Early Autonomic and Repolarization Abnormalities Contribute to Lethal Arrhythmias in Chronic Ischemic Heart Failure

Characteristics of a Novel Heart Failure Model in Dogs With Postmyocardial Infarction Left Ventricular Dysfunction

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OBJECTIVES

Using a model of arrhythmias associated with ischemic left ventricular (LV) dysfunction, this study investigated autonomic and electrophysiologic mechanisms associated with sudden cardiac death (SCD) in chronic heart failure (HF).

BACKGROUND

Left ventricular dysfunction from ischemic heart disease is associated with many instances of SCD. Electrophysiologic and autonomic derangements occur in HF, but their contribution to SCD risk is poorly understood.

METHODS

Sudden death risk was assessed in 15 dogs with a healed anterior myocardial infarction (MI) during submaximal exercise and brief acute circumflex ischemia. Left ventricular dysfunction was then induced by repetitive circumflex microembolization until LV ejection fraction reached 35%. Before embolization, six dogs were susceptible to SCD, and nine were resistant.

RESULTS

Baroreflex sensitivity was lower in susceptible dogs (10 ms/mm Hg ± 4 ms/mm Hg vs. 20 ms/mm Hg ± 11 ms/mm Hg, p = 0.04). QT intervals from susceptible dogs were longer after MI (246 ms ± 26 ms susceptible vs. 231 ms ± 20 ms resistant, p < 0.001) and prolonged within eight weeks after LV dysfunction was established (from 246 ms ± 26 ms to 274 ms ± 56 ms, +11%, p < 0.01). Heart rate in susceptible dogs increased during transient ischemia (+20%) and with progressive LV dysfunction (102 beats/min ± 28 beats/min baseline to 154 beats/min ± 7 beats/min LV dysfunction, p = 0.003). All susceptible dogs had spontaneous sustained ventricular tachycardia culminating in SCD. In contrast, QT intervals in resistant dogs prolonged after 24 ± 6 weeks (from 231 ms ± 20 ms to 238 ms ± 15 ms, p < 0.05), and heart rates were unchanged. Only one resistant dog died suddenly with LV dysfunction.

CONCLUSIONS

Depressed vagal and elevated sympathetic control of heart rate coupled with abnormal repolarization are associated with high SCD risk when post-MI LV dysfunction develops.

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Patients with chronic heart failure (HF), most commonly the result of ischemic heart disease (1), are at risk for sudden cardiac death (SCD) arising from malignant ventricular tachyarrhythmias (2,3). A large body of evidence describes left ventricular (LV) mechanical remodeling leading to death from irreversible pump failure (4–6), but little is known about electrical remodeling of the ischemic heart with LV dysfunction leading to SCD in patients who otherwise have few congestive symptoms (7). Recent trials (8–10) document the efficacy of antiadrenergic therapy in reducing SCD in patients with HF and demonstrate a critical role of sympathetic activity in arrhythmogenesis in the failing ventricle. However, specific mechanisms by which antiadrenergic therapy reduces lethal arrhythmias are not clear.

This limited understanding of SCD mechanisms is partly due to a lack of appropriate animal models of HF suitable for studying the arrhythmia process over time (11). The most common large animal model of HF induces LV dysfunction with high-rate pacing (11). Pacing-induced mechanical dysfunction, however, spontaneously resolves shortly after pacing is terminated (11), which makes it less suitable for chronic evaluation. Furthermore, the very nature of tachycardia-induced LV dysfunction directly relates to nonischemic dilated cardiomyopathies, and extrapolation of arrhythmia mechanisms from this model to ischemic HF may be inappropriate. Finally, seldom is SCD predictable in most large animal models of HF, which hinders accurate conclusions about mechanisms that actually lead to lethal arrhythmias. Canine models of chronic ischemic heart disease and LV dysfunction have described important aspects of ventricular remodeling and nonlethal arrhythmogenesis (12–15), but several important questions about life-threatening arrhythmias in chronic LV dysfunction remain unanswered.

This report describes the characteristics of a new canine model designed to address mechanisms of SCD during moderate chronic LV dysfunction arising from ischemic heart disease. Autonomic and repolarization abnormalities

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associated with spontaneous SCD were investigated in two discrete groups of dogs with very different spontaneous arrhythmia development during progression from myocardial infarction (MI) to HF.

METHODS

**Surgical preparation.** The novel model of chronic ischemic HF reported here is partly based on an established conscious dog model of post-MI SCD (16). Mongrel dogs were instrumented with an anterior MI by ligation of the left anterior descending artery during thoracotomy under general anesthesia. A Doppler flow probe and vascular occluder were placed around the circumflex, and a catheter was placed in the descending aorta. Each animal received immediate postoperative short-term analgesia (pentazocine lactate, 1 mg/kg, intramuscularly) followed by a longer acting analgesic (nalbuphine HCl, 0.5 mg/kg, intramuscularly). Guidelines outlined by the National Institutes of Health, American Physiological Society and the American Heart Association pertaining to the appropriate care and use of animals were strictly followed. Thirty days of recovery and acclimation to the facilities and personnel were allowed before any testing was started.

**Baroreflex sensitivity (BRS).** All animals were acclimated to the testing environment and personnel. Baroreflex sensitivity was measured according to published methods (17). The slope of a regression line relating phenylephrine-induced blood pressure rise to RR interval slowing defined BRS, and only significant correlations (R > 85%) were used. Baroreflex sensitivity testing was performed 30 days after MI but before the exercise and ischemia test and before the microembolization procedures.

**Risk for ventricular fibrillation after MI with normal LV function.** To evaluate the post-MI risk for lethal arrhythmias during transient ischemia 30 days after MI, each dog exercised in a submaximal stress test protocol as previously published (16). When target heart rate was reached (210 to 220 beats/min), the circumflex occluder was inflated for 2 min: the animals continued to run during the first minute and exercise was stopped during the second minute. Dogs developing ventricular fibrillation (VF) during the 2 min of myocardial ischemia lost consciousness and then were defibrillated. These animals were labeled “susceptible” or “high-risk” for VF. The remaining animals did not develop sustained ventricular arrhythmias and were labeled “resistant” or “low-risk” for VF. This protocol is associated with a >90% reproducibility of risk status (18).

**Microembolization to produce chronic LV dysfunction.** Ischemic LV dysfunction was produced by repeated microembolization of the circumflex coronary artery using a method previously described (12). Instrumented and characterized post-MI dogs were sedated for each embolization procedure with propofol (1 mg/kg, to effect, intravascularly). The left or right femoral artery was isolated using sterile technique over local anesthesia with lidocaine (2 to 3 cc, 1% solution, SQ). A purse-string suture (4-O silk) was placed in the artery wall, and a small arteriotomy was made with an 18-gauge needle. A 6.5F introducer sheath system (Daig Corp., Minnetonka, Minnesota) was advanced into the lumen of the artery over a wire. Using a 6.0F Judkins left 3.5 diagnostic coronary catheter (Medtronic, Inc., Danvers, Minnesota), the left main coronary artery was engaged under fluoroscopic guidance. The left circumflex coronary artery was identified and subselected using angiographic contrast.

Once the circumflex coronary artery was engaged, 1.5 to 3 cc of 90 µ warmed and sonicated latex beads (2.53% solids, Polysciences, Inc., Warrington, Pennsylvania) were slowly injected and flushed into the artery. The catheter was removed and the purse-string suture closed to ensure hemostasis. The wound was closed with 2-0 absorbable suture, and skin staples were placed. The electrocardiogram (ECG) was monitored during the procedure and for 30 min after embolization. Dogs recovered in a special high-flow oxygen cage and received postprocedure analgesia (nalbuphine 0.5 mg/kg, intramuscularly). The procedure was repeated weekly until the target LV ejection fraction (LVEF) was reached (LVEF approximately 35%).

**LV function assessment.** To monitor decline of LV function, modified two and four chamber two-dimensional echocardiogram images of the heart were made from the apex of the sternum along the long-axis of the LV using a 3.5 MHz transducer. Endocardial definition was maximized and traced in both the two and four chamber views. Systolic and diastolic LV volumes and calculated LVEF were averaged from both orthogonal views. The mitral valve was interrogated using color Doppler to identify the presence of significant mitral regurgitation.

**Arrhythmia monitoring and QT interval measurements.** Transthoracic modified lead I ECG was digitally acquired at baseline and three times a week after the target ejection fraction was reached and daily after arrhythmias appeared. Electrocardiogram recordings were performed while the animal was supine at rest during the midmorning time. The ECGs were digitized, and QT intervals were measured from QRS complexes at various cycle lengths and used to compare resistant and susceptible dogs. Eight to ten QT intervals were measured at matched RR intervals (±5 ms) after MI and periodically after LVEF reached 35 ± 4%, including the last recording before death. QT intervals were consistently available in five resistant and five susceptible
dogs. In order to match the higher heart rate of susceptible dogs with LV dysfunction, QT intervals in resistant dogs were measured during increased heart rate in response to routine activity in the laboratory. Additionally, QT interval measurements were made in 10 normal dogs at different heart rates obtained during routine recordings in the same conditions used for the study groups. This was done to generate normal distribution curves relating QT and RR intervals. This design avoided potential confounding influences of heart rate correction algorithms and increased the likelihood of reflecting real changes in QT measurements.

Pathological examination. Postmortem analysis was performed in four susceptible and three resistant dogs to assess infarct size and pathological consequences of the microembolization. Resistant animals were euthanized by overdose of sodium pentobarbitol, and the heart was immediately excised and put in a 10% formalin solution. In susceptible dogs, hearts were excised as soon as the dog was found dead. Hearts were cut into five to six slices, 1 cm thick, were photographed, and the images were digitized. The total areas of the LV and the infarct zone were calculated using computerized software (Autodesk AutoCAD R14.0, Manchester, UK). Wall thickness was also measured.

Serum catecholamines. Serum catecholamine analysis was performed by the hospital laboratory service at the University Hospital of the University of Oklahoma Health Sciences Center. Whole blood was centrifuged, and serum was eluted. The serum was immediately measured to measure total catecholamines, norepinephrine, epinephrine and dopamine. Blood was obtained after two weeks of reaching the target LVEF.

Statistical analyses. Differences in QT intervals and heart rates in the same animals under different conditions (post-MI and HF) were compared with analysis of variance (ANOVA) for repeated measures. Differences between groups for these parameters were evaluated with ANOVA. Other comparisons, such as ejection fraction, ventricular volumes, size of MI, BRS and catecholamines were evaluated with a Student t test for group comparison. Frequency of SCD within the two groups was analyzed using the Fisher exact test. To describe the normal QT and RR interval relationship, nonlinear regression analysis was used to generate the best-fit line illustrating the relationship in normal dogs only. An alpha level of p < 0.05 was considered significant. Data are presented as mean ± SD unless otherwise noted.

RESULTS

Risk for post-MI ventricular fibrillation with normal LV function. Fifteen dogs underwent the exercise and myocardial ischemia test 30 days after MI. Six dogs developed VF and were successfully defibrillated (susceptible). Nine dogs had no sustained ventricular arrhythmias (resistant) during acute myocardial ischemia.

Susceptible dogs had a sustained tachycardia in response to acute myocardial ischemia with heart rates averaging 212 beats/min ± 15 beats/min at the onset of coronary occlusion and accelerating to 255 beats/min ± 16 beats/min (p < 0.001) after 30 s of coronary occlusion or just before VF if it occurred before 30 s. Resistant dogs were characterized by a lack of cardioacceleration during acute ischemia with heart rates averaging 203 beats/min ± 28 beats/min at the onset of coronary occlusion and 200 beats/min ± 29 beats/min after 30 s of myocardial ischemia (p = 0.1). No resistant dog had ventricular ectopy during myocardial ischemia. These findings are consistent with previously published characteristics of the model (16,19–21).

BRS. Baroreflex sensitivity after MI, but before microembolization, was lower in susceptible dogs compared with the resistant group. Baroreflex sensitivity in susceptible dogs averaged 10 ms/mm Hg ± 7 ms/mm Hg compared with 20 ms/mm Hg ± 11 ms/mm Hg in resistant dogs (p = 0.04).

Microembolization to produce LV dysfunction. Embolization of the circumflex coronary artery successfully impaired LV function in the resistant (LVEF from 62 ± 4% to 35 ± 3%) and the susceptible (LVEF from 66 ± 3% to 35 ± 4%) dogs. Left ventricular end diastolic volume was not changed by the time the susceptible dogs died suddenly (45 cc ± 16 cc to 46 cc ± 15 cc, p > 0.1). In fact, by the time susceptible dogs developed SCD, there was no significant ventricular dilation in either group. However, after six to eight months of follow-up, the ventricles in resistant dogs eventually dilated from an LV end diastolic volume of 43 cc ± 20 cc to 60 cc ± 21 cc (p < 0.001). Left ventricular ejection fraction remained 35 ± 3% in resistant dogs throughout the follow-up period despite progressive dilation. No animal developed severe mitral regurgitation, and there were no differences in the amount (trace to mild) of mitral regurgitation between the two groups of dogs. No animal developed ascites, rales or any finding consistent with New York Heart Association class IV HF.

Incidence of spontaneous arrhythmias. Incidence of SCD was significantly higher in susceptible dogs (p = 0.001). This group developed premature ventricular contractions (PVCs) within days of reaching an LVEF of 35% and progressed rapidly to nonsustained then sustained ventricular tachycardia (Fig. 1 and 2). By eight weeks, all susceptible dogs died suddenly after having sustained or nonsustained ventricular tachycardia before their death. All animals were noted to be alive at the end of the day and were found dead in their cage the next morning.

Resistant dogs developed only PVCs over a much longer period of time (24 weeks ± 6 weeks of observation, Fig. 1B), and only one dog died suddenly three weeks after reaching the target LVEF. No sustained arrhythmias were documented in the resistant dogs after production of LV dysfunction.

Repolarization abnormalities, heart rate and SCD. Arrhythmia progression in susceptible dogs was associated with a persistent sinus
tachycardia from 102 beats/min at baseline to 144 beats/min when LVEF was 35% and 154 beats/min ± 7 beats/min immediately before SCD (p < 0.01). Resistant dogs did not have a change in heart rate throughout the six month follow-up (96 beats/min ± 7 beats/min at baseline, 106 beats/min ± 19 beats/min at an LVEF of 35%, 101 beats/min ± 23 beats/min at six months after an LVEF of 35%, p = 0.1). After induction of LV dysfunction, five susceptible and five resistant dogs had a strikingly different QT interval evolution. QT interval analyses were performed using absolute QT values at comparable RR intervals to avoid the risk of overcorrection that may occur when using the Bazett formula at high heart rates.

The opposite pattern of arrhythmia progression between the two groups might have been, in part, predicted by examination of QT values after MI and before the occurrence of LV dysfunction. QT intervals were longer in susceptible dogs (246 ms ± 26 ms susceptible vs. 231 ms ± 20 ms resistant, p < 0.01) one month after MI with normal LV function. Three weeks after LVEF 35% was obtained, the QT interval of resistant dogs became slightly prolonged from 231 ms ± 20 ms to 238 ms ± 13 ms (p = 0.03, Fig. 3). By six months, the resistant dogs’ QT interval was 247 ms ± 20 ms (p < 0.05 vs. post-MI). In contrast, the QT interval lengthened in susceptible dogs from 246 ms ± 26 ms to 274 ms ± 56 ms within three weeks of ischemic LV dysfunction (p < 0.01, Fig. 3).

**Pathological findings.** The size of the MI from the left anterior descending coronary artery ligation was not different in the two groups (5 ± 2% vs. 4 ± 2%, p = NS). Fibrotic tissue in the embolized LV posterior-lateral wall was similarly distributed in high- and low-risk dog hearts. However, susceptible dogs tended to have more hypertrophy in the anterior wall (1.2 cm ± 0.3 cm susceptible vs. 0.9 cm ± 0.2 cm resistant, p = 0.10) and posterior wall (1.3 mm ± 0.2 mm susceptible vs. 0.9 mm ± 0.2 mm, p = 0.08).

**Serum catecholamines.** Serum catecholamines were available in four susceptible and four resistant animals. Total catecholamines were higher in susceptible dogs (1,017 ng/dl ± 323 ng/dl vs. 661 ng/dl ± 191 ng/dl, p < 0.05) accounted for by increased norepinephrine in susceptible dogs (715 ng/dl ± 276 ng/dl vs. 463 ng/dl ± 183 ng/dl, p = 0.05). Epinephrine and dopamine were not different between the groups.

**DISCUSSION**

This work reports two characteristics that promise to shed light on the mechanisms of SCD in ischemic HF. First, dogs with autonomic imbalances favoring the sympathetic nervous system after MI, but before LV dysfunction, rapidly developed lethal arrhythmias as ischemic heart disease progressed. Since the high-risk animals had very little “congestive” symptoms, we describe their progression as “arrhythmogenic HF.” Second, dogs that died suddenly lost the ability to adapt ventricular repolarization to shortening cycle length. Left ventricular dysfunction did not differ between the two groups of animals, so the observed alteration in repolarization adaptation may represent a primary electrical remodeling specifically occurring in susceptible animals but not in resistant dogs. These findings suggest a link between pathologic autonomic control of heart rate and abnormalities of repolarization leading to SCD early in the progression of ischemic heart disease into HF.

**Novel animal model of ischemic HF.** The model described in this study is new and combines a chronic infarct matrix with ongoing small vessel coronary disease to produce moderate levels of LV dysfunction. Stable LV dysfunction typical of this preparation allowed time to analyze repolarization abnormalities and unambiguously associate...
them with lethal arrhythmia development. This animal model is partially based on the experimental preparation of SCD after MI (normal LV function) developed by Schwartz, Billman and Stone (16). Several general principles of arrhythmia mechanisms after a first MI were described in that model (16,19–22) and were subsequently clinically validated (23,24).

Considering the etiology of LV, dysfunction in an experimental HF model is important since the clinical characteristics of arrhythmias in ischemic heart disease may be different from those of nonischemic origin. Ischemic HF has regional abnormalities of mechanical and electrical function adjacent to normal or hypertrophied myocardium. This heterogeneous substrate contrasts with nonischemic cardiomyopathies, which generally have global homogeneous dysfunction (11). An important and widely used model of HF, pacing induced cardiomyopathy, accurately duplicates a homogeneous dilated cardiomyopathic process, and findings from that model are applicable to human disease (11). However, what is lacking in the literature is a large animal HF model that duplicates the heterogeneity of ischemic HF accompanied with predictable risk for lethal arrhythmias. The model presented in this study was designed to address this gap in understanding and demonstrated very distinct differences in arrhythmia characteristics and risk based on individual autonomic control of heart rate and repolarization kinetics.

Autonomic mechanisms of arrhythmogenic HF. Autonomic mechanisms found in this study challenge the traditional paradigm that sympathetic activation arises only from reflex adaptation to a loss of cardiac output in HF. The traditional neurohumoral view of HF explains why there is a relationship between circulating catecholamines, functional class and total mortality (25,26). Unfortunately, after

Figure 2. An example of spontaneous arrhythmia progression over time in a susceptible (A) and resistant (B) dog with ischemic heart disease and moderate left ventricular dysfunction (EF approximately 35%). Note the rapid progression to sustained, incessant ventricular tachycardia in the susceptible dog within three months. In contrast, the resistant dog had isolated premature ventricular contractions after six months of follow-up. Left ventricular ejection fractions in the two dogs were similar. EF = ejection fraction; MI = myocardial infarction.
20 years of understanding HF as a neurohumoral disease process, there are still few clues to explain why patients with similar LV dysfunction may or may not have autonomic derangements and, more importantly, may or may not die of arrhythmic death.

This study further supports the hypothesis that cardio-cardiac reflexes (27) originating from the damaged ventricle initiate adverse sympathetic activation coupled with vagal withdrawal (28) independent from hemodynamic effects. In this study, a clear association between abnormal cardiac autonomic control in susceptible subjects and a rapid progression of electrophysiological alterations was found. Although cause and effect cannot be established, these findings suggest that abnormal autonomic control of the heart may be linked to electrophysiologic derangements leading to SCD in ischemic LV dysfunction.

Clinical application of these results must be done carefully and tempered by the limitations of animal models in general. For example, epicardial vessel ligation and microembolization are intended to duplicate chronic, combined with subacute, large and small vessel coronary artery disease. This process does not exactly duplicate the chronic process of atherosclerotic coronary disease, nor does it approximate the length of human disease. Even with these limitations, however, the results suggest that there may be two groups of patients that develop ischemic LV dysfunction differentiated by their risk for SCD. Patients who exhibit "arrhythmic HF" may not survive LV dysfunction long enough to be involved in clinical trials. If this were true, traditional approaches to risk stratification would be applied to the survivors, who are, by definition, at relatively low-risk for SCD. In fact, the lack of accurate risk stratification by traditional means has led to large clinical trials examining the hypothesis that all patients with LV dysfunction should receive an implantable defibrillator (29). Hopefully, a better understanding about the target populations coupled with elaboration of autonomic and electrophysiologic mechanisms of arrhythmias in ischemic HF will lead to better risk assessment.

Abnormalities of repolarization. Normally, ventricular repolarization shortens when the heart rate increases. This important characteristic of the ventricle reduces the chance of excessively long QT intervals at short cycle lengths with the attendant risk for life-threatening arrhythmias. In this study, susceptible dogs quickly developed abnormal QT interval prolongation with shorter cycle lengths, which may reflect a defect in ion channels responsible for ventricular repolarization found in this group. Depressed expression of repolarizing currents occurs in ventricular myocytes both from experimental HF preparations and from human

![Figure 3](image-url)
Lethal Arrhythmias in Ischemic HF

 hearts with cardiomyopathy. Specifically, the transient outward potassium channel, $I_{to}$, is underexpressed in cardiomyopathic human hearts and pacing induced animal HF, leading to prolongation of cellular action potential (30,31). Additionally, $I_{la}$ current is reduced in LV hypertrophy induced by chronic atrioventricular block (32). More recent studies demonstrate abnormalities in cellular calcium kinetics, which are also important mechanisms in action potential prolongation of HF (33).

Additionally, tissue heterogeneity in the current model, which duplicates clinical reality, probably produced regional abnormalities of ion channels responsible for repolarization. This type of heterogeneity alters action potential duration rate dependence (34) and may critically increase dispersion of repolarization, thus setting the stage for lethal arrhythmias.

Conclusions. This study describes a new chronic animal model of ischemic LV dysfunction in which dogs at high-risk for spontaneous SCD can be reliably identified. The main findings are that autonomic imbalances, present before HF develops in subjects with ischemic heart disease, are associated with lethal arrhythmias as LV dysfunction progresses. QT interval prolongation and the loss of repolarization adaptation to short cycle lengths complete the high-risk matrix. Overall this model provides novel information to understand SCD in HF and sets the stage for further studies aimed at describing neural and cellular mechanisms of arrhythmogenic HF.

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