2 (PerkinElmer Sciex, Inc.**,** Rodgau, Germany). The HPLC system comprised an L-7612 degasser (Merck, Reinbek Hamburg, Germany), Column oven Jetstream II Plus (Merck, Reinbek Hamburg, Germany) and AOC 5000 autosampler (Shimadzu GmbH, Dusiburg, Germany). Samples (20µL injections) were separated on a reversed phase Kromasil 100 C18 (150 mm x 2 mm, 5 µm) with a Phenomenex C18 pre-column (4 x 2

mm; Phenomex Inc., Aschaffenburg, Germany). Eluents were 630 mg of formic acid ammonium salt, 500 g of water, 300 g of methanol, 335 µL of trifluoroacetic acid. The flow rate remained constant at 0.2 ml/min throughout the run.

Ivabradine, S-18982 and IS were ionized via electrospray ionization (ESI) and fragmented with collision gas for analysis using multiple reaction monitoring (MRM) in positive-ion mode. Parameters were adjusted to optimize fragment ion intensities, and proposed reaction mechanisms and fragment ion structures were generated with Analyst Version 1.4.2. Instrument settings were as follows: Nebulizer gas 8 instrument units, curtain gas 8 instrument units, collision gas 4 instrument units, turbo ion spray gas 8 L/min, ion spray voltage 5000 V. Temperature settings were as follows: autosampler 10ºC, column 50 ºC , instrument 400 ºC . Mass transitions monitored were 469 > 117 amu (Ivabradine), 455>262 amu (S-18982) and 483 > 262 amu (IS), [M+H]+.

Peak areas of analytes and internal standard were determined by the Analyst 1.4.2 software and the chromatographic data were then transferred on-line to the analytical database dbLabCal 2.1 by AAIPharma. From the generated calibration curve, the concentrations of quality control samples and study samples were determined and reported in nanograms of analyte per milliliter of cat plasma.

Data evaluation: For calculating accuracy and precision the following formulas were used:

$$\text{Accuracy} = 100\,\% \text{ x } \frac{\text{mean calculated - nominal}}{\text{nominal}}$$

$$\text{Precision} = 100\,\% \text{ x } \frac{\text{standard deviation}}{\text{mean calculated}}$$

where mean calculated is the mean of the observed concentrations calculated from the standard curve, nominal is the amount added to the sample, and standard deviation is that of the calculated concentrations.

Pharmacokinetic calculations: Concentrations of ivabradine and S-18982 were determined in each plasma sample collected according the analytical method protocol. Concentration-time profiles for ivabradine and S-18982 were generated from the data and used for PK modeling. Non-compartmental PK parameter estimates for plasma ivabradine and S-18982 were initially generated in WinNonlin (v. 5.2, Pharsight Corp., Mountain View, CA). Linear up/log down calculations for area under the curve (AUC) and 1/Y weighting was employed for all cats. For compartmental PK analysis; one-, two-, and three-compartment models were evaluated for each ivabradine and S-18982. The goodness of fit for each model was assessed by evaluation of diagnostic parameters by Akaike Information Criteria (AIC) and Schwarz Criteria (SBC), residual plots, and standard errors of estimate. All PK parameter estimates were then used as initial values in a combined 4-compartment PK model.

For statistical comparison of maximum plasma concentration (Cmax) and AUC from 4 to 24 hours on day one and day four for either parent drug or metabolite, a paired Student t-test was used (Excel, Microsoft Corp., Redmond,WA).

Results

Ivabradine was well tolerated in all animals. Physical examinations, clinical observations, and clinical and hematological data did not indicate any adverse effects related to the

treatment.

Cross-validation of analytical method: Calibration curves for the parent drug and its metabolite were linear from 0.250 to 250 ng/mL with a limit of quantitation of 0.250 ng/mL in cat plasma. Validation data indicated accuracy and precision within acceptable limits. The inter-day validation data for calibration standards and for quality control (QC) samples are presented for ivabradine and S-18982 in **Tables 1.1** to 1.**4**. Inter-day accuracy and precision for QC samples of both compounds were evaluated at the lower limit of quantification (LLOQ) and found to be within 12%, and results are presented in **Table 1.5**. Similarly, **Table 1.6** summarizes results from intra-day assessments of the LLOQ QC samples.

Pharmacokinetics of ivabradine and S-18982: Plasma ivabradine and S-18982 concentration-time profiles on day 1 and day 4 are illustrated in **Figures 1.1** and **1.2**. In three cats, plasma levels of ivabradine were below the LLOQ after 36 hours. Plasma concentrations of S-18982 were below the LLOQ in 7 cats after 12 to 24 hours and in all cats after 36 hours. Concentration-time plots for ivabradine indicated biphasic profiles for all the cats on day 1 and day 4, and profiles for S-18982 indicated monophasic profiles for all cats on both days. For ivabradine, a two-compartment model with first-order input and elimination provided the best fit to the data. For S-18982, a one-compartment model with first-order input and elimination provided the best fit. There was no significant difference between day 1 and day 4 $AUC_{4\text{-}24hrs}$ (calculated between 4 and 24 hours) or C_{max} (**Table 1.7**), which indicates a lack of appreciable accumulation with this dosing schedule and duration.

Pharmacokinetic modeling: Ivabradine disposition was identified by two-compartmental kinetics with first-order absorption and elimination. The active metabolite S-18982 was characterized by first-order rate constants of formation and elimination with a single compartment disposition (**Fig 1.3**). When given orally, ivabradine was anticipated to be subject to first-pass metabolism and to reach systemic circulation intact or as S-18982.[9] Differential equations used in the combined model to describe the rate of change of ivabradine and S-18982 in various compartments were as follows:

Equation 1 $$\frac{d[I]}{dt} = -k_A \times I$$

Equation 2 $$\frac{d[I_1]}{dt} = k_A \times I - (k_{10} + k_{12} + k_{13}) \times I_1 + k_{21} \times I_2$$

Equation 3 $$\frac{d[I_2]}{dt} = k_{12} \times I_1 - k_{21} \times I_2$$

Equation 4 $$\frac{d[S]}{dt} = k_{13} \times I_1 - k_{30} \times S$$

Compartment 0 (C0) represents the gut receiving ivabradine, and k_a is the rate constant for ivabradine absorption into systemic circulation; Compartments 1 and 2 (C1 and C2) are the central plasma and peripheral tissue compartments, respectively, with k_{12} and k_{21} rate constants for inter-compartmental transfer of ivabradine; k_{10} is the rate constant associated with elimination for ivabradine from compartment 1 (excluding conversion to S-18982); Compartment 3 (C3) is the plasma compartment for S-18982 with k_{13} and k_{30} the respective rate constants for formation and elimination. Estimated parameter values are summarized in **Table 1.8**.

Table 1.1 – Inter-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of ivabradine using calibration standards (n=12 samples).

Concentration [ng/mL]	0.250	1.00	2.50	10.0	25.0	100	250
Mean	0.249	1.00	2.49	10.2	24.6	99.5	251
SD	0.0151	0.0684	0.131	0.529	1.05	4.87	7.99
Precision	6.1	6.8	5.3	5.2	4.3	4.9	3.2
Accuracy	-0.2	0.1	-0.5	2.4	-1.6	-0.5	0.3

Table 1.2 – Inter-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of S-18982 using calibration standards (n=12 samples).

Concentration [ng/mL]	0.250	1.00	2.50	10.0	25.0	100	250
Mean	0.244	1.01	2.45	10.3	25.1	99.3	250
SD	0.0179	0.0606	0.165	0.463	1.59	3.25	7.09
Precision	7.3	6.0	6.7	4.5	6.3	3.3	2.8
Accuracy	-2.4	1.4	-1.9	3.1	0.4	-0.7	0.1

Table 1.3 – Inter-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of ivabradine using quality control samples (n=12 samples).

Concentration [ng/mL]	0.75	20	200
Mean	0.741	20.9	193
SD	0.0565	1.80	11.7
Precision	7.6	8.6	6.0
Accuracy	-1.2	4.3	-3.4

Table 1.4 – Inter-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of S-18982 using quality control samples (n=12 samples).

Concentration [ng/mL]	0.75	20	200
Mean	0.775	20.9	191
SD	0.0573	1.92	13.8
Precision	7.6	9.2	7.2
Accuracy	0.6	4.6	-4.4

Table 1.5 – Inter-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of ivabradine and S-18982 using quality control samples at the lower limit of quantification (LLOQ, 0.25 ng/mL; n=10 samples).

	Ivabradine	S18982
Mean	0.242	0.260
SD	0.0152	0.0287
Precision	6.3	11.1
Accuracy	-3.1	3.9

Table 1.6 – Intra-day accuracy (deviation from nominal [%]) and precision (coefficient of variation [%]) of ivabradine and S-18982 using quality control samples at the lower limit of quantification (LLOQ, 0.25 ng/mL; n=6 samples).

	Ivabradine	S18982
Mean	0.243	0.279
SD	0.0116	0.0106
Precision	4.8	3.8
Accuracy	-3.0	11.5

Table 1.7 – Mean and SD of maximum plasma concentrations (Cmax) and area under the plasma concentration-time curves (AUC) comparison between day 1 and day 4 for ivabradine and S-18982.

	Ivabradine		S18982	
	Cmax (ng/mL)	AUC4_24 (ng/mL*hr)	Cmax (ng/mL)	AUC4_24 (ng/mL*hr)
Day1	103 ± 57.8	102 ± 42.7	3.86 ± 2.41	6.09 ± 2.45
Day4	60.6 ± 40.2	183 ± 140	2.89 ± 1.62	13.9 ± 12.2

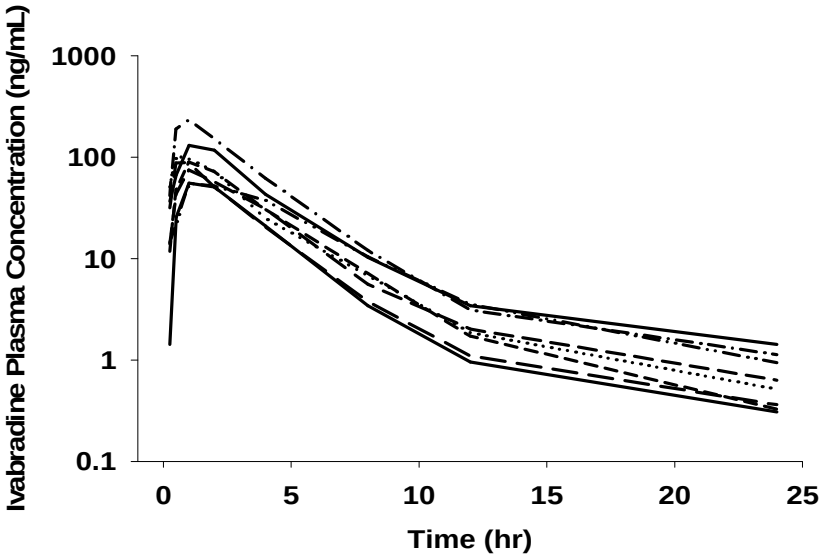
AUC4_24, AUC from 4 hrs to 24hrs.

Table 1.8 – PK parameters (mean±SD; n= 8 cats) from linked ivabradine and S-18982 pharmacokinetic model.

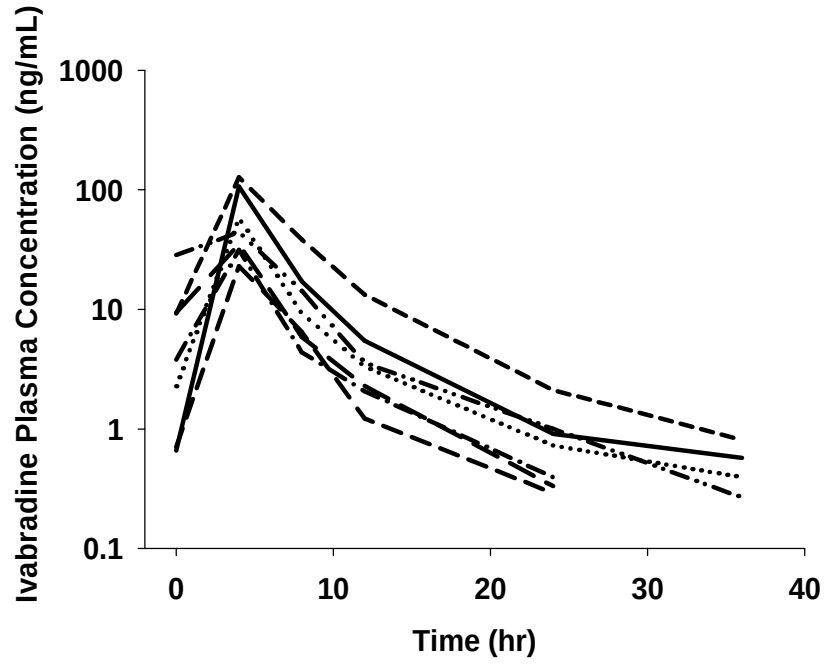
KA (1/hr)	K12 (1/hr)	K21 (1/hr)	K10 (1/hr)	K13 (1/hr)	K30 (1/hr)	V1 (L/kg)	V2 (L/kg)	V3 (L/kg)
0.88 ±0.31	0.11 ±0.09	0.14 ±0.13	0.62 ±0.14	0.36 ±0.18	1.12 ±0.58	1.78 ±1.15	1.46 ±0.89	34.16 ±24.64

K_A, ivabradine first-order absorption rate from oral depot to central compartment; K12, transfer from central to peripheral compartment of ivabradine; K21, transfer from peripheral to central compartment of ivabradine; K13, transfer from central compartment of ivabradine to central compartment of S-18982; K10, first-order elimination rate of ivabradine from central compartment; K30, first-order elimination rate of S-18982 from central compartment; V1, volume of distribution of the central compartment of ivabradine; V2, volume of distribution of peripheral compartment of ivabradine; V3, volume of distribution of central compartment of S-18982.

(A)



(B)

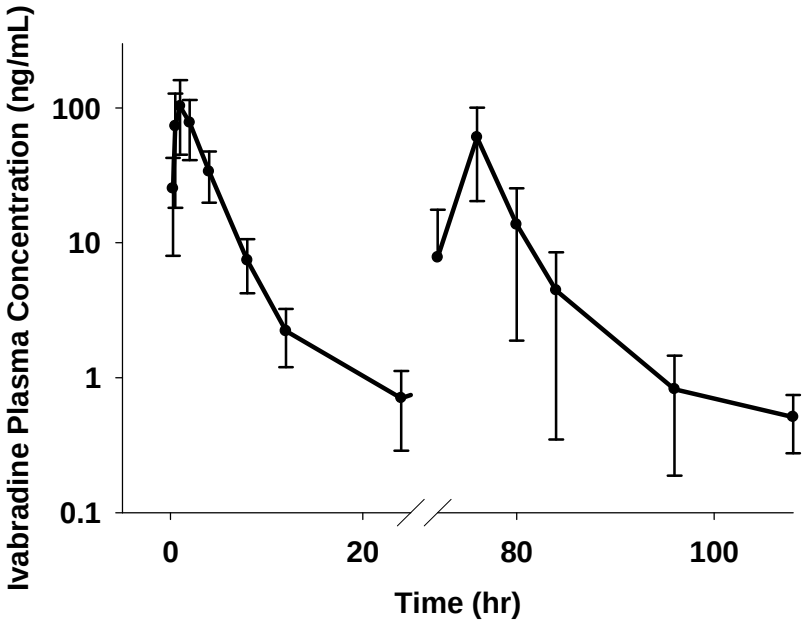


Continued

Figure 1.1 – Concentration-time profiles of ivabradine in eight cats on day 1 (panel A) and day 4 (panel B).

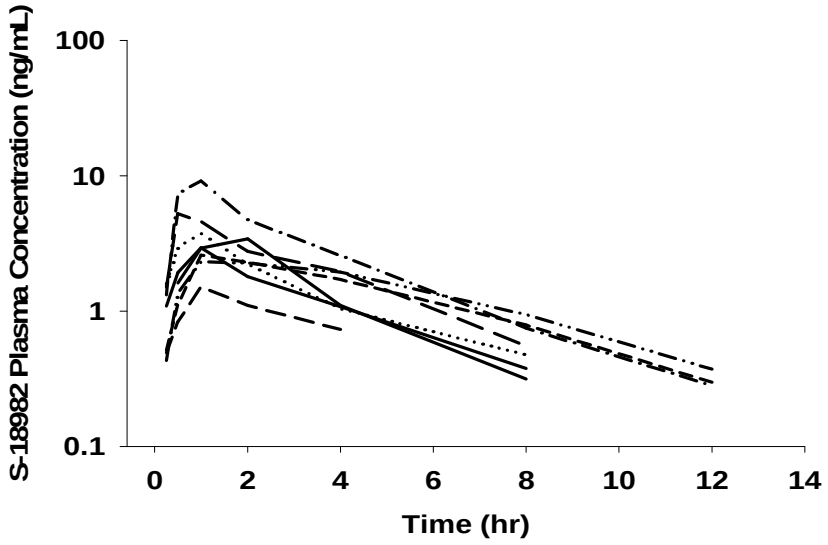
Figure 1.1 – Continued

(C)

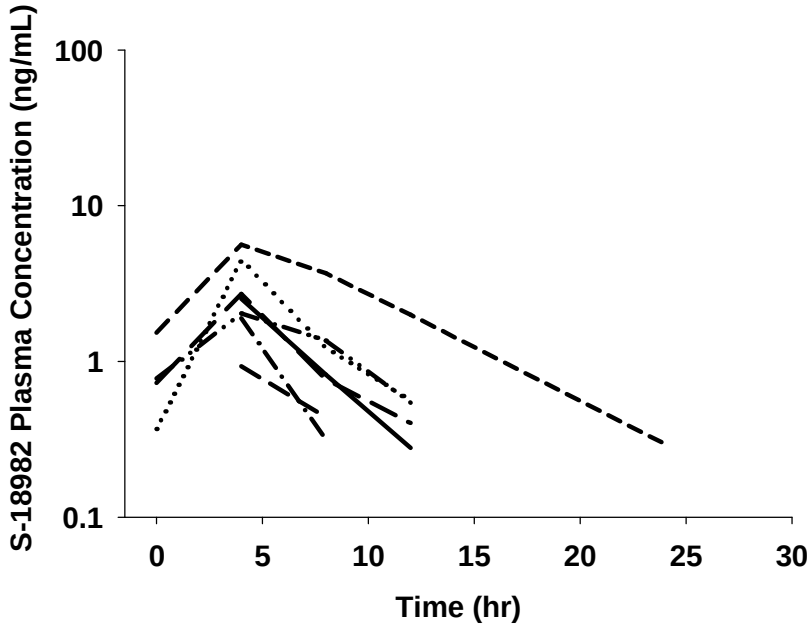


Panel C, average concentration-time profile from all cats. Dots and error bars represent mean and SD.

(A)



(B)

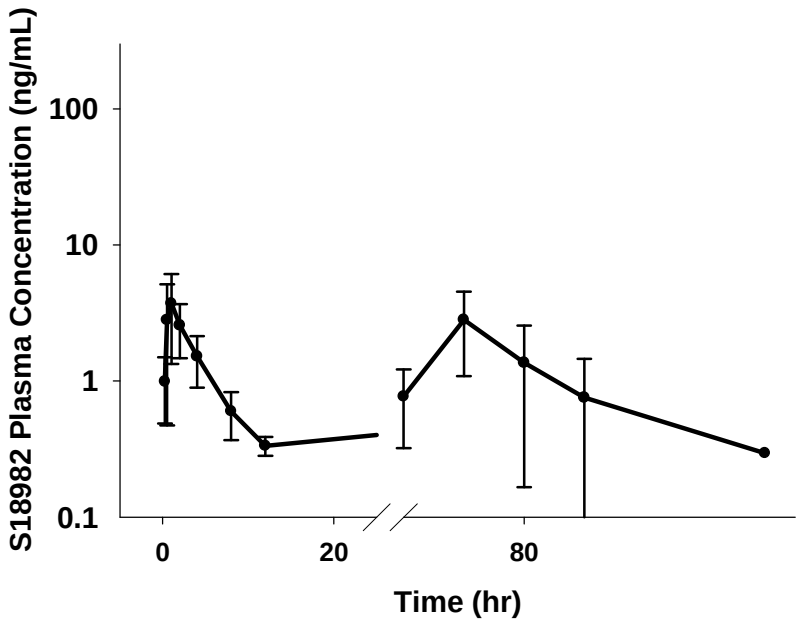


Continued

Figure 1.2 – Concentration-time profile of S-18982 in eight cats on day 1 (panel A) and day 4 (panel B).

Figure 1.2 – Continued

(C)



Panel C, average concentration-time profile from all cats. Dots and error bars represent mean and SD.

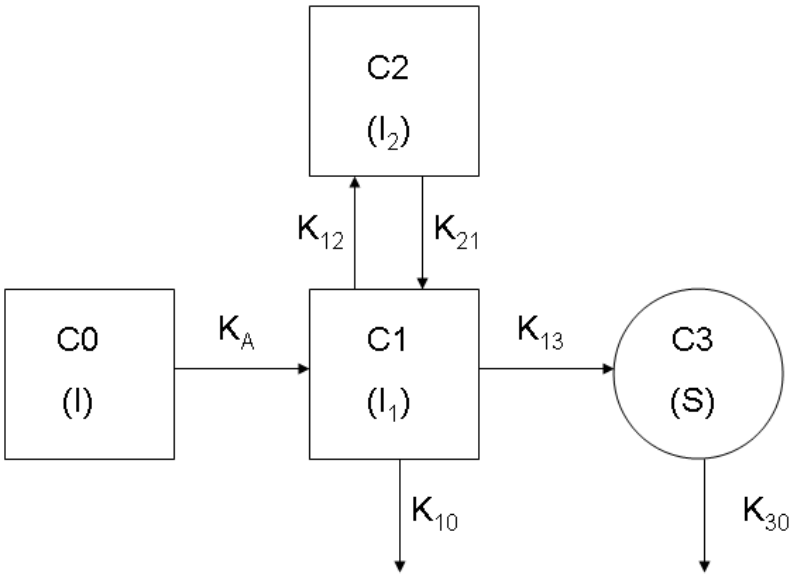


Figure 1.3 – Structural pharmacokinetic model for ivabradine (I) and its metabolite, S-18982 (S).

Squares represent the oral depot (C0), central (C1), and peripheral (C2) compartments for ivabradine. *Circle* represents central compartment (C3) for S-18982. Arrows represent first-order constants. K_A, transfer from oral depot to central compartment; K12, transfer from central to peripheral compartment of ivabradine; K21, transfer from peripheral to central compartment of ivabradine; K13, transfer from central compartment of ivabradine to central compartment of S-18982; K10, first-order elimination rate of ivabradine from central compartment; K30, first-order elimination rate of S-18982 from central compartment.

Discussion

This paper describes the validation of an analytical method for the determination of ivabradine and S-18982 in feline plasma, the pharmacokinetics of ivabradine and its major metabolite following single and repeated oral administration, and the development of a combined 4-compartment PK model.

The pharmakocinetics of ivabradine and its metabolite has been previously reported from healthy dogs, rats, pigs, and humans.[4] It has been shown in rat, dog, and human plasma, and human urine that the mean intra-assay precision and mean relative error were within acceptable limits regardless of the biological matrix studied.[4] Accuracy and precision were also found to be within acceptable limits in this study. The intra- and inter-assay precision at 0.250 ng/mL did not exceed 12% for ivabradine and S-18982. Therefore, the LLOQ was set at 0.250 ng/mL for both the parent drug and its metabolite which is in agreement with those determined in other species.[4, 6]

The pharmacokinetics of ivabradine has not previously been determined in cats. In other species, non-compartmental and compartmental approaches were used to characterize the pharmacokinetics of ivabradine, while S-18982 has only been studied using a non-compartmental approach.[5] In the present study, a two-compartmental model with first-order input and elimination and a one-compartmental model with first-input and elimination provided the best fit to the data for ivabradine and for S-18982, respectively. These models were combined to form a single model characterizing both ivabradine and S-18982 pharmacokinetics.

Compared to dogs,[4, 5] the Cmax and tmax after oral administration of ivabradine at comparable doses were similar to those observed in our cats. In cats, ivabradine was eliminated with a plasma half-life of 1.12 hours, which is similar to the plasma half-life of approximately 2 hours observed both in humans and dogs.[5, 10] Estimated absorption rate constants ranged from 0.88 to 1.19 hr^{-1} in our study. Although the accuracy of these parameter estimates is limited without PK data from an IV route of administration, variability in the rate of absorption from the gastrointestinal tract may have been due in part to food effects since cats were fed ad libitum during the entire study period.

Ivabradine undergoes metabolic conversion by cytochrome P450, including CYP 3A4. Because cats are deficient in CYP pathways, the relative importance of oxidation versus reduction pathways in cats may be different than in other species.[11-13] Both, ivabradine and its active metabolite were present in feline plasma. Since our findings were not markedly different compared to previous observations in humans and dogs[5, 7, 10] we conclude that biotransformation of ivabradine is either not affected or only minimally affected by differences in the CYP pathways in cats. Comparison of concentration-time profiles of ivabradine and its metabolite obtained on day 1 and day 4 did not reveal a cumulative effect for maximal plasma concentration or area under the plasma concentration-time curve, which is consistent with the half-life and with previous findings in humans, dogs, and pigs.[5, 7, 10]

The dose of ivabradine used was selected based on the results of a previous dose-finding study in healthy cats using 24-hour radio-telemetry.[a] In this placebo-controlled, randomized, fully-crossed study, the effects of a single dose of ivabradine at 0.1 mg/kg,

0.3 mg/kg, and 0.5 mg/kg PO were studied. Heart rate decreased significantly ($p<0.05$) in a dose-dependent manner compared to placebo (Mean±SD 24-h heart rate for placebo: 144±20 min^{-1}; ivabradine 0.1 mg/kg: 133±22 min^{-1}; ivabradine 0.3 mg/kg: 112±20 min^{-1}; ivabradine 0.5 mg/kg, 104±11 min^{-1}) with peak negative chronotropic effects observed approximately 3 hours after ivabradine administration. Overall, pharmacodynamic effects of ivabradine at 0.3 mg/kg [a, b] were similar in cats to those observed in humans, dogs, rats, and pigs. [5, 7, 10] In addition, the heart rate lowering actions of ivabradine were similar to those of atenolol, a drug commonly used in cats with hypertrophic cardiomyopathy. MacGregor et al.[14] and Quinones et al.[15] evaluated the pharmacokinetic and pharmacodynamic effects of orally administered atenolol on heart rate in healthy cats. Our results revealed a similar effect of ivabradine on mean heart rate in healthy cats with a slightly different peak effect, but a comparable duration of action thereby encouraging the oral use of ivabradine in the clinical setting.

In summary, an analytical method for quantification of ivabradine and its major metabolite in feline plasma was cross-validated and revealed acceptable accuracy and precision. This assay was used to characterize and model the pharmacokinetics of both ivabradine and S-18982 given at 0.3 mg/kg PO as a single dose and repeated oral doses. Ivabradine was clinically well tolerated and indicated a pharmacokinetic profile suitable for 12 hour dosing intervals in healthy cats. Further studies in cats with HCM are needed to assess the pharmacokinetic profile of ivabradine in the target population.

Footnotes

[a]Cober, R.E., Schober, K.E., Buffington C.A.T., Riesen, S.C. & Bonagura, J.D. (2010) Effects of ivabradine, a selective I_f channel inhibitor, on heart rate in healthy cats. *J Vet Intern Med* 2010;24:695.
[b]Riesen, S.C., Schober, K.E., Smith, D.N., Otoni, C.C. & Bonagura, J.D. (2010) Effects of ivabradine on invasive indices of LV function in anesthetized cats with hypertrophic cardiomyopathy. *J Vet Intern Med* 2010;24:692-693.

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CHAPTER 2

MYOCARDIAL EXPRESSION OF HYPERPOLARIZATION-ACTIVATED, CYCLIC NUCLEOTIDE-GATED PROTEINS IN HEALTHY CATS AND CATS WITH HYPERTROPHIC CARDIOMYOPATHY

Cardiac pacemaking is the result of the electrical activity of pacemaker cells, determined by the interplay of several ionic currents, pumps, and exchangers.[1-4] Spontaneous electrical pacemaker activity may occur in several regions of the heart. However, under normal physiological conditions, the intrinsic pacemaker rate is fastest in the sinoatrial node, which therefore determines overall heart rate.[1-4] Of the cellular and molecular mechanisms involved, the funny current (I_f) plays an important role.[1, 2, 5, 6] The molecular correlates of native funny channels are the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels, of which four isoforms are known (HCN1 to 4).[7-10] Of these isoforms, HCN4 is the most abundant and predominantly expressed in the sinoatrial node, while HCN1 and HCN2 are less expressed.[11-14]

The density of HCN channels in the heart does change during development,[15-17] in disease states,[18-22] and in response to hormonal stimuli.[23, 24] In the early embryonic heart, all cardiomyocytes display automaticity.[1] Maturation of myocytes toward the adult myocardial phenotype is associated with the disappearance of diffuse automaticity and progressive loss in I_f expression.[1, 15, 17] Atrial and ventricular myocytes can re-express I_f

during adult life under conditions such as hypertrophy, aging, and heart failure.[18-22] From a clinical point of view, mislocalized expression and/or overexpression of HCN channels may predispose myocytes from failing hearts to enhanced automaticity, a condition associated with an increased risk for ventricular and potentially lethal tachyarrhythmias.[16, 25] Sudden death is a common sequelae of hypertrophic cardiomyopathy (HCM) in cats[26] and humans.[27]

To our knowledge, cardiac expression of HCN proteins has not been previously described in cats. Therefore, the objectives of the study reported herein were to 1) evaluate the myocardial expression of different HCN isoforms in myocardial tissue from control cats and cats with HCM and to 2) study the effect of age on the expression of HCN by comparing kittens and adult healthy cats. We hypothesized that expression of myocardial HCN2 and HCN4 is significantly higher in ventricular myocardium of cats with HCM and that channel density is increased in healthy kittens as compared to adult healthy cats.

Material and Methods

Myocardial tissue: Myocardial samples (6 to 8 transmural tissue blocks of approximately 2x2x2 mm) were obtained from healthy control cats and cats with hypertrophic cardiomyopathy (HCM). Myocardial samples included tissue from the right atrium (RA) taken from the area of the sinoatrial node, and the right mid-ventricular free wall (RV) and the left ventricular mid-posterior wall (LV). Control cats were grouped into: adult cats (cats >1 year old) and kittens (2 to 3 months old). Cats with idiopathic LV

hypertrophy (end-diastolic dimension of the interventricular septum or the left ventricular posterior wall of > 6 mm determined on echocardiographic examinations[a]) were classified as cats with HCM.[28-30] Control cats were acquired from a local research laboratory undergoing only observational studies and euthanasia. Hearts of cats with HCM were acquired from the Veterinary Medical Center from cats that were euthanized due to clinically significant HCM (moderate to severe) after owner consent was obtained. The study protocol was reviewed and approved by the Animal Care and Use Committee (2008 A 0195) and the Institutional Review Board of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

Western blot analysis: The magnitude of feline HCN expression was determined by immunoblot analysis. Myocardial samples were homogenized in ice-cold buffer containing 10mM Tris-maleate,[b] 0.9% NaCl (pH 6.8),[c] and protease inhibitor cocktail[d] and centrifuged (40 000 g for 30 min at 4 C). Only fresh tissue samples harvested immediately after euthanasia and processed within two hours of sampling were used. Tissue blocks were homogenized and cell lysate proteins (10 μg) were subjected to 4 % to 20 % SDS-PAGE, blotted onto nitrocellulose membranes,[e] and probed with four antibodies specific for rabbit polyclonal HCN2[f,g] and HCN4[h,i] protein. Blots were developed with Super Signal West Pico,[j] scanned, and quantified in a blinded manner using gel analysis software.[k,l,m] For validation of the four different antibodies tested in this study, cerebral tissue (fresh and frozen) of healthy cats, where HCN2 and HCN4 is abundant,[31] was used as a positive control.

Tissue analyses: In 12 adult control cats and in 4 cats with HCM myocardial tissue could be obtained at the same time and cell lysate proteins from LV myocardial samples were analyzed simultaneously on the same nitrocellulose membranes. For these samples, optical densities of HCN bands were determined and compared side by side.

If tissue samples from only one group (control adult cats [n=4], control kittens [n=4], or cats with HCM [n=4]) were obtained at one time, cell lysate proteins from their RA, RV and RV myocardial samples were analyzed on one nitrocellulose membrane. For sample comparison, optical densities of Western blot target bands (RA, RV, and LV) of each cat were determined and RV and LV bands were then expressed quantitatively by normalization to the RA band on the same lane. Thus, in total cardiac HCN expression in tissue stemming from 16 adult control cats, 4 control kittens, and 8 cats with HCM was determined.

Statistical analysis: Statistical analysis was performed by use of commercially available software.[n] Normal distribution of variables was determined by the Kolmogorov-Smirnov test. Descriptive statistics were calculated from control cats and cats with HCM and data presented as mean and SEM. Comparisons between groups were performed by using an unpaired t-test or one-way ANOVA with Holm-Sidak post-hoc analyses, when appropriate, and significance was defined at $P < 0.05$.

Results

Of the four different antibodies evaluated, only one identifying HCN4 (anti-HCN4[i]) revealed consistent results in both cerebral and cardiac tissue. Using this antibody, a band

of ~ 150 kDa was observed for HCN4 in all samples tested (**Figure 2.1** and **2.2**). The three remaining antibodies did not perform reliably in positive control (brain) samples and

were therefore, considered unsuitable for inclusion.

Comparison of gels containing LV tissue from both cats with HCM and control cats: Cats with HCM revealed a 1.98-fold higher HCN4 expression compared to adult control cats ($P = 0.036$; **Figure 2.1**).

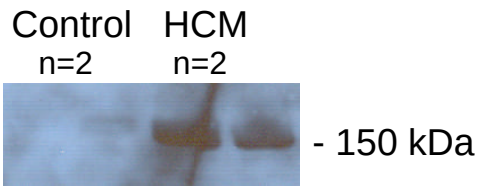
Comparison of gels containing tissue (RA, RV, and LV) from either control adult cats, control kittens, or cats with HCM: Expression of HCN4 in RV and LV myocardial samples was not different between adult control cats and kittens. No significant differences in RV and LV tissue were found when normalized to their respective RA band. However, a trend was observed for higher HCN4 expression in RV tissue from cats with HCM compared to adult control cats ($P = 0.055$, statistical power 0.224). Results of the optical densities observed are summarized in **Table 2.1** and are graphically illustrated in **Figure 2.2**.

Table 2.1 – Optical densities (OD) of HCN4 from right atrial and left and right ventricular myocardial tissue obtained from adult control cats (n=4), control kittens (n=4), and cats with hypertrophic cardiomyopathy (HCM; n=4).

Samples	**Control**	**Kittens**		**HCM**	
	OD	OD	*P**	OD	*P**
RA	1 ± 0.151	1 ± 0.411	n.d.	1 ± 0.239	n.d.
RV norm	0.287 ± 0.041	0.541 ± 0.168	0.260	0.752 ± 0.192	0.055
LV norm	0.666 ± 0.361	0.522 ± 0.088	0.714	2.347 ± 1.311	0.263

Values are expressed as mean ± SEM. *, compared to control; n.d., not determined. RA, right atrium; RV, right ventricle; LV, left ventricle; norm, normalized to right atrial OD.

A



B

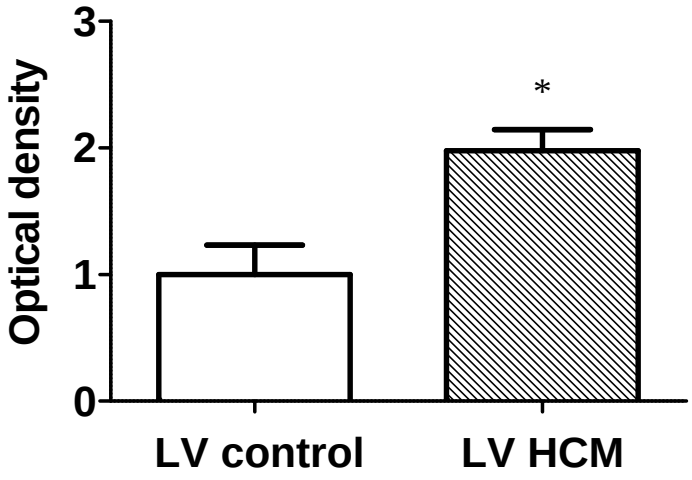


Figure 2.1 – Myocardial expression of HCN4 in left ventricular tissue (LV) from 12 control cats and 4 cats with hypertrophic cardiomyopathy (HCM).

A: Representative immunoblots of HCN4 in left ventricular myocytes from control cats (n=2) and cats with HCM (n=2). **B**: Optical densities of HCN4 of left ventricular tissue obtained from control cats (n=12) and cats with HCM (n=4). Results are expressed as mean and SEM. *, $P < 0.05$.

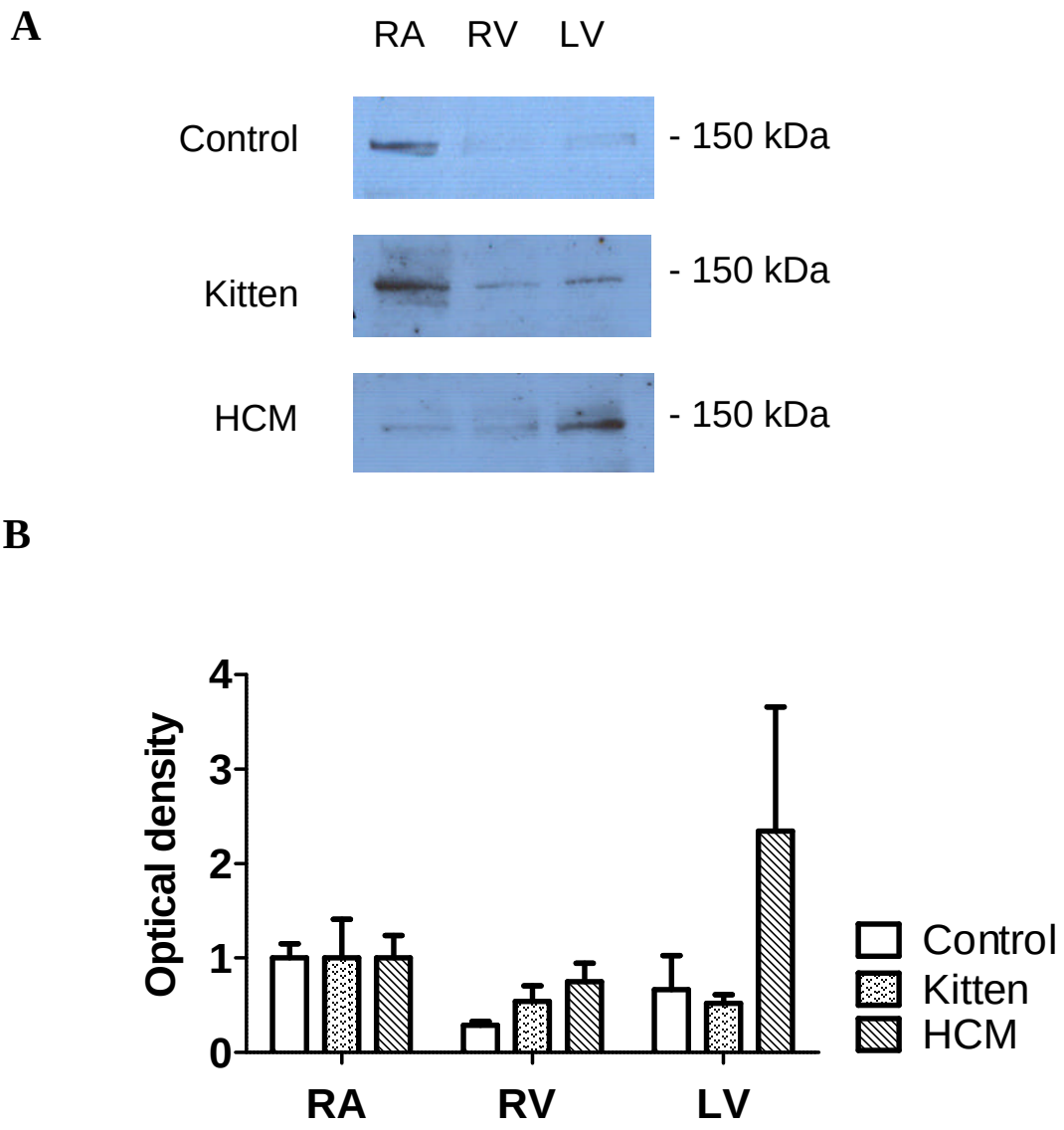


Figure 2.2 – Myocardial expression of HCN4 in right atrial (RA), right ventricular (RV), and left ventricular (LV) tissue obtained from 4 adult control cats, 4 control kittens, and 4 cats with hypertrophic cardiomyopathy (HCM).

A: Representative immunoblots of HCN4 in right atrial (RA), right ventricular (RV), and left ventricular (LV) tissue obtained from an adult control cat, a control kitten, and a cat with HCM. **B**: Optical densities of HCN4 right atrial (RA), right ventricular (RV), and

left ventricular (LV) tissue obtained from adult control cats (n=4), control kittens (n=4), and cats with HCM (n=4). Results are expressed as mean and SEM.

Discussion

The principal finding of this study is that HCN4 is significantly upregulated in LV myocardial tissue of cats with HCM, whereas HCN4 expression in RV myocardium revealed only a trend toward upregulation. In addition, we did not find differences in HCN4 expression between kittens and adult healthy cats indicating that HCN4 expression is completed within a few weeks after birth.

Funny channels are abundantly expressed in all cardiac regions during fetal development, but soon after birth, their expression in the working myocardium decreases and the voltage range of activation shifts to values more negative than the resting membrane potential.[15-17] During conditions such as heart failure, atrial and ventricular myocytes can re-express I_f during the adult life as a consequence of electrophysiological remodeling.[18-22] Previous studies in humans[18, 19, 32] and rats[20, 22, 33, 34] reveal an increase in both expression and density of HCN in hypertrophied ventricular cardiomyocytes. The degree of hypertrophy is positively correlated with I_f density[33] and the magnitude of HCN and I_f upregulation is most pronounced in cardiac regions with the highest pressure load.[35, 36] This indicates that the development of hypertrophy and channel expression are interrelated. Moreover, it has been reported that changes in cardiac I_f density are related to disease etiology with I_f overexpression being greater in ischemic cardiomyopathy compared to idiopathic dilated cardiomyopathy.[19]

To our knowledge, expression of HCN proteins has not been reported in healthy cats or cats with HCM. Overexpression of HCN4 in LV tissue was confirmed in cats with HCM in this study when compared side-by-side to control tissue run simultaneously on the same membranes. However, when the optical density of LV samples was normalized to their respective RA bands as done in 12 cats, no significant differences in HCN4 expression could be demonstrated. The latter may be explained, at least in part, by sample differences with regard to severity of LV hypertrophy, degree of tissue fibrosis, and magnitude of prior myocardial ischemia. Nevertheless, a trend toward HCN4 upregulation was observed in RV tissue normalized to RA HCN4 expression of cats with HCM ($P = 0.055$). Furthermore, we could not demonstrate a difference in myocardial HCN4 expression in normalized RV and LV tissue obtained from control adult cats and control kittens. This indicates that maturation toward the adult myocardial phenotype with regard to I_f expression is completed prior to the age of 2-3 months in healthy cats.

We evaluated the potential use of four different antibodies specific for HCN2[f,g] or HCN4,[h,i] which all have been utilized in prior studies in various species.[37-40] Only one antibody detecting HCN4 (anti-HCN4[i]) revealed consistent results in our study. Using this antibody, a strong ~ 150-kDa signal was detected in all myocardial and cerebral samples studied which is in agreement with previous findings.[37] The three other antibodies (two different HCN2[f,g] and another HCN4[h] antibody) used in our study failed to consistently detect their corresponding HCN bands in positive control (brain) samples. Therefore, no conclusion with regard to HCN2 expression in feline myocardial tissue can

be drawn. A possible explanation for the inability of these three antibodies to detect HCN may be related to structural differences of these proteins between species.

From a clinical point of view control of HR and prevention of unwanted tachycardia may be relevant in cats with HCM.[28, 41] Conflicting results with regard to the impact of HR on prognosis have been reported previously in cats. In one study[41], tachycardia (HR > 200 min^{-1}) was found to be associated with a shorter survival time whereas another study[26] failed to demonstrate any association between HR and outcome. In human patients, high HR and tachycardic spells have been linked to myocardial ischemia leading to clinical signs particular in patients with limited coronary flow reserve.[42, 43] Therefore, pharmacological reduction of HR, with or without auxiliary effects on the cardiovascular system, may be of therapeutic benefit in such patients.

Heart rate in general and I_f channels in particular are directly modulated by sympathetic activity.[5, 44-46] A resulting increase of cyclic adenosine monophosphate (cAMP) shifts the I_f activation curve to a more positive voltage and thus accelerates activation and slows deactivation kinetics[9, 31, 46, 47] Therefore, beta-receptor blockers, which reduce intracellular cAMP, and I_f inhibitors, which bind directly on the inside of the HCN channel, may modulate the I_f current and thereby HR. A recently performed study[o] in anesthetized cats with HCM done by the authors demonstrated that the tachycardic response induced by dobutamine administration was blunted by ivabradine, a selective I_f current inhibitor; whereas esmolol, an ultra-short acting selective beta-receptor blocker, failed to blunt the chronotropic response to dobutamine. This indicates

that the negative chronotropic effect of ivabradine is maintained under sympathetic stimulation in cats with HCM.[o]

Furthermore, overexpression of HCN channels in ventricular myocardium may predispose myocytes of failing hearts to enhanced automaticity,[25] an arrhythmogenic mechanism that may be important in the pathogenesis of sudden cardiac death. Along with reduced expression of the inwardly rectifying K^+ current as observed in failing hearts of rats[22] and humans[21, 48] leading to increased diastolic excitability, the higher myocardial expression of functional HCN channels may further contribute to electrical inhomogenicity and thus, electrical instability.[25] This may result in the development of fatal arrhythmias especially in presence of high sympathetic tone.[25] Thus, in addition to anti-ischemic properties related to HR reduction, selective I_f inhibition may reduce the risk of ventricular tachyarrhythmias and/or sudden death, although the latter has not yet been substantiated by clinical evidence. Studies in cats with HCM are needed to validate the potential benefits of selective I_f blockade on arrhythmogenicity and outcome.

Certain limitations of the study should be emphasized. First, the number of cats included was small, rendering the study underpowered to detect smaller differences between the three groups studied. Due to the low number of cats, no attempt was made to evaluate the HCN expression in various regions of the LV (e.g., papillary muscles or LV posterior wall). Furthermore, we evaluated HCN protein expression but not mRNA expression or I_f current. Therefore, no conclusions can be drawn regarding ionic conductance of the upregulated HCN4 channels in cats. The stage of cardiac disease and the severity of LV hypertrophy in cats with HCM were variable and no attempts were

made to specifically relate HCN4 optical densities to disease stage or LV mass. Finally, HCN proteins degrade rapidly in feline myocardial tissue. Therefore, detection is limited to fresh, un-frozen myocardium, and only cell lysates from freshly harvested samples can be used. As HCN degradation was not specifically studied, ant the time between animal death and preparation of cell lysate varied between 30 minutes adn two hours, mild protein degradation may have been occured in some samples possibly affecting quantitative HCN4 analysis. In addition, owing to instability of HCN, optical density of LV and RV myocardial tissue had to be normalized to their respective RA band in a subset of cats when only tissue from one of the groups studied (adult control cats, control kittens, and cats with HCM) was available. This normalization may have affected the interpretation of our findings.

In summary, detection and quantification of myocardial HCN4 was feasible in cats. Results indicate that HCN4 expression is upregulated in ventricular myocardium in cats with HCM. Moreover, HCN4 expression does not differ between kittens and adults. Although still hypothetical, myocardial HCN4 upregulation may predispose myocytes from failing hearts to enhanced automaticity and may possibly increase the risk of sudden cardiac death. Further studies are needed to evaluate the potential clinical benefits of I_f blockade on pathogenesis, clinical signs, and outcome in cats with HCM.

Footnotes

[a]Vivid 7 Vantage, GE Medical Systems, Milwaukee, WI.
[b]Trise-maleate, Sigma-Aldrich, St. Louis, MO.
[c]Sodium Chloride, Sigma-Aldrich, St. Louis, MO.
[d]Protease inhibitor cocktail, Sigma-Aldrich, St. Louis, MO.

[e]Nitrocellulose membranes, Bio-Rad Laboratories Headquarters, Hercules, CA.
[f]Rabbit polyclonal to HCN2, Abcam Inc., Cambridge, MA.
[g]Anti-HCN2, Alomone Labs Ltd., Jerusalem, Israel.
[h]Rat monoclonal to HCN4, Abcam Inc., Cambridge, MA.
[i]Anti-HCN4, Alomone Labs Ltd., Jerusalem, Israel.
[j]Super Signal West Pico, PIERCE Rockford, IL.
[k]Image J-1.37, National Institute of Health, Bethesda, MD.
[l]Visage 2000 Blot Scanning, BioImage Systems, Jackson, MI.
[m]Origin 7.0, OriginLab Corporation, Northampton, MA.
[n]SigmaStat, Version 3.5, SPSS Inc., Chicago, IL.
[o]Riesen SC, Schober KE, Smith DN, Otoni CC, Bonagura JD. Effects of ivabradine on invasive indices of LV function in anesthetized cats with hypertrophic cardiomyopathy. *J Vet Intern Med* 2010;24:692-693 (abstract).

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CHAPTER 3

EFFECTS OF IVABRADINE ON HEART RATE AND LEFT VENTRICULAR FUNCTION IN HEALTHY CATS AND CATS WITH HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats and is characterized by left ventricular (LV) hypertrophy, myofiber disarray, myocardial fibrosis, intramural coronary arterial narrowing, and myocardial ischemia, all factors that compromise diastolic LV function.[1-3] Treatment of cats with preclinical HCM remains controversial, but has been directed at relieving obstruction of the LV outflow tract, improving LV diastolic function, and preventing arterial thromboembolism and sudden cardiac death. Beta-adrenoreceptor blockers and less frequently calcium channel antagonists have been used in the management of cats with preclinical HCM.[4-8] However, concerns have been raised regarding potential adverse effects of these drugs in cats including lethargy, inappetence, salivation, weight loss, and reduced left atrial function.[4-8] Beta-blockers may also be contraindicated in congestive heart failure, cats with allergic airway disease, hypotension, and arterial thromboembolism.[4-8]

Several prognostic indicators have been proposed in cats with HCM including LV diastolic function, left atrial (LA) size, and heart rate (HR)[1, 9] which is a major

determinant of myocardial oxygen consumption.[10] Tachycardia is poorly tolerated in people with HCM. Consequences of unwanted tachycardia can include reduced LV filling, decreased myocardial perfusion and increased myocardial oxygen demand with worsening of preexisting diastolic dysfunction and myocardial ischemia.[10] Evidence in human patients suggests that ischemia is a

major contributor to clinical signs, disease progression, and fatal outcome. Furthermore, ischemia can be present in asymptomatic patients with HCM, with syncope and sudden cardiac death caused by ischemia, rather than to a primary arrhythmogenic substrate.[11] The association between ischemia, cellular calcium overload, and LV diastolic dysfunction is well established. Hence, pharmacological modulation of HR is an important mechanism in the treatment of pathological conditions characterized by a mismatch between oxygen supply and demand of the myocardium, such as coronary artery disease, hypertension, and heart failure,[12-15] and may also be important to management of HCM.

Recent advances related to the molecular basis of cardiac pacemaker function and the physiology of associated ion channels have led to novel approaches in the selective control of HR.[16, 17] One such cellular target is the pacemaker funny current (I_f) of the sinoatrial node. To date, several I_f -current inhibitors have been studied, but only ivabradine (Iva) has been approved for clinical use in human patients with ischemic heart disease.[17]

To the authors' knowledge, the hemodynamic effects of Iva have not yet been studied in cats. Therefore, the objectives of the current pilot study were 1) to evaluate the acute effects of Iva on HR, LV function, and LA performance by use of catheter-based

and echocardiographic techniques; and 2) to study the effects of sympathetic stimulation on central hemodynamics under the influence of a beta blocker (esmolol, Esm) and Iva. These studies were performed under general anesthesia in healthy cats (controls) and in cats with preclinical HCM. We hypothesized that Iva would exhibit minimal effects on LV and LA function but would significantly blunt positive chronotropic responses induced by catecholamine administration.

Material and Methods

Animals: Subjects included six healthy domestic shorthair cats (controls) from two to six year of age (3.7 to 5.0 kg bodyweight) and six domestic shorthair cats with idiopathic symmetric or asymmetric LV hypertrophy. Cats with hypertrophy were classified as affected by HCM and defined by an end-diastolic dimension of the interventricular septum (IVSd) or the LV posterior wall (LVPWd) of > 6 mm determined over three or more different echocardiographic examinations.[2, 18, 19] Cats with HCM were three to eight years old and weighed 2.8 to 8.3 kg. These cats were acquired from a commercial vendor[a] (n=8) or from an in-house research colony. Three of the cats with HCM were male, and nine cats were female.

The study protocol was reviewed and approved by the Animal Care and Use Committee and the Institutional Review Board of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University. All animals were treated in compliance with the National Institutes of Health guidelines on the care and use of laboratory animals.

Anesthesia, instrumentation, and hemodynamic measurements: Following sedation with acepromazine[b] (0.025 mg/kg, IM) and butorphanol[c] (0.25 mg/kg, IM), each cat was anesthetized with propofol[d] (5 μg/kg, IV) and intubated. Anesthesia was maintained with isoflurane[e] (0.5 to 2.0 %) in 100 % oxygen by use of mechanical ventilation with a tidal volume of 10-15 mL/kg and a respiratory frequency of 12 breaths/min. The cats were positioned in left lateral recumbency on a fluoroscopy table designed to allow echocardiographic examinations during cardiac catheterization. Drugs and fluids were infused into the right cephalic vein as boluses or at a constant rate by use of syringe pumps[f]. Cefazolin[g] (20 mg/kg, IV) was administered immediately before and 90 minutes and eight hours after induction of anesthesia. Heparin[h] (100 IU/kg, IV) was given once after completion of instrumentation. The right external jugular vein and the right carotid artery were surgically exposed according to standard techniques and 2% lidocaine[i] was infiltrated to provide additional local anesthesia. A 3-F, high-fidelity, dual-micromanometer-tipped catheter[j] was advanced into the LV through the right carotid artery under fluoroscopic guidance and positioned to simultaneously record LV and aortic pressures. Digital sampling rate for the micromanometer catheter was 500 samples/s. As previously described by our laboratory,[20] the transducers were placed in a water bath for 30 minutes, then balanced at atmospheric pressure, and finally calibrated against a mercury manometer prior to use. A 5-F, flow-directed, fluid filled, thermistor-tipped catheter[k] connected to a pressure transducer and a cardiac output (CO) computer[l] was positioned in the pulmonary artery (PA) via the right external jugular vein. The proximal catheter port was positioned in the right atrium or cranial vena cava, the thermistor in the main PA, and the distal tip in the main or proximal left or right PA

branch. This catheter was used to measure PA pressures and CO by thermodilution. For measurement of CO, a 1 mL bolus of room-temperature saline[m] was rapidly injected into the right atrium through the proximal port of the PA catheter. Body temperature of the cats was monitored from the thermistor of the Swan-Ganz catheter, and maintained with a circulating warm water blanket and an external warming unit. Body temperature, ECG, HR, LV pressures, aortic pressures, and PA pressures were monitored continuously and recorded simultaneously during each treatment period. Cardiac output was measured at end expiration during each intervention simultaneously with echocardiographic recordings.[20]

Echocardiography: Each cat underwent repeated transthoracic two-dimensional (2-D), spectral and color Doppler, and pulsed wave tissue Doppler imaging (TDI) echocardiographic examinations using digital probes with a transducer array of 5.0 to 7.0 MHz nominal frequency. [n] Recordings for TDI and 2-D strain analyses were made at end-expiration. Echocardiography was performed by a single operator (SCR). Data were stored digitally, and echocardiographic data analysis was performed off-line by use of a commercial analysis software package.[o] The mean of three cardiac cycles was calculated for each variable measured. A simultaneous one-lead ECG was recorded.

Right parasternal long and short-axis and left apical long-axis imaging views were acquired to allow for optimal recording of the LA, the LV, transmitral and pulmonary venous flow, left atrial appendage flow (LAA), aortic outflow, isovolumic relaxation time (IVRT), and tissue Doppler-derived velocity of the lateral mitral annulus.[20-22] All Doppler-derived velocity measurements were performed from the left apical 4-chamber view. Two-dimensional and M-mode variables of the LV and LA were obtained as

previously described.[20-23] Ejection fraction was measured by use of the modified Simpson method from a left apical transducer site. [24] Left atrial size was assessed from the right parasternal long-axis view.[21, 22] In addition, fractional area change of the LA (FAC) and left atrial shortening fraction (LA SF) were calculated.[21, 22] Left atrial appendage flow was obtained from a left apical tilted 3-chamber view and with a sample volume of 3 to 5 mm placed in the proximal third of the appendage.[21, 22] Pulmonary venous flow was obtained from the left apical 4-chamber view with minimized low velocity filtering and with a sample volume of 5 mm placed 2 to 4 mm within the pulmonary vein. Transmitral flow was recorded with a sample volume of 2 mm placed between the tips of the opened mitral valve leaflets. Peak velocities (Peak E and Peak A), deceleration time of E, and A-wave duration were measured. Fused E and A waves were measured but data were excluded from final statistical analysis. E:IVRT was calculated as the ratio of Peak E (in m/ms) to IVRT (in ms) and reported as dimensionless number.

Pulsed wave Doppler-derived velocities of myocardial motion were also recorded from the left apical view.[25] The Doppler gain was minimized to generate a clear tissue signal with minimum background noise. Frame rate was optimized (>160 frames/s) by narrowing the tissue Doppler imaging sector. A sample volume of 5 mm was placed at the lateral mitral annulus and the peak systolic and diastolic velocities (Peak Sa, Peak Ea, and Peak Aa) were measured. The E:Ea ratio was calculated. Data were excluded from statistical analysis when Ea and Aa waves were fused.

The LV was imaged in a right parasternal mid-ventricular short-axis plane for quantification of peak systolic radial strain (radial SR Peak S), peak systolic and diastolic radial strain rate (radial SrR Peak S, radial SrR Peak E, and radial SrR Peak A), and

global peak systolic and diastolic circumferential strain rate (circ SrR Peak S, circ SrR Peak E, and circ SrR Peak A) all recorded at end-expiration. Care was taken to obtain a frame rate between 60 and 200 per second (depending on HR) with the sector width and image depth optimized. The off-line analysis of strain was obtained using a 2D speckle-tracking methodology as previously described in dogs.[26] In brief, aortic valve opening and closure were defined based on the pulsed wave aortic outflow signal. Once a suitable 2D-image was identified, the LV endocardium was manually traced in systole or diastole, and an optimal region of interest was then chosen for automated determination of myocardial deformation using speckle tracking. Radial strain, $SR_{R,}$ and SR_C were determined as the average of six corresponding myocardial segments.[26]

Hemodynamic interventions: After baseline measurements were made, four treatment periods (Esm,[p] esmolol and dobutamine [Esm+Dob],[q] Iva,[r] and ivabradine and dobutamine [Iva+Dob]) were studied. A ten to 20-min time period was allowed for hemodynamic stabilization between each study period. Once heart rate was stable, hemodynamic variables and echocardiographic data were acquired simultaneously, with data collection taking approximately 15 min for each treatment period. The ultra short-acting beta-blocker (Esm) was administered (one to six loading doses of 200 μg/kg, followed by one to four doses of 400 μg/kg each administered slowly over one minute, IV, followed by CRI of 200 to 600 μg/kg/min, IV) to lower HR. Once HR reduction was achieved, the dose of Esm was maintained at 200-400 μg/kg/min, and measurements were performed after hemodynamic stabilization. Thereafter, Esm was continued (200-400 μg/kg/min, IV) and Dob (5.0 μg/kg/min, IV) was added to study the effects of

sympathoadrenergic stimulation during β-adreneoreceptor blockade (Esm+Dob). Following data collection, Esm+Dob were discontinued and HR, LV pressure, and aortic pressure were allowed to return to near-baseline values. A single bolus of Iva[r] (0.3 mg/kg, IV) was then administered. The dose of Iva used was selected based on the results of a previous dose-finding study in healthy cats using 24-hour radio-telemetric method and is similar to doses used in dogs and humans.[s] Ivabradine for injection (0.2 mg/mL) was reconstituted by adding sterile water to 1 and 2 mg vials of ivabradine hydrochloride immediately before administration and the solution was injected through a diposable microfilter device.[t] Finally, Dob (5.0 μg/kg/min, IV) was administered to study the effects of sympathoadrenergic stimulation under the influence of Iva (Iva+Dob).

At the conclusion of the experiment, drug administration was stopped, all catheters and monitoring equipment were removed, the cannulated vessels and the skin incision were surgically closed, and the cats were closely observed until extubated and completely recovered from anesthesia. Butorphanol (0.35 mg/kg, SC) was administered every eight hours for postoperative control of pain. Subsequently, the cats were transferred back to their original study protocol and the cats were later placed in adoption homes.

Hemodynamic Data: Pressures[u], CO[l], echocardiographic[o] data, and a single lead ECG were simultaneously recorded and stored digitally for subsequent analyses. Pressures were measured from the digitized recordings at end-expiration. Catheter-derived variables included HR per minute, systolic LV pressure (LVSP), LV end-diastolic pressure (LVEDP), aortic systolic pressure (AoSP), aortic diastolic pressure (AoDP), peak rate of rise of LVP ($+dP/dt_{max}$), systolic PA pressure (PASP), diastolic PA pressure

(PADP), and mean PA pressure (PAP_{mean}). LVEDP was defined as the LVP immediately preceding the onset of LV contraction. Tau was calculated by the method of Weiss et al[27] assuming a zero asymptote.

Cardiac output was determined with a commercial CO computer.[l] The mean of five measurements with a maximum variance of 15 % was used as the determination for each study period. Left ventricular end-diastolic and systolic wall stress was calculated using a cylindrical model[10, 28] as: stress = 1.36 x (LVP x D/2h), where D is the maximum internal short-axis diameter and h is wall thickness of the LV with each of these parameters being measured at end-diastole and end-systole. Considering that some cats with HCM had asymmetric LV hypertrophy, 2h was calculated as the sum of the thickness of the intraventricular septum and the LV posterior wall.

The Rate-Pressure Product was calculated as an estimate of myocardial oxygen consumption as: Rate-Pressure Product = LVSP x HR.[29]

Statistical analysis: Statistical analysis was performed by use of commercially available software.[v,w] All data were graphically evaluated, and descriptive statistics were calculated for all variables. Data are presented as mean and standard deviation, unless stated otherwise. Normal distribution of variables was determined by the Kolmogorov-Smirnov test. To identify differences between treatments and between controls and cats with HCM a two-way repeated measures (RM) ANOVA was used with cardiac status (HCM and control) and animal as within-subject factors and three of the treatment periods (baseline, Esm, and Iva) as a between-subject factor. If significant statistical differences were identified ($P<0.05$), a Holm-Sidak test was used for pairwise comparisons. If statistical assumptions for the use of 2-way RM ANOVA were not met, linear mixed effects models

and change scores[30] were used for group comparison. The models included fixed effects of diagnosis (HCM and Control), and treatment stage, the interaction of these two factors, and the baseline measure as a covariate.

In addition, changes of variables induced by dobutamine (Esm+Dob versus Esm; Iva+Dob versus Iva; and Esm+Dob minus Esm, and Iva+Dob minus Iva) were compared using a 2-way RM ANOVA with Holm-Sidak post-hoc analyses. Finally, univariate and multiple linear regression analyses, and logistic regression were used to identify associations between tau and the independent variables of HR, CI, $+dP/dt_{max}$, LVSP, LVEDP, and treatment using pooled data. For all analyses, a $P \leq 0.05$ was considered statistically significant.

Results

Cats with HCM and control cats were not different ($P>0.05$) with regard to age and body weight. Mean (±SD) of HR, hemodynamic data, and echocardiographic variables of cats of both groups are summarized in **Tables 3.1 to 3.4**. At baseline, only CI, PASP, IVSd, Peak E, and S:D differed significantly ($P<0.05$) between groups (**Tables 3.1 and 3.2**). Although HR ($P=0.19$), tau ($P=0.09$), the rate-pressure product ($P=0.26$), and IVRT ($P=0.21$) appeared to be higher and end-systolic WS ($P=0.17$), end-diastolic WS ($P=0.21$), and Peak Ea ($P=0.08$) appeared to be lower in cats with HCM compared to controls, the observed differences did not attain statistical significance. Data on the changes induced by Dob with regard to HR and selected variables of LV and LA function are summarized in **Table 3.5**. Only variables in which significant differences were found are shown.

Effects of ivabradine on HR and rate-pressure product: Diagnosis (HCM or control) did not have a fixed effect on HR (P=0.61), whereas treatment (P<0.001) and baseline values (P<0.001) did. Compared to baseline, HR was significantly reduced by Iva in cats with HCM and control cats (both P<0.001), whereas the negative chronotropic effect of Esm did not reach statistical significance (HCM P=0.44; control P=0.59). Administration of Dob increased HR in cats with HCM when given concurrently with Esm and Iva. However, the mean and maximum HR observed during Esm+Dob (164 min^{-1} and 204 min^{-1}) were significantly higher (P<0.001) compared to the mean and maximum HR observed during Iva+Dob (122 min^{-1} and 140 min^{-1}; **Fig 3.1** and **Tables 3.1 and 3.5**). Similar observations were made in control cats, although dobutamine did not significantly increase HR during Iva+Dob in this group. Mean (± SD) relative change (in per cent) of HR compared to baseline in cats with HCM and control cats were -12% (± 25%) and +3% (± 13%) for Esm; +12% (± 19%) and +24% (± 15%) for Esm+Dob; -33% (± 4%) and -22% (± 10%), for Iva; and -17% (± 12%) and -16% (± 12%) for Iva+Dob.

With regard to the rate-pressure product, diagnosis (HCM or control; P=0.79) and baseline characteristics (P=0.24) did not have a fixed effect on outcome, whereas treatment (P<0.001) did. In contrast to Esm (P=0.40), Iva significantly reduced the rate-pressure product in cats with HCM (P<0.001).

Effects of ivabradine on variables of LV contractility and LV systolic *function:* Diagnosis (HCM or control; P=0.39) and baseline values (P=0.19) did not have a fixed effect on $+dP/dt_{max}$, whereas treatment (P<0.001) did. Both Iva and Esm reduced $+dP/dt_{max}$ in cats with HCM, but with Iva the decline was not statistically significant

when compared to Esm (Iva *P*=0.45; Esm *P*=0.005; **Table 3.1 and Fig 3.2**). Dobutamine administered simultaneously with Esm and Iva increased $+dP/dt_{max}$ in both HCM and control cats with a significant difference in absolute change between groups (*P*=0.044; **Tables 3.1 and 3.5 and Fig 3.2).**

Overall, cats with HCM had lower CI compared to controls (**Table 3.1**), although statistical significance was only reached with at baseline and with Esm and Iva for CI. Stroke volume was not different between treatment groups and disease groups (Esm, *P*=0.62; Iva, *P*=0.088). Stroke volume increased after Dob and was not different between HCM and control (**Table 3.1**).

In terms of echocardiographic changes, LVEF was not affected by Iva whereas Esm reduced EF in both groups **(Table 3.2)**. Segmental myocardial analysis using 2-D speckle tracking techniques revealed that systolic strain indices were not significantly affected by Iva or Esm in both groups. However, in contrast to Esm+Dob, most of the radial and circumferential systolic strain and strain rate indices were significantly increased (*P*<0.05) during Iva+Dob in HCM and control cats with only circ SrR Peak S showing significant differences (*P*<0.05) between HCM and control **(Table 3.4)**. Cats with HCM had a larger increase of circ SrR Peak S at Iva+Dob compared to Esm+Dob than control cats (**Table 3.5**).

Effects of ivabradine on variables of LV diastolic function: *Variables of LV relaxation* **–** Diagnosis (HCM or control) did not have a fixed effect on tau (*P*=0.183), whereas treatment (*P*<0.001) and baseline values (*P*<0.001) did. Tau was slightly prolonged (mean, +4 ms) after Iva in cats with HCM (HCM *P*<0.009; control *P*=0.61) in contrast to tau after Esm (*P*>0.05; **Table 3.1**). Dobutamine administered simultaneously with Esm

and Iva decreased tau in both groups. However, there was a significant difference (*P*=0.019) in the magnitude of mean reduction of tau at Iva+Dob compared to Esm+Dob in cats with HCM (**Table 3.5 and Fig 3.3**). Moreover, there was a significant difference (*P*=0.029) in the mean reduction of tau at Iva+Dob between cats with HCM (mean, -12ms) and in controls (mean, -6ms; **Table 3.5**). Multivariate logistic regression analysis revealed that tau could be predicted from a combination of $+dP/dt_{max}$ (*P*<0.001), animal (*P*=0.001), treatment (*P*=0.039), and LVEDP (*P*=0.011) in cats with HCM. Using forward stepwise regression, a cumulative R^2 of 0.51 was determined (step 1, $+dP/dt_{max}$, R^2=0.37 and step 2, animal, change of R^2=0.14, cumulative R^2=0.51, *P*<0.001; power of 0.997 at alpha =0.05). Heart rate did not enter the final prediction model. In control cats, multivariate logistic regression analysis demonstrated that tau could be predicted from a combination of HR (*P*=0.009), animal (*P*=0.028), and treatment (*P*=0.047). Using forward stepwise regression, a cumulative R^2 of 0.55 was determined (step 1, HR, R^2=0.19; step 2, treatment, change of R^2=0.22; and step 3, animal, change of R^2=0.41; cumulative R^2=0.55, *P*<0.001; power of 0.998 at alpha =0.05).

Overall, IVRT was prolonged in HCM cats compared to controls with statistical significance (*P*<0.05) observed at Esm+Dob, Iva, and Iva+Dob (**Table 3.3**). Neither Iva nor Esm affected IVRT in either cat group when compared to baseline. Ivabradine, but not Esm, increased Peak Ea in both groups (*P*<0.05) when compared to baseline. Mean increase in Ea was 8 % in cats with HCM and 34 % in control cats. There were no significant differences of 2-D strain-derived indices of LV diastolic function between the two groups (**Table 3.4**).

Variables of LV compliance – Left ventricular end-diastolic pressure (HCM, *P*=0.006 and control, *P*<0.001) and end-diastolic wall stress (HCM, *P*=0.001 and control, *P*=0.01; **Table 3.1**) were significantly elevated with Iva in both groups of cats. Overall, calculated end-diastolic wall stress was lower in cats with HCM compared to control cats, although statistical significance was only observed during Esm and Esm+Dob. LVEDP and end-diastolic wall stress increased under the influence of Dob in all treatment groups with no statistical difference evident between HCM and control groups. Doppler variables of early LV compliance (MV $DecT_E$) and late LV compliance (Aduration:ARduration) were unaffected by treatment or disease group.

Variables of LV Filling and LV filling pressure – At baseline and during Esm+Dob, S:D was significantly (*P*=0.045) different between HCM and controls. Ivabradine did not affect Peak E, E:A, or S:D in either group (**Table 3.3**). Ivabradine increased LVEDP (*P*<0.05) by a mean of 4.2 mmHg in cats with HCM and by a mean of 4.9 mmHg in the control group (**Table 3.1**). Dobutamine increased LVEDP and PADP at Esm+Dob and Iva+Dob at a comparable magnitude in both groups with no difference between HCM and control (LVEDP, HCM *P*=0.93 and control *P*=0.59; PADP, HCM *P*=0.50 and control *P*=0.07). Similar to LVEDP, E:IVRT was increased at Esm+Dob and Iva+Dob (**Table 3.3**).

Effects of ivabradine on variables of LA function: In contrast to control cats, LA_{max} was not increased in cats with HCM after Iva (**Table 3.2**). Although Esm did not change left atrial FAC, Iva increased FAC in both HCM (*P*=0.045) and control (*P*=0.009; **Table 3.2**). Peak AR, Peak LAA, Peak Aa lat, and circumferential and radial strain variables derived at atrial contraction were not significantly altered by Iva. Dobutamine led to an

increase (P<0.05) of FAC, LA SF, and Peak LAA in both groups during Esm+Dob and Iva+Dob; whereas, Peak A did show a tendency to increase (P=0.056) at Iva+Dob when compared to Esm+Dob in cats with HCM (**Tables 3.3 to 3.5).**

Table 3.1 – Heart rate and invasively-derived indices of left ventricular systolic and diastolic function under five different hemodynamic conditions in six control cats and six cats with hypertrophic cardiomyopathy.

Variable	Group	Baseline	Esm	Esm+Dob	Iva	Iva+Dob
HR (min^{-1})	Control	129 ± 23	132 ± 17	160 ± 20 [b]	101 ± 12 [a]	108 ± 16
	HCM	147 ± 30	135 ± 37	164 ± 28 [b]	99 ± 6 [a]	122 ± 18 [c]
LVSP (mmHg)	Control	79 ± 9	78 ± 11	104 ± 17 [b]	87 ± 16	110 ± 23 [c]
	HCM	82 ± 14	77 ± 16	102 ± 27 [b]	84 ± 23	118 ± 40 [c]
LVEDP (mmHg)	Control	7.8 ± 3.9	9.8 ± 3.2	12.7 ± 3.2 [b]	12.9 ± 3.2 [a]	16.8 ± 4.5 [c]
	HCM	7.7 ± 2.8	7.2 ± 3.2	11.9 ± 3.0 [b]	11.8 ± 4.7 [a]	16.7 ± 5.0 [c]
AoSP (mmHg)	Control	82 ± 6	77 ± 10	102 ± 18 [b]	86 ± 15	108 ± 22 [c]
	HCM	81 ± 15	78 ± 16	102 ± 29 [b]	85 ± 24	111 ± 40 [c]
AoDP (mmHg)	Control	57 ± 7	52 ± 9	53 ± 16	51 ± 11	52 ± 12
	HCM	62 ± 14	59 ± 15	61 ± 22	55 ± 17	63 ± 20

Continued

Table 3.1 – Continued

PASP (mmHg)	Control	15.2 ± 3.7	15.3 ± 1.7	27.4 ± 4.9 [b]	17.7 ± 3.3	26.5 ± 4.4 [c]
	HCM	11.0 ± 2.7 [#]	12.6 ± 2.0	22.6 ± 3.8 [b,#]	15.4 ± 3.7 [a]	23.2 ± 6.2 [c]
PADP (mmHg)	Control	11.3 ± 2.4	10.9 ± 2.8	19.5 ± 7.0 [b]	11.2 ± 3.3	15.2 ± 2.9 [c]
	HCM	8.1 ± 4.4	10.5 ± 1.8	17.2 ± 3.8 [b]	11.0 ± 4.1	16.1 ± 5.3 [c]
PAP_{mean} (mmHg)	Control	13.4 ± 2.9	13.3 ± 1.8	23.5 ± 5.5 [b]	14.9 ± 2.8	20.8 ± 2.9 [c]
	HCM	9.7 ± 3.5	11.2 ± 1.7	20.0 ± 3.6 [b]	13.2 ± 3.6	19.8 ± 5.7 [c]
$+dP/dt_{max}$ (mmHg/s)	Control	2,098 ± 430	1,712 ± 246 [a]	2,687 ± 1,089	1,954 ± 430	3,107 ± 1,092 [c]
	HCM	2,101 ± 661	1,569 ± 391 [a]	2,449 ± 1,311	1,488 ± 343 [a]	3,456 ± 1,627 [c]
Tau (ms)	Control	30 ± 3	31 ± 3	24 ± 5 [b]	31 ± 4	26 ± 3 [c]
	HCM	34 ± 7	34 ± 7	29 ± 8 [b]	38 ± 5 [a]	26 ± 4 [c]
SV (ml)	Control	3.5 ± 0.8	3.5 ± 0.6	4.1 ± 0.6	4.0 ± 0.8 [a]	5.2 ± 0.9 [c]
	HCM	2.8 ± 1.0	2.7 ± 0.8	3.5 ± 1.2 [b]	3.2 ± 0.8	4.5 ± 1.2 [c]
CO (l/min)	Control	0.44 ± 0.07	0.46 ± 0.05	0.66 ± 0.11 [b]	0.41 ± 0.11	0.56 ± 0.15 [c]
	HCM	0.39 ± 0.11	0.34 ± 0.05 [#]	0.56 ± 0.18 [b]	0.31 ± 0.07 [a,#]	0.55 ± 0.20 [c]

Continued

Table 3.1 – Continued

CI (ml/kg/min)	Control	99.9 ± 15.3	103.9 ± 14.4	149.5 ± 20.6 [b]	93.8 ± 27.6	129.0 ± 40.1 [c]
	HCM	69.6 ± 17.8 [#]	63.2 ± 19.0 [#]	100.6 ± 31.3 [b,#]	57.2 ± 19.1 [#]	99.6 ± 33.2 [c]
Endsystol WS (g/cm^2)	Control	70.1 ± 18.5	88.3 ± 19.6	73.6 ± 27.3	73.6 ± 16.4	62.8 ± 13.3 [c]
	HCM	54.8 ± 25.6	57.3 ± 27.8	57.8 ± 36.9	65.0 ± 34.9	66.6 ± 35.3
Enddiast WS (g/cm^2)	Control	15.2 ± 7.8	20.9 ± 6.4 [a]	28.4 ± 6.8 [b]	27.9 ± 7.4 [a]	36.1 ± 10.4 [c]
	HCM	9.8 ± 3.4	9.7 ± 5.3 [#]	17.3 ± 8.8 [b,#]	19.7 ± 10.7 [a]	28.2 ± 11.3 [c]
Rate-pressure product	Control	10,146 ± 1,750	10,179 ± 1,752	16,690 ± 3828 [b]	8,720 ± 1,630	11,677 ± 2,367
(mmHg/min)	HCM	12,140 ± 3,659	10,781 ± 5,047	16,961 ± 6,231 [b]	8,429 ± 2,513 [a]	14,794 ± 5,968 [c]

Values are expressed as mean ± SD. Esm (esmolol), Esm+Dob (esmolol and dobutamine), Iva (ivabradine), and Iva+Dob (ivabradine and dobutamine).

[a], Within a row, values of Esm and Iva differ significantly ($P < 0.05$) from baseline value. [b], Within a row, values of Esm+Dob differ significantly ($P < 0.05$) from Esm. [c], Within a row values of Iva+Dob differ significantly ($P < 0.05$) from Iva.

[#] = Within a variable within a column, values in cats with HCM differ significantly ($P < 0.05$) from control cats.

See Appendix B 'Abbreviations Chapter 3' for reminder of key.

Table 3.2 – Echocardiographically-derived 2-D and M-mode indices of left ventricular and left atrial function under five different hemodynamic conditions in six control cats and six cats with hypertrophic cardiomyopathy.

Variable	Group	Baseline	Esm	Esm+Dob	Iva	Iva+Dob
IVSd (mm)	Control	4.7 ± 0.1	4.7 ± 0.3	4.6 ± 0.3	4.6 ± 0.2	4.7 ± 0.2
	HCM	6.8 ± 1.5 #	6.8 ± 1.2 #	6.8 ± 1.3 #	6.7 ± 1.5 #	6.4 ± 1.6 #
LVIDd (mm)	Control	13.4 ± 0.6	14.4 ± 1.3	14.7 ± 0.9	14.4 ± 0.4	14. 9 ± 0.9
	HCM	12.1 ± 2.8	11.9 ± 2.9 #	12. 1 ± 4.1	13.9 ± 1.6 a	14.1 ± 1.7
LVPWd (mm)	Control	4.8 ± 0.4	4.5 ± 0.2	4.3 ± 0.5	4.5 ± 0.3	4.7 ± 0.3
	HCM	6.1 ± 1.8	6.1 ± 1.6	6.1 ± 1.7 #	5.7 ± 1.5	5.7 ± 1.6
FS (%)	Control	38 ± 10	33 ± 10	52 ± 12 b	42 ± 7	58 ± 6 c
	HCM	39 ± 17	37 ± 16	50 ± 16 b	41 ± 7	54 ± 7 c
EF (%)	Control	64 ± 4	53 ± 5 a	68 ± 6 b	71 ± 4	79 ± 4 c
	HCM	62 ± 12	53 ± 14 a	68 ± 12 b	67 ± 4	77 ± 5 c

Continued

Table 3.2 – Continued

FAC (%)	Control	41 ± 10	39 ± 5	52 ± 5 [b]	50 ± 8 [a]	55 ± 4
	HCM	39 ± 7	36 ± 6	46 ± 8 [b]	4 6 ± 3 [a]	52 ± 4 [c]
LA_{max} (cm)	Control	1.4 ± 0.2	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.1 [a]	2.5 ± 0.5
	HCM	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.2 [c]
LA SF (%)	Control	23 ± 6	21 ± 5	31 ± 3 [b]	26 ± 5	32 ± 4
	HCM	22 ± 6	21 ± 4	28 ± 4 [b]	25 ± 4	32 ± 6 [c]

Values are expressed as mean ± SD. Esm (esmolol), Esm+Dob (esmolol and dobutamine), Iva (ivabradine), and Iva+Dob (ivabradine and dobutamine). *See* Appendix B ‘Abbreviations Chapter 3’ and **Table 3.1** for reminder of key.

Table 3.3 – Doppler-derived echocardiographic indices of left ventricular and left atrial function under five different hemodynamic conditions in six control cats and six cats with hypertrophic cardiomyopathy.

Variable	Group	Baseline	Esm	Esm+Dob	Iva	Iva+Dob
Peak E (m/s)	Control	0.61 ± 0.12	0.60 ± 0.05	0.84 ± 0.12	0.64 ± 0.15	0.76 ± 0.12
	HCM	0.50 ± 0.05 [#]	0.51 ± 0.09	0.67 ± 0.06	0.51 ± 0.17	0.69 ± 0.19
MV $DecT_E$ (ms)	Control	63 ± 13	61 ± 10	45 ± 15	60 ± 8	59 ± 11
	HCM	77 ± 10	75 ± 7	66 ± 11	71 ± 12	57 ± 12
Peak A (m/s)	Control	0.37 ± 0.11	0.39 ± 0.10	0.50 ± 0.02	0.41 ± 0.13	0.58 ± 0.14
	HCM	0.36 ± 0.05	0.38 ± 0.09	0.36 ± 0.13	0.34 ± 0.07	0.53 ± 0.10
E:A	Control	1.81 ± 0.72	1.66 ± 0.64	1.70 ± 0.22	1.71 ± 0.76	1.36 ± 0.33
	HCM	1.41 ± 0.18	1.46 ± 0.66	1.99 ± 0.83	1.50 ± 0.44	1.33 ± 0.43
IVRT (ms)	Control	63 ± 11	64 ± 17	45 ± 13 [b]	57 ± 10	47 ± 12
	HCM	75 ± 15	85 ± 23 [#]	68 ± 12 [b,#]	78 ± 16 [#]	63 ± 20 [c]

Continued

Table 3.3 – Continued

E:IVRT	Control	9.9 ± 1.9	9.9 ± 3.0	18.3 ± 6.7 [b]	11.5 ± 4.0	16.7 ± 3.8 [c]
	HCM	6.8 ±1.7	6.5 ± 2.7	10.7 ± 3.8 [b,#]	7.1 ± 3.6 [#]	12.3 ± 5.5 [c]
S/D	Control	1.09 ± 0.84	0.81 ± 0.34	0.96 ± 0.41	0.73 ± 0.15	1.03 ± 0.37
	HCM	1.80 ± 0.89 [#]	1.45 ± 0.52	1.57 ± 0.63 [#]	1.18 ± 0.40	1.47 ± 0.90
Peak AR (m/s)	Control	0.18 ± 0.05	0.17 ± 0.06	0.22 ± 0.04	0.14 ± 0.06	0.24 ± 0.08
	HCM	0.18 ± 0.08	0.15 ± 0.06	0.28 ± 0.21	0.12 ± 0.03	0.26 ± 0.18 [c]
Aduration :	Control	0.78 ± 0.13	0.78 ± 0.10	0.91 ± 0.06	0.71 ± 0.07	0.76 ± 0.08
ARduration	HCM	0.91 ± 0.24	1.02 ± 0.30	1.07 ± 0.30	0.92 ± 0.23	0.83 ± 0.09
Peak LAA (m/s)	Control	0.43 ± 0.17	0.32 ± 0.07	0.51 ± 0.10 [b]	0.37 ± 0.10	0.57 ± 0.13 [c]
	HCM	0.44 ± 0.16	0.39 ± 0.12	0.54 ± 0.19 [b]	0.34 ± 0.08	0.63 ± 0.14 [c]

Values are expressed as mean ± SD. Esm (esmolol), Esm+Dob (esmolol and dobutamine), Iva (ivabradine), and Iva+Dob (ivabradine and dobutamine). *See* Appendix B 'Abbreviations Chapter 3' and **Table 3.1** for reminder of key.

Table 3.4 – Tissue velocity imaging and speckle tracking-derived indices of systolic and diastolic left ventricular function under five different hemodynamic conditions in six control cats and six cats with hypertrophic cardiomyopathy.

Variable	Group	Baseline	Esm	Esm+Dob	Iva	Iva+Dob
Peak Ea lat (cm/s)	Control	7.25 ± 0.62	7.84 ± 1.78	10.47 ± 3.33	9.73 ± 3.09 [a]	10.88 ± 1.99
	HCM	6.20 ± 1.41	5.58 ± 1.05	7.87 ± 1.02	6.75 ± 2.29 [a,#]	8.73 ± 2.47 [c]
Peak Aa lat (cm/s)	Control	4.13 ± 0.73	3.62 ± 0.62	5.23 ± 1.14	5.13 ± 1.97	7.75 ± 1.69 [c]
	HCM	3.23 ± 1.41	3.28 ± 1.13	4.90 ± 2.04	4.42 ± 1.95	6.52 ± 1.85 [c]
E:Ea lat	Control	8.44 ± 1.40	8.03 ± 1.35	8.47 ± 2.46	6.74 ± 1.08	7.15 ± 1.44
	HCM	8.91 ± 2.24	9.76 ± 1.91	8.89 ± 0.63	7.70 ± 1.11	7.84 ± 0.44
circ SrR Peak S (1/s)	Control	1.73 ± 0.17	1.74 ± 0.34	1.86 ± 0.37	1.63 ± 0.44	2.10 ± 0.20 [c]
	HCM	1.64 ± 0.53	1.55 ± 0.42	1.54 ± 0.40	1.56 ± 0.31	2.63 ± 0.62 [c,#]
circ SrR Peak E (1/s)	Control	1.96 ± 0.67	2.50 ± 0.69	2.08 ± 0.49	2.36 ± 0.47	2.09 ± 0.63
	HCM	1.48 ± 0.01	1.76 ± 1.07	2.58 ± 0.65	2.39 ± 0.86	2.62 ± 1.59

Continued

Table 3.4 – Continued

circ SrR Peak A (1/s)	Control	0.87 ± 0.40	0.86 ± 0.45	1.09 ± 0.20	0.74 ± 0.45	1.06 ± 0.54
	HCM	0.41 ± 0.16	0.79 ± 0.49	1.34 ± 0.39	0.83 ± 0.33	1.17 ± 0.54
radial SrR Peak S (1/s)	Control	2.31 ± 0.34	2.03 ± 0.49	2.85 ± 0.98 [b]	1.94 ± 0.18	3.13 ± 0.61 [c]
	HCM	2.19 ± 0.41	1.95 ± 0.53	2.48 ± 0.75	1.85 ± 0.27	3.44 ± 1.32 [c]
radial SrR Peak E (1/s)	Control	2.70 ± 0.22	3.12 ± 1.15	3.75 ± 0.59	3.26 ± 1.13	3.32 ± 0.94
	HCM	2.06 ± 0.28	1.92 ± 0.41	3.69 ± 0.67	2.94 1.20	4.01 ± 1.81 [c]
radial SrR Peak A (1/s)	Control	1.29 ± 0.32	1.41 ± 0.22	1.60 ± 0.64	0.98 ± 0.27	1.84 ± 0.70 [c]
	HCM	1.16 ± 0.50	1.39 ± 0.96	2.16 ± 1.09	1.28 ± 0.53	2.07 ± 0.94 [c]
radial SR Peak S (%)	Control	42.2 ± 10.3	44.1 ± 18.7	44.2 ± 13.2	38.7 ± 4.6	51.8 ± 12.7 [c]
	HCM	32.3 ± 9.7	27.6 ± 8.9 [#]	39.2 ± 12.8	37.1 ± 7.7	48.3 ± 10.3 [c]

Values are expressed as mean ± SD. Esm (esmolol), Esm+Dob (esmolol and dobutamine), Iva (ivabradine), and Iva+Dob (ivabradine and dobutamine). *See* Appendix B 'Abbreviations Chapter 3' and **Table 3.1** for reminder of key.

Table 3.5 – Changes induced by dobutamine when administered concurrently with esmolol and ivabradine in six control cats and six cats with hypertrophic cardiomyopathy.

Variable	**Group**	**Esm+Dob**	**Iva+Dob**	***P***
HR (min^{-1})	Control	+28 ± 16	+7 ± 8	0.034
	HCM	+29 ± 21	+23 ± 17	ns
$+dP/dt_{max}$ (mmHg/s)	Control	+975 ± 1172	+1,153 ± 980	0.568
	HCM	+880 ± 1063	+1,968 ± 1,398	0.044
Tau (ms)	Control	-7 ± 2	-6 ± 6	ns
	HCM	-5 ± 5	-12 ± 6 [#]	0.019
EF (%)	Control	+15 ± 4	+8 ± 8	0.02
	HCM	+15 ± 8	+10 ± 5	0.044
FAC (%)	Control	+14 ± 6	+5 ± 6	0.030
	HCM	+10 ± 7	+6 ± 6	ns
circ SrR Peak S (1/s)	Control	+0.11 ± 0.28	+0.47 ± 0.30	ns
	HCM	-0.01 ± 0.68	+1.07 ± 0.39 [#]	0.002

Values are expressed as mean ± SD. Esm+Dob (esmolol and dobutamine) and Iva+Dob (ivabradine and dobutamine). *See* Appendix B 'Abbreviations Chapter 3' and **Table 3.1** for reminder of key.

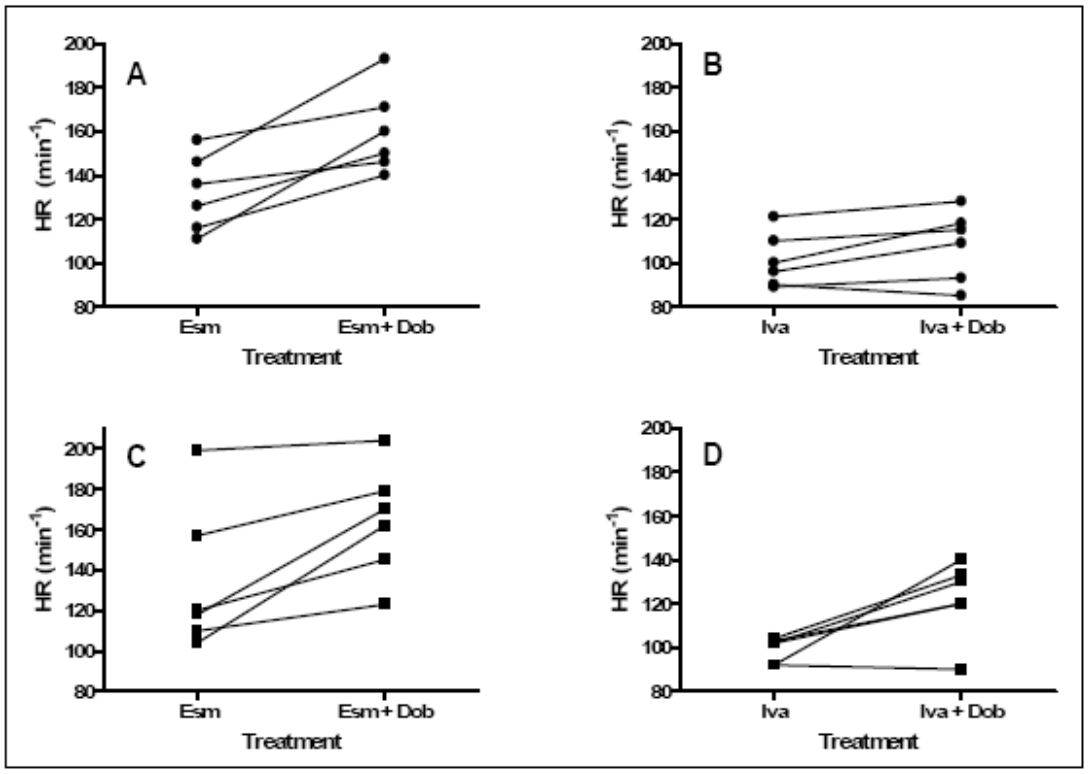

Figure 3.1 – Effects of dobutamine on heart rate.

Effects of dobutamine (Dob) on heart rate (HR) in six control cats (panels A and B; •) and six cats with hypertrophic cardiomyopathy (HCM; panels C and D; ■) after prior administration of esmolol (Esm) and ivabradine (Iva). There was a significant difference (P=0.034) in the mean increase of HR after treatment with Dob between Esm (28 min^{-1}) and Iva (7 min^{-1}) in control cats. Please notice that in panels B and D, peak HR is < 140 min^{-1} in all cats.

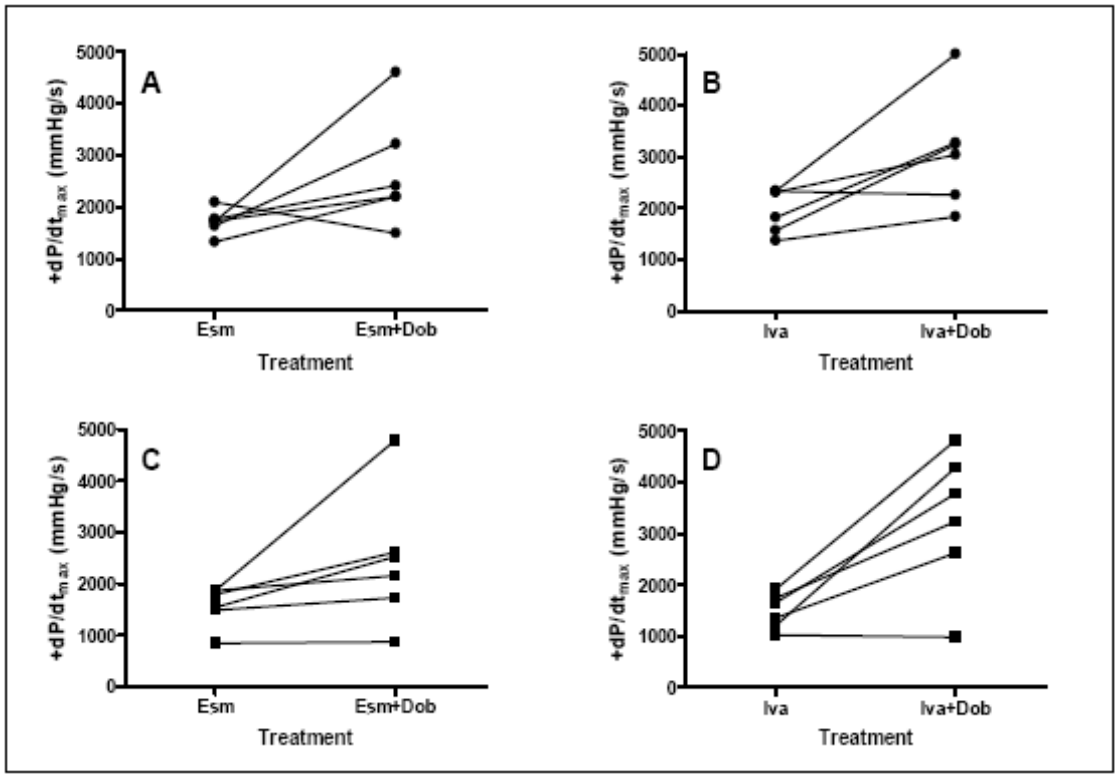

Figure 3.2 – Effects of dobutamine on peak positive change of pressure over time. Effects of dobutamine (Dob) on peak positive change of pressure over time ($+dP/dt_{max}$) in six control cats (panels A and B; ●) and six cats with hypertrophic cardiomyopathy (HCM; panels C and D; ■) after prior administration of esmolol (Esm) and ivabradine (Iva). There was no difference in the mean increase of $+dP/dt_{max}$ between treatments in control cats (panel A, + 975 mmHg/s and panel B, + 1153 mmHg/s, $P>0.05$). There was a significant difference in the mean increase of $+dP/dt_{max}$ between treatments in cats with HCM (panel C, + 880 mmHg/s and panel D, + 1968 mmHg/s, $P=0.044$). There was no significant difference ($P>0.05$) in the mean increase of $+dp/dt_{max}$ between control cats and cats with HCM at each treatment.

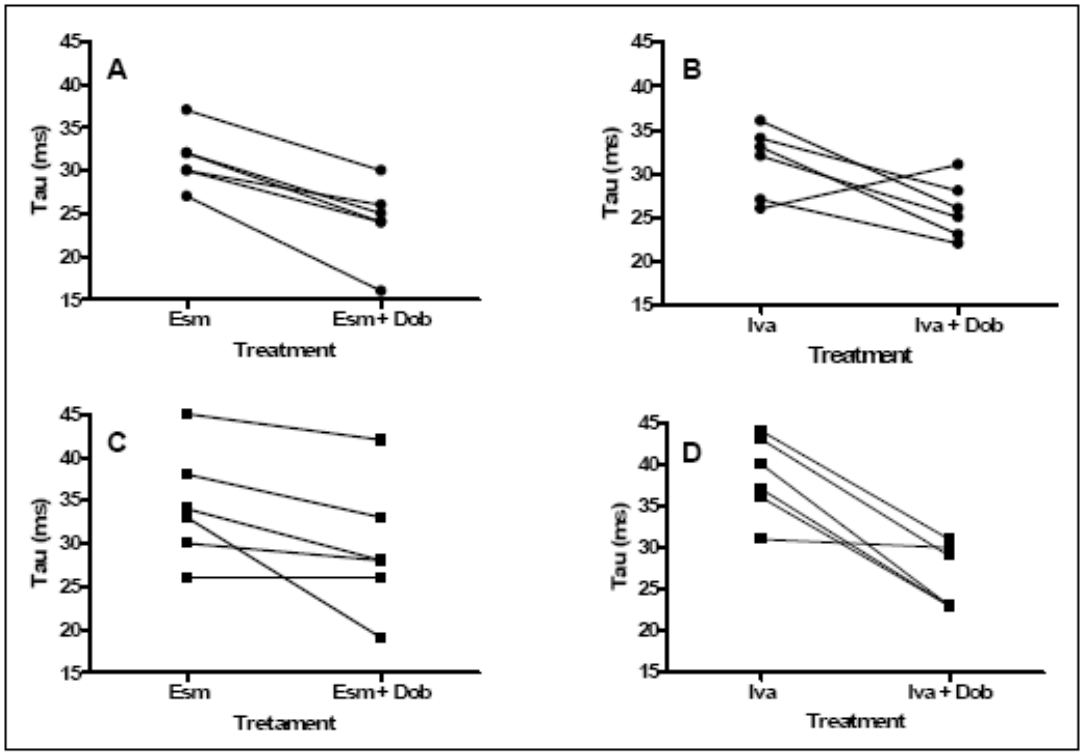

Figure 3.3 – Effects of dobutamine on the time constant of isovolumic relaxation. Effects of dobutamine (Dob) on the time constant of isovolumic relaxation (Tau) in six control cats (panels A and B; ●) and six cats with hypertrophic cardiomyopathy (panels C and D; ■) after prior administration of esmolol (Esm) and ivabradine (Iva). There was a significant difference (P=0.019) in the mean reduction of Tau after Esm+Dob (-5 ms) compared to Iva+Dob (-12 ms) in cats with HCM. There was also a significant difference (P=0.029) in the mean reduction of Tau after to Iva+Dob between control (-6 ms) and HCM (-12 ms).

Discussion

The results of this study demonstrate that intravenous administration of Iva reduces HR and the rate pressure product in anesthetized cats with HCM. LV systolic and diastolic function as well as LA performance was either unchanged or minimally affected by Iva. Tachycardia induced by Dob was significantly blunted by Iva but not by Esm at the dosages studied, indicating that the negative chronotropic activity of ivabradine is maintained under both resting conditions and sympathetic stimulation.

Effects of ivabradine on HR and rate-pressure product : Previous studies have established the selective HR-reducing effect of Iva in various species, including rats, pigs, dogs and humans when given intravenously[31-35] and orally.[36] Although Iva reduces HR at rest and during exercise,[32-34, 36] only limited information is available relative to its negative chronotropic effects during stress or enhanced sympathoadrenergic activity. Control of HR could be important in management of HCM considering the anecdotal evidence that stress and unwanted tachycardia may contribute to clinical signs, disease progression, and fatal outcome.[3, 37]

In this study, we investigated the effects of intravenous Iva on HR in anesthetized cats with HCM with and without sympathetic stimulation. The mean reduction of HR in HCM cats observed after Iva was 33 % relative to baseline. Heart rate was still reduced by 17 % compared to baseline during Dob infusion in these cats. These results are in accordance with previous studies in dogs and humans where subjects were studied at rest and during exercise[31-35] and could have important clinical implications. In contrast to Esm, Iva reduced HR consistently and maximum HR observed after Dob did not exceed

140 min^{-1} in any of the cats studied. Thus, our results suggest that the negative chronotropic effect of Iva in the setting of sympathetic stimulation is more pronounced than that of a moderately high dose of Esm in anesthetized cats with HCM. However, during Iva infusion the sinus rate was still responsive to Dob. Complete suppression of the HR response to sympathetic stimulation is undesirable considering a higher HR is needed during stress and exercise. It should be emphasized that we did not critically evaluate dose-responses of either Iva or Esm in this pilot study and it is likely that different dosages of either Esm or Iva could have blunted the HR response to Dob in a similar manner.

The effects of ivabradine on *myocardial perfusion and oxygen consumption* were not directly measured in this study. However, HR was determined, the rate-pressure product calculated,[38-41] and systolic and diastolic wall stress estimated.[10, 28] Systolic wall stress was not different between treatments or between cat groups, whereas calculated end-diastolic wall stress was lower in cats with HCM during treatments with Esm and Esm+Dob when compared to controls. In contrast to previous studies in healthy dogs,[10, 28] we found a significant increase in diastolic wall stress following Iva in both groups due to an increase of LVEDP and LV end-diastolic dimension. Increased diastolic wall stress in combination with hypertrophy may favor the development of myocardial ischemia, especially in the subendocardium.[42] However, the increase in end-diastolic wall stress was low, and the consequences would likely be balanced by the concurrent reduction of HR and the rate-pressure product in cats with HCM. Both HR and rate-pressure product decreased with ivabradine, indicating a lower myocardial oxygen demand.[38-41] Studies in

awake cats with HCM are needed to further elucidate the clinical importance of increased LV end-diastolic wall stress after Iva.

Effects of ivabradine on variables of LV contractility and systolic function: As expected, LV contractility decreased under the influence of Esm in all cats. Similar observations were identified in cats with HCM after Iva; however, other variables of systolic function (e.g., FS, EF, and radial and circumferential strain rate and radial strain) were not altered. This finding is in contrast to previous studies in healthy laboratory dogs and in dogs with coronary artery ligation[31-34] in which no significant changes in inotropic state were observed after Iva. In the study presented, mean reduction of HR after Iva was 33 % in cats with HCM and

22 % in control cats. Thus, the decrease of LV $+dP/dt_{max}$ observed in cats with HCM could simply be related to the negative staircase effect as previously suggested.[32] According to Colin et al.,[10, 28] the presence of an intrinsic effect of Iva on myocardial contractility is unlikely and was not believed to directly reduce LV $+dP/dt_{max}$ in cats with HCM. Although the mechanism for the mild decrease of LV contractility could not be determined, this observation may indeed be of benefit for cats with HCM and LV outflow obstruction. More than 50 % of cats with HCM have dynamic obstruction of the LV outflow tract,[19] and negative inotropy is well known mechanism for relief of the obstruction.[4-6] In contrast, none of the variables characterizing LV systole were reduced in the control group cats after Iva, indicating preserved LV systolic performance in this group, a finding consistent with previous reports.[31-34]

Effects of ivabradine on variables of LV diastolic function: LV diastolic function has been characterized conceptually by two distinct components, relaxation and compliance.[20] Relaxation may be quantified by tau, the time constant of isovolumic relaxation.[43] Assessment of ventricular relaxation has clinical value, especially in cats with HCM in which abnormal relaxation is a characteristic feature. Ventricular relaxation in normal hearts is affected by beta receptor blockade and improved by sympathetic activation.[27, 44, 45] Heart rate may influence tau directly via the reversed relaxation-frequency relationship.[46] However, only small and clinically non-relevant changes were observed after abrupt changes in HR from 120 to 170 min^{-1} in healthy dogs.[46, 47] Our data suggest a mild prolongation of tau in cats with HCM after Iva (mean, +4ms, $P<0.009$). This finding is in contrast to previous studies in healthy dogs and dogs with reduced coronary perfusion[31-34] and may be related to our experimental design (e.g., effects of anesthetic drugs; duration of anesthesia; mild hypothermia; or sequence of drug administration) or the mild depression of LV contractility found after Iva. In accordance to previous observations in healthy anesthetized cats,[20] there was a significant linear association between LV $+dP/dt_{max}$ and tau in cats with HCM. LV $+dP/dt_{max}$ was a major determinant of tau in this study confirming that systolic and diastolic function are closely intertwined. [21, 48] Since regression analysis did not reveal an association between HR and tau in cats with HCM, the pure HR-lowering effects of Iva could not explain the prolongation of tau. Other estimates of ventricular relaxation suggested improvement (Ea) or no change in relaxation (IVRT and variables of regional relaxation from strain

analysis) in cats with HCM. In contrast, tau was unchanged after Iva in control cats. The reason for this discrepancy between cat groups deserves further study.

Sympathetic stimulation achieved a more profound reduction in tau in cats with HCM when administered simultaneously with Iva as compared to Esm. This suggests an enhanced positive lusitropic effect of Dob under the influence of Iva (**Figure 3.3**). In addition to tau, other estimates of ventricular relaxation such as IVRT, Peak Ea, and radial strain rate-derived Peak E also improved during Iva+Dob. This effect may be of benefit in cats with HCM in situations characterized by sympathetic stimulation including stress and excitement.

Variables of LV compliance, LV Filling, and LV filling pressure – Treatment with Iva resulted in a significant increase LVEDP and LV end-diastolic wall stress in both cats with HCM and control cats. These observations are in contrast to results of previous studies performed in healthy dogs at rest.[10, 49] However, in the same studies during exercise, Iva also lead to an increase of LVEDP and LV end-diastolic wall stress.[10, 49] The mild elevation of LVEDP may be explained by the concept of preload reserve, a mechanism by which filling pressure increases in response to blunted HR response to maintain CO.[28] This was confirmed by normalization of the increased LVEDP and end-diastolic wall stress after Iva in humans using atrial pacing.[28, 50] The significant (P=0.006) increase of LVIDd found in our study strengthens this assumption. Other variables related to LV compliance and LV filling including MV $DecT_E$, Aduration:ARduration, Peak E, E:A, E:Ea, and S:D were not significantly altered by ivabradine in cats of either group. We concluded from these findings that a mild increase of preload due to the

negative chronotropic effects of Iva may lead to an increase of LVED and LV end-diastolic wall stress observed in both anesthetized cats with HCM and control cats. The clinical significance of such observations needs to be determined.

Effects of ivabradine on variables of LA function: LA performance and LA size were not significantly altered after treatment with Iva, supporting the concept that I_f -current inhibition does not affect LA function and size in anesthetized cats with HCM. Due to the design of our study, only acute and possibly only very marked changes could have been detected with the known effects of anesthesia on LA function[51] potentially complicating the interpretation of our results. Therefore, to further elucidate the effects of I_f -current inhibitors and beta-blockers on LA function, long-term studies in awake cats are needed addressing this issue. As expected, sympathetic stimulation with Dob led to an augmentation of LA performance with all treatments and in all groups.

Certain limitations of this pilot study should be emphasized. First, gender was not equally balanced between groups. The diagnosis of HCM was made by 2D echocardiography using wall thickness and generally accepted decision thresholds. However, LV wall thickness may be affected by factors other than idiopathic myocardial growth, and histopathologic studies needed for a definitive diagnosis were not performed. In addition, the number of cats in each group was small, rendering the study underpowered to detect smaller differences between groups or treatments. We did not measure LA or LV pressure-volume loops which are usually considered the gold standard for assessing compliance and LV contractile function. The order of drug administration was not randomized owing to the long-lasting hemodynamic and HR-reducing effects of

Iva. Furthermore, the resultant HR responses in the Esm versus the Iva treatments were not similar, and the influence of HR on outcome variable, while not statistically evident, cannot be completely discounted. Observers were not blinded to the pharmacological interventions during the studies. However, to reduce the effects of observer bias on interpretation of data, images and recordings were coded during data analysis, making observers blind to animal and treatment during measurement of data. Repeatability of echocardiographic and hemodynamic measurements was not specifically addressed. However, previous data reported from our laboratory revealed clinically acceptable reproducibility of most variables determined.[21, 48] Finally, owing to the study design, the effects of anesthesia on cardiac function and possible interactions with the effects of the drugs could not be eliminated. Therefore, caution is advised when extrapolating our findings to conscious cats.

In summary, this pilot study revealed that Iva, given intravenously at 0.3 mg/kg, reduces HR significantly with only minimal effects on LV and LA function in anesthetized cats with HCM. Ivabradine also blunts the positive chronotropic response to Dob in healthy cats and cats with HCM cats but still allows for responsiveness to catecholamines which may be important for response to physiologic stress. These results are promising for potential clinical use of Iva in cats. Further studies in awake cats with HCM using oral Iva administration are needed to clinically validate our findings.

Footnotes

[a]Liberty Research Inc., Waverly, NY.
[b]Acepromazine maleate injection, Boehringer Ingelheim Vetmedica Inc., St Joseph, MO.

[c]Torbugesic, Fort Dodge Laboratories, Fort Dodge, Iowa.
[d]PropoFlo, Abbott Laboratories, North Chicago, IL.
[e]Isoflurane, Abbott Laboratories, North Chicago, IL.
[f]Medfusion 2010i syringe pump, Medexine, Duluth, GA.
[g]Cefazolin, Sandoz Inc., Princeton, NJ.
[h]Heparin Sodium, APP Pharmaceuticals LLC, Schaumburg, IL.
[i]Lidocaine HCL 2%, Abbott Laboratories, Chicago, IL.
[j]Model SPC-751 Millar Micro-tip catheter pressure transducer and Model TCB-600 control unit, Millar Instruments Inc., Houston, TX.
[k]Arrows Thermodilution Balloon catheter, Model AI-07165, Arrow International Inc., Reading, PA.
[l]Cardiomax III, Columbus Instruments, Columbus, OH.
[m]0.9% Sodium chloride injection, Baxter Healthcare Corp., Deerfield, IL.
[n]Vivid 7 Vantage, GE Medical Systems, Milwaukee, WI.
[o]EchoPac software package, Version BT06, GE Medical Systems, Milwaukee, WI.
[p]Esmolol HCL, Bedford Laboratories, Bedford, OH.
[q]Dobutamine, Hosperia Inc., Lake Forrest, IL.
[r]Ivabradine HCL, Franck's Pharmacy, Ocala, FL.
[s]Cober RE, Schober KE, Buffington CAT, Riesen SC, Bonagura JD. Effects of ivabradine, a selective I_f channel inhibitor, on heart rate in healthy cats. J Vet Intern Med 2010;24:695 (abstract).
[t]Puradisc Syringe Filter, Whatman International Ltd, Maidstone, England, UK.
[u]DATAQ work station and WINDAQ/200, Windows Acquisition and Analysis Software and Advanced CODAS, DATAQ Instruments Inc., Akron, OH.
[v]SigmaStat, Version 3.5, SPSS Inc, Chicago, IL.
[w]PC SAS Version 9.2, SAS Institute, Cary, NC.

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CHAPTER 4

COMPARISON OF SHORT-TERM EFFECTS OF IVABRADINE AND ATENOLOL ON HEART RATE AND ECHOCARDIOGRAPHIC VARIABLES OF LEFT HEART FUNCTION IN HEALTHY CATS

Hypertrophic cardiomyopathy (HCM) is the most commonly observed myocardial disease in cats.[1-3] The progression of HCM is variable with most cats having a long asymptomatic period that may end abruptly with the development of congestive heart failure, arterial thromboembolism, or sudden cardiac death. Although the exact mechanisms leading to clinical decompensation are not fully understood, several risk factors have been identified including severity of left ventricular (LV) hypertrophy, degree of diastolic dysfunction, increased left atrial (LA) size, and elevated heart rate (HR).[1, 3, 4] Tachycardia induced by stress or exercise seems to be an important trigger event leading to decompensation and death in human patients with previously occult HCM.[5-7] These outcomes may be associated with abbreviated coronary filling time, decreased myocardial perfusion, and myocardial ischemia suddenly aggravating ongoing diastolic dysfunction and favoring the development of malignant arrhythmias.[5]

Beta adrenergic blockers and calcium channel blockers are the most frequently used drugs in preclinical feline HCM. General use of these two drugs remains

controversial since a beneficial effect on disease progression or survival has not been demonstrated with either drug. [8-11] Atenolol is generally preferred to diltiazem in subclinical HCM by veterinary cardiologists, based on a recent on-line survey performed by Rishniew.[a]

The use of bradycardic agents is predicated on their antiischemic properties and potential to enhance left ventricular ventricular diastolic function, control sinus tachycardia, and reduce dynamic outflow tract obstruction. However, there are concerns related to adverse effects of diltiazem and atenolol, including weakness, lethargy, salivation, weight loss, and reduced LA function. These effects may limit their clinical utility.[8-11]

Heart rate, a major determinant of myocardial oxygen consumption and cardiac work load, has become a novel treatment target in people with ischemic heart disease. Ivabradine is a highly selective funny current (I_f) inhibitor that acts directly on the sinoatrial node to induce a use- and dose-dependent reduction of HR. This negative chronotropic activity occurs without significant effects on inotropy, lusitropy, or dromotropy.[12, 13] Ivabradine has been shown to have favorable effect on cardiac output, coronary blood flow, and myocardial oxygen consumption in experimental animals.[14, 15]

Preliminary studies in cats using intravenous and oral ivabradine have demonstrated hemodynamic,[b] pharmacokinetic,[c] and pharmacodynamic[d] effects that recommend consideration of this drug for oral use in the clinical setting. To the author's knowledge, no short-term data on the clinical effects of I_f -inhibitors in cats are available. Accordingly, the objectives of the study reported herein were to 1) evaluate the short-term clinical tolerability of ivabradine and 2) study the short-term effects of ivabradine

on HR, blood pressure, ECG, LV function, and LA performance. These investigations were conducted in healthy cats after repeated oral dosing of both ivabradine and atenolol. We hypothesized that ivabradine would be non-inferior to atenolol. The dose of ivabradine selected was based on prior pharmacologic and pharmacokinetic studies in our laboratory,[c,d] and the dose of atenolol evaluated was based on common clinical usage.[16]

Material and Methods

Animals: Ten healthy, female-spayed, domestic shorthair cats were studied. The cats were 2 to 7 years old (mean, 4.6; SD, 1.7) and weighed 2.8 to 6.4 kg (mean, 4.3; SD, 1.0). All cats were acquired from a commercial vendor.[e] The study protocol was reviewed and approved by the Animal Care and Use Committee and the Institutional Review Board of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University. All animals were treated in compliance with the National Institutes of Health guidelines on the care and use of laboratory animals.

Study design: This was a prospective noninferiority study of two months duration. The design was further classified as double-blind, randomized,[f] active-control, and fully crossed. Each cat was assigned to either ivabradine[g] (0.3 mg/kg, q12h, PO; group 1) or atenolol[h] (6.25 mg/cat, q12h, PO; group 2) for the first four weeks and then switched to the alternative treatment for another four weeks. The dose of atenolol was selected based on clinical usage, since it is uncommon to compound this drug to a mg/kg dose. In this study, the mean dose of atenolol was 1.3 mg/kg (range: 1.0 to 1.7 mg/kg). Prior to randomization exact doses of both drugs were prepared for each cat and subsequently filled in opaque capsules[i] assuring blinding. The capsules were administered manually

twice daily by the investigators (SCR and RMC) to assure proper drug administration. No other drug was administered during the study period.

At baseline, each cat was subjected to a complete clinical evaluation, including a physical exam, a CBC and blood biochemistry panel, a 6-lead ECG,[j] an indirect measurement of systolic blood pressure (SBP),[k] and a complete transthoracic echocardiogram.[l] Follow up evaluations were performed at four and eight weeks after inclusion. Physical exam, ECG recording, BP measurement, and transthoracic echocardiography were performed three hours after drug administration when the maximum negative chronotropic effect of each drug was anticipated.[d] Echocardiographic recordings were labeled with random numbers, allowing subsequent offline measurements in a blinded fashion. In addition, the overall well-being of the cats including clinical variables such as appetite, behavior, activity, interaction, defecation, urination, vomiting, grooming, and respiration was monitored daily during the entire study period. Findings were summarized once weekly, and a Clinical Composite Score (CCS) was determined for each cat and each week (*See* Appendix C). Each variable was scored with either 1 point (observation within the cat's normal range) or 2 points (any abnormal findings). Points of all variables were then added to calculate the CCS (minimum 9 to maximum 18 points). The CCS was then graded as: normal (CCS of 9 points), mildly to moderately abnormal (CCS of 10-15 points), and severely abnormal (CCS of 16-18 points).

ECG: A standard six-lead ECG[j] was recorded with the cats in right lateral recumbency, and ECG traces were examined by analysis of rhythm, heart rate (HR_{ECG}), time intervals,

and amplitude of the complexes.[17] Duration of the QT interval was corrected for cycle length according to Fridericia ($QT_F = QT / RR^{1/3}$).[18]

Heart rate, rate pressure product and systolic wall stress: At clinical exam, heart rate (HR_{CE}) by auscultation and SBP were determined three hours after drug administration in conscious cats, and the rate-pressure product (RPP), an estimate of myocardial oxygen consumption, was calculated ($RPP = SBP \times HR_{CE}$).[19] Left ventricular end-systolic wall stress was calculated using a cylindrical model[15, 20] as stress = 1.36 x (SBP x D/2h), where D is the internal short-axis diameter and h is wall thickness of the LV at end-systole.

Echocardiography: Following sedation with acepromazine[m] (0.1 mg/kg, IM) and butorphanol[n] (0.25 mg/kg, IM), each cat underwent a transthoracic two-dimensional (2D), M-mode, spectral and color Doppler, pulsed wave tissue Doppler imaging (TDI), and 2D-strain echocardiographic examinations. In five cats, the sedative effects were insufficient for quality examinations and ketamine[o] (1 mg/kg, IM) was added. All cats received the same type and dose of the tranquilizers at each examination. Echocardiographic examinations were performed with a transducer array of 5.0 to 7.0 MHz nominal frequency.[l] A simultaneous one-lead ECG was recorded and used for heart rate (HR_{Echo}) measurement. Data were stored digitally and echocardiographic data analysis was performed off-line by use of a commercial analysis software package.[p] The mean of three cardiac cycles was calculated for each variable measured.

Right parasternal long and short-axis and left apical long-axis imaging views were acquired to allow for optimal recording of the left atrium, the left ventricle, transmitral and pulmonary venous flow, left atrial appendage flow, aortic outflow, isovolumic

relaxation time (IVRT), tissue Doppler-derived myocardial velocities of the lateral mitral annulus, and 2D-strain based indices of LV deformation.[4, 21, 22] Two-dimensional and M-mode variables of the LV and LA were obtained as previously described.[4, 21-23] LV ejection fraction was measured by use of the modified single plane Simpson's method from left apical long-axis images.[24] Maximum and minimum LA dimensions and area were assessed from the right parasternal long-axis view.[4, 22] In addition, fractional area change of the LA (FAC) and left atrial shortening fraction (LA SF) were calculated. [4, 22] Left atrial appendage (LAA) flow velocity was obtained from a left apical tilted 3-chamber view and with a sample volume of 3 to 5 mm placed in the proximal third of the appendage.[4, 22] Pulmonary venous flow was obtained from a right parasternal short-axis heart base view with minimized baseline filter and with a sample volume of 5 mm placed 2 to 4 mm within the right upper pulmonary vein. Transmitral flow was recorded with a sample volume of 2 mm placed between the tips of the opened mitral valve leaflets, and peak velocities (Peak E and Peak A) as well as A-wave duration were measured. In case of complete fusion of E and A waves, or partial fusion of the waveforms (E-at-A velocity > 20 cm/s), peak diastolic velocity of summated waves was reported as Peak EA fus.

Pulsed wave Doppler-derived velocities of myocardial motion were also recorded from the left apical view using the tissue Doppler imaging application of the echocardiographic system.[25] The Doppler gain was minimized to generate a clear tissue signal with minimum background noise. Frame rate (>160 frames/s) was optimized by narrowing the tissue Doppler imaging sector. A sample volume of 5 mm was placed at the lateral mitral annulus and the peak systolic and diastolic velocities (Peak Sa, Peak Ea, and Peak Aa) were measured. In case of complete fusion of Ea and Aa, velocity of the

summated waves was reported as Peak EaAa fus. The E:Ea ratio or alternatively the EA fus:EaAa fus ratio were calculated.

The right parasternal mid-ventricular short-axis plane of the LV was imaged for quantification of the peak systolic radial strain (radial SR Peak S), and peak systolic and diastolic radial strain rate (radial SrR Peak S, radial SrR Peak E, and radial SrR Peak A). In case of fusion of early and late diastolic myocardial deformation waves, diastolic radial strain rate was reported as radial SrR EA fus. Optimal frame rate was obtained by adjusting the sector width and image depth to achieve frame rates of 86 to 251 per second using the formula: 0.8 x HR (Stoylen A., personal communication). Off-line analysis of 2D speckle imaging was performed as previously described in dogs.[26] Briefly, timing of aortic valve opening and closure were defined using spectral Doppler outflow signals. The LV endocardium was traced manually using a single frame with well delineated endocardial borders. Then the region of interest for speckle tracking was optimized and tracked automatically using the software algorithm. Adequate tracking of each of the six equally sized myocardial segments was visually verified before approval of the measurements. For LV radial strain and strain rate values reported were determined as the average of six corresponding myocardial segments.[26]

Non-inferiority: To determine that treatment with ivabradine was at least as effective as treatment with atenolol (non-inferiority) with regard to HR_{CE}, RPP, systolic wall stress, and echocardiographic indices of LV and LA function, a non-inferiority margin was set *a-priori* at 50% ($f = 0.5$). This criterion was based on predicted clinical relevance, statistical reasoning related to estimated random effects, and observer measurement variation,[27-30] and is in agreement with FDA guidelines for this analysis.[31-33] The non-

inferiority margin is defined in terms of some fraction (*f*) of the treatment effect observed with the alternative treatment (i.e., atenolol).[31-33] That is, non-inferiority of ivabradine compared to atenolol was present if the effects of ivabradine were at least 50% of the treatment effects observed with atenolol.

Statistical analysis: Statistical analysis was performed by use of commercially available software.[q] Normal distribution of variables was determined by the Kolmogorov-Smirnov test. Descriptive statistics were calculated for all clinical and echocardiographic variables, and presented as mean and standard deviation (SD) unless stated otherwise. To compare the effects of ivabradine and atenolol, a repeated measures linear model (2-way repeated measures ANOVA) was used with sequence effect (group 1: ivabradine followed by atenolol; group 2: atenolol followed by ivabradine) with cat as a within-subject factor and treatment as a between-subjects factor (to identify differences between groups and treatments). Differences between treatments and baseline were compared by use of the Holm-Sidak post hoc test. If more than 4/10 observations per treatment were missing, statistical analyses were not performed and data were only reported descriptively. Bonferroni correction was used for multiple comparisons. For all analyses, $P \leq 0.05$ was considered significant.

Results

Animals and Clinical Tolerance: Both ivabradine and atenolol were well tolerated in this study, and no cat was withdrawn owing to adverse effects. Neither clinically relevant changes in body weight nor hematologic or serum biochemical abnormalities were detected. Moreover, the overall well being of the cats as assessed by appetite, behavior,

activity, interaction, defecation, urination, vomiting, grooming, and respiration was not altered by either treatment. The CCS remained within baseline limits during the entire study period in all cat.

At baseline, cats of both groups were not different with regard to age, body weight, HR, SBP, or any of the echocardiographic and electrocardiographic variables determined. Nor was any significant sequence effect observed for any of the recorded variables.

Heart rate, RPP, Systolic Wall Stress, and ECG: Results of HR_{CE}, SBP, RPP, systolic wall stress, and electrocardiographic variables are summarized **Tables 4.1** and **4.2** and **Figure 4.1**. Ivabradine decreased HR_{CE} and RPP significantly ($P<0.001$), with no statistical difference between treatments (HR_{CE}, $P=0.721$; RPP, $P=0.847$). Ivabradine had no significant effect on systolic wall stress compared to baseline, however the difference between treatments was significant ($P=0.009$). Ivabradine caused a reduction of mean systolic wall stress whereas atenolol caused an increase, indicating a more favorable effect of ivabradine on this variable. Systolic BP was not changed by ivabradine, with no difference between treatments ($P=0.083$).

Ivabradine prolonged the PQ interval ($P=0.04$), QT duration ($P<0.001$), and QT_F interval ($P<0.001$), with no difference between treatments (QT duration, $P=0.269$; and QT_F, $P=0.880$). The PQ interval was significantly increased after atenolol compared to ivabradine ($P=0.006$).

Echocardiography: Results regarding echocardiographically-derived variables are summarized in **Tables 4.3** to **4.6**.

Effects on LA and LV size – Ivabradine did not change variables of LA size significantly, and no differences between treatments were observed (**Table 4.3**). M-mode derived indices of the LV size were not changed by ivabradine, but a significant difference for LVIDs (P=0.013) between treatments was evident (**Fig 4.1** and **Table 4.3)**. Ivabradine increased estimated LV end-systolic volume (ivabradine, P=0.005) and LV end-diastolic volume (ivabradine, P<0.001), with no differences between treatments (ESV, P=0.773; EDV, P=0.097).

Effects on variables of LV systolic and diastolic function – Ivabradine increased FS (P<0.001) and radial SR Peak S (P<0.001), with significant differences between treatments (FS, P<0.001; radial SR Peak S, P=0.025). The remaining variables of LV systolic function (EF, AO vmax, radial SrR Peak S) were not significantly altered by ivabradine (**Fig 4.1** and **Table 4.4)**, however differences were observed between treatments for Ao Vmax (P<0.008) and radial SrR Peak S (P<0.003).

At baseline, 80% of the cats had fusion of early and late diastolic waves derived by PW spectral Doppler and PW TDI, and all cats had fused E and A waves derived by 2D strain imaging (**Table 4.5** and **4.6**). Ivabradine led to separation of early and late diastolic waves assessed by PW spectral Doppler, PW TDI, and 2D strain imaging in all cats. Atenolol reduced fusion of E and A from 80% to 60% for both PW Doppler and PW TDI, and from 100% to 30% for 2D strain imaging.

Variables of LV relaxation: IVRT was not changed by ivabradine, with no difference between treatments. However, IVRT was significantly prolonged by atenolol (P=0.006, **Fig 4.1**) compared to baseline. Other estimates of LV relaxations such as Peak

Ea lat could not be statistically evaluated due to low numbers of observations secondary to fusion of the early and late diastolic waves at baseline.

Variables of LV Compliance, filling, and filling pressures: Ivabradine decreased E:IVRT (P<0.003) and S:D (ivabradine, P<0.001), with no difference between treatments (E:IVRT, P=0.328; S:D P=0.234). Other estimates of LV compliance and filling such as Peak E, E:A, E:Ea, and Aduration:ARduration could not be statistically evaluated due to low numbers of observations owing to fusion of the early and late diastolic waves at baseline.

Effects on variables of LA function – Ivabradine did not change Peak AR and Peak LAA; however significant differences were observed between treatments (Peak AR, P=0.005; Peak LAA, P<0.009; **Table 4.6** and **Fig 4.1**). A significant difference (P=0.012) was observed for radial SrR Peak A between treatments, with higher values observed after ivabradine compared to atenolol. Other variables of LA systolic function such as Peak A and Peak Aa could not be statistically assessed due to low numbers of observations secondary to fusion of the early and late diastolic waves at baseline. Ivabradine did not change LA SF and LA FAC; however a significant difference was observed between treatments for LA SF (P=0.001, **Table 4.6**).

Non-inferiority: Ivabradine was non-inferior ($f \geq 0.5$) to atenolol with regard to the following variables: HR, RPP, LADs, LA area s, LVIDd, EDV, ESV, IVRT, E:IVRT, Peak D, S:D, ARduration, and radial SR Peak S. The following variables could not be evaluated due to low number of observations: Peak E, Peak EA fus, E:A, Aduration, Aduration:ARduration, Peak Ea lat, Peak Aa lat, Peak Aa lat, EaAa fus, E:Ea lat, Peak

Aa lat, EaAa fus, E:Ea lat, EA fus:EaAafus, radial SrR Peak E, radial SrR Peak A, and radial SrR Peak EA fus (**Tables 4.1** and **4.3** to **4.6**).

Table 4.1 – Heart rate (HR) at clinical exam, systolic arterial blood pressure (SBP), rate-pressure product (RPP), and systolic LV wall stress at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	**Baseline**	**Atenolol**	**% Δ**	**Ivabradine**	**% Δ**
HR_{CE} (min^{-1})	204 (32)	144 (20) [a]	-27 (10)	135 (30) [a]	-34 (15) [§]
SBP (mmHg)	148 (18)	156 (16)	+5 (11)	164 (20)	+11 (14)
RPP (mmHg x min^{-1})	30,048 (5229)	22,616 (4859) [a]	-25 (16)	22,245 (6063) [a]	-26 (20) [§]
Systolic WS (g/cm^2)	94.1 (17.7)	103.6 (10.6) [b]	+10 (11)	84.0 (15.3) [b]	-11 (16)

Values are expressed as mean (SD) and percent change (% Δ±SD).

[a] = Within a row, values differ significantly ($p < 0.05$) from baseline value.

[b] = Within a row, values differ significantly ($p < 0.05$) between atenolol and ivabradine.

[§]= Effects of ivabradine compared to atenolol within the non-inferiority margins of 50% (*f*=0.5).

WS, wall stress.

Table 4.2 – Electrocardiographically-derived indices at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	Baseline	Atenolol	Ivabradine
HR_{ECG} (min^{-1})	238 (45)	177 (34) [a]	155 (21) [a]
P duration (ms)	28 (6)	30 (5)	29 (6)
P amplitude (mV)	0.15 (0.04)	0.13 (0.05)	0.16 (0.04)
PQ interval (ms)	68 (6)	80 (9) [a,b]	73 (7) [a,b]
QRS duration(ms)	31 (7)	33 (5)	33 (5)
R amplitude (mV)	0.51 (0.34)	0.49 (0.20) [b]	0.62 (0.31) [b]
QT duration (ms)	145 (14)	179 (15) [a]	185 (17) [a]
QT_F (ms)	22 (2)	25 (1) [a]	25 (2) [a]

Values are expressed as mean (SD).

[a] = Within a row, values differ significantly ($p < 0.05$) from baseline value.

[b] = Within a row, values differ significantly ($p < 0.05$) between atenolol and ivabradine.

Table 4.3 – Echocardiographically-derived indices of left atrial and left ventricular size at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	Baseline	Atenolol	% Δ	Ivabradine	% Δ
HR_{Echo} (min^{-1})	247 (33)	165 (25) [a]	-33 (10)	161 (28) [a]	-35 (11) [§]
LAD_{min} (mm)	10.4 (1.7)	10.8 (0.1)	+4 (1)	9.8 (0.1)	-6 (1)
LADs (mm)	13.9 (1.4)	13. 9 (0.1)	0 (1)	14.0 (0.2)	+1 (1) [§]
LA area d (cm2)	1.02 (0.31)	1.10 (0.23)	+8 (23)	1.00 (0.21)	-1 (20)
LA area s (cm2)	1.76 (0.32)	1.91 (0.33)	+9 (19)	1.98 (0.46)	+13(26) [§]
LVIDd (mm)	11.0 (0.9)	11.4 (1.6)	+4 (15)	12.3 (2.2)	+12 (20) [§]
LVIDs (mm)	6.8 (1.7)	7.0 (1.1) [b]	+3 (15)	5.2 (1.0) [b]	-24 (15)
EDV (ml)	1.89 (0.68)	2.24 (0.71) [a]	+19 (37)	2.80 (1.02) [a]	+49 (54) [§]
ESV (ml)	0.63 (0.26)	0.86 (0.28) [a]	+37 (45)	0.84 (0.29) [a]	+33 (46) [§]

Values are expressed as mean (SD) and percent change (% Δ±SD). *See* Appendix D 'Abbreviations Chapter 4' and **Table 4.1** for reminder of key.

Table 4.4 – Echocardiographically-derived indices of left ventricular systolic function at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	Baseline	Atenolol	% Δ	Ivabradine	% Δ
FS (%)	39 (14)	38 (10) [b]	-3 (10)	57 (10) [a,b]	+46 (25)
EF (%)	67 (6)	61 (6)	-9 (8)	68 (11)	+1 (16)
Ao Vmax (m/s)	1.31 (0.65)	0.74 (0.12) [a,b]	-44 (9)	1.16 (0.33) [b]	-11 (25)
Radial SrR Peak S (1/s)	4.81 (1.73)	3.55 (0.84) [a,b]	-26 (17)	4.98 (0.97) [b]	+4 (20)
Radial SR Peak S (%)	38.5 (11.1)	48.1 (13.9) [b]	+25 (36)	60.2 (10.7) [a,b]	+56 (28) [§]

Values are expressed as mean (SD) and percent change (% Δ±SD). *See* Appendix D 'Abbreviations Chapter 4' and **Table 4.1** for reminder of key.

Table 4.5 – Echocardiographically-derived indices of left ventricular diastolic function at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	Baseline	n	Atenolol	% Δ	n	Ivabradine
Peak E (m/s) *	0.57; 0.45	2	0.55 (0.07)	+8 (14)	4	0.61 (0.11)
E:A*	0.8; 0.7	2	1.2 (0.1)	+59 (16)	4	0.9 (0.2)
IVRT (ms)	55 (12)	10	66 (7) [a]	+20 (12)	10	62 (7)
E:IVRT (m/s^2)	15.9 (6.5)	10	9.6 (2.5) [a]	-40 (16)	10	10.0 (2.0) [a]
Peak Ea lat (cm/s) *	5.1; 4.6	2	4.85 (0.66)	0 (14)	4	5.79 (1.41)
E:Ea lat*	11,2; 9.8	2	11.5 (1.8)	+10 (17)	4	11.1 (3.3)
Peak S (m/s)	0.72 (0.16)	10	0.48 (0.25) [a]	-33 (35)	10	0.62 (0.14)
Peak D (m/s)	0.35 (0.11)	10	0.44 (0.36)	+26 (100)	10	0.45 (0.07)
S/D	2.18 (0.46)	10	1.60 (0.49) [a]	-27 (9)	10	1.37 (0.23) [a]
Radial SrR Peak E (1/s)	Nd	0	4.54 (1.54)	Nd	7	4.74 (1.20)

Values are expressed as mean (SD) and percent change (% Δ±SD). In case of less than 3 observations, individual values are reported.

n = numer of observations; Nd = not determined. * = no statistical analysis performed due to low number of observations.

See Appendix D ‘Abbreviations Chapter 4’ and **Table 4.1** for reminder of key.

Table 4.6 – Echocardiographically-derived indices of left atrial function at baseline and after four weeks of treatment with atenolol and ivabradine in ten healthy cats.

Variable	Baseline	n	Atenolol	% Δ	n	Ivabradine
FAC (%)	43 (8)	10	42 (6)	-2 (14)	10	49 (6)
LA SF (%)	25 (6)	10	22 (3) [b]	-12 (10)	10	29 (5) [b]
Peak AR max (m/s)	0.35 (0.09)	10	0.18 (0.04) [a,b]	-49 (11)	10	0.29 (0.09) [b]
ARduration (ms)	59 (10)	10	58 (9)	-2 (15)	10	50 (8)
Peak LAA flow (m/s)	0.78 (0.22)	10	0.51 (0.10) [a,b]	-35 (13)	10	0.71 (0.14) [b]
Radial SrR Peak A (1/s)	Nd	0	2.34 (0.37) [b]	Nd	7	3.49 (0.78) [b]

Values are expressed as mean (SD) and percent change (% Δ±SD). In case of less than 3 observations, absolute values are reported. *See* Appendix D 'Abbreviations Chapter 4' and **Table 4.1 and 4.4** for reminder of key.

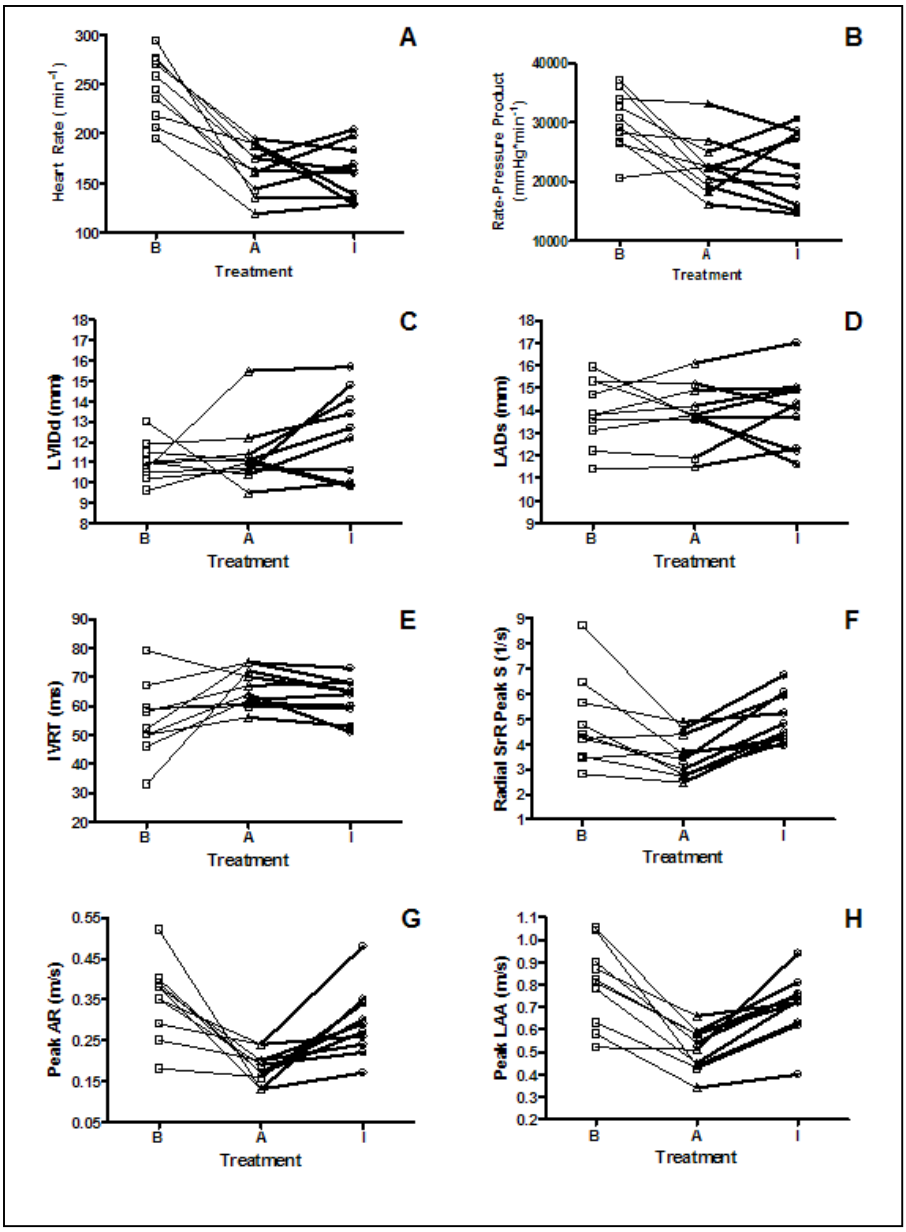

Figure 4.1 – Effects of ivabradine and atenolol on selected variables.

Heart rate obtained at clinical exam (HR; panel A), Rate-Pressure product (RPP; panel B), left ventricular internal dimension at end-diastole (LVIDd; panel C), maximum left atrial dimension (LADs; panel D), isovolumic relaxation time (IVRT; panel E), radial strain rate peak systolic strain (radial SrR Peak S; panel F), peak velocity of pulmonary vein flow at

atrial contraction (AR; panel G), and peak left atrial appendage flow velocity (LAA; panel H) in ten healthy cats recorded at baseline (B) and after four weeks each of treatment with atenolol (A) and ivabradine (I). There was no significant difference ($P > 0.05$) between the response to atenolol and ivabradine for HR, RPP, LVIDd, LADs, and IVRT. There was a significant difference ($P < 0.05$) between the response to atenolol and ivabradine for radial SrR Peak S, Peak AR, and Peak LAA.

Discussion

In this short-term study with healthy laboratory cats, the non-inferiority of ivabradine was established compared to a commonly-used clinical dosage of atenolol. Non-inferiority was evident with respect to HR_{CE}, RPP, and several variables of LA and LV function. Moreover, ivabradine demonstrated more favorable effects on several echocardiographic variables, including indices of LV systolic wall stress, LV relaxation, LA function, and left auricular flow velocity. This makes ivabradine potentially attractive in the treatment of feline HCM, although its effects in cats with HCM and in particular with dynamic LV outflow obstruction require investigation. Additionally, summated filling waves, a common hindrance in the assessment of LV diastolic function in many cats during an echocardiographic examination, was uncommon in cats treated with ivabradine. The drug was clinically well tolerated, did not affect SBP, and did not lead to withdrawal of any cat from treatment owing to adverse effects.

Active-control, non-inferiority trials are performed with increasing frequency in people, especially in cardiovascular and oncologic applications. Placebo-controlled trials

may be considered unethical and new drugs with similar effectiveness may offer advantages to established treatments with regard to adverse effects, dosing frequency, or costs.[31-33] The critical step in determining therapeutic non-inferiority is the selection of the marginal difference. Statistical reasoning and clinical judgment are commonly used to choose this margin.[31-33] The margin of non-inferiority was set at 50% in this study. This value based on predicted clinical relevance, known observer measurement variation,[27-30] and published FDA guidelines.[31-33] Admittedly, the selection of this margin is somehow arbitrary, and selection of a different value could alter the interpretation of our findings. The effects of a new drug can also fall outside of the non-inferiority margin; however, this does not presume that the new drug is clinically inferior to the active control. The opposite effect (i.e., a more favorable action) may occur, as was seen for echocardiographic variables of LA performance in this study. Atenolol led to a decrease of mean LA SF of 12% whereas ivabradine led to an increase of 16% (P=0.001). Even though the changes were outside the non-inferiority margin, they most likely represent a favorable effect of ivabradine, as an improvement of LA performance may be of clinical benefit in cats with HCM. Similar effects of ivabradine compared to atenolol also were observed for LV systolic wall stress, LAD_{min}, LAD area d, radial SrR Peak S, radial SR Peak S, FAC, Peak AR max, and Peak LAA flow at the dosages compared. Ivabradine was potentially less favorable with regard to LVIDd and EDV compared to atenolol, which is in accordance with previous findings observed by our group.[b] The nonsignificant difference in LVIDd may have explained the slightly greater QRS voltages observed with that treatment.

Ivabradine and atenolol had similar negative chronotropic effects and reduced the RPP, an estimate of myocardial oxygen consumption, by approximately 25%. The HR-lowering effect of ivabradine observed is in accordance with previous studies in dogs and humans indicating similar negative chronotropic efficacy of ivabradine in cats compared to other species. [14, 34-37] A reduction of HR per se reduces myocardial oxygen demand and increases diastolic filling time and thus oxygen supply and ventricular filling. The energy expended by the heart occurs mainly during isovolumetric ventricular contraction and relaxation.[38] When HR accelerates and exceeds the physiologic optimum of the force-frequency relationship, cardiac work becomes uneconomical.[39] As a consequence, high HR may increase oxygen demand even when the external work performed by the heart is relatively constant.[38, 39] By lowering HR, myocardial oxygen demand is decreased and myocardial oxygen supply is increased, both of which may prevent the development of regional myocardial ischemia. Evidence suggests that ischemia is a major contributor to clinical signs, disease progression, and fatal outcome and may be present even in asymptomatic patients with HCM.[5]

Left ventricular systolic function was improved after ivabradine indicating absence of negative effects on LV systolic performance whereas atenolol resulted in depression of the LV systolic function. The latter is in agreement with previous findings in cats with HCM after propranolol and is a proposed beneficial mechanism in cats with HCM and obstruction of the LV outflow tract.[8] More than 50% of cats with HCM may have dynamic obstruction of the LV outflow tract,[40] and negative inotropy is well known mechanism for relief of the obstruction.[8-10] Since ivabradine also resulted in a significant

increase of both EDV and ESV in this study, we do not expect the improved systolic function to further deteriorate dynamic LV outflow tract obstruction as mild chamber dilatation may offset the effects of ivabradine on LV systolic function with regard to favoring the development of obstruction. Moreover, we could demonstrate in a previous study in anesthetized cats with HCM,[c] that ivabradine has mild negative inotropic effects as determined by direct measurement of $+dP/dt_{max}$. In the same study, echocardiographic variables such as LV SF and LV EF were not influenced by ivabradine or were even mildly elevated supporting the concept that variables of LV function (LV SF and LV EF) may not necessarily be good indicators of LV contractility.[41, r] Studies focusing on the effect of ivabradine on LV outflow tract obstruction in cats with spontaneous HCM are needed to address this issue.

Diastolic function is routinely assessed in cats with cardiomyopathy by spectral and tissue Doppler imaging.[8, 21, 42] Unfortunately, fusion of diastolic filling E and A waves may be observed in more than 50% of cats with HCM creating a clinical relevant hindrance for diagnosing and staging of LV diastolic dysfunction.[4, 43] In this study, the effects of the two negative chronotropic agents on some echocardiographic variables of LV diastolic function could not be fully evaluated due to fusion of E and A waves. At baseline, separated E and A waves could be identified in only two cats using spectral Doppler and PW TDI and in none of the cats by 2D strain. Undoubtedly this represented the effects of high sympathetic tone, which may have been further augmented by the use of low-dose ketamine in 5/10 cats. Following treatment with ivabradine, E and A waves could be identified in all cats allowing for assessment of LV diastolic function (despite

the same sedative dosages). Atenolol also increased the number of cats in which isolated early and late diastolic flow waves could be observed, however, to a lesser extent. Had higher dosages of atenolol been selected, however, the effects might have been comparable. Although HR reduction leads to separation of early and late diastolic filling waves, the direct effect of HR on Doppler variables of LV diastolic function appears to be negligible within the range of physiologic HR.[30] Therefore, ivabradine also may be of diagnostic benefit in the assessment of LV diastolic function in cats since at the 0.3 mg/kg dose, it consistently separated the filling waves.

Abnormalities of LV relaxation are a hallmark of HCM, and drugs with negative lusitropic properties such as beta blockers may further deteriorate LV relaxation. Ivabradine did not change IVRT, an estimate of LV relaxation,[44] possibly indicating no or lesser effects on lusitropy as compared to atenolol which prolonged the IVRT. The clinical relevance of this potential benefit deserves further study.

Left atrial size and function are known to be altered in cats with HCM and may contribute the development of intracardiac thrombi and arterial thromboembolism (ATE), a devastating consequence of myocardial disease in cats.[45] As the atrium enlarges, LA systolic function often declines,[46] facilitating blood stasis, local activation of the coagulation cascade, endothelial cell damage, and thus increased risk of thrombus formation. It has been shown recently that cats with HCM have reduced LAA function as measured by LAA flow velocities and that presence of ATE and spontaneous echocardiographic contrast, increased LA size, and reduced LAA flow velocities are interrelated.[4] Therefore, any drug that increases LA size or reduces LAA flow velocity

may potentially favor the development of ATE. It was demonstrated previously that atenolol reduces a number of echocardiographic indices characterizing LA systolic function in cats with HCM.[s] A reduction of LAA flow velocity and LAA ejection fraction was also observed in humans with heart disease treated with beta-blockers.[47, 48] In the present study, Peak LAA flow velocity, Peak S, S:D, and Peak AR max, all estimates of left atrial function,[49] were significantly decreased by atenolol, whereas only S:D was decreased by ivabradine. Between treatments, significant differences were observed with regard to LA SF, Peak AR max and Peak LAA flow indicating preserved systolic and global LA function only after ivabradine at the dosages studied. We conclude, that atenolol in contrast to ivabradine, may cause deterioration of LA function. Therefore, caution is advised if treatment with atenolol is considered in cats with significant LA enlargement, LA dysfunction, or evidence of spontaneous echocardiographic contrast. Theoretically, under these circumstances, ivabradine may be preferred for HR control as it does not affect echocardiographic variables of LA size and LA and LAA function as found in our study. To the authors' knowledge, the effects of I_f -inhibition on LA performance in human patients with structural heart disease have not yet been demonstrated. However, preliminary data reported from our laboratory[b] revealed absence of negative effects of ivabradine on echocardiographic variables of the LA function in cats with HCM after intravenous administration. Studies addressing the long-term effects of ivabradine on LA performance, thromboembolic risk, and overall mortality in cats with HCM are needed.

Cardiac auscultation, 6-minute surface ECG recordings, and 15-minute single lead

ECG recordings obtained during the echocardiographic studies did not reveal any arrhythmogenic potential with either drug. Both agents led to a significant prolongation of PQ, QT, and QT_F duration. It has been previously demonstrated that ivabradine has no direct negative dromotropic effects.[37] In humans, administration of ivabradine using similar doses revealed that the mean QT interval does increase after ivabradine but the corrected QT interval does not.[37] this would be in support of the known dependency of QT interval on HR. However, it is in contrast to our findings. A possible explanation for this discrepancy could be that in the latter study a different formula was used to correct the QT interval for HR whereas in our study Fridericia's method was applied.[18] In addition, a direct effect of ivabradine on QT in cats cannot be completely ruled out. Our results also reveal that the increase of QT_F after ivabradine was not different to that observed after atenolol. For both drugs, the increase of QT_F was very mild and below the limit of 5 ms used to define an increased torsadogenic risk as recommended by a consensus of the International Conference on Harmonisation and the Clinical Evaluation of the QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-arrythmogenic Drugs.[50] Finally, previous data reported by the authors[d] revealed absence of proarrhythmogenic effects of ivabradine using 24-hour radiotelemetry electrocardiography in healthy cats.

Certain limitations of this study require emphasis. Only spayed female cats were studied, all cats were healthy, and the number studied was small. These aspects render the study underpowered to detect minor differences between groups or to evaluate drug effects in the putative clinical population. Repeatability of echocardiographic

measurements was not specifically addressed; however, previous data reported by our laboratory indicates acceptable reproducibility.[22] The dose of ivabradine used was selected based on the results of a previous dose-finding study in healthy cats using 24-hour radio-telemetric method.[d] Whether or not this dose is equipotent to the dose of atenolol administered in terms of chronotropic effect is unknown. Based on the similar negative chronotropic effects of ivabradine and atenolol relative to the baseline values, the influence of HR as an explanation for these drug differences is unlikely.

Since a study goal was a demonstratration that ivabradine preserves at least some fraction of the effect of the active control (atenolol), a non-inferiority margin, a function of the active control effect, was chosen. The selected value of $f = 0.5$ that was selected *a priori* represents an arbitrary cut-off, but was based on clinical and statistical relevance. Selection of a different value could have produced a different interpretation of our results. Finally, the effects of sedation on cardiac function, including drug intereactions, could not be eliminated. No overt safety concerns were raised by this trial. However, drug efficacy and safety can only be assessed in an appropriately monitored clinical trial, in the target population with HCM, and with sufficient post-approval drug monitoring.

In summary, the results of this study demonstrate that ivabradine, given at 0.3 mg/kg PO twice daily for four weeks, is non-inferior to 6.25 mg of oral atenolol administered twice daily in healthy cats. Ivabradine was non-inferior to atenolol with regard to its negative chronotropy, reduction of RPP, and clinical tolerance. Moreover, ivabradine appeared to exert more beneficial effects on LV systolic wall stress, LV diastolic function, LA performance, and left auricular appendage flow velocity. The

apparent enhancements in LV systolic function and increased LV end-diastolic volume identified with ivabradine deserve further evaluation. Studies in cats with HCM, in particular those with dynamic LV outflow tract obstruction, are needed to further elucidate the potential value of ivabradine in clinical practice.

Footnotes

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[c]Riesen SC, Schober KE, Lindsey KJ, Carnes CA, Ni W, Phelps MA. Pharmacokinetics of oral ivabradine in healthy cats. *J Vet Intern Med* 2010;24:732 (abstract).
[d]Cober RE, Schober KE, Buffington CAT, Riesen SC, Bonagura JD. Effects of ivabradine, a selective I_f channel inhibitor, on heart rate in healthy cats. *J Vet Intern Med* 2010;24:695 (abstract).
[e]Liberty Research Inc., Waverly, NY.
[f]ClinPro software, Clinical Systems, Garden, NY City.
[g]Procoralan®, Les Laboratoires Servier, 22 Rue Garnier, 92200 Neuilly-sur-Seine, France.
[h]Atenolol, Mallinckrodt Inc, St Louis, MO.
[i]Capsules 4 blue, Gallipot Inc, St. Paul, MN.
[j]MAC 8 resting ECG analysis system, Marquette Electronics, Milwaukee, WI.
[k]Ultrasonic Doppler Flow Detector 811-AL, Parks Medical Electronics Inc, Aloha, OR.
[l]Vivid 7 Vantage, GE Medical Systems, Milwaukee, WI.
[m]Acepromazine maleate injection, Boehringer Ingelheim Vetmedica Inc, St Joseph, MO.
[n]Torbugesic, Fort Dodge Laboratories, Fort Dodge, Iowa.
[o]Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa.
[p]EchoPac software package, Version BT06, GE Medical Systems, Milwaukee, WI.
[q]SigmaStat, Version 3.5, SPSS Inc, Chicago, IL.
[r]Schober KE, Bonagura JD, Luis-Fuentes V, Hatfield D. Invasive validation of Doppler echocardiographic indices of left ventricular systolic function in healthy cats. J Vet Intern Med 2006;20:1534-1535 (abstract).
[s]Schober KE, Bonagura JD, Luis-Fuentes V. Atenolol versus diltiazem for LV diastolic function in cats with occult hypertrophic cardiomyopathy. *Proceedings*, Annual Forum of ACVIM, 2007, Seattle, WA.

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CHAPTER 5

EFFECTS OF TREATMENT WITH IVABRADINE AND ATENOLOL ON REPRODUCIBILITY OF ECHOCARDIOGRAPHIC INDICES OF LEFT HEART FUNCTION IN HEALTHY CATS

Assessment of myocardial function in cats with cardiomyopathy is primarily based on echocardiographic measurements. Echocardiography is also widely used to demonstrate efficacy of cardiac drugs in clinical trials and to monitor effects of treatment in individual cats with cardiac disease.[1-7] Since both acquisition and quantification of echocardiographic data are operator dependent, variations in imaging and measurement technique may impact interpretation of data. The reproducibility of echocardiographically-derived variables has been studied excessively in healthy cats and cats with hypertrophic cardiomyopathy (HCM).[8-13] Although important information regarding inter- and intraobserver variability has been generated, the threshold by which echocardiographic indices must change to demonstrate drug-induced changes that lie beyond methodological variability on sequential examinations has not yet been reported.

Alterations in heart rate (HR) may influence echocardiographic indices of chamber size and function,[5, 12, 14] thereby further enhancing the biological variability of such indices. Specifically, high HR and sudden changes of HR, often observed during an echocardiographic exam in cats, may be a relevant cause of measurement variability.[12]

Therefore, drugs that reduce HR should decrease observer variation. To the authors' knowledge, the effects of HR-lowering drugs on reproducibility of echocardiographically-derived variables in cats have not yet been studied.

Therefore, the objective of this study was to determine the effects of ivabradine, a funny current inhibitor, and atenolol, a selective beta-1 receptor antagonist, on reproducibility of echocardiographic variables used to characterize LV and LA size and function. We hypothesized that both agents would reduce observer variability and improve reproducibility of echocardiographic data in healthy cats.

Material and Methods

Animals: Eight experimental healthy, female-spayed, domestic shorthair cats that were 2 to 7 years old and weighed 2.8 to 6.5 kg were studied. All cats were acquired from a commercial vendor.[a] The study protocol was reviewed and approved by the Animal Care and Use Committee (2008 A 0154) and the Institutional Review Board of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

Study design: This was a prospective, double-blind, randomized study of four weeks duration. Four cats each were randomly assigned to a treatment group (Group 1: ivabradine[b] 0.3mg/kg, q12h, PO; Group 2: atenolol[c] 6.25mg/cat, q12h, PO) by use of a randomization software.[d] Prior to randomization, individual doses of both drugs based on body weight were prepared for each cat and subsequently filled in opaque capsules[e] assuring blinding. The capsules were administered manually twice daily by the investigators (SCR and RMC) to assure proper drug administration.

At baseline (stage 1, Day 1 to 3) and after four weeks of treatment (stage 2, Day 31 to 33; **Fig 5.1**), each cat underwent repeated echocardiographic[f] examinations. Transthoracic echocardiography was performed approximately three hours after drug administration when the maximum negative chronotropic effect was anticipated.[g,h] All echocardiographic recordings were labeled with random numbers selected by a person not involved in the study, allowing subsequent offline measurements in a blinded fashion.

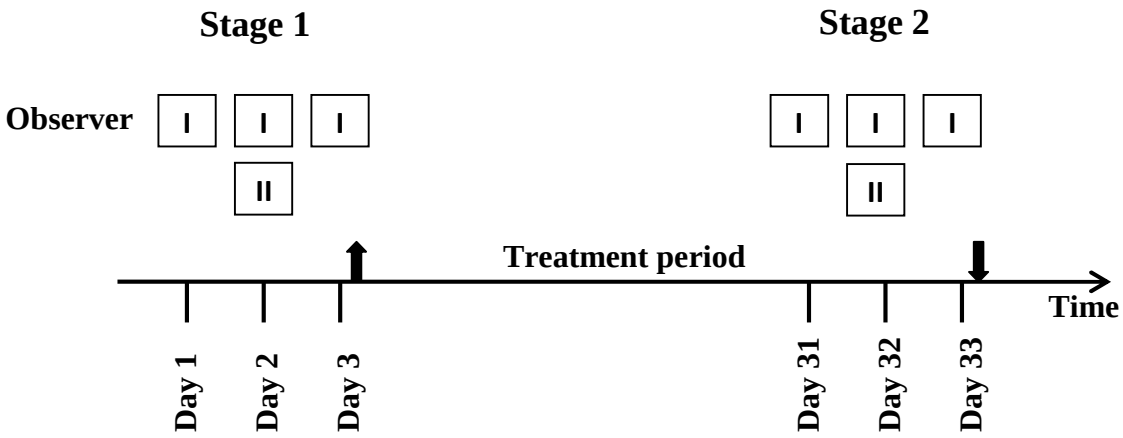

Figure 5.1 – Timeline of the study.

Stage 1, baseline; Stage 2, at the end of treatment period; I, observer one; II, observer two.

↑, begin of treatment period after echocardiogram on Day 3.

↓, end of treatment period after echocardiogram on Day 33.

Echocardiography: After sedation with acepromazine[i] (0.1 mg/kg, IM) and butorphanol[j] (0.25 mg/kg, IM), each cat underwent a transthoracic two-dimensional (2D), M-mode, spectral and color Doppler, pulsed wave (PW) tissue Doppler imaging (TDI), and 2D-strain echocardiographic examination. Cats were positioned in lateral recumbency, mildly restrained, and imaged from underneath. Echocardiographic examinations were performed with a transducer array of 7.0 to 10.0 MHz nominal frequency.[f] Data were stored digitally, and data analysis was performed off-line by use of a commercially available data analysis software package.[k] For each variable, the mean of five measurements was determined and used for statistical analyses. A simultaneous one-lead ECG was recorded and HR was calculated from preceding RR intervals.

Standard right and left parasternal imaging views were acquired, and two-dimensional, M-mode, tissue Doppler, and 2-D strain variables were obtained.[8, 14-16] Left atrial dimension and area were assessed from the right parasternal long-axis view[8, 15] and fractional area change (FAC) and shortening fraction (LA SF) were calculated. Left atrial appendage (LAA) flow, pulmonary venous flow, and transmitral flow were recorded and quantified as recently described.[8, 15] Pulsed wave Doppler-derived velocities of myocardial motion (Peak Sa, Peak Ea, and Peak Aa) were recorded from the left apical imaging view using a sample volume of 5 mm placed at the lateral mitral annulus.[17] The right parasternal mid-ventricular short-axis plane of the LV was imaged for quantification of the peak systolic radial strain (Radial SR Peak S), and peak systolic and diastolic radial strain rate (Radial SrR Peak S, Radial SrR Peak E, and Radial SrR Peak A). If diastolic LV deformation waves were fused, Radial SrR Peak EA fus was measured. Optimal

frame rate was obtained by adjusting the sector width and image depth to achieve frame rates of 96 to 251 per second using the formula: 0.8 x HR (Stoylen A., personal communication). Off-line analysis of 2D speckle imaging was performed as previously described in dogs.[18] The LV radial strain and strain rate values reported were determined as the average of six corresponding myocardial segments.[18] Fused or partially fused diastolic filling and wall motion waves obtained by PW Doppler and PW TDI were eliminated from statistical analyses.

Reliability of echocardiographic variables: All cats underwent repeated echocardiographic examinations by two board certified cardiologists. One observer (SCR) examined each cat once on three consecutive days both at baseline (stage 1, Day 1 to 3) and after four weeks of treatment (stage 2, Day 31 to 33; **Fig 5.1**). On one occasion at each stage, a second independent observer (KES) examined each cat immediately before (four cats) or after (four cats) the other observer (**Fig 5.1**). Thus, each cat underwent a total of four echocardiographic examinations at both stages.

The *intraobserver* measurement variability was determined by a single observer (SCR) measuring one study of each cat at baseline and after four weeks of treatment repeatedly on three different days. For determination of the *interobserver* measurement variability, a second observer (KES) blinded to the results of the first observer measured the same cardiac cycles of the same studies. For determination of the *within-day interobserver* variability, one observer (SCR) measured the two studies of each cat that were recorded consecutively on the same day. The *between-day intraobserver* variability was measured was determined by one blinded observer (SCR) measuring each cat's two studies that were recorded by SCR on two different days. All measurements were

performed within three weeks using stored images in random order and with the observers blinded to the cat's identification and results of previous measurements.

Data analysis and statistics: Statistical analysis was performed by use of commercially available software.[1] Descriptive statistics (mean ± SD) were calculated for all echocardiographic variables based on the first study of each cat at both stages (Day 1 and Day 31; **Fig 5.1**). For baseline comparison of the two groups studied, a one-way ANOVA was used after normal distribution of data was confirmed.

Analyses of variability were performed as previously described by Schwarzwald et al.[19, 20] Briefly, test reliability was quantified by the within subject variance for repeated measurements (residual mean square) determined by one-way ANOVA with treatments as groups. The within-subject standard deviation (s_w) was calculated as the square root of the residual mean square. Measurement variability and recording variability was reported in two ways: 1) the within-subject coefficient of variation (CV) expressed as a percent value was calculated as CV = s_w/mean x 100 in order to compare the reliability of the various indices in this study. The degree of variability was arbitrary defined as follows: CV < 5%, excellent (class 1); CV = 5 to 15%, good (class 2); and CV > 15%, poor (class 3). 2) The absolute value below which the difference between two measurements will lie with 95% probability was estimated following guidelines recommended by the British Standards Institution (BSI): BSI = 2.77 x s_w. The BSI was reported to provide a clinically applicable measure of variability, hence an absolute value that allow comparison with measured changes in echocardiographic variables on a case-by-case basis.

To determine the effect of treatment on reproducibility, a change from one variability class to another (e.g., from class 2 to class 1) was arbitrarily defined as "improvement" whereas "deterioration" was defined as a change in the opposite direction (e.g., from class 2 to class 3). In addition, linear mixed effects models ANOVA and Fisher's exact test were used to determine the effects of treatment on observer variation and to compare categorical (frequency) data. For all analyses, $P \leq 0.05$ was considered significant.

Results

At baseline, cats of both groups were not different ($P > 0.05$) with regard to HR and any of the echocardiographic variables determined. After treatment, only Peak AR (P=0.035) and Peak LAA (P=0.009) differed significantly between treatment groups (**Tables 5.1**). Although Radial SR Peak S (P=0.070) appeared to be higher after ivabradine compared to atenolol, the observed difference did not attain statistical significance. Of the 64 echocardiograms recorded from all cats, recordings were of sufficient quality to allow for proper analysis. Also, adequate tracking for 2D strain signals were achieved for all cycles analyzed by both observers. At baseline, fusion of early and late diastolic waves Doppler (PW and PW TDI) and 2D strain was observed in 90.6% and in 81.3% of echocardiograms, respectively. After ivabradine and atenolol, fused early and late waves were reduced ($P < 0.05$) and observed in only 12.5% and 25%, respectively. The CVs of all variables determined and their corresponding BSI-values are summarized in **Table 5.1**.

Heart rate: Only within-day variability and between-day variability were determined (**Table 5.1**). At baseline, variability of HR was low (all CVs < 15%) for both groups. Ivabradine increased the between-day variability from 9.8% to 20.0%, but had no effect on the within-day variability. Compared with ivabradine, atenolol resulted in a more consistent HR reduction (all CVs < 8%).

Reproducibility at baseline: Coefficients of variation ranged from 0.5 to 50.6% at baseline. Overall, reliability of 10 indices was determined of which 82.2% showed excellent to good reproducibility (CV < 15%; **Table 5.1 and 5.2**). With regard to 2D strain-derived indices, this number was slightly lower (66.7%). *Intra-* and *interobserver* variability was below 15% for all CVs determined. Reproducibility was poor with regard to following *within-day interobserver* CVs: Peak AR, Peak LAA, Radial SR Peak S, Radial SrR Peak S, Radial SrR EAfus, and Radial SR Peak S, and *between-day intraobserver* CVs: Peak LAA, Radial SrR EAfus, Radial SR Peak S, Radial SrR Peak S and Radial SR Peak S, respectively (**Table 5.1 and 5.2**).

Reproducibility after Ivabradine: Coefficients of variation ranged from 0.5 to 45.5% after ivabradine. Overall, reliability of 17 indices was determined of which 83.8% showed excellent to good variability (CV < 15%; **Table 5.1 and 5.2**). With regard to 2D strain-derived indices, this number was slightly lower (62.5%). *Intraobserver* variability was below 15% for all variables determined. Reproducibility was poor with regard to following *interobserver* CVs: Radial SrR Peak S and Radial SR Peak S, *within-day interobserver* CVs: ARduration, E:Ea lat, Radial SrR Peak E, and Radial SR Peak A, and *between-day intraobserver* CVs: E:IVRT, Peak AR, Peak Ea lat, Radial SrR Peak S and Radial SrR Peak E, respectively (**Table 5.1 and 5.2**).

Reproducibility after Atenolol: Coefficients of variation ranged from 0.5 to 23.3% after atenolol. Overall, reliability of 17 indices was determined of which 85.0% showed excellent to good variability (CV < 15%; **Table 5.1 and 5.2**). Reproducibility of 2D strain-derived indices was similar (81.3%) to the overall reliability. *Intra-* and *interobserver* variability were below 15% for all variables determined. Reproducibility was poor with regard to following *within-day interobserver* CVs: Peak Ea lat, Peak Aa lat, and Radial SR Peak S, and *between-day intraobserver* CVs: Peak AR, ARduration, Peak LAA, Peak Ea lat, E:Ea lat, Radial SrR Peak E, and Radial SR Peak S, respectively (**Table 5.1 and 5.2**).

Effect of treatment on reproducibility: Overall, CVs of nine indices were evaluated before and after ivabradine and atenolol, of which 10/36 (28%) and 7/36 (19%) CVs revealed an improvement of the reliability and 12/36 (33%) and 5/36 (14%) CVs a deterioration, respectively. Overall, reproducibility of all variables determined did neither improve nor deteriorate consistently after either treatment ($P > 0.05$). Atenolol compared to ivabradine exhibited a trend toward higher reliability with none of the echocardiographic CVs above 24% (**Table 5.1** and 5.**2**) and improved more CVs than it deteriorated (7 versus 5 CVs). However, its effects on *intraobserver*, *interobserver*, *within-day interobserver* and *between-day intraobserver* CVs of individual echocardiographic indices were inconsistent and did therefore not reach statistical significance.

Table 5.1 – Data on reproducibility (mean [SD]) of echocardiographic indices of left atrial and left ventricular size and function determined at baseline (BL) and after four weeks of treatment with ivabradine (Iva; 0.3mg/kg, q12h, PO; n=4) or atenolol (Aten; 6.25mg/cat, q12h, PO; n=4) in healthy cats.

Variables	Group	Mean (SD)	Measurement Variability				Within-Day Variability		Between-Day Variability	
			Intraobserver		Interobserver		Interobserver		Intraobserver	
			CV (%)	BSI	CV (%)	BSI	CV (%)	BSI	CV (%)	BSI
HR (min^{-1})	BL Iva	247 (42)	nd	nd	nd	nd	6.9	51.19	9.8	68.93
	Iva	154 (22)	nd	nd	nd	nd	5.5	26.88	20.0	79.80
	BL Aten	244 (23)	nd	nd	nd	nd	1.8	11.81	7.3	46.14
	Aten	148 (22)	nd	nd	nd	nd	2.8	13.13	8.0	34.98
LVIDd (mm)	BL Iva	10.8 (1.2)	1.8	0.55	5.8	1.85	4.6	1.29	5.4	1.56
	Iva	12.5 (2.5)	1.1	0.37	3.9	1.45	0.9	0.32	11.5	4.10
	BL Aten	9.9 (1.1)	2.3	0.65	2.5	0.73	2.1	0.63	4.0	1.12
	Aten	11.5 (1.1)	0.7	0.21	1.5	0.52	3.0	0.94	8.2	2.55

Continued

Table 5.1 – Continued

LADs (mm)	BL Iva	14.7 (1.3)	0.7	0.27	1.4	0.59	4.7	1.67	1.9	0.76
	Iva	14.1 (2.2)	1.0	0.38	2.2	0.84	0.5	0.19	6.9	2.78
	BL Aten	13.4 (1.6)	0.5	0.19	1.6	0.59	1.7	0.63	2.6	0.96
	Aten	13.4 (1.4)	0.6	0.22	2.0	0.72	1.8	0.67	4.6	1.71
Peak E (m/s) *	Iva	0.63 (0.18)	1.4	0.02	1.1	0.02	6.6	0.12	10.1	0.19
	Aten	0.53 (0.06)	1.0	0.02	2.1	0.03	4.8	0.07	2.7	0.04
Peak A (m/s) *	Iva	0.72 (0.22)	0.8	0.02	1.0	0.02	7.0	0.14	7.4	0.15
	Aten	0.47 (0.09)	0.7	0.01	0.9	0.01	3.6	0.05	6.7	0.09
E:A*	Iva	0.9 (0.2)	2.2	0.05	1.9	0.05	4.4	0.12	8.9	0.23
	Aten	1.15 (0.11)	0.5	0.02	1.5	0.05	1.4	0.05	5.3	0.17
IVRT (ms)	BL Iva	45 (9)	3.2	4.05	11.1	13.04	4.4	6.19	0.9	1.13
	Iva	63 (7)	2.3	4.00	2.2	3.75	1.2	1.96	5.3	9.10
	BL Aten	58 (2)	2.6	4.36	3.3	5.30	4.7	7.42	3.8	6.27
	Aten	66 (7)	0.8	1.53	2.2	3.92	2.8	5.06	4.0	7.39

Continued

Table 5.1 – Continued

E:IVRT (m/s²)	BL Iva	20.0 (9.6)	3.5	1.93	7.7	4.51	4.9	2.28	6.3	3.47
	Iva	10.3 (3.1)	3.4	0.96	3.5	0.99	8.2	2.70	17.4	5.32
	BL Aten	17.0 (7.1)	1.2	0.42	7.7	2.92	7.0	3.44	6.4	2.38
	Aten	9.3 (2.9)	0.9	0.22	4.8	1.24	5.8	1.56	8.7	2.22
Peak AR (m/s)	BL Iva	0.36 (0.14)	2.2	0.02	4.4	0.04	15.1	0.15	14.0	0.14
	Iva	0.30 (0.06)	1.9	0.02	2.3	0.02	5.8	0.05	21.9	0.17
	BL Aten	0.33 (0.08)	1.4	0.01	2.8	0.03	6.5	0.05	13.8	0.13
	Aten	0.20 (0.04)	1.8	0.01	4.5	0.03	6.4	0.04	18.9	0.10
ARduration (ms)	BL Iva	63 (7)	2.3	4.10	3.4	5.85	6.1	8.54	3.4	5.63
	Iva	49 (9)	2.8	3.81	1.4	1.88	36.3	49.78	9.2	13.03
	BL Aten	58 (12)	2.4	4.10	5.8	9.78	6.9	9.66	7.2	11.70
	Aten	57 (11)	1.5	2.40	7.8	12.64	3.7	5.29	17.2	27.19
Peak LAA (m/s)	BL Iva	0.78 (0.22)	1.8	0.04	4.2	0.09	18.7	0.34	9.5	0.21
	Iva	0.74 (0.18)	0.8	0.01	1.9	0.03	4.4	0.08	5.2	0.09

Continued

Table 5.1 – Continued

	BL Aten	0.69 (0.31)	1.2	0.02	1.9	0.04	6.6	0.12	17.3	0.34
	Aten	0.54 (0.11)	0.8	0.01	5.3	0.07	2.8	0.04	15.8	0.22
Peak Ea lat (cm/s)*	Iva	5.5 (0.5)	1.2	0.19	0.7	0.10	13.9	2.65	22.8	4.16
	Aten	5.5 (1.5)	0.8	0.11	2.2	0.30	19.5	2.72	15.5	2.26
Peak Aa lat (cm/s)*	Iva	7.2 (2.6)	1.7	0.33	7.2	1.33	10.3	1.87	1.5	0.32
	Aten	4.8 (1.0)	0.7	0.09	6.0	0.74	23.0	2.64	4.6	0.63
E:Ea lat*	Iva	11.3 (3.4)	2.0	0.64	2.3	0.77	16.1	4.53	7.7	2.20
	Aten	10.1 (1.6)	1.2	0.34	8.4	2.56	13.6	4.08	18.5	5.36
Radial SrR Peak S (1/s)	BL Iva	4.58 (1.74)	9.5	1.18	6.9	0.91	50.6	6.06	5.3	0.66
	Iva	4.43 (0.56)	3.9	0.48	15.7	2.02	5.2	0.74	32.4	3.97
	BL Aten	5.35 (2.24)	8.9	1.33	7.0	1.07	8.7	0.99	28.5	3.93
	Aten	3.50 (0.83)	4.3	0.43	5.3	0.55	14.4	1.40	13.2	1.38
Radial SrR Peak E (1/s)*	Iva	4.69 (0.98)	3.9	0.50	7.9	1.04	29.2	4.42	11.5	1.50
	Aten	3.45 (0.94)	2.8	0.32	3.6	0.41	13.9	1.56	23.3	2.50

Continued

Table 5.1 – Continued

Radial SrR Peak A (1/s)*	Iva	3.08 (0.60)	3.3	0.28	8.6	0.72	18.0	1.83	45.5	4.12
	Aten	2.27 (0.58)	3.4	0.18	4.7	0.26	9.5	0.75	11.5	0.72
Radial SrR EA fus (1/s)#	BL Iva	7.93 (2.40)	7.0	1.54	4.1	0.91	27.5	5.00	17.2	3.67
	BL Aten	6.64 (1.86)	6.4	1.26	8.7	1.68	11.6	1.50	14.4	2.70
Radial SR Peak S (%)	BL Iva	34.0 (11.8)	9.2	8.72	5.7	5.47	28.7	27.81	20.1	17.36
	Iva	59.0 (8.2)	1.4	2.31	18.2	28.99	9.2	13.23	13.9	22.25
	BL Aten	41.1 (10.8)	9.4	10.20	12.1	12.76	19.1	17.52	38.8	43.38
	Aten	44.5 (10.3)	4.2	5.48	3.5	4.80	17.0	23.49	20.6	26.50

BL Iva, baseline prior to treatment with ivabradine; BL Aten, baseline prior to treatment with atenolol.

*, missing BL information for both Iva and Aten due to summated early and late diastolic waves.

#, missing information for both Iva and Aten due to isolated early and late diastolic waves after treatment. nd= not determined.

CV (%), coefficient of variation in per cent. BSI, the absolute value below which the difference between two measurements will lie with 95% probability was estimated following the British Standards Institution (BSI) recommendations. *See* Appendix E ‘Abbreviations Chapter 5’ for reminder of key.

Table 5.2 – Data on reproducibility at baseline (BL) and after four weeks of treatment with ivabradine (Iva; 0.3mg/kg, q12h, PO; n=4) or atenolol (Aten; 6.25mg/cat, q12h, PO; n=4) in healthy cats.

Group	n	Excellent CV (< 5%)		Good CV (5-15%)		Poor CV (> 15%)	
		Observations	Total (%)	Observations	Total (%)	Observations	Total (%)
BL Iva	40	7/6/4/3	20 (50%)	3/4/1/5	13 (33%)	0/0/5/2	7 (18%)
BL Aten	40	7/5/3/3	18 (45%)	3/5/6/4	18 (45%)	0/0/1/3	4 (10%)
Iva	68	17/12/5/1	35 (51%)	0/3/8/11	22 (32%)	0/2/4/5	11 (11%)
Aten	68	17/12/8/4	41 (60%)	0/5/6/6	17 (25%)	0/0/3/7	10 (15%)

BL Iva, baseline prior to treatment with ivabradine; BL Aten, baseline prior to treatment with atenolol; observations. number of coefficients of variation.

Values are presented as absolute number of CVs and relative (%) frequencies of intraobserver/ interobserver /within-day interobserver /between-day intraobserver coefficients of variation. The degree of variability was defined as excellent (CV < 5%), good (CV = 5 to 15%), and poor (CV > 15%).

Discussion

This study presents for the first time data illustrating acquisition and measurement variability of echocardiographic variables before and after treatment in healthy cats. Overall, the majority of variables obtained in this study had excellent to good reproducibility justifying their use for objective quantification of LV and LA function in cats. Reproducibility of all variables determined did neither improve nor deteriorate consistently after either treatment.

Coefficients of variation and BSI-values: Studies on the assessment of the reproducibility of echocardiographic variables using CVs and the BSI method were previously published in horses.[19, 20] For determination of the threshold by which echocardiographic indices must change to demonstrate that changes relate to progression of underlying heart disease or the effects of a cardiac drug and not to biological and measurement variability, the absolute value below which the difference between two measurements will lie with 95% probability (BSI-value) can be used. In particular day-to-day variability seems to be of clinical relevance when methodological variability versus drug-induced changes are studied. For example, mean IVRT obtained at baseline in the ivabradine group was 45 ms with a between-day BSI-value of 1.13 ms. Hence, the absolute value within the two measurements will lie with 95% probability owing to biological and measurement variability is between 43.87 to 46.13 ms. After administration of ivabradine, a mean IVRT of 63 ms with a BSI-value of 5.3 ms was observed, which is clearly outside this baseline range. We conclude therefore that the

change in IVRT is most likely drug related and not due to biological and between-day measurement variability. Similar findings were observed for HR, LVIDd, E:IVRT, ARduration, and radial SR Peak S in the ivabradine group and for HR, LVIDd, IVRT, and E:IVRT in the atenolol group. Although the between-day reproducibility was the most relevant source of variability in this study, the latter approach may be over simplified. In the worst case scenario, the added effects of all variabilities (biological, *intraobserver* measurement, *interobserver* measurement, *within-day interobserver* recording, and *between-day intraobserver* measurement) as investigated in our study may occur, neglecting the use of a single CV or a single BSI-value in the decision-making process. Since the combined effect of each of these CVs on overall variability has not yet been determined, we used the *between-day* BSI-value to evaluate a possible drug effect.

Ivabradine and atenolol resulted in an improvement of 10/36 and 7/36 CVs and in a deterioration of 12/36 and 5/36 CVs, respectively. While atenolol improved more CVs than it deteriorated, ivabradine deteriorated more CVs than it improved. However, the changes within one variable where often counter-directional (e.g., treatment improved *within-day interobserver* variability of Peak AR whereas it deteriorated its *between-day intraobserver* variability) leading to an almost neutral effect on overall reproducibility. Thus, the overall reproducibility of echocardiographic data was not significantly improved after either treatment thereby rejecting our study hypothesis. None of the CVs recorded after atenolol exceeded 24% whereas much higher values (up to 45.5%) were obtained after ivabradine. However, the effects after atenolol as mentioned above were inconsistent with regard to *intraobserver, interobserver, within-day interobserver* and

between-day intraobserver CVs and did therefore not reach statistical significance. The slight differences in variabilities observed after atenolol and ivabradine may partially be explained by the high between-day HR variability observed after ivabradine, by differences in individual responses to sedation, and by a higher physiologic variation in the sympathetic tone after ivabradine compared to atenolol. Improvement was defined as change from one variability class to a more favorable (e.g., from class 2 to class 1) whereas deterioration was defined as change in the opposite direction (e.g., change from class 2 to class 3). Alterations within the same class of variability (e.g. from 2% to 4%) most likely represent variations of low relevance and were therefore not considered as changes. Our definition of improvement or deterioration represents a somehow arbitrary approach, and election of different cut-offs could have changed the interpretation of our findings.

Intraobserver variability was below 15% for all variables studied indicating good reproducibility when measured by the same observer. *Interobserver* variability was above 15% for two indices of LV systolic function only (Radial SrR Peak S and Radial SR Peak S, both after ivabradine); thereby, demonstrating reliable assessment of LA and diastolic LV function by two different operators whereas the evaluation of systolic function by 2D strain imaging seems to be less reproducible. *Within-day interobserver* and *the between-day intraobserver* variabilities ranged from excellent to poor at all treatment stages. The high *within-day interobserver* variability may be explained partially by fading of the sedative effect when the second echocardiogram was performed and by differences in imaging technique (e.g., cursor position) by the two operators. The high *between-day*

intraobserver variability may relate to differences in HR control after ivabradine and atenolol and individual differences in between-day responses to sedation.

Left ventricular and atrial size: The two variables (LVIDd and LADs) quantified in this study revealed excellent to good reproducibility. Although M-mode recordings and subsequent measurements were repeatedly derived from real time video loops and not previously recorded M-mode images, CVs were reasonably low indicating minor influence of cursor position. In addition, within-day interobserver variability was low demonstrating small operator differences in image acquisition. Similar findings were also observed for LADs. Overall, reproducibility of 2D and M-mode derived indices was better than that of Doppler-derived indices in our study. This observation is in agreement with previous reports[9, 21] and may be explained in part, by generally less fluctuation of dimensional echocardiographic variables as opposed to hemodynamic, Doppler-based indices that tend to change almost instantaneously in response to changes of HR and loading conditions.

Indices of LV diastolic function: At baseline, separation of the early and late diastolic waves were observed in only 6/32 (18.7%) echocardiograms. Following treatment with ivabradine, E and A waves could be identified in 14/16 (87.5%) of the echocardiograms allowing for assessment of LV diastolic function in most of the cases. Atenolol also increased the number of cats with isolated early and late diastolic flow waves, however, to a lesser extent compared to ivabradine (12/16 echocardiograms; 75%). Previously, fusion of the diastolic filling waves has been observed in 14 to 35% in healthy cats[12, 22] and in 33 to 53% in cats with HCM[15, 22] being a clinical relevant hindrance for diagnosing

and staging of LV diastolic function. In contrast to these reports, we observed a higher percentage of cats with summation of E and A waves at baseline. One potential reason for such observation may be the higher HR observed in our study at basleine. Although HR reduction may lead to separation of early and late diastolic filling waves, the direct effect of HR on Doppler variables of LV diastolic function seems to be negligible in the range of physiologic heart rates.[12] In addition to facilitating staging of the LV diastolic function, atenolol as well as ivabradine demonstrated good reproducibility of PW Doppler-derived diastolic filling waves (CVs < 11%) rendering their measurement useful for quantification of LV diastolic function. In contrast to PW Doppler-derived filling waves, E and A waves derived by PW TDI and 2D strain revealed much higher variabilities, possibly suggesting a less reliable use of these methods in the clinical setting. Moreover, IVRT could be obtained in each cat over a wide range of HR, making it more robust for assessing LV diastolic function in tachycardic cats where E and A waves are commonly fused. [9, 15, 22] Coefficients of variation of IVRT were below 11% at all times rendering its quantification useful with both high and low HR.

Indices of left atrial function: Reproducibility of Peak LAA ranged between good and poor and was not changed by atenolol. Ivabradine resulted in an improvement of the reliability of Peak LAA with none of the CVs reported above 6%. The higher variability observed after atenolol may be explained by the significant lower absolute value of mean Peak LAA velocity compared to ivabradine (P= 0.009). A similar negative effect of low velocity values on measurement variabilities was previously demonstrated for TDI

derived indices.[9] The variabilites of the Peak LAA and Peak AR at baseline were below 19% and were in agreement with previous published data.[8, 12]

Indices of 2D strain: Coefficients of variation of speckle-derived indices ranged from 4.1% to 50.6% at baseline, from 1.4 to 45.5% after ivabradine, and from 2.8 to 23.3% after atenolol. In human patients, 2D strain imaging data are highly reproducible, and analysis is affected by only small intraobserver (mean±SD, 4.4±1.6%) and interobserver variability (mean±SD, 7.3±2.5%).[23] In studies performed in dogs and horses, within and between day CVs of less than 20%[12] and 17%[19, 24] were reported. In contrast, much higher variabilities were observed in our study. Although the reason for this discrepancy is not obvious, it might be speculated that the differences relate to the different frame rates for 2D strain imaging used in our study. We took recommendations from the laboratory that developed the speckle tracking technique for use in people in consideration by which optimal frame rate is calculated based on HR present (optimal frame rate = 0.8 x HR). At baseline, this target frame rate could not be achieved in all cats owing to their high HRs, whereas after atenolol and ivabradine it was consistently achieved. The sub-optimal frame rates obtained at baseline may have resulted in undersampling thereby affecting the reproducibility of the echocardiographic variables analyzed. However, high measurement variability was also observed with optimal frame rates after ivabradine and atenolol thereby excluding undersampling as the sole factor affecting reproducibility. Administration of atenolol exhibited a trend toward improvement of the reproducibility of speckle-derived indices with 81.3% of all speckle-derived indices CVs below 15%. This effect of atenolol may relate to the more consistent

reduction of HR observed. Despite the improvement of 2D strain derived indices in cats by atenolol, reproducibility of some CVs was still beyond (CV > 15%) clinically acceptable limits calling the general applicability of this method to clinical circumstances into question.

Certain weaknesses of this study need emphasis. Only healthy cats were used which does not represent the target population of cats for use of bradycardic agents. The number of cats included was low. The use of a cross-over design would have potentially offered a more objective assessment of the changes in reliability induced by the bradycardic agents. Although a standardized protocol was used for sedation, large differences in individual responses were observed. Moreover, the HR response to ivabradine and atenolol was not uniform, and a more consistent control of HR by atenolol was found. This may offer a possible explanation for the relative high observer variability seen after ivabradine. Finally, summation of early and late diastolic flow waves limited the comparison of the reliability of several indices prior and after HR reduction.

In summary, treatment of healthy cats with either atenolol or ivabradine only had minor effects on reproducibility of echocardiographic data. Treatment with ivabradine failed to reduce observer and measurement variation of echocardiographic variables whereas atenolol exhibited a trend toward improvement of their reproducibility. Both bradycardic agents consistently led to separation of summated diastolic Doppler signals which may be of potential use in the assessment of LV diastolic function in cats. If these findings can be extrapolated to cats with HCM deserves further study.

Footnotes

[a]Liberty Research Inc., Waverly, NY.
[b]Procoralan®, Les Laboratoires Servier, 22 Rue Garnier, 92200 Neuilly-sur-Seine, France
[c]Atenolol 25mg®, Mallincrodt Inc, St Louis, MO.
[d]ClinPro® software, Clinical Systems, Garden, NY City.
[e]Capsules 4 blue, Gallipot Inc, St. Paul, MN.
[f]Vivid 7 Vantage, GE Medical Systems, Milwaukee, WI.
[g]Riesen SC, Schober KE, Smith DN, Otoni CC, Bonagura JD. Effects of ivabradine on invasive indices of LV function in anesthetized cats with hypertrophic cardiomyopathy (abstract*). J Vet Intern Med* 2010;24:692-693.
[h]Cober RE, Schober KE, Buffington CAT, Riesen SC, Bonagura JD. Effects of ivabradine, a selective I_f channel inhibitor, on heart rate in healthy cats (abstract*). J Vet Intern Med* 2010;24:695.
[i]Acepromazine maleate injection®, Boehringer Ingelheim Vetmedica Inc, St Joseph, MO.
[j]Torbugesic®, Fort Dodge Laboratories, Fort Dodge, Iowa.
[k]EchoPac software package, Version BT06, GE Medical Systems, Milwaukee, WI.
[l]SigmaStat, Version 3.5, SPSS Inc, Chicago, IL.

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SUMMARY AND CONCLUSIONS

This project served two overall goals. Firstly, we intended to study a new therapeutic option for heart rate (HR) control in cats with hypertrophic cardiomyopathy (HCM). The idea for these studies arose from the current lack of available drugs that effectively and safely control HR in cats without affecting the left ventricular (LV) systolic and diastolic function and left atrial (LA) performance. Therefore, we investigated the pharmacology of ivabradine and its hemodynamic effects in healthy cats and cats with HCM in order to lay the foundation for its future use for HR control in cats with HCM. Then, we compared its effect to those of atenolol, a commonly used drug in the treatment of feline HCM.

Secondly, we intended to advance the current knowledge on myocardial expression of hyperpolarization-activated, cyclic nucleotide-gated (HCN) proteins in myocardial tissue from control cats and cats with HCM.

The first study (**Chapter 1**) described the validation of an analytical method for the determination of ivabradine and its major metabolite S-18982 in feline plasma; the pharmacokinetics (PK) of ivabradine and S-18982 following single and repeated oral administration; and the development of a combined 4-compartment PK model. Ivabradine at 0.3 mg/kg PO was administered as a single dose on Day 1, followed by twice daily dosing on Days 2 through 4. Plasma was collected at various time points,

frozen and analyzed by liquid chromatography-mass spectrometry-mass (LC/MS/MS) methods. Both, concentrations of the parent compound (S-16257) and S-18982 were measured. Individual plasma concentrations versus time from each cat were analyzed and used in a non-compartmental and compartmental model using the Phoenix WinNonlin 6.1 software. Accuracy and precision were found to be within acceptable limits in this study, and the lower limit of quantification (LLOQ) was determined at 0.250 ng/mL for both the parent drug and S-18982. Ivabradine and S-18982 reached their maximum concentrations of 103.33 and 3.86 ng/mL within 1 hour of administration. Concentration-time plots for ivabradine indicated biphasic profiles for all the cats on day 1 and day 4. Profiles for S-18982 indicated monophasic kinetics for all cats on both days. Following repeated administration, the areas under the plasma concentration-time curves for ivabradine and S-18982 did not significantly increase. Two-compartmental and one-compartmental models with first-order input and elimination provided the best fit to the data for ivabradine and S-18982. Subsequently, both models were combined to produce a single 4-compartment model characterizing ivabradine and S-18982 pharmacokinetics.
The results of this study indicate that repeated oral doses of ivabradine produced plasma drug concentrations suitable for 12-hour dosing intervals in healthy cats. Furthermore, the analytical assay and combined ivabradine/S-18982 model provide tools for further evaluation of the pharmacokinetics and pharmacodynamics of ivabradine in future studies in cats.

Myocardial HCN expression was then determined in LV and right ventricular (RV) myocardial tissue samples from healthy cats and cats with HCM (**Chapter 2**).

Myocardial expression of feline HCN was investigated in 16 adult control cats, 4 control kittens and 8 cats with HCM by immunoblot analysis using four different HCN antibodies against HCN2 and HCN4. For validation of the four different antibodies used in this study, cerebral and retinal tissue (fresh and frozen) of healthy cats were used as positive controls. Of the four antibodies evaluated only one identifying HCN4 revealed consistent results in both positive controls (brain and retina) and cardiac tissue. HCN4 expression was increased (1.98-fold) in LV myocardial samples of cats with HCM (P=0.036) compared to control cats, while in RV samples only a trend was observed (P=0.055, statistical power 0.224). Furthermore, expression of HCN4 in samples from the RV and LV was not different between adult control cats and control kittens. The results of this study inidcate that LV and RV myocardial HCN4 expression is upregulated in cats with HCM and that maturation toward the adult myocardial phenotype of HCN4 expression is completed prior to the age of 2 to 3 months.

The third study (**Chapter 3**) determined the effects of ivabradine on HR, LV systolic and diastolic function, and LA performance in six healthy cats and six cats with HCM. After induction of anesthesia cats were positioned in left lateral recumbency on a fluoroscopy table. For continuous recording of the LV and aortic pressures a 3-F, high-fidelity, dual-micromanometer-tipped catheter was advanced into the LV. For measurement of pulmonary artery pressures and cardiac output by thermodilution, a 5-F, flow directed, fluid-filled, thermistor-tipped catheter was placed into the left pulmonary artery. Simultaneously, echocardiographic studies were performed at each treatment to assess the cardiac function. After baseline measurements were made, four consecutive

treatment periods were studied: 1) esmolol (200-400 µg/kg/min, CRI, IV); 2) esmolol and dobutamine (5 µg/kg/min, CRI, IV); 3) ivabradine, (0.3 mg/kg, bolus, IV); and 4) ivabradine and dobutamine.

Treatment with ivabradine resulted in a significant reduction of HR (P<0.001), the rate-pressure product (P<0.001), and LV contractile function (P=0.01) and a significant increase in LVEDP (P=0.006), LV end-diastolic wall stress (P=0.001), and tau (P=0.009) in cats with HCM. Concurrent administration of ivabradine and dobutamine resulted in a significant increase of LV contractility and lusitropy (P<0.05), with blunted chronotropic effects of the catecholamine. (Peak HR < 140 min^{-1} in all cats as opposed to HRs between 164 min^{-1} and 204 min^{-1} under the influence of esmolol). Regression analysis revealed no association between tau and HR in cats with HCM. The results of this study revealed that intravenous administration of ivabradine reduces HR and the rate-pressure product in anesthetized cats with HCM. LV systolic and diastolic function as well as LA performance were either unchanged or only minimally affected by ivabradine justifying its use in clinical trials.

The fourth study of our project served to characterize the clinical use of ivabradine in cats (**Chapter 4**). To obtain that aim, ten experimental healthy, female-spayed, domestic shorthair cats were used in a prospective, double-blind, randomized, active-control, fully crossed study. Each cat was randomized to either ivabradine (0.3 mg/kg, q12h) or atenolol (6.25 mg/cat, q12h) for four weeks and then switched to the alternative treatment for another four weeks. At each stage, cats underwent a thorough clinical evaluation including a physical exam, a complete blood cell count, and a blood

biochemistry panel, an indirect blood pressure measurement, ECG, and a transthoracic echocardiogram. Ivabradine was clinically well tolerated with no adverse events observed. HR (ivabradine, $P<0.001$; atenolol, $P<0.001$; ivabradine vs. atenolol, $P=0.721$) and the rate-pressure product (ivabradine, $P<0.001$; atenolol, $P=0.001$; ivabradine vs. atenolol, $P=0.847$) were not different between treatments. At the dosages used, ivabradine was non-inferior to atenolol with regard to its negative chronotropy, reduction of the rate-pressure product, and clinical tolerance. Moreover, ivabradine appeared to exert more beneficial effects on LV systolic wall stress, LV diastolic function, LA performance, and left auricular appendage flow velocity. However, the apparent enhancements in LV systolic function and increased LV end-diastolic volume identified with ivabradine deserve further evaluation in particular in cats with dynamic LV outflow tract obstruction.

In the final study, the effects of ivabradine and atenolol on the reproducibility of echocardiographic indices of left heart function and cardiac chamber size were investigated (**Chapter 5**). Repeated echocardiographic examinations were performed by two observers in mildly sedated cats at baseline and after four weeks of treatment (Group 1, ivabradine 0.3 mg/kg, q12h, PO, n=4; Group 2, atenolol 6.25 mg/cat, q12h, PO, n=4) in a prospective, double-blind, randomized study. Test reliability was determined by estimating measurement variability, within-day interobserver variability, and between-day intraobserver variability of all echocardiographic indices. Variability was expressed as coefficient of variation (CV) and the absolute value below which the difference between two measurements will lie with 95% probability. Overall, CVs ranged from 0.5-

50.6% at baseline, 0.5-45.5% after ivabradine, and 0.5-23.3% after atenolol. The majority of echocardiographic variables obtained in this study had excellent to good reproducibility justifying their use in the clinical setting. While ivabradine and atenolol improved the reproducibility of selected echocardiographic variables, they also deteriorated the reproducibility of others, leading to an almost neutral effect on overall reproducibility. However, both drugs consistently led to separation of fused diastolic filling and myocardial motion waves, facilitating assessment of LV diastolic function. This study revealed that treatment of healthy cats with either ivabradine or atenolol had only minor effects on reproducibility of echocardiographic data. However, treatment may influences reproducibility of single echocardiographic variables and thereby be of relevance in clinical trials assessing the effects of drugs on cardiovascular function. Both bradycardic agents consistently led to separation of summated diastolic Doppler signals which may be of potential use in the assessment of LV diastolic function in cats.

In conclusion, the results of our studies provide data on a potentially new therapeutic option for cats with HCM. We found that ivabradine reduces HR in a predictable manner, blunts chronotropic effects of catecholamines, and affects LV systolic and diastolic function as well as LA performance only minimally in cats with HCM. In addition, we demonstrated that ivabradine is non-inferior to atenolol with regard to its negative chronotropy, reduction of the rate-pressure product, and clinical tolerance. Moreover, ivabradine appeared to exert more beneficial effects on LV systolic wall stress, LV diastolic function, LA performance, and left auricular appendage flow

velocity. Furthermore, our results revealed that HCN4 expression is upregulated in LV myocardial tissue in cats with HCM. This may predispose cardiomyocytes from failing hearts to enhanced automaticity, an arrhythmogenic mechanism which may predispose to sudden cardiac death. It needs to be proven, if selective I_f inhibiton reduce the risk of sudden death in feline HCM.

Based on our studies, it appears that ivabradine is clinically well tolerated, safe with regard to central hemodynamics and effects on blood pressure and cardiac function, and useful for effective HR control in cats. This makes ivabradine attractive in the treatment of feline HCM, although its long-term effects in cats with HCM and in particular in cats with dynamic LV outflow obstruction require further investigation.

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Appendix A: Abbreviations Chapter 2

cAMP	Cyclic adenosine monophophate
HCM	Hypertrophic cardiomyopathy
HCN	Hyperpolarization-activated, cyclic nucleotide-gated
I_f	Funny current
LV	Left ventricle
RA	Right atrium
RV	Right ventricle

Appendix B: Abbreviations Chapter 3

Aduration	Duration of the late diastolic transmitral flow wave
Aduration:ARduration	Ratio between duration of the late diastolic transmitral flow wave to duration of the late diastolic pulmonary vein atrial reversal wave
AoDP	Aortic diastolic pressure
AoSP	Aortic systolic pressure
ARduration	Duration of the late diastolic pulmonary vein atrial reversal wave
BL	Baseline
CI	Cardiac Index
Circ SrR peak A	Global late diastolic circumferential strain rate
Circ SrR peak E	Global early diastolic circumferential strain rate
Circ SrR peak S	Global systolic circumferential strain rate
CO	Cardiac output determined by thermodilution
Dob	Dobutamine
E:A	Ratio between peak velocity of early diastolic transmitral flow to peak velocity of late diastolic transmitral flow

E:IVRT	Ratio between peak early transmitral flow velocity to isovolumic relaxation time
EF	Ejection fraction
Esm	Esmolol
Esm+Dob	Concurrent administration of esmolol and dobutamine
FAC	Fractional area change
FS	Fractional shortening
HCM	Hypertrophic cardiomyoathy
HR	Heart rate
Iva	Ivabradine
Iva+Dob	Concurrent administration of ivabradine and dobutamine
IVRT	Isovolumic relaxation time
IVSd	Thickness of the intraventricular septum in diastole
LA	Left atrium
LA SF	Left atrial shortening fraction
LA_{max}	Maximum left atrial antero-posterior dimension
LV	Left ventricle
LVEDP	Left ventricular end-diastolic pressure
LVIDd	Left ventricular internal dimension in diastole
LVPWd	Thickness of the left ventricular posterior wall in diastole
LVSP	Left ventricular systolic pressure

MV $DecT_E$	Deceleration time of the early diastolic transmitral flow wave
PADP	Pulmonary artery diastolic pressure
PAP_{mean}	Pulmonary mean artery pressure
PASP	Pulmonary artery systolic pressure
Peak A	Peak velocity of late diastolic transmitral flow
Peak Aa lat	Peak velocity of late diastolic motion of the lateral mitral annulus
Peak AR	Peak velocity of the late diastolic pulmonary vein atrial reversal wave
Peak E	Peak velocity of early diastolic transmitral flow
Peak Ea lat	Peak velocity of early diastolic motion of the lateral mitral annulus
Peak LAA	Peak velocity of left arterial appendage flow
Radial SR Peak S	Peak radial systolic strain
Radial SrR Peak A	Peak radial late diastolic strain rate
Radial SrR Peak E	Peak radial early diastolic strain rate
Radial SrR Peak S	Peak radial systolic strain rate
S:D	Ratio between peak velocity of systolic pulmonary vein flow to peak velocity of diastolic pulmonary vein flow
SV	Stroke volume
WS	Wall stress

Appendix C: Clinical Composite Score on tolerance of treatments

Variable	Assessment		
Appetite:	□ decreased	□ unchanged	□ increased
Behavior:	□ nervous	□ unchanged	□ anxious
Activity:	□ decreased/quiet	□ unchanged	□ increased/restless
Interaction:	□ hiding	□ unchanged	□ attention seeking
Defecation:	□ normal	□ abnormal	
Urination:	□ normal	□ abnormal	
Vomiting:	□ yes	□ no	
Grooming:	□ normal	□ abnormal	
Respiration:	□ normal	□ abnormal	

Appendix D: Abbreviations Chapter 4

Aduration	Duration of the late diastolic transmitral flow wave
Aduration:ARduration	Ratio between duration of the late diastolic transmitral flow (A wave) to duration of the late diastolic pulmonary vein atrial reversal wave (AR wave)
Ao Vmax	Maximum aortic velocity
ARduration	Duration of the late diastolic pulmonary vein atrial reversal flow wave
CCS	Clinical Composite Score
E:Ea	Ratio between peak velocity of early diastolic transmitral flow (E wave) to peak velocity of early diastolic motion (Ea wave) of the lateral mitral annulus
EA fus:EaAa fus	Ratio between peak velocity of fused early and late transmitral flow waves (EA fus) to peak velocity of fused early and late diastolic motion waves of the lateral mitral annulus (EaAa fus)
E:A	Ratio between peak velocity of early diastolic transmitral flow (E wave) to peak velocity of late diastolic transmitral flow (A wave)

E:IVRT	Ratio between peak early transmitral flow velocity (E wave) to isovolumic relaxation time (IVRT)
EDV	End-diastolic volume
EF	Ejection fraction
ESV	End-systolic volume
FAC	Fractional area change
FS	Fractional shortening
HCM	Hypertrophic cardiomyopathy
HR	Heart rate
HR_{CE}	Heart rate obtained at clinical exam
HR_{ECG}	Heart rate obtained by ECG
HR_{ECH}	Heart rate obtained at echocardiographic exam
IVRT	Isovolumic relaxation time
IVSd	Thickness of the intraventricular septum in diastole
IVSs	Thickness of the intraventricular septum in systole
LA	Left atrial
LA area d	Left atrial area in diastole
LA area s	Left atrial area in systole
LA SF	Left atrial shortening fraction
LADs	Maximum left atrial antero-posterior dimension
LAD_{min}	Minimum left artrial antero-posterior dimension
LV	Left ventricular

LVIDd	Left ventricular internal dimension in diastole
LVIDs	Left ventricular internal dimension in systole
LVPWd	Thickness of the left ventricular posterior wall in diastole
LVPWs	Thickness of the left ventricular posterior wall in systole
MV $DecT_E$	Deceleration time of the early diastolic transmitral flow
Peak A	Peak velocity of late diastolic transmitral flow
Peak Aa lat	Peak velocity of late diastolic motion of the lateral mitral annulus
Peak AR	Peak velocity of late diastolic pulmonary vein atrial reversal flow wave
Peak E	Peak velocity of early diastolic transmitral flow
Peak EA fus	Peak velocity of fused early and late transmitral flow
Peak EaAa fus	Peak velocity of fused early and late diastolic motion of the lateral mitral annulus
Peak Ea lat	Peak velocity of early diastolic motion of the lateral mitral annulus
Peak LAA flow	Peak velocity of left atrial appendage flow
Peak S	Peak velocity of systolic pulmonary vein flow
Peak D	Peak velocity of diastolic pulmonary vein flow
Radial SR Peak S	Peak radial systolic strain
Radial SrR Peak A	Peak radial late diastolic strain rate
Radial SrR Peak E	Peak radial early diastolic strain rate

Radial SrR EA fus	Peak radial strain rate of fused early and late diastolic strain waves
Radial SrR Peak S	Peak radial systolic strain rate
RPP	Rate-Pressure Product
SBP	Systolic blood pressure
S:D	Ratio between peak systolic (S wave) to peak diastolic (D wave) pulmonary vein flow velocity

Appendix E: Abbreviations Chapter 5

ARduration	Duration of the late diastolic pulmonary vein atrial reversal flow wave
BSI	British Standards Institution
CV	Coefficient of variation
E:A	Ratio between peak velocity of early diastolic transmitral flow (E wave) to peak velocity of late diastolic transmitral flow (A wave)
E:Ea	Ratio between peak velocity of early diastolic transmitral flow (E wave) to peak velocity of early diastolic motion of the lateral mitral annulus (Ea wave)
E:IVRT	Ratio between peak early transmitral flow velocity (E wave) to isovolumic relaxation time (IVRT)
HCM	Hypertrophic cardiomyopathy
HR	Heart rate
IVRT	Isovolumic relaxation time
LA	Left atrial
LADs	Maximum left atrial antero-posterior dimension
LV	Left ventricular

LVIDd	Left ventricular internal dimension in diastole
Peak A	Peak velocity of late diastolic transmitral flow
Peak Aa lat	Peak velocity of late diastolic motion of the lateral mitral annulus
Peak AR	Peak velocity of late diastolic pulmonary vein atrial reversal flow wave
Peak E	Peak velocity of early diastolic transmitral flow
Peak Ea lat	Peak velocity of early diastolic motion of the lateral mitral annulus
Peak LAA	Peak velocity of left atrial appendage flow
PW	Pulsed wave
Radial SR Peak S	Peak radial systolic strain
Radial SrR Peak A	Peak radial late diastolic strain rate
Radial SrR Peak E	Peak radial early diastolic strain rate
Radial SrR EA fus	Peak radial strain rate of fused early and late diastolic strain waves
Radial SrR Peak S	Peak radial systolic strain rate
TDI	Tissue Doppler imaging
2D	Two-dimensional