EFFECT OF IVABRADINE, A NOVEL I$_{f}$ CURRENT INHIBITOR, ON DYNAMIC OBSTRUCTION OF THE LEFT VENTRICULAR OUTFLOW TRACT IN CATS WITH PRECLINICAL HYPERTROPHIC CARDIOMYOPATHY: A SINGLE-DOSE STUDY

Master’s Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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ABSTRACT

A relevant subset of cats with hypertrophic cardiomyopathy (HCM) develop dynamic obstruction of the left ventricular outflow tract and mitral regurgitation secondary to hypertrophy of the left ventricle and systolic anterior motion (SAM) of the mitral valve. This leads to an increase in left ventricular systolic pressure, and thus wall stress, that may ultimately lead to progression of the disease. While prospective studies documenting the morbidity and mortality associated with dynamic obstruction have not been performed in feline HCM, it is possible that the results would be similar to human studies, in which SAM has been proven repeatedly to be a negative prognostic indicator in HCM, as well as an independent predictor of disease progression and sudden death. Beta-blockers are the primary medication used to reduce heart rate and relieve dynamic left ventricular outflow tract obstruction in cats with HCM, however, side effects or contraindications sometimes limit their use. Ivabradine is a highly selective If current inhibitor that exerts negative chronotropic effects without significant effects on inotropy, lusitropy, or dromotropy as documented in multiple species. Ivabradine has been studied in cats, and has been shown to have unique heart rate lowering properties. The drug is clinically well tolerated without any discernable side effects, has only minimal effects on central hemodynamics, has pharmacokinetic and pharmacodynamic properties that allow for twice daily dosing, and enhances left atrial function. The purpose of this study was to determine whether ivabradine, at a single dose of 0.3 mg/kg PO, could reduce or eliminate dynamic obstruction of the left ventricular outflow tract in cats with HCM.
In this prospective, randomized, double-blind, active-control single dose study, 21 cats with preclinical HCM and dynamic left ventricular outflow tract obstruction received one dose of atenolol at approximately 2 mg/kg PO or one dose of ivabradine at 0.3 mg/kg PO. Baseline and 3-hour post-treatment heart rate, echocardiographic variables, and blood pressure were recorded. Statistical comparisons were made using analysis of covariance (ANCOVA).

Peak velocity in the left ventricular outflow tract was significantly decreased compared to baseline for both drugs, however the mean change in velocity was more reduced for atenolol (2.54 m/s; 95% confidence interval 1.83 m/s to 3.25 m/s) compared to ivabradine (0.51 m/s; 95% confidence interval 0.01 m/s to 1.01 m/s; \( P<0.0001 \)). Echocardiographic indices of systolic function were largely unchanged by ivabradine, but reduced by atenolol. Doppler systolic blood pressure was not affected by either drug. Predictably, heart rate was reduced by both drugs.

These findings indicate that a single dose of ivabradine at 0.3 mg/kg mildly reduces dynamic left ventricular outflow tract obstruction in cats with HCM while preserving systolic function, but this reduction is inferior compared to that achieved by atenolol and is likely not clinically relevant. Further studies evaluating the utility of ivabradine in reducing dynamic obstruction over a longer time period are needed.
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CHAPTER 1

INTRODUCTION

Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats, characterized by an increase in left ventricular (LV) wall thickness of unknown cause, with histomorphologic changes such as myofiber disarray and narrowing of intramural coronary arteries, which finally lead to myocardial ischemia and interstitial and replacement fibrosis.\textsuperscript{1-6} Diagnosis is most commonly achieved via transthoracic two-dimensional (2D) echocardiography, with many cats being recognized in the subclinical stage due to the presence of a murmur, a gallop sound, or an arrhythmia.\textsuperscript{7-10} The development of HCM in most cats is likely due to mutations in one or more genes encoding sarcomeric proteins, resulting in symmetric or regional hypertrophy of the interventricular septum, the LV posterior wall, and/or the papillary muscles.\textsuperscript{4,5,11,12} The resultant impaired ventricular relaxation and increased wall stiffness impede diastolic filling, which leads to elevated LV filling pressures, left atrial (LA) enlargement, neurohormonal activation, blood stasis, arrhythmias, congestion, and ultimately decompensation to congestive heart failure. Potential adverse outcomes in cats with HCM include the development of congestive heart failure, arterial thromboembolism, and sudden cardiac death, all of which may develop acutely following a long
asymptomatic period that may last for years. While the progression of HCM is variable, large-scale studies have shown that most cats with HCM will eventually die of their cardiac disease.\textsuperscript{6,9,13} A number of studies have identified risk factors such as LA size and LV diastolic function as negative prognostic indicators, with conflicting reports regarding the effect of heart rate (HR) and left ventricular outflow tract obstruction on prognosis.\textsuperscript{6,13,14}

**Dynamic Obstruction of the Left Ventricular Outflow Tract**

The majority of cats with HCM develop dynamic obstruction of the LV outflow tract (LVOT) and secondary mitral regurgitation as a result of hyperdynamic LV function and systolic anterior motion (SAM) of the septal (anterior), and rarely posterior, mitral valve leaflet. This contributes to increases in LV systolic pressure that may ultimately lead to clinical signs and progression of the disease. A complete understanding of the initiating mechanism of SAM has yet to be elucidated, but most studies indicate a diverse, multifactorial etiology. The initially favored hypothesis speculated that SAM was the result of the Venturi effect, which develops when a narrowing of a conduit causes a high velocity stream that results in a pressure gradient. Applying this mechanism to the LV outflow tract, the Venturi effect argues that a narrowed LV outflow tract, possibly due to hypertrophy of the basilar interventricular septum (IVS), causes a high velocity stream during ejection, which produces a low pressure region in accordance with Bernoulli’s law, that “sucks” the anterior mitral valve leaflet into the outflow tract, thus obstructing it and further increasing the outflow tract velocity.\textsuperscript{15,16} A central premise of the Venturi theory is that high velocity flow during ejection is the driving force behind SAM, however, echocardiographic data comparing M-mode tracings with continuous wave (CW) and pulsed wave (PW) Doppler tracings in humans with SAM clearly demonstrate that SAM begins in early systole when LVOT velocity is normal. The low velocity flow at the onset of SAM is not high enough to generate significant Venturi forces, indicating that the Venturi mechanism is not the main cause of SAM.\textsuperscript{17} Additionally, the Venturi
theory fails to explain the clinical observation that SAM can occur in patients without a narrowed outflow tract, as the high velocity flow required to generate Venturi forces can only be achieved secondary to LVOT narrowing. Finally, investigators have shown that sufficient papillary muscle restraint could prevent SAM, even in the presence of a narrowed LVOT, suggesting that factors other than the Venturi effect are more likely to play a primary role in the development of SAM.

While the determinants of SAM are likely numerous, there is increasing evidence that abnormal papillary muscle-mitral valve geometry plays an important role in its development, creating drag forces that propagate anterior motion of the mitral leaflets. Indeed, anterior displacement of the papillary muscles experimentally created in dogs, in the absence of hypertrophy or marked outflow tract narrowing, has been shown to induce SAM and obstruction. In this model, papillary muscle displacement is thought to result in SAM by the following mechanisms: 1) by decreasing the ability of the papillary muscles to restrain the valve posteriorly as a result of reduced papillary muscle tension; 2) by interposing the leaflets anteriorly into the outflow stream causing drag forces (pushing forces parallel to flow experienced by the leaflets) with an anterior component to develop; and 3) by creating a geometry of mitral valve coaptation that favors SAM, whereby anterior papillary muscle displacement pulls the posterior mitral valve leaflet upward so that it meets the anterior leaflet closer to its base, creating a long, overlapping residual leaflet that is relatively free to move anteriorly (increased slack), unrestrained by the pressure difference between the left atrium and ventricle that keeps normally coapted leaflet bodies closed. Reduced papillary muscle tension is thought to be a result of ineffective chordal restraint. Chordal slack created by papillary muscle displacement is necessary for SAM to occur, as this slack prevents tethering of the leaflets during systole. This hypothesis of reduced chordal tension promoting anterior motion of the mitral valve leaflets has gained support experimentally, in which an in vitro model of a horizontal leaflet mounted in a flow chamber was shown to generate anterior
motion of the leaflet by altering the distribution or effectiveness of tension tethering the leaflet, which was attached by chords at its distal end to a series of upstream screws, and that the shape achieved by the leaflet depended on the direction of chordal tension. Results of these experiments suggest that the abnormal leaflet geometry seen in patients with HCM can be reproduced in a membrane model simply by altering the distribution of tension on a leaflet exposed to flow, and that this leaflet geometry can be altered by changing the amount of chordal tension. Specifically, systolic anterior leaflet motion, as seen in patients with HCM, could be reproduced by decreasing central chordal restraint while tension on the leaflet edges was maintained, and greater degrees of anterior motion at earlier stages in the release of chordal restraint could be achieved by directing chordal tension anteriorly. Once the leaflet has moved anteriorly into the path of flow, the impact of fluid on its undersurface could propagate the anterior motion by generating drag forces, as was shown in the previously mentioned study by Levine.

Yet another finding that supports the hypothesis of drag forces propagating systolic anterior motion of the mitral leaflets is the orientation of the mitral valve leaflets in patients with obstructive HCM, in which there is a high “angle of attack” of the ejection flow stream onto the valve leaflets. This orientation implicates drag, while precluding significant Venturi effects, as the low velocity flow pushes the mitral valve into the septum and initiates SAM. As the mitral valve is pushed further toward the septum as systole progresses, the angle of attack relative to flow increases, thereby increasing drag forces. Additionally, it has been shown in a porcine mitral valve model that not only does mitral leaflet elongation promote the development of SAM in response to papillary muscle displacement by creating long overlapping residual leaflets capable of moving anteriorly, thus providing a favorable geometry for obstruction to occur, but that the degree of SAM is directly related to the residual leaflet length for any given degree of papillary muscle malposition.
The recent introduction of real-time 3-dimensional echocardiography (3DE) has allowed for a more comprehensive analysis of the determinants of SAM, specifically changes in the mitral valve apparatus that provide a mechanism that determines leaflet slack and anteriorly directed motion. In a study examining 47 human patients with asymmetrical septal hypertrophy, 20 of which had LVOT obstruction, it was demonstrated that mitral annular area and annular height were larger in patients with obstruction compared to those without obstruction and normal controls, and that mitral leaflet area in patients with LV outflow obstruction was more than twice that of controls. This same study also demonstrated significant medial and anterior displacement of both papillary muscles in patients with LV outflow obstruction, but not in controls or patients with septal hypertrophy and no obstruction, similar to previous reports and again supporting the concept that abnormal papillary muscle-mitral valve geometry best explains the development of SAM. Further support for the importance of geometric changes of the mitral apparatus as a primary determinant of SAM can be found in another recent study utilizing 3DE, which investigated how spatial dynamics of the mitral apparatus could affect the hemodynamics of the LV outflow tract in humans with the obstructive form of HCM. The principal finding of this study was that the mitral coaptation point, mitral tenting, and papillary muscles all interacted with each other to contribute to the development of SAM by creating an altered distribution of tension to the mitral leaflets. Specifically, tethered mitral valve leaflets produced by the close proximity of both papillary muscles as a result of anterior and inward displacement, and movement of the coaptation point towards the outflow tract participate in provoking obstruction by propelling the residual leaflet into the outflow tract stream at mid-systole.

**Mitral Regurgitation**

Mitral regurgitation (MR) is a common secondary finding in patients with SAM. Transesophageal echocardiography has been instrumental in identifying the mechanism of MR in these patients, illustrating that the development of SAM in early systole results in leaflet-septal
contact and incomplete mitral leaflet coaptation in mid-systole, which causes the formation of a
funnel composed of the distal parts of both mitral leaflets, allowing a jet of posteriorly directed MR
to pass through this gap caused by the lack of leaflet coaptation in mid- and late systole. Failure
to effectively coapt depends on the motion of both mitral leaflets, rather than motion of the anterior
leaflet alone. As such, for any given degree of SAM, patients can have a wide spectrum of MR
severity, as the length of effective coaptation depends primarily on posterior leaflet length and
mobility. It has been shown that mitral valve geometry with limited ability of the posterior leaflet to
move anteriorly prevents effective valve coaptation, resulting in more severe mitral regurgitation for
any given degree of SAM. The ability of the posterior leaflet to move anteriorly is determined by
its length, which needs to be sufficiently elongated to follow the anterior leaflet, and by the range of
motion dictated by its chordal and papillary muscle connections.

**Dynamic Outflow Tract Obstruction in Cats**

A recent study performed in our laboratory showed that abnormalities of the mitral valve
apparatus may play a role in the pathogenesis of dynamic outflow tract obstruction in cats with
HCM. In this study, cats with HCM and dynamic outflow tract obstruction were shown to have a
higher prevalence of mitral valve and LVOT abnormalities compared to control cats and cats with
HCM but no obstruction. Among these abnormalities were elongation of the anterior mitral valve
leaflet, papillary muscle hypertrophy, and a higher prevalence of false tendons in the LVOT.
Furthermore, the results of this study revealed that there was a direct association between the
presence and severity of SAM and the magnitude of LV hypertrophy, suggesting that LV
hypertrophy may be, at least in part, a consequence of LV pressure overload from dynamic
obstruction.

It is estimated that one half to two thirds of cats with HCM develop dynamic outflow tract
obstruction, though a recent study involving 127 cats with HCM detected SAM in only 45.7%.
and in another, 31 of 111 cats (27.9%) with HCM were detected to have SAM.\textsuperscript{30} The most likely reason for this discrepancy is related to the definition of SAM (i.e., threshold LV-to-aortic root systolic pressure gradient) and the techniques used for detection, as accurate velocity measurement is highly dependent upon the Doppler angle of interrogation. A study in humans with HCM reported that approximately 37\% of patients have “resting” LVOT obstruction, defined as a LV outflow gradient $\geq$ 50 mmHg under resting conditions, while an additional 33\% of patients have “inducible”, or latent, LVOT obstruction, wherein these patients develop mechanical obstruction to LV outflow resulting from mitral valve-septal contact following exercise.\textsuperscript{31} Furthermore, latent obstruction may be identified in human HCM patients following the use of a number of provocative maneuvers intended to activate the sympathetic nervous system or decrease LV afterload.\textsuperscript{32} In cats, activation of the sympathetic nervous system while in the hospital may induce SAM in a certain percentage of cats that lack resting obstruction, making the detection of latent SAM in these patients more likely.

The clinical significance of SAM in cats is an area of continuous debate. Earlier retrospective studies in cats have suggested that SAM is associated with better survival and is an overall positive prognostic indicator.\textsuperscript{8,29} More recently, SAM was not shown to be of prognostic importance in cats with congestive heart failure or in asymptomatic cats with HCM.\textsuperscript{14} Moreover, recently unpublished data from our laboratory on 5-year survival after diagnosis in 63 cats with preclinical HCM failed to identify SAM as an independent predictor of outcome.\textsuperscript{a} Only LA size and age predicted 5-year mortality in these cats. A possible explanation for these findings is that most cats with SAM are diagnosed early in the asymptomatic stage of the disease due to the presence of a heart murmur during cardiac auscultation. In contrast, many cats without SAM are first diagnosed with HCM after presenting to the veterinarian late in the disease process due to congestive heart failure (CHF) or a thromboembolic event and are, therefore, less likely to survive as long as
asymptomatic cats, thus biasing the survival data. Furthermore, SAM may simply be a representation of mitral valve dysplasia, a relatively common congenital cardiac abnormality in cats with a good prognosis, with secondary LV hypertrophy, and thus may only mimic the presence of HCM.\textsuperscript{33}

To the contrary, it has been proven repeatedly that SAM is a negative prognostic indicator of survival in human HCM patients, and is an independent predictor of progression of heart disease status and risk of worsening exercise intolerance, CHF, stroke, and cardiac mortality.\textsuperscript{34,35} Interestingly, it is not the severity of LVOT obstruction that predicted outcome in these studies, but rather, only the presence of outflow tract obstruction defined as a resting LV-aortic root pressure gradient greater than 30 mmHg. While similar studies documenting the morbidity and mortality associated with dynamic obstruction are sparse in cats, it is possible that the results would be similar to the human studies, making reduction of SAM a critical therapeutic target in the treatment of asymptomatic cats. Finally, moderate and severe SAM may lead to clinical signs including weakness, syncope, and CHF, and relief of obstruction using negative inotropic agents has been shown to alleviate syncope and improve activity levels in humans with dynamic obstruction and anecdotally in cats as well.

**Dynamic Mid-Left Ventricular Obstruction**

Although SAM with mitral-septal contact is the most common cause of dynamic LV outflow tract obstruction in cats with HCM, other variants do occur, most notably mid-LV obstruction. This occurs most commonly in cats secondary to hypertrophy of the dorsal interventricular septum, which protrudes into the LV outflow tract during systole. Additionally, hypertrophied papillary muscles and hyperdynamic chamber function have been postulated as causes.\textsuperscript{36} Much like SAM, mid-LV obstruction creates an intraventricular pressure gradient that can lead to increased systolic
intraventricular pressure (and thereby increase myocardial wall stress); exacerbate subendocardial ischemia; increase myocardial oxygen demand; and stimulate ventricular hypertrophy.

**Treatment of Preclinical Feline Hypertrophic Cardiomyopathy**

Given the potential morbidity and mortality associated with dynamic LV outflow tract obstruction, a major goal in the treatment of preclinical feline HCM includes relief of moderate to severe or clinically relevant obstruction. The most commonly prescribed medications used to achieve this goal include beta-adrenergic receptor blockers. Atenolol, a hydrophilic β₁-selective adrenergic receptor antagonist, is the most commonly used drug to relieve dynamic obstruction in cats at our institution. Oral bioavailability of the drug in cats is around 90% with a peak beta-blocking effect occurring at approximately one hour after administration.\(^{37}\) Beta-blockers exhibit both negative inotropic and chronotropic effects, thus leading to reduction of LV outflow tract obstruction, as well as improvement in LV filling and coronary perfusion. However, these drugs may also induce adverse effects such as weakness, lethargy, salivation, inappetence, weight loss, as well as reduced LA function that might predispose to the development of thromboembolic disease.\(^{38,39}\)

Additionally, beta-blockers may also be contraindicated in certain conditions such as bronchoconstrictive pulmonary disease, hypotension, CHF, and thromboembolic disease due to their potential to exacerbate such conditions. The long-term use of beta-blockers continues to be controversial due to lack of efficacy data on disease progression or survival.\(^{40}\) In fact, as previously referenced, a recent study conducted in our laboratory showed no survival benefit in cats with preclinical HCM treated with atenolol compared to non-treated cats followed for a period of five years.\(^{a}\)

**Ivabradine**

Recently, new medications have been developed that exert purely negative chronotropic effects by targeting specific ion channels in the sinoatrial node (SAN), and thus HR, in the absence
of effects on myocardial performance. Some of these medications are currently in clinical use in human patients; for example, ivabradine, an inhibitor of the pacemaking $I_f$ current in SAN cells, is approved in Europe for the prevention of tachycardia-induced aggravation of myocardial ischemia in human patients with coronary artery disease, and has recently been shown to be an effective and safe alternative to beta blockers and calcium channel blockers for the reduction of heart palpitations, exercise intolerance, and other associated clinical signs in patients with inappropriate sinus tachycardia. Should these pure HR reducers also relieve dynamic LV outflow tract obstruction, they may become a treatment option in cats with preclinical HCM.

The SAN forms a wedge-shaped group of cells set into the junction of the cranial vena cava, right auricular orifice, and body of the right atrium in the region of the crista terminalis, a thick ridge of muscle between the cranial vena cava and atrioventricular opening. It is separated from the atrial myocytes by a connective tissue barrier, primarily composed of collagen and fibroblasts. The node is composed of histologically discrete, specialized cells that are responsible for initiating cardiac impulses. The transmembrane resting potential of the sinus node pacemaker cells gradually decreases during diastole until threshold potential is attained. Diastolic depolarization in the sinus node begins from a trough potential of about -60 millivolts (mV), and is produced by the combined actions of several ionic channels. The major ion channels that appear to contribute to spontaneous diastolic depolarization in SAN cells are the delayed rectifier potassium current ($I_{K,v}$), inward calcium channels ($I_{Ca-L}$ and $I_{Ca-T}$), inward background current sodium current ($I_b$), and the inward “funny” current ($I_f$).

Ivabradine, as previously mentioned, is a novel HR lowering agent that acts by selectively inhibiting the pacemaking $I_f$ (“funny” inward) current in the SAN in a dose-dependent manner by reducing the slope of diastolic depolarization of SAN cells. The “funny” current, originally described in SAN cells, is an inward (depolarizing) current activated by hyperpolarization of...
pacemaker cells at the end of action potential repolarization. It is likely that this current is composed of both sodium and potassium ions, although sodium ions may be dominant under physiologic conditions. This current is also known as the hyperpolarization-activated cyclic nucleotide-gated current (HCN) based on its ability to be activated directly by cyclic adenosine 3',5'-monophosphate (cAMP), independent of protein kinase A phosphorylation, and as such, the channel carrying this current is known as the HCN channel. HCN channels are located predominantly in cardiac tissue and neurons, with those in the heart giving rise to the formation and regulation of pacemaker activity in the SAN. While four HCN channel subunits are currently known (HCN 1-4), HCN 4 is the dominant isoform found within the SAN and conduction system in all species studied (rabbit, guinea pig, mouse, and dog), having a midpoint of voltage dependent activation of -100 mV. The If pacemaker function occurs at end repolarization when the diastolic potential is most negative, causing activation of the If current, diastolic depolarization, and a less negative membrane potential. When the membrane potential reaches the activation threshold of the L-type calcium channels, a nodal action potential occurs. The sole HR reducing effects of ivabradine appear to be due to its high binding affinity for HCN 4 within the SAN, thus inhibiting the If channel at hyperpolarized states and reducing the slope of diastolic depolarization. Ivabradine has minimal effects on myocardial contractility, lusitropy, blood pressure, and intracardiac conduction. In fact, in spontaneously beating healthy mouse left atria, ivabradine was shown to exert a pronounced positive inotropic effect. To the contrary, while ivabradine did not influence the rate of LV pressure increase (+dP/dt\text{max}), a marker of myocardial contractility, in anesthetized healthy cats, a significant reduction was observed in cats with HCM. Ivabradine has been shown to improve LV function, increase stroke volume, preserve cardiac output, improve myocardial ischemia, decrease myocardial oxygen consumption and increase its supply, and to prevent LV electrical and structural remodeling in humans and experimental animals.
of studies in experimental healthy cats and cats with HCM completed in our lab over the past five years have demonstrated that ivabradine has unique HR lowering properties and has only minimal effects on central hemodynamics including LV relaxation and contractility.\textsuperscript{52,56} The drug is clinically well tolerated without any discernable side effects.\textsuperscript{56} Pharmacokinetic and pharmacodynamic properties of ivabradine allow for twice daily dosing at 0.3 mg/kg to effectively control HR and prevent a sudden increase of HR due to stress.\textsuperscript{57,58} Ivabradine has also been shown to enhance LA function, and that short- and long-term administration is not inferior to atenolol with regard to effects on cardiac function in healthy cats and cats with HCM.\textsuperscript{b,56,59} However, the effects of ivabradine on dynamic outflow tract obstruction in cats with HCM have not been studied, and comparative data from studies in other species are lacking. To study the effect on dynamic LVOT obstruction in cats with HCM seems to be of clinical importance. If ivabradine aggravates dynamic outflow tract obstruction it may not be suitable in the treatment of feline HCM. However, if ivabradine were able to eliminate, or at least reduce, LV outflow obstruction to a clinically relevant degree, it may become a potential new treatment option for cats with preclinical HCM. The objectives of this study were to compare the effects of a single dose of ivabradine or atenolol on dynamic LVOT obstruction in cats with preclinical HCM, as well as compare each drug’s effect on HR, echocardiographic indices of systolic and diastolic function, and SBP. We hypothesized that ivabradine reduces dynamic obstruction of the LVOT in cats with HCM comparable to that achieved by atenolol. Additionally, we hypothesized that ivabradine achieves this goal without a significant effect on LV systolic function.
CHAPTER 2

MATERIAL AND METHODS

**Animals** – Twenty-one consecutive client-owned cats examined between 2011 and 2013 with preclinical HCM and dynamic LV outflow tract obstruction were enrolled in this study. For all cats, HCM was previously diagnosed based upon idiopathic concentric LV hypertrophy (global or regional diastolic LV wall thickness > 6 mm) as determined by 2D echocardiography, with Doppler echocardiographic (DE) evidence of LV diastolic dysfunction. Dynamic obstruction of the LVOT was defined as the presence of SAM with resting LV-to-aortic root systolic pressure gradient > 25 mmHg or mid-ventricular obstruction with a mid-ventricular systolic pressure gradient > 25 mmHg as determined by 2D and DE. Echocardiographic criteria for SAM included an abrupt bend of the tips of the anterior or both mitral valve leaflets, with the distal tip of the respective leaflet approaching or contacting the IVS in systole, and evidence of flow turbulence and increased LVOT velocities with a dagger-shaped flow signal reflecting dynamic obstruction. Criteria for mid-LV obstruction included the presence of a mid-LV gradient unrelated to SAM as detected by DE. Turbulence in the LVOT was determined using color flow Doppler with turbulence mapping and a Nyquist limit between 0.9 and 1.2 m/s. Peak flow velocity in the LVOT (LVOT $V_{max}$) was
measured from a left apical five chamber view using CW Doppler. Contamination of the outflow signal with the jet of MR was avoided.

Prior to enrollment, health status was determined in all cats based on a thorough physical examination; 2D, M-mode and DE; SBP measurement; and plasma T4 analysis in cats 6 years of age or older, or younger if clinical signs suggestive of hyperthyroidism were observed. Exclusion criteria included the presence of any cardiac disease other than HCM based on established echocardiographic criteria, systemic hypertension (SBP > 170 mmHg), hyperthyroidism, concurrent pulmonary or bronchial disease, heartworm disease, any other systemic disease, a resting HR < 120 bpm or resting SBP < 100 mmHg, brady- and tachyarrhythmias, CHF, intracardiac thrombi, LA spontaneous echocardiographic contrast, ongoing treatment with other cardiac medications, and poor animal compliance. All screening examinations were performed by either the principal investigator (K.A.B.) or co-investigator (K.E.S.). The study protocol was reviewed and approved by the Animal Care and Use Committee of The Ohio State University (2010A00000157).

**Study Design** – The study was a prospective, randomized, double-blind, active-control single dose study. After echocardiographic phenotyping aimed at identifying suitable cats, animals were randomly assigned and divided into two groups: one group received a single dose of the active control atenolol (target dose 2 mg/kg PO), and the other group received a single dose of the study drug ivabradine (target dose 0.3 mg/kg PO). In order to ensure that an acceptable number of cats received the study drug, randomization was performed in a 2:1 manner in favor of ivabradine. To assure blinding of the investigators and objectivity of the data, assignment of drug was randomized by one investigator not involved in the data acquisition process with the help of a random numbers generator. Moreover, both drugs were placed in identical opaque capsules. All cats were assessed at baseline and post-drug administration via physical examination, indirect SBP measurement, and
echocardiography by a single investigator (K.A.B.). Auscultation was performed at rest, immediately after 5 to 8 times up and down lifting, and immediately after inhalation of amyl nitrite. The latter two activities were performed in random order as chosen by a third party, a registered veterinary technician not involved in the study, though the investigator was not blinded as to the order of these stressors. Amyl nitrite administration was performed as follows: a single glass capsule containing 0.3 mL of 98% amyl nitrite liquid (294 mg) was crushed between the fingertips, with the liquid immediately transforming into a vapor. The crushed capsule was held in front of the cat's nasal planum over a time period of one minute so that several inhalations were allowed. After a 10-minute rest period to allow the effects of both provocative maneuvers to subside, an echocardiographic examination was performed, followed by DE measurement of LVOT $V_{\text{max}}$ after the introduction of noise intended to invoke stress on the cats (Doppler audio signal maximized for 10 to 30 sec). After an additional 5-minute rest period, SBP was measured. Cats were then randomized to receive the active control (atenolol target dose 2 mg/kg PO) or the study drug (ivabradine target dose 0.3 mg/kg PO). Three hours after administration of either drug, the above-mentioned procedures were repeated. Cats were housed in a quiet, feline-only ward between examinations.

**Echocardiography** - Cats were gently restrained in right and left lateral recumbency without the use of sedation and imaged from underneath. All echocardiographic studies were performed by one investigator (K.A.B.) using a digital high-end ultrasound system with 10 MHz and 7 MHz nominal frequency transducers preset for optimal feline imaging and DE studies. 2D images were recorded at >80 frames/s. Simultaneous ECG and PW, as well as CW, DE were recorded at 150 mm/sec sweep speed. PW and CW DE flow recordings were guided by 2D color-coded DE imaging with appropriate settings to observe low velocity signals. Assessments, measurements, and calculations were performed off-line from digitized still images or cine loops as an average of
three cardiac cycles, irrespective of the phase of respiration, using the embedded software and calculation packages by a single investigator (K.A.B.) blinded to treatment group.

Echocardiographic variables included: From the right parasternal four and five chamber long-axis or short-axis views – subjective assessment of the presence of dynamic obstruction of the LVOT based on 2D, M-mode and color flow Doppler; pattern of LV hypertrophy (symmetrical or asymmetrical, region affected if asymmetrical); assessment of LV hypertrophy severity using subjectively determined categories based on maximum diastolic wall thickness measurements (mild: 6.0 – 6.9 mm; moderate: 7.0 – 7.9 mm; severe: > 8.0 mm); dimensions of the IVS and left ventricular posterior wall (LVPW) at end-diastole from both long- and short-axis images; distance of the anterior mitral valve leaflet (AMVL) coaptation point to the IVS at end-systole and end-diastole; the presence, direction, and subjective severity of MR based on color flow Doppler; the maximum LA antero-posterior dimension from the long axis 4 chamber view (LAD); and the maximum LV internal dimension at end-systole (LVIDs) and end-diastole (LVIDd) from a short-axis image. From the left parasternal apical three, four, or five chamber views – LV isovolumic relaxation time (IVRT); Peak early diastolic transmitral flow (TMF) velocity (E); Ratio of peak early diastolic TMF velocity to late diastolic TMF flow velocity (E:A); Peak velocity of a fused diastolic transmitral wave (EA fused); Peak early diastolic velocity of the lateral mitral annulus by tissue Doppler (TDI) (Ea); Peak systolic velocity of the lateral mitral annulus (Sa); the ratio of E to Ea (E:Ea); LV pre-ejection period (PEP); LV ejection time (ET); the ratio of PEP to ET (PEP:ET); LVOT Vmax using CW DE; mid-LV Vmax using CW DE; the maximum flow velocity at the level of the aortic valve (Ao Vmax) using PW Doppler; and the maximum MR velocity using CW DE. The only echocardiographic variables measured during “noise stress” were LVOT Vmax and Ao Vmax using both PW and CW Doppler from the left apical three or five chamber view. LV shortening fraction (SF) was calculated as {(LVIDd - LVIDs)/LVIDd x 100}, and the maximum LA area in ventricular
systole (LA\_area s) was determined by planimetry along the endocardial borders of the LA, excluding the pulmonary veins.

Measurement reliability was determined for continuous echocardiographic variables. Previously recorded echocardiograms from six cats (3 from each study group) were randomly selected to undergo repeated analyses three times by one observer (K.A.B.) to determine intraobserver measurement variability. The same studies were analyzed once by a second independent observer (K.E.S) to determine interobserver measurement variability. Both investigators were blinded to the results of the prior echocardiographic analyses.

**Assessment** - Dynamic LVOT obstruction was assessed using 1) loudness of the systolic murmur (Grades I-VI/VI), 2) presence of SAM or mid-LV obstruction from 2DE and color Doppler images, 3) severity of MR (+, ++, +++),\(^65\) and 4) LVOT \(V_{\text{max}}\), which included peak velocity of mid-LV obstruction if SAM was not present. LV systolic function was assessed using SF, \(S_a\), and PEP:ET. LV diastolic function was assessed using IVRT, E:A, \(E_a\), and E:E\(_a\).\(^67\) However, owing to the rapid feline heart rate during the baseline echocardiographic studies, TMF and mitral annular TDI early- and late-diastolic waveforms were fused in the majority of studies precluding analysis of LV diastolic function.

**Blood Pressure Measurement** – Cats were gently restrained in right lateral recumbency in a quiet room. Non-invasive SBP measurements were obtained by the same registered veterinary technician who randomly selected the order of auscultation following either stress or amyl nitrite inhalation using a Doppler ultrasonic flow detector.\(^1\) The left or right forelimb was randomly selected, with the same limb used again for the post-pill measurement. Blood pressure cuff width was chosen to be approximately 40% of the circumference of the limb at the placement site. Average SBP for each cat was calculated as the mean of 3 to 7 consecutively obtained, consistent (<20% variability) values.\(^68\)
**Statistical Analysis** – Statistical analyses were performed with commercially available software. All data were graphed and visually inspected and tested for normality (Kolmogorov-Smirnov test) and equal variance (F-test), and transformed if necessary to normal distribution using logarithmic transformation. Descriptive statistics were calculated for all clinical and echocardiographic variables; these are presented as mean ± standard deviation (SD) except for data that were not normally distributed, in which case median and range (minimum to maximum) are presented. The main statistical procedures included a paired t-test to assess the effect of treatment on variables within each treatment group and analysis of covariance (ANCOVA) to assess differences in response pattern (change scores) between treatment groups, with post-treatment measurement serving as the outcome variable and both treatment group (atenolol and ivabradine) baseline measurements serving as covariates. Differences in baseline measurements between treatment groups were also compared using an unpaired t-test if normally distributed. Data that was not normally distributed was analyzed using a Mann-Whitney rank sum test. Categorical and frequency data were compared using Fisher’s exact test and McNemar’s test. Statistical significance was determined at alpha=0.05. In addition to the previously described analyses, observer variability was calculated using the formula: Coefficient of variation (CV) = mean difference between measurements/average of measurements x 100 and expressed in percent.69
CHAPTER 3

RESULTS

No major adverse effects were seen during the study. Four cats experienced ptyalism during inhalation of amyl nitrite, which rapidly resolved in all four cats following withdrawal of the ampule. No tachy- or bradyarrhythmias were noted in any of the cats during auscultation or echocardiography.

All cats were subjected to auscultation at rest and under stress, had full baseline and post-treatment echocardiographic studies, and had baseline and post-treatment Doppler SBP measured. Inhalation of amyl nitrite could not be performed in one cat due to a temporary lack of drug availability.

Twenty-one cats, nineteen of which were of mixed breeds, were enrolled in the study. One Maine Coon cat was enrolled in the ivabradine group, while one Persian cat was enrolled in the atenolol group. Demographic data and results of physical examination under all three auscultation conditions both before and after treatment are summarized in Table 1. The majority of cats enrolled were male, with only two females represented in the atenolol group. In the cats receiving ivabradine, there was no difference in baseline heart murmur grade at rest and after up and down lifting ($P=0.50$), nor was there a difference between resting murmur grade and that after inhalation of amyl nitrite ($P=0.75$). Similarly, in the cats receiving atenolol, there was no difference in baseline heart murmur grade at rest and after up and down lifting ($P=0.98$), nor was there a difference
between resting murmur grade and that after inhalation of amyl nitrite \((P=0.50)\). A gallop sound was diagnosed in two cats at rest, three cats after stress, and three cats after inhalation of amyl nitrite in the ivabradine group and in none of the cats receiving atenolol. Baseline and post-treatment categorical echocardiographic findings are summarized in Table 2. Eighteen of 21 cats had evidence of SAM during the baseline echocardiogram, while 2 cats from the ivabradine group and 1 cat from the atenolol group had evidence of dynamic mid-LV obstruction without SAM. All cats with SAM during the baseline echocardiogram had color-flow Doppler evidence of MR, while none of the cats with only mid-LV obstruction did. MR was directly posteriorly in all cats. The majority of cats in both groups had symmetric LV hypertrophy. There was no difference in categorical severity of LV hypertrophy between treatment groups \((P=0.062)\). Owing to rapid heart rate, TMF \((n=13)\) and mitral diastolic TDI waves \((n=12)\) were fused in the majority of cats at baseline, precluding further comparative analyses.

**Baseline Variables**

A comparison of mean physical examination findings, echocardiographic measurements, and blood pressure measurements at baseline between each treatment group is shown in Table 3. **Demographic data:** There were no significant differences in age or body weight between treatment groups. **Heart rate:** There were no significant differences in HR between treatment groups under any of the three auscultation conditions: at rest, after being lifted up and down 5-8 times, and after inhalation of amyl nitrite. **Peak blood flow velocity:** There were no significant differences in LVOT \(V_{\text{max}}\), including peak velocity of mid-LV obstruction if SAM was not present, or Ao \(V_{\text{max}}\) both at rest and during noise stress between treatment groups. **Indices of LV systolic function:** There were no significant differences in LV SF or peak \(S_a\) between
treatment groups. PEP was significantly longer in the ivabradine group ($P=0.023$). There was no
difference in ET ($P=0.518$) between treatment groups. There was a significant difference in
PEP:ET ($P=0.049$), with the ivabradine group being higher than the atenolol group.

*Indices of LV diastolic function:* There was no significant difference for IVRT between treatment
groups ($P=0.679$). For those cats with fused early and late diastolic TMF waveforms, there was no
difference of peak inflow velocity between treatment groups ($P=0.446$).

*2-D measurements and calculations:* There were no significant differences between treatment
groups for LVPW and IVS thickness in both long- and short-axis imaging planes, distance from the
mitral valve coaptation point to the IVS in both systole and diastole, LAD, LA area s, and short-axis
LVIDs and LVIDd.

*Doppler systolic blood pressure:* There was a significant difference between treatment groups for
Doppler SBP measurement, with baseline SBP being higher in the atenolol group (mean difference
± SD, 14 ± 8 mmHg, $P=0.001$).

**Effects of Treatment on Variables**

*Treatment effects on heart murmur grade:* Both ivabradine and atenolol significantly lowered heart
murmur grade at rest (ivabradine $P=0.004$; atenolol $P=0.016$), after up and down lifting (ivabradine
$P=0.031$; atenolol $P=0.016$), and after inhalation of amyl nitrite (ivabradine $P=0.016$; atenolol
$P=0.002$; **Table 1**). When comparing the change scores of heart murmur grade at rest between
treatment groups, atenolol lowered it significantly more compared to ivabradine ($P=0.0036$; **Table
10**; **Figure 1**).

*Treatment effects on qualitative echocardiographic variables:* Neither ivabradine ($P=1.00$) or
atenolol ($P=0.56$) had an effect on the presence of SAM, nor did either drug have an effect on the
presence of mid-LV obstruction (ivabradine $P=1.00$; atenolol $P=1.00$; **Table 2**). Neither drug had an
effect on the presence of MR (ivabradine $P=1.00$; atenolol $P=0.266$) or the severity of MR
Both ivabradine ($P=0.002$) and atenolol ($P=0.021$) significantly decreased the presence of fusion of TMF E and A waves, while both drugs significantly increased the presence of distinct $E_a$ waves ($ivabradine P=0.016$; atenolol $P=0.005$; Table 2).

Treatment effects on heart rate: Ivabradine significantly lowered HR at rest, and HR response to up and down lifting and inhalation of amyl nitrite. Similarly, atenolol significantly lowered HR under all three conditions (Table 4; Figures 2 to 4). When comparing treatment-induced change scores of resting HR between treatment groups, there was no significant difference between the two treatments (Table 10; Figure 5).

Treatment effects on peak blood flow velocity: Both ivabradine and atenolol significantly lowered LVOT $V_{\text{max}}$, including $V_{\text{max}}$ associated with mid-LV obstruction in patients without SAM, both without and during noise stress compared to baseline (Table 5; Figures 6 and 7). However, when comparing pre-post change scores of LVOT $V_{\text{max}}$ between treatment groups, atenolol lowered LVOT $V_{\text{max}}$ significantly more ($P<0.0001$) compared to ivabradine both without (Table 10; Figure 8) and during (Table 10; Figure 9) noise stress. Ivabradine did not significantly change Ao $V_{\text{max}}$ both without ($P=0.246$) and during ($P=0.272$) noise stress. Atenolol significantly lowered Ao $V_{\text{max}}$ both without ($P=0.015$) and during ($P=0.015$) noise stress (Table 5; Figures 10 and 11).

Treatment effects on indices of LV systolic function: Ivabradine did not significantly change LV SF, while atenolol significantly decreased it ($P=0.029$; Table 6; Figure 12). The change score between groups was significantly different ($P=0.0142$; Table 10; Figure 13). Ivabradine did not change $S_a$ compared to baseline, while atenolol significantly lowered it ($P=0.007$; Table 6; Figure 14). Ivabradine had no effect on PEP ($P=0.281$), while atenolol significantly prolonged it ($P=0.001$; Table 6; Figure 15). The change score for PEP was significantly different between treatments, with atenolol significantly increasing PEP compared to ivabradine ($P=0.0022$; Table 10; Figure 15).
Both ivabradine \((P<0.001)\) and atenolol \((P=0.048)\) significantly increased ET \((\text{Table 6, Figure 17})\). The change score was no different between treatment groups for ET \((P=0.159; \text{Table 10}; \text{Figure 18})\). Ivabradine significantly decreased LV PEP:ET compared to baseline \((P=0.010)\), while atenolol significantly increased it \((P=0.014; \text{Table 6}; \text{Figure 19})\), with a significant difference \((P=0.0048; \text{Table 10}; \text{Figure 20})\) between treatments.

**Treatment effects on indices of LV diastolic function:** Both drugs led to unfusion of summed TMF E and A waves and TDI E\(_a\) and A\(_a\) waves, however meaningful comparisons of their effects on LV diastolic function could not be made due to summation in the majority of cats at baseline. Ivabradine did not significantly change LV IVRT compared to baseline, while atenolol significantly prolonged it \((\text{Table 7}; \text{Figure 21})\). For those cats with separation of mitral annular TDI waveforms at baseline, ivabradine had no effect on E\(_a\) \((\text{Table 7}; \text{Figure 22})\). Ivabradine also had no effect on E:E\(_a\) \((P=0.464; \text{Table 7}; \text{Figure 23})\).

**Treatment effects on 2-D measurements:** Both ivabradine and atenolol did not significantly change LVPWd or IVSd wall thickness compared to baseline in both long-axis \((\text{Table 8}; \text{Figures 24 and 25})\) and short-axis \((\text{Table 8}; \text{Figures 26 and 27})\) imaging planes. Both ivabradine and atenolol did not significantly change the distance from the mitral valve coaptation point to the IVS compared to baseline in both systole \((\text{Table 8}; \text{Figure 28})\) and diastole \((\text{Table 8}; \text{Figure 30})\). When comparing the change scores between treatment groups, there was no difference for the distance of the mitral valve coaptation point to the IVS in both systole \((P=0.637; \text{Table 10}; \text{Figure 29})\) and diastole \((P=0.241; \text{Table 10}; \text{Figure 31})\). Both ivabradine \((P=0.142)\) and atenolol \((P=0.214)\) did not change LAD compared to baseline \((\text{Table 8}; \text{Figure 32})\). However, when comparing the change scores between treatment groups, significant differences were found \((P=0.037; \text{Table 10}; \text{Figure 33})\). Both ivabradine \((P=0.371)\) and atenolol \((P=0.111)\) did not change LA\(_{area}\) s compared to baseline \((\text{Table 8}; \text{Figure 34})\), with no difference of change scores between
treatments observed ($P=0.120$; Table 10; Figure 35). Both ivabradine ($P=0.036$) and atenolol ($P=0.045$) significantly increased the short-axis LVIDs compared to baseline (Table 8; Figure 36). When comparing the change scores, there was no difference between treatment groups for LVIDs ($P=0.205$; Table 10; Figure 37). Ivabradine significantly increased the short-axis LVIDd compared to baseline ($P=0.026$), while atenolol had no effect on LVIDd ($P=0.417$; Table 8; Figure 38).

Treatment effects on Doppler systolic blood pressure: Both ivabradine ($P=0.160$) and atenolol ($P=0.135$) did not affect the mean Doppler SBP measurement (Table 9; Figure 39), with no difference between change scores ($P=0.394$; Table 10; Figure 40).

Repeatability Studies

Results of the reproducibility studies are presented in Table 11. For all variables, the CV for inter- and intraobserver measurement variability was less than 10%.
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Table 1: Demographic data and results of physical examination in 21 cats with hypertrophic obstructive cardiomyopathy at baseline (Pre) and three hours after (Post) oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). Age and body weight presented as mean +/- SD. 1, at rest; 2, after up and down lifting; 3, after inhalation of amyl nitrite.
<table>
<thead>
<tr>
<th></th>
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<td>6/7 sym</td>
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<tr>
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<td>4/14 asym</td>
<td>1/7 asym</td>
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<tr>
<td>Pattern of LV hypertrophy</td>
<td>7 mild</td>
<td>-</td>
<td>5 mild</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mod</td>
<td>1 mod</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 sev</td>
<td>1 sev</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral regurgitation (n)</td>
<td>12/14</td>
<td>11/14</td>
<td>6/7</td>
<td>3/7</td>
<td></td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>2 cats 0</td>
<td>3 cats 0</td>
<td>1 cats 0</td>
<td>4 cats 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 cats 1+</td>
<td>3 cats 1+</td>
<td>2 cats 1+</td>
<td>3 cats 1+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 cats 2+</td>
<td>8 cats 2+</td>
<td>4 cats 2+</td>
<td>0 cats 2+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 cats 3+</td>
<td>0 cats 3+</td>
<td>0 cats 3+</td>
<td>0 cats 3+</td>
<td></td>
</tr>
<tr>
<td>Direction of mitral regurgitation</td>
<td>All posteriorly</td>
<td>All posteriorly</td>
<td>All posteriorly</td>
<td>All posteriorly</td>
<td></td>
</tr>
<tr>
<td>Fused transmtral E and A waves (n)</td>
<td>8/14</td>
<td>0/14</td>
<td>5/7</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td>Fused TDI Eₐ and Aₐ waves (n)</td>
<td>6/14</td>
<td>0/14</td>
<td>6/7</td>
<td>0/7</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 – Selected echocardiographic variables in 21 cats with hypertrophic obstructive cardiomyopathy at baseline (Pre) and three hours after (Post) oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). TDI, tissue Doppler imaging; Eₐ, mitral annular tissue Doppler early diastolic motion; Aₐ, mitral annular tissue Doppler late diastolic motion.
Table 3: Comparison of baseline measurements between cats prior to oral administration of either ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). Variables are expressed as mean ± SD unless stated otherwise. Variables expressed as median (min to max) due to lack of normal distribution. Baseline values for each treatment group were compared using an unpaired t-test if normally distributed and a Mann-Whitney rank sum test if not normally distributed. yrs, years; kg, kilograms; bpm, beats per minute; Lax, long-axis; mm, millimeters; Sax, short-axis; MV, mitral valve; IVS, interventricular septum; ms, milliseconds; Sa, systolic velocity of the lateral mitral annulus; s, seconds; PEP:ET, ratio of left ventricular pre-ejection period to ejection time; Vmax, peak velocity; m/s, meters per second; mmHg, millimeters of mercury.

<table>
<thead>
<tr>
<th>Baseline Variable</th>
<th>Ivabradine</th>
<th>Atenolol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>6.0±3.7</td>
<td>6.0±3.1</td>
<td>0.983</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>5.64±1.04</td>
<td>5.54±1.21</td>
<td>0.857</td>
</tr>
<tr>
<td>Heart rate at rest (bpm)</td>
<td>200(164-244)</td>
<td>215±29</td>
<td>0.572</td>
</tr>
<tr>
<td>Heart rate after lifting (bpm)</td>
<td>218±20</td>
<td>226±32</td>
<td>0.627</td>
</tr>
<tr>
<td>Heart rate after amyl nitrite (bpm)</td>
<td>214±23</td>
<td>230±34</td>
<td>0.217</td>
</tr>
<tr>
<td>Left ventricular posterior wall thickness in diastole – Lax (mm)</td>
<td>6.44±1.00</td>
<td>6.28±0.71</td>
<td>0.714</td>
</tr>
<tr>
<td>Interventricular septum wall thickness in diastole – Lax (mm)</td>
<td>7.14±0.86</td>
<td>6.63±0.59</td>
<td>0.156</td>
</tr>
<tr>
<td>Left ventricular posterior wall thickness in diastole – Sax (mm)</td>
<td>6.48±0.85</td>
<td>6.21±1.07</td>
<td>0.546</td>
</tr>
<tr>
<td>Interventricular septum wall thickness in diastole – Sax (mm)</td>
<td>6.88±0.95</td>
<td>6.40±0.77</td>
<td>0.264</td>
</tr>
<tr>
<td>Distance from anterior MV leaflet to IVS in systole (mm)</td>
<td>3.51±0.62</td>
<td>3.93±0.83</td>
<td>0.205</td>
</tr>
<tr>
<td>Distance from anterior MV leaflet to IVS in diastole (mm)</td>
<td>7.39±1.38</td>
<td>7.63±1.59</td>
<td>0.722</td>
</tr>
<tr>
<td>Left atrial area at end-systole (cm²)</td>
<td>2.43±0.50</td>
<td>2.54±0.88</td>
<td>0.709</td>
</tr>
<tr>
<td>Left atrial diameter at end-systole (mm)</td>
<td>15.7±1.43</td>
<td>16.0±3.50</td>
<td>0.792</td>
</tr>
<tr>
<td>Left ventricular end-systolic diameter (mm)</td>
<td>4.49±1.00</td>
<td>4.70±1.26</td>
<td>0.687</td>
</tr>
<tr>
<td>Left ventricular end-diastolic diameter (mm)</td>
<td>13.64±2.67</td>
<td>15.52±1.60</td>
<td>0.104</td>
</tr>
<tr>
<td>Length of anterior mitral valve leaflet (mm)</td>
<td>12.19±0.90</td>
<td>11.43±1.49</td>
<td>0.158</td>
</tr>
<tr>
<td>Shortening fraction (%)</td>
<td>70(53-77)</td>
<td>70±8</td>
<td>0.332</td>
</tr>
<tr>
<td>Isovolumic relaxation time (ms)</td>
<td>50±7</td>
<td>49±5</td>
<td>0.679</td>
</tr>
<tr>
<td>Fused diastolic transmitral waveforms (m/s)</td>
<td>1.15±0.17</td>
<td>1.25±0.29</td>
<td>0.446</td>
</tr>
<tr>
<td>Sa (cm/s)</td>
<td>7.63±2.35</td>
<td>8.87±1.83</td>
<td>0.237</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>34.82±4.23</td>
<td>30.10±3.91</td>
<td>0.023</td>
</tr>
<tr>
<td>ET (ms)</td>
<td>133.52±15.38</td>
<td>129.34±8.97</td>
<td>0.518</td>
</tr>
<tr>
<td>PEP:ET</td>
<td>0.26±0.04</td>
<td>0.23±0.02</td>
<td>0.049</td>
</tr>
<tr>
<td>Aortic Vmax (m/s)</td>
<td>1.16±0.27</td>
<td>1.22±1.17</td>
<td>0.575</td>
</tr>
<tr>
<td>Left ventricular outflow tract Vmax including mid-LV obstruction (m/s)</td>
<td>4.30±0.98</td>
<td>4.29±1.14</td>
<td>0.980</td>
</tr>
<tr>
<td>Aortic Vmax during noise (m/s)</td>
<td>1.18±0.26</td>
<td>1.26±0.17</td>
<td>0.467</td>
</tr>
<tr>
<td>Left ventricular outflow tract Vmax during noise (m/s)</td>
<td>4.39±0.96</td>
<td>4.28±1.23</td>
<td>0.820</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110±8</td>
<td>124±9</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 4: Mean +/- SD heart rate (min^-1) in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). * indicates median (min to max) used as data not normally distributed.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-Pill</th>
<th>P</th>
<th>Baseline</th>
<th>Post-Pill</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>200(164-244) *</td>
<td>146±19</td>
<td>&lt;0.001</td>
<td>215±29</td>
<td>158±26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stress</td>
<td>218±20</td>
<td>161±16</td>
<td>&lt;0.001</td>
<td>226±32</td>
<td>162±21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amyl Nitrite</td>
<td>214±23</td>
<td>155±12</td>
<td>&lt;0.001</td>
<td>230±34</td>
<td>160±22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 5: Mean +/- SD maximum blood flow velocity in the left ventricular outflow tract, including mid-LV obstruction in patients without SAM, as measured by continuous wave Doppler, and aorta as measured by pulsed wave Doppler, with and without noise in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). LVOT, left ventricular outflow tract; Ao, aorta. See Table 3 for remainder of key.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-Pill</th>
<th>P</th>
<th>Baseline</th>
<th>Post-Pill</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVOT V_{max} (m/s)</td>
<td>4.30±0.98</td>
<td>3.87±1.23</td>
<td>0.022</td>
<td>4.29±1.14</td>
<td>1.69±0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVOT V_{max} Noise (m/s)</td>
<td>4.39±0.96</td>
<td>3.99±1.42</td>
<td>0.03</td>
<td>4.28±1.23</td>
<td>1.72±0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ao V_{max} (m/s)</td>
<td>1.16±0.27</td>
<td>1.13±0.29</td>
<td>0.272</td>
<td>1.22±1.17</td>
<td>0.98±0.14</td>
<td>0.015</td>
</tr>
<tr>
<td>Ao V_{max} Noise (m/s)</td>
<td>1.18±0.26</td>
<td>1.14±0.28</td>
<td>0.246</td>
<td>1.26±0.17</td>
<td>0.97±0.13</td>
<td>0.015</td>
</tr>
</tbody>
</table>
### Table 6: Echocardiographic indices of left ventricular systolic function in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). Values are listed as mean +/-SD unless indicated otherwise. * indicates median (min to max) used as data not normally distributed. SF, left ventricular shortening fraction. See Table 3 for remainder of key.

<table>
<thead>
<tr>
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<th>Ivabradine</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-Pill</td>
<td>P</td>
<td>Baseline</td>
</tr>
<tr>
<td>SF (%)</td>
<td>70(53-77)†</td>
<td>68±8</td>
<td>0.643</td>
<td>70±8</td>
</tr>
<tr>
<td>SA (cm/s)</td>
<td>7.63±2.35</td>
<td>7.37±1.63</td>
<td>0.646</td>
<td>8.87±1.83</td>
</tr>
<tr>
<td>PEP (ms)</td>
<td>34.82±4.23</td>
<td>35.94±3.83</td>
<td>0.281</td>
<td>30.10±3.91</td>
</tr>
<tr>
<td>ET (ms)</td>
<td>133.52±15.38</td>
<td>155.46±12.19</td>
<td>&lt;0.001</td>
<td>129.34±8.97</td>
</tr>
<tr>
<td>PEP:ET</td>
<td>0.26±0.04</td>
<td>0.23±0.03</td>
<td>0.010</td>
<td>0.23±0.02</td>
</tr>
</tbody>
</table>

### Table 7: Echocardiographic indices of left ventricular diastolic function in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14 for IVRT, n=8 for E_a, n=6 for E:E_a) or atenolol (2 mg/kg, n=7). Values are listed as mean +/-SD unless indicated otherwise. IVRT, isovolumic relaxation time; E_a, early diastolic velocity of the lateral mitral annulus; E, early diastolic velocity of transmitral flow. See Table 3 for remainder of key.

<table>
<thead>
<tr>
<th></th>
<th>Ivabradine</th>
<th></th>
<th>Atenolol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-Pill</td>
<td>P</td>
<td>Baseline</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>50±7</td>
<td>54±7</td>
<td>0.09</td>
<td>49±5</td>
</tr>
<tr>
<td>E_a (cm/s)</td>
<td>5.06±1.15</td>
<td>5.61±1.61</td>
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</tr>
<tr>
<td>E:E_a</td>
<td>13.12±4.87</td>
<td>11.62±2.77</td>
<td>0.464</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ivabradine</td>
<td>Atenolol</td>
<td></td>
<td>Ivabradine</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-Pill</td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVPWd Lax (mm)</td>
<td>6.44±1.00</td>
<td>6.36±0.93</td>
<td>0.098</td>
<td>6.28±0.71</td>
</tr>
<tr>
<td>IVSd Lax (mm)</td>
<td>7.14±0.86</td>
<td>7.09±0.88</td>
<td>0.187</td>
<td>6.63±0.59</td>
</tr>
<tr>
<td>LVPWd Sax (mm)</td>
<td>6.48±0.85</td>
<td>6.45±0.95</td>
<td>0.583</td>
<td>6.21±1.07</td>
</tr>
<tr>
<td>IVSd Sax (mm)</td>
<td>6.88±0.95</td>
<td>6.84±0.83</td>
<td>0.416</td>
<td>6.40±0.77</td>
</tr>
<tr>
<td>IVS-Coapt systole (mm)</td>
<td>3.51±0.62</td>
<td>3.66±0.58</td>
<td>0.061</td>
<td>3.93±0.83</td>
</tr>
<tr>
<td>IVS-Coapt diastole (mm)</td>
<td>7.39±1.38</td>
<td>7.71±1.53</td>
<td>0.241</td>
<td>7.63±1.59</td>
</tr>
<tr>
<td>LAD Lax systole (mm)</td>
<td>15.7±1.43</td>
<td>16.0±1.37</td>
<td>0.142</td>
<td>16.0±3.50</td>
</tr>
<tr>
<td>LAarea Lax systole (cm²)</td>
<td>2.43±0.50</td>
<td>2.53±0.41</td>
<td>0.371</td>
<td>2.54±0.88</td>
</tr>
<tr>
<td>LVIDs Sax (mm)</td>
<td>4.49±1.00</td>
<td>4.96±1.19</td>
<td>0.036</td>
<td>4.70±1.26</td>
</tr>
<tr>
<td>LVIDd Sax (mm)</td>
<td>13.64±2.67</td>
<td>15.46±1.48</td>
<td>0.026</td>
<td>15.52±1.60</td>
</tr>
</tbody>
</table>

Table 8: Mean +/- SD transthoracic echocardiographic values from both long- (Lax) and short-axis (Sax) views in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). LVPWd, left ventricular posterior wall thickness in diastole; IVSd, interventricular septum thickness in diastole; IVS-Coap, distance of the mitral valve coaptation point to the interventricular septum; LAD, left atrial diameter, LAarea, left atrial area; LVIDs, left ventricular internal dimension in systole; LVIDd, left ventricular internal dimension in diastole. See Table 3 for remainder of key.

<table>
<thead>
<tr>
<th></th>
<th>Ivabradine</th>
<th>Atenolol</th>
<th></th>
<th>Ivabradine</th>
<th>Atenolol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-Pill</td>
<td></td>
<td>Baseline</td>
<td>Post-Pill</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110±8</td>
<td>114±12</td>
<td>0.160</td>
<td>124±9</td>
<td>115±14</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Table 9: Mean +/- SD systolic blood pressure (BP) measurement values in 21 cats at baseline and three hours after oral administration of a single dose of ivabradine (0.3 mg/kg, n=14) or atenolol (2 mg/kg, n=7). See Table 3 for key.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Change</th>
<th>95% CI of Change</th>
<th>P (within group)</th>
<th>P (between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate at rest (bpm)</td>
<td>Iva</td>
<td>63</td>
<td>53, 72</td>
<td>&lt; 0.0001</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>55</td>
<td>41, 68</td>
<td>&lt; 0.0001</td>
<td>0.338</td>
</tr>
<tr>
<td>Heart murmur grade</td>
<td>Iva</td>
<td>0.625</td>
<td>0.230, 1.020</td>
<td>0.0037</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>1.75</td>
<td>1.18, 2.32</td>
<td>&lt; 0.0001</td>
<td>0.0036</td>
</tr>
<tr>
<td>Leaflet coaptation point diastole (mm)</td>
<td>Iva</td>
<td>-0.301</td>
<td>-0.794, 0.190</td>
<td>0.215</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>0.188</td>
<td>-0.509, 0.887</td>
<td>0.577</td>
<td>0.241</td>
</tr>
<tr>
<td>Leaflet coaptation point systole (mm)</td>
<td>Iva</td>
<td>-0.141</td>
<td>-0.289, 0.005</td>
<td>0.057</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>-0.201</td>
<td>-0.412, 0.009</td>
<td>0.059</td>
<td>0.637</td>
</tr>
<tr>
<td>Left atrial area systole (cm²)</td>
<td>Iva</td>
<td>-0.080</td>
<td>-0.238, 0.079</td>
<td>0.304</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>0.134</td>
<td>-0.091, 0.359</td>
<td>0.226</td>
<td>0.120</td>
</tr>
<tr>
<td>Left atrial diameter systole (mm)</td>
<td>Iva</td>
<td>-0.257</td>
<td>-0.609, 0.101</td>
<td>0.143</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>0.399</td>
<td>-0.099, 0.399</td>
<td>0.110</td>
<td>0.037</td>
</tr>
<tr>
<td>LVIDs Sax</td>
<td>Iva</td>
<td>0.117</td>
<td>-0.402, 0.635</td>
<td>0.642</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>-1.32</td>
<td>-2.103, 0.539</td>
<td>0.0023</td>
<td>0.205</td>
</tr>
<tr>
<td>SF (%)</td>
<td>Iva</td>
<td>-0.334</td>
<td>-2.720, 2.053</td>
<td>0.772</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aten</td>
<td>5.051</td>
<td>1.663, 8.444</td>
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<tr>
<td>PEP (ms)</td>
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<td>-3.93, 0.58</td>
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<td></td>
<td>Aten</td>
<td>-8.90</td>
<td>-12.24, -5.56</td>
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<td>ET (ms)</td>
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<td>-30, -16</td>
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<td></td>
<td>Aten</td>
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<td>-24, -5</td>
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<td>PEP:ET</td>
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<td>0.006, 0.042</td>
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<td>-0.054, 0.001</td>
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<td>LVOT Vmax including mid-LV obstruction (m/s)</td>
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<td>-0.041, 0.707</td>
<td>0.0773</td>
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<tr>
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<td>Aten</td>
<td>2.597</td>
<td>2.069, 3.126</td>
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<td>LVOT Vmax including mid-LV obstruction during noise (m/s)</td>
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<td>Systolic BP (mmHg)</td>
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<td>-7.7, 16.4</td>
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Table 10: Mean change score (pre minus post) and 95% confidence interval (CI), for selected variables in 21 cats after oral administration of a single dose of ivabradine (Iva; 0.3 mg/kg, n=14) or atenolol (Aten; 2 mg/kg, n=7). See Tables 3, 5, 6, 8, 9 for key.
Table 11: Interobserver and intraobserver measurement variability, expressed as mean standard deviation (SD) and coefficient of variation (CV), of selected 2D and Doppler echocardiographic variables obtained from 6 randomly selected cats. See tables 3 and 7 for key.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Interobserver</th>
<th>Intraobserver</th>
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<tr>
<td></td>
<td>SD (mm)</td>
<td>CV (%)</td>
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<td>Interventricular septum wall thickness in diastole – Lax</td>
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<td>1.80</td>
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<tr>
<td>Left ventricular posterior wall thickness in diastole – Sax</td>
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<td>4.11</td>
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<tr>
<td>Interventricular septum wall thickness in diastole – Sax</td>
<td>0.16</td>
<td>3.33</td>
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<tr>
<td>Distance from anterior MV leaflet to IVS in systole</td>
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<td>9.17</td>
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<tr>
<td>Distance from anterior MV leaflet to IVS in diastole</td>
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<td>8.83</td>
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<td>Left atrial area at end-systole (cm²)</td>
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<tr>
<td>Left atrial diameter at end-systole (mm)</td>
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<td>Left ventricular end-systolic diameter (mm)</td>
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<td>Left ventricular end-diastolic diameter (mm)</td>
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<td>Shortening fraction (%)</td>
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<td>Isovolumic relaxation time (ms)</td>
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<tr>
<td>Ea (cm/s)</td>
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<td>Sa (cm/s)</td>
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<tr>
<td>Aortic Vmax (m/s)</td>
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<tr>
<td>Left ventricular outflow tract Vmax including mid-LV obstruction (m/s)</td>
<td>0.12</td>
<td>3.67</td>
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**FIGURES**

Figure 1: Mean change of heart murmur grade (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 2: Resting heart rate (HR) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 3: Heart rate (HR) after being lifted up and down five to eight times before (Pre) and after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 4: Heart rate (HR) after inhalation of amyl nitrite before (Pre) and after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 5: Mean change in resting heart rate (HR; baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy receiving either Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 6: Peak velocity in the left ventricular outflow tract (LVOT $V_{max}$), including mid-LV obstruction in patients without SAM, before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 7: Peak velocity in the left ventricular outflow tract (LVOT $V_{\text{max}}$), including mid-LV obstruction in patients without SAM, during noise stress before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 8: Mean change of peak velocity in the left ventricular outflow tract, including peak velocity of mid-LV obstruction if SAM not present, (LVOT $V_{\text{max}}$; baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).
Figure 9: Mean change of peak velocity in the left ventricular outflow tract, including peak velocity of mid-LV obstruction if SAM not present, (LVOT $V_{\text{max}}$) during noise stress (baseline value minus 3-hour post-pill value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 10: Peak velocity at the level of the aortic valve (Ao $V_{\text{max}}$) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 11: Peak velocity at the level of the aortic valve (Ao $V_{\text{max}}$) during noise stress before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 12: Left ventricular shortening fraction (SF) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 13: Mean change of left ventricular shortening fraction (SF; baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 14: Peak velocity of lateral mitral annular systolic motion ($S_a$ Lat) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 15: Left ventricular pre-ejection period (PEP) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 16: Mean change of left ventricular pre-ejection period (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (mg/kg PO; n=7).
Figure 17: Left ventricular ejection time (ET) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 18: Mean change of left ventricular ejection time (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).
Figure 19: Ratio of left ventricular pre-ejection period to ejection time (PEP:ET) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 20: Mean change of the ratio of left ventricular pre-ejection period to ejection time (PEP:ET) (baseline value minus 3-hour post-pill value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).
Figure 21: Left ventricular isovolumic relaxation time (IVRT) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 22: Early diastolic velocity of the lateral mitral annulus (Ea_Lat) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg) in cats with hypertrophic cardiomyopathy.
Figure 23: Ratio of the early diastolic velocity of transmitral flow to the early diastolic velocity of the lateral mitral annulus (E/Eₐ Lat) before (Pre) and after (Post) oral administration of Ivabradine (0.3 mg/kg) in cats with hypertrophic cardiomyopathy.

Figure 24: Long-axis left ventricular posterior wall thickness (LVPWd Lax) in diastole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 25: Long-axis interventricular septal wall thickness (IVSd Lax) in diastole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 26: Short-axis left ventricular wall thickness in diastole (LVPWd Sax) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 27: Short-axis interventricular septal wall thickness in diastole (IVSd Sax) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 28: Distance from the mitral valve coaptation point to the interventricular septum during systole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 29: Mean change of the distance from the mitral valve coaptation point to the interventricular septum during systole (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 30: Distance from the mitral valve coaptation point to the interventricular septum during diastole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 31: Mean change of the distance from the mitral valve coaptation point to the interventricular septum during diastole (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 32: Left atrial diameter (LAD Lax) at end-systole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 33: Mean change of left atrial diameter (LAD Lax) at end-systole (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 34: Left atrial area (LA area Lax) at end-systole before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 35: Mean change of left atrial area ($\text{LA}_{\text{area}}$) at end-systole (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 36: Short-axis systolic left ventricular internal dimension (LVIDs Sax) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 37: Mean change of systolic left ventricular internal dimension (LVIDs Sax) (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).

Figure 38: Short-axis diastolic left ventricular internal dimension (LVIDd Sax) before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.
Figure 39: Indirect Doppler systolic blood pressure before (Pre) and 3 hours after (Post) oral administration of Ivabradine (0.3 mg/kg; left panel) and Atenolol (2 mg/kg; right panel) in 21 cats with hypertrophic cardiomyopathy.

Figure 40: Mean change of Doppler systolic blood pressure (baseline value minus 3-hour post-drug value) with 95% confidence intervals in 21 cats with hypertrophic cardiomyopathy after Ivabradine (0.3 mg/kg PO; n=14) or Atenolol (2 mg/kg PO; n=7).
CHAPTER 4

DISCUSSION

The results of this study indicate that a single dose of oral ivabradine has a statistically significant, but clinically negligible, effect on the degree of dynamic LVOT obstruction in cats with preclinical HCM, in contrast to the active control atenolol, which consistently reduces, and in some cases relieves, outflow obstruction. Using a $P$ value of 0.01, rather than the arbitrarily chosen value of 0.05, would have more clearly demonstrated the statistical difference between the two drugs, as only atenolol would have demonstrated a significant reduction in LVOT $V_{\text{max}}$, though the difference in change scores indisputably establishes the clinical superiority of atenolol at reducing obstruction. Furthermore, while the desired effect of markedly reducing obstruction by ivabradine was not achieved in this study, there was no evidence that ivabradine aggravated obstruction in any of the cats. Additional findings support the conclusions of other recent studies examining the use of ivabradine in cats, notably that ivabradine predictably lowers HR, while having minimal effects on LV function and SBP.\textsuperscript{b,52,56} Lastly, while neither treatment had a significant effect on LA size, there was a counterdirectional change between the two treatments that reached significance, with a decrease in LAD following atenolol administration compared to ivabradine administration.
Currently, the most commonly used medications to relieve dynamic LV outflow obstruction in cats include beta-adrenergic receptor blockers such as atenolol, which was used as the active control in this study. Ivabradine is similar to beta-blockers in that they both are negative chronotropic agents that decrease HR response to sympathetic stimulation, resulting in an increase in diastolic filling time and, thus, decreased hydrodynamic force on the mitral valve. As SAM seemingly results from altered hydrodynamic forces on the mitral valve leaflets secondary to abnormal geometry of the valve apparatus and the LVOT,\textsuperscript{15,17,19-21} it is reasonable to speculate that ivabradine might be able to reduce obstruction as a result of its negative chronotropically-mediated decrease in such forces. Moreover, additional mechanisms have been proposed to explain why ivabradine might have the potential to reduce obstruction in patients with HCM. The increase in diastolic filling time achieved with ivabradine results in better LV filling, thus increasing the distance between the AMVL and the LVOT at the beginning of systole. Furthermore, the negative chronotropic effect of ivabradine should result in a negative Bowditch effect,\textsuperscript{70} in which myocardial contractility decreases with decreasing HR, presumably due to a decrease in cytosolic calcium. Indeed, in a group of anesthetized cats with HCM, direct measurement of the rate of LV pressure increase (+dP/dt\textsubscript{max}) showed ivabradine to have mild negative inotropic effects, even though echocardiographic indices of LV systolic function were unchanged or mildly improved by ivabradine.\textsuperscript{52}

However, as ivabradine lacks potent negative inotropic effects, the failure of ivabradine to reduce obstruction in this study suggests that a substantial negative inotropic effect is required to alter the mitral valve-outflow tract spatial relationship sufficiently to prevent the formation of SAM. In contrast to ivabradine, both beta-blockers and calcium channel blockers exert a pronounced negative inotropic effect in addition to their negative chronotropic effect, and it is this additional property that has been thought to explain their effectiveness at reducing obstruction. In human
patients with hypertrophic cardiomyopathy with obstruction, negative inotropic agents have been shown echocardiographically to decrease LV ejection acceleration, which in turn is thought to decrease the hydrodynamic force on the protruding mitral valve leaflet in early systole, thereby reducing or eliminating mitral-septal contact and obstruction, and lowering the pressure gradient across the LVOT.\textsuperscript{71} A proposed explanation of pressure gradient development in patients with SAM states that an amplifying feedback loop is generated, as early mitral-septal contact induced by rapid LV ejection acceleration creates a narrowed orifice, resulting in a pressure difference, which further forces the leaflet against the septum, decreasing the orifice size and further increasing the pressure difference.\textsuperscript{71} A working hypothesis explaining how a decrease in ejection acceleration may lead to relief of obstruction revolves around the principles of fluid mechanics, which state that the force of flow is directly related to the square of velocity.\textsuperscript{72} Negative inotropic agents, by decreasing the ejection acceleration, decrease the force on the mitral valve leaflets in early systole, which delays the development of SAM. Mitral-septal contact occurs later during the systolic ejection period, leaving less time for the aforementioned feedback loop to narrow the orifice, reducing the final pressure difference.\textsuperscript{71} Interestingly, this is achieved without a change in the peak LV ejection velocity or the distance between the mitral coaptation point and the IVS,\textsuperscript{71} a finding achieved in the present study as well. Additionally, delaying the trigger to SAM may allow more time for papillary muscle shortening to increase chordal tension, possibly completely preventing SAM from developing.\textsuperscript{21,73} Therefore, the lack of a pronounced negative inotropic effect of ivabradine should explain why it unlikely to successfully relieve SAM, as HR reduction alone does not decrease LV ejection acceleration and, thus, is unable to decrease the force on the mitral valve leaflets that promote SAM. The minor reduction of LVOT velocity achieved in this study following ivabradine administration is likely due to the increased distance between the AMVL and the LVOT
at the beginning of systole secondary to prolongation of diastolic filling as a result of ivabradine’s pronounced reduction of HR, with a mild contribution from the negative Bowditch effect.

In order to evaluate the effect of ivabradine under stress, three provocative maneuvers intended to induce dynamic obstruction (inhalation of amyl nitrite, lifting cat up and down, and noise stress) were performed. Amyl nitrite is a peripheral and coronary venous and arterial vasodilator that also acts to decrease venous return, resulting in afterload reduction, reflex tachycardia, and a secondary positive inotropic response. The combination of these effects leads to a small but consistent increase in Doppler LVOT V\text{max}, which is why amyl nitrite has been routinely used in human medicine to identify HCM patients with latent LVOT obstruction. Amyl nitrite can be conveniently administered and has a rapid onset and offset of action following inhalation due to rapid absorption through the pulmonary alveoli and rapid metabolism, probably by hydrolytic denitration. Drug effect should be auscultable within 30 seconds to one minute and should last no more than 3 to 5 minutes. In humans, side effects of amyl nitrite inhalation are usually mild and transient, and may include dizziness, nausea, vomiting, restlessness, or fainting. There is no known drug interaction between amyl nitrite and atenolol or ivabradine.

To the author’s knowledge, there are no studies evaluating the use of amyl nitrite in cats. The present study failed to show any effect of amyl nitrite on heart rate or murmur intensity regardless of which drug was administered. A likely explanation for this is the fact that virtually all cats turned their head or backed away from the amyl nitrite capsules, as the inhalant form has an unpleasant odor, and possibly did not inhale a high enough concentration to experience relevant hemodynamic effects. In humans, in addition to inhalation of amyl nitrite, provocative maneuvers such as treadmill exercise, the Valsalva maneuver, and dobutamine infusion are commonly used to induce latent obstruction or to assess the range of dynamic outflow tract gradients in patients with HCM. These provocative maneuvers, or stressors, aggravate obstruction via activation of the sympathetic
nervous system, with an associated increase in the inotropic state. Moreover, human patients that have their provoked gradients reduced or abolished by medications most often exhibit relief or at least a reduction in their symptoms, thus proposing another argument supporting the relief of obstruction in these patients.\textsuperscript{31,74,75}

Lifting of cats up and down and introduction of noise seem to be attractive provocations because they reproduce the symptomatic state of a fight-or-flight situation. In this study, HR was significantly reduced by both ivabradine and atenolol at rest and in response to both provocative maneuvers, presumably due to ivabradine’s ability to inhibit the I\textsubscript{f} current and atenolol’s beta-blocking effect. However, whereas both drugs had a similar effect on HR reduction, only atenolol significantly reduced murmur intensity under both stress conditions. This difference can likely be attributed to the significant negative inotropic effect achieved by atenolol in this study, which, as previously described, is a crucial property that decreases LV ejection acceleration, and, in turn, reduces dynamic LVOT obstruction. The lack of a pronounced negative inotropic effect observed by ivabradine in this study suggests that ivabradine should have no, or only a minor, effect on LV ejection acceleration, and thus, should be unable to alter the forces that promote the development of dynamic obstruction.

Importantly, this study showed no evidence that a single dose of ivabradine worsens the severity of obstruction, indicating that ivabradine may potentially be safe for use in cats with the obstructive form of HCM. Results from a previous study in our lab showed a significant increase in LV SF in healthy cats given ivabradine,\textsuperscript{56} suggesting that improvement of LV systolic function produced by ivabradine may further deteriorate dynamic outflow obstruction. However, the latter study also demonstrated that ivabradine results in a significant increase of LV volumes, which may, at least in part, offset the effects of increased systolic function with regard to favoring the development of obstruction. While the present study showed no significant effect of ivabradine on
LV SF or $S_a$ in cats with HCM, it did demonstrate that ivabradine significantly reduced LV PEP:ET, suggesting a possible improvement of LV systolic function, which theoretically could worsen the severity of dynamic outflow obstruction. However, similar to the previous study in healthy cats in which ivabradine increased LV volumes, this study demonstrated a significant increase in both LV systolic and diastolic dimensions following the administration of ivabradine, which may prevent the worsening of an already present obstruction. Moreover, the reduction in LV PEP:ET achieved in this study was due solely to ivabradine’s effect on LV ET, an expected consequence of a reduction in heart rate, and is not indicative of an effect on LV systolic function. Furthermore, as previously referenced, another study in our lab demonstrated that, in a group of anesthetized cats with HCM, ivabradine has mild negative inotropic effects as determined by direct measurement of the rate of LV pressure increase ($+dP/dt_{max}$).\textsuperscript{52} In this study, echocardiographic indices of systolic function, including both LV SF and ejection fraction, were unchanged or even mildly increased by ivabradine, suggesting that echocardiographic variables of LV systolic function may not necessarily be good indicators of LV contractility.\textsuperscript{1,76} The very mild negative inotropic effect of ivabradine likely prevents aggravation of obstruction, and potentially mildly reduces it, as was seen in the present study.

While the results of this study suggest that ivabradine is unlikely to be a first line treatment for the reduction of dynamic outflow obstruction, it may still have a role in the treatment of cats with HCM, as its effect on HR reduction is thought to be beneficial and it may be safer to use than beta blockers in certain situations, such as in cats with impaired LA function. Furthermore, the effects of a single dose of ivabradine may not be representative of its long-term effects, with further study needed to evaluate ivabradine’s ability to reduce dynamic LVOT obstruction over a longer period of time.
Heart rate has been suggested as a negative prognostic indicator in cats with HCM in one study. Tachycardia is a major contributor to increased myocardial oxygen consumption and may lead to increased LV systolic function and worsening LV outflow obstruction, reduced coronary perfusion, myocardial ischemia, delayed myocardial relaxation, reduced LV filling, and finally deterioration of LV diastolic function. Intolerance to episodes of tachycardia induced by stress is thought to be an important triggering event leading to acute decompensation of previously stable cats with HCM. Therefore, control of HR and prevention of tachycardic spells seem to be important goals of patient management. While beta-blockers do reduce HR and lead to reduced myocardial oxygen consumption, they have also been shown to possibly cause coronary arterial vasoconstriction at rest and during exercise when given at high doses or intravenously, potentially exacerbating myocardial ischemia. Ivabradine, on the other hand, has been shown to effectively reduce HR without causing coronary vasoconstriction in dogs, humans, and rats, thus potentially making it a more attractive alternative to beta-blockers, especially if comparable effects can be demonstrated in cats. In the present study, all cats receiving ivabradine experienced a significant reduction in HR at rest, and in response to both provocative maneuvers, suggesting that ivabradine may effectively prevent tachycardic episodes that can potentially serve as a trigger for acute decompensation of previously stable disease.

Arterial thromboembolism (ATE) due to thrombus formation in the LA is a common and potentially devastating outcome in cats with HCM. Proposed pathogenic factors that favor clot formation include stagnation of blood flow, endothelial damage, and activation of platelets and coagulation factors associated with LA dilation and poor contractile function. Furthermore, the development of spontaneous echocardiographic contrast (SEC) within the LA and/or left atrial appendage (LAA) appears to be a marker of a prothrombotic state and a risk factor for the development of ATE. This pattern, characterized by visible echogenic swirling of blood
components, is often observed in cats with advanced cardiomyopathy. Schober et al\textsuperscript{6} demonstrated that decreased LAA flow velocity, a marker of impaired LAA function, as determined by transthoracic echocardiography in cats with cardiomyopathy, could reliably predict the presence of SEC, and thus, an increased risk for the development of ATE. Drugs that decrease LA function could potentially predispose a cardiomyopathic cat to the development of thromboembolic complications. A previous study in healthy cats showed that ivabradine results in minimal effects on LA and LAA function, while atenolol, in contrast, results in a reduction in LA and LAA function,\textsuperscript{j} indicating that ivabradine may be a safer negative chronotropic agent compared to atenolol in cats with HCM, particularly in the face of LA dilation and dysfunction.

Changes in LV diastolic function were difficult to assess in this study due to the rapid baseline HR, which resulted in fused early- and late-diastolic waveforms of TMF and mitral annular TDI velocities in the majority of studies. The only index of diastolic function available for comparison was IVRT, which was mildly, but not significantly, prolonged by ivabradine, and was significantly prolonged by atenolol. Given the similar reduction in HR produced by both treatments, change in HR alone cannot explain the significant prolongation in IVRT by atenolol when compared to ivabradine, suggesting that, while beta-blockade predictably results in worsening of LV relaxation, ivabradine has no such negative effect. Indeed, ivabradine was shown to have a minimal effect on IVRT in both control cats and cats with HCM in a previous study, though in that study other estimates of ventricular relaxation suggested either improved ($E_a$) or impaired ($\tau$) diastolic function following ivabradine administration.\textsuperscript{52} In the present study, those cats with separation of early and late diastolic waveforms at baseline showed no change in both $E_a$ and $E:E_a$ following ivabradine administration, again suggesting that ivabradine has minimal effects on LV diastolic function. Furthermore, summated filling waves, a common obstacle in the assessment of LV diastolic function in cats during echocardiographic examination, were absent in all cats treated with
ivabradine in the present study, making ivabradine potentially attractive as a diagnostic aid.

While neither ivabradine nor atenolol significantly changed maximum LAD compared to baseline for each drug, interestingly, atenolol did significantly decrease LAD when compared to ivabradine, which mildly increased LAD. This finding is not consistent with that previously observed in normal cats, in which there was no significant difference between the response to atenolol and ivabradine after 4 weeks of treatment, both of which resulted in no change in LAD. While the difference in treatment period between these two studies may, in part, explain this discrepancy, a compelling argument could be made that in HCM cats with obstruction, as opposed to normal cats, by reducing dynamic LVOT obstruction, forward flow is increased, resulting in better LV filling and less MR, both of which could result in a decrease in LA systolic volume. Indeed, in the present study, three of six cats with baseline MR in the atenolol group had no MR after drug administration, with the three remaining having only mild MR; to the contrary, only one of twelve cats with baseline MR in the ivabradine group had no MR after drug administration, with eight of the cats considered to have moderate MR and only three cats having mild MR post-treatment.

Doppler SBP was unchanged by ivabradine in this study, consistent with previously described findings in healthy cats and experimental studies in humans, dogs, and rats, while BP was mildly, but not significantly, reduced by atenolol. This finding is likely due to the absence of direct effects on cardiac output or blood pressure produced by ivabradine’s inhibition of the I f current, while atenolol has been known to consistently reduce blood pressure via multiple mechanisms, mainly through reduction in cardiac output and inhibition of the renin-angiotensin-aldosterone system.

This study has several limitations. Firstly, the relatively small sample size did not allow us to assign the required number of cats to each group based on our a priori statistical power analysis, which indicated that a total of 36 cats would be needed to identify a statistically significant
reduction of the peak outflow tract gradient beyond 35% biological variability (preliminary data),
assuming a desired $P$ value of 0.05 and a statistical power of 0.80. However, this study showed a
difference between the two treatment groups far beyond what would be expected if due only to
biological variability, therefore our a priori power calculation overestimated the number of cats
needed for each group to show the pre-and post-pill difference in LVOT velocity achieved in this
study. If our power calculation had considered a larger effect size, such as a 50% reduction in
Doppler LVOT velocity, the sample sizes used in this study would have achieved an acceptable
power of 0.80. Secondly, the lack of a cross-over design did not allow for the evaluation of the
effect each drug would have had on each individual cat, which would have eliminated between-
subject variability, and thus, strengthened our results. Thirdly, the dosage of each drug used was
based on clinical experience for atenolol and experimental data for ivabradine. Whether these
dosages are equimolar, and therefore, equivalent in their potential effect, is unknown. Fourthly, no
placebo was used in this study, only an active control. The lack of a placebo control theoretically
could increase the risk of a type I error, i.e., concluding that a treatment has a significant effect
when in fact it does not. While the goal of this study was to examine whether ivabradine was not
inferior to atenolol at reducing dynamic LVOT obstruction, the results could potentially lead to
erroneous conclusions about ivabradine’s true effect compared to no treatment at all. Fifthly, all
cats enrolled in this study were presumed to be affected with HCM, however in the absence of
definitive genetic or histopathologic confirmation, it is possible that some of the cats may have
been affected with mitral valve dysplasia or a secondary cardiomyopathy, and should not have
been included in the study. Finally, as only a single dose of the study medications were given in
this study, our results cannot be extrapolated to assume that chronic usage of either drug would
achieve results similar to those achieved in this study.

In conclusion, this study indicates that a single dose of ivabradine is ineffective at reducing
dynamic LVOT obstruction to a clinically relevant degree in cats with preclinical HCM, and is inferior to the standard therapy with atenolol for this purpose. Ivabradine does not appear to aggravate obstruction in this population of cats, and therefore, appears safe to administer. Whether ivabradine can be an effective long-term therapy in cats with preclinical HCM due to its ability to reduce HR and its minimal influence on cardiac function requires further study.
FOOTNOTES


\(^b\) Cober RE. Effects of ivabradine, a new selective If current inhibitor, on heart rate in cats. Master’s Thesis, 2010. The Ohio State University, Columbus, OH


\(^d\) Atenolol, Mallinckrodt Inc., St. Louis, MO

\(^e\) Procoralan®, Les Laboratoires Servier, 22 Rue Garnier, 92200 Neuilly-sur-Seine, France

\(^f\) Amyl Nitrite Inhalants USP, X-Gen Pharmaceuticals Inc., Northport, NY

\(^g\) Vivid 7 Dimension™, GE Medical Systems, Milwaukee, WI

\(^h\) EchoPac software package, Version BT06, GE Medical Systems, Milwaukee, WI

\(^i\) Ultrasonic Doppler Flow Detector, Model 811-B, Parks Medical Electronics Inc., Aloha, OR

\(^j\) SAS version 9.1, SAS Institute, Cary, NC

\(^k\) Kent AM. Effects of atenolol, ivabradine and pimobendan on left atrial and left atrial appendage function: An echocardiographic study in healthy cats. Master’s Thesis, 2011. The Ohio State University, Columbus, OH

REFERENCES


