Mechanism of Sudden Cardiac Death in Pigs with Viable Chronically Dysfunctional Myocardium and Ischemic Cardiomyopathy

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Supported by the Department of Veterans Affairs; the American Heart Association; the National Heart, Lung and Blood Institute (HL-55324, HL-61610, HL-75324); the Albert and Elizabeth Rekate Fund; the Mae Stone Goode Trust; and the John R. Oishei Foundation.

Running Head – Sudden Death in Pigs with Ischemic Cardiomyopathy

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ABSTRACT

Pigs with viable chronically dysfunctional myocardium and ischemic cardiomyopathy are at high risk of sudden cardiac death (SCD). We performed the present study to identify the arrhythmic mechanism of SCD, the relation to changes in LV function, and inducibility of malignant arrhythmias prior to SCD. Juvenile pigs (n=72) were instrumented with chronic stenoses on the proximal left anterior descending and circumflex arteries. Survival was only 29% three months after instrumentation and all of the deaths were sudden and without prodromal symptoms of heart failure. TTC staining demonstrated necrosis in only 9 animals averaging 2.3±0.9% of the LV, with no difference between SCD and survivors. Implantable loop recorders (n=13) documented both ventricular fibrillation (n=6) and brady-asystole (n=2) as the arrhythmic mechanism of death. While regional and global function were depressed (anteroseptal wall-thickening 1.8±0.2 vs. 4.2±0.2 mm in shams, p<0.001; fractional shortening 21±2 vs. 31±1% in shams, p<0.01), there were no differences between SCD animals and survivors. LV mass increased in animals with ischemic cardiomyopathy and was greater in animals with SCD (4.0±0.2 vs. 3.1±0.1 g/kg in survivors, p<0.001). Serial programmed ventricular stimulation failed to induce any sustained arrhythmias. We conclude that pigs with viable dysfunctional myocardium and globally reduced LV function have a high rate of SCD with a spectrum of arrhythmias similar to patients with ischemic cardiomyopathy. The risk is independent of necrosis, but appears to increase with LV hypertrophy. Like patients with ischemic cardiomyopathy, programmed stimulation is insensitive to predict SCD when viable dysfunctional myocardium is the pathological substrate.

Key Words – Hibernating myocardium, cardiomyopathy, sudden death, arrhythmias
INTRODUCTION

Patients with chronic coronary artery disease and ischemic cardiomyopathy develop left ventricular dysfunction that is disproportionate to the mass of infarcted myocardium (7, 26). This is due to viable dysfunctional myocardium arising either from repetitive episodes of reversible ischemia (chronically stunned and hibernating myocardium) or remodeling in response to LV dysfunction (11). Viable, chronically dysfunctional myocardium with both normal (chronically stunned myocardium) (17) and reduced resting perfusion (hibernating myocardium) (22) are common in patients with ischemic cardiomyopathy and present in at least half of all patients (2). While the amount of hibernating myocardium impacts functional improvement and symptoms of heart failure after revascularization, it is also an extremely important determinant of survival. This appears to be independent of LV dysfunction since patients with hibernating myocardium have a higher mortality than patients with irreversible scar (1). While cause specific mortality has not been widely studied, small investigations have suggested that the poor prognosis is related to arrhythmic death (16, 35). While indirect, this is consonant with the profound effect of coronary revascularization on improving survival in the setting of global LV dysfunction which may largely negate the potential benefit of an implantable cardiac defibrillator (9).

In support of viable dysfunctional myocardium being a pathological substrate increasing the risk of SCD independently of scar, we have recently demonstrated that pigs with hibernating myocardium and normal global LV function develop SCD in the absence of heart failure or significant infarction (12). Sudden death in this model is due to ventricular tachycardia degenerating into ventricular fibrillation, with total coronary occlusion and collateral-dependent hibernating myocardium frequently present prior to sudden death (12). When the volume of viable dysfunctional myocardium was increased by placing chronic stenoses on both the proximal left anterior descending (LAD) and circumflex coronary arteries (18), animals developed global LV dysfunction and a hemodynamic picture similar to patients with ischemic cardiomyopathy and compensated LV failure. While this was associated with trivial myocardial necrosis, the development of global LV dysfunction markedly amplified the risk of SCD in comparison to animals instrumented with a single LAD stenosis (18).
We performed the present study to determine whether the physiological substrate and mechanism of SCD in animals with global LV dysfunction from 2-vessel coronary disease were similar to or differed from those with hibernating myocardium and normal LV dysfunction from a single LAD stenosis. We used Reveal Plus implantable loop recorders to identify the spectrum of arrhythmic mechanisms of SCD. Serial echocardiography was used to identify whether LV dysfunction preceded SCD and to determine if regional or global dysfunction differed in animals with SCD as compared to survivors. Finally, a chronically implantable right ventricular pacing lead was used to perform serial programmed electrical stimulation to identify whether inducible tachyarrhythmias were present prior to SCD.

METHODS

Coronary Artery Instrumentation

All experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research. Juvenile farm-bred pigs were fasted and premedicated with a Telazol (tiletamine 50 mg/ml and zolazepam 50 mg/ml) and xylazine (100 mg/ml) mixture (0.022 ml/kg IM) and given prophylactic antibiotics (cefazolin 0.5mg IV and gentamicin 40 mg IV). Pigs were intubated, and sedation was maintained with isoflurane (1 - 2%) and oxygen (balance). Through a thoracotomy (fourth left intercostal space), the proximal left anterior descending (LAD) and left circumflex coronary arteries were exposed with a limited pericardiotomy, leaving the rest of the pericardium intact. Fixed diameter Delrin stenoses (1.5 mm i.d.) were secured around both arteries. The wound was closed and the pneumothorax was evacuated. A single postoperative dose of antibiotics was repeated, and an intercostal nerve block (marcaine) and analgesics (stadol 2.2mg/kg IM and banamine 1.0-2.5mg/kg IM) were given postoperatively to alleviate pain. Survival analysis was performed on a total of 72 pigs which includes 17 animals used to characterize hemodynamics, flow and function in survivors in a previous publication(18). Myocardial necrosis was assessed by triphenyltetrazolium chloride staining of 3 - 4 concentric rings of the left ventricle (n = 51 animals), and quantified as a percent of LV mass(12). Point counting was used to quantify connective tissue staining of trichrome stained histological sections (n = 17 animals)(22).
Implantable Loop Recorder (n=13)

Implantable loop recorders (Reveal Plus Model 9526, Medtronic Inc.) were used to establish the cardiac rhythm responsible for sudden death. The recorders were placed in a subcutaneous pocket along the upper thoracic spine, as previously described (12). Implantation was performed at the time of coronary artery instrumentation in 8 animals, and in 5 animals a second surgical procedure was performed 28 ± 5 days later. The loop recorder was programmed to activate as follows: # of subject-activated events = 0, # of auto-activated events = 13, storage mode = 42 minutes, consecutive beats required to record an event = 16, bradycardia detection <30 bpm, and asystole duration >3.0 seconds. The tachycardia detection rate was initially set at >230 bpm. Since the maximal heart rate decreased with age, it was lowered to 180 bpm one month after initial coronary instrumentation. Gain and sensitivity settings were manually adjusted for optimal detection of the QRS complex without triggering on the T wave. Loop recorders were interrogated every two weeks after implantation (after sedation with Telazol/xylazine) and following sudden death to document arrhythmias.

Transthoracic Echocardiography

Regional and global left ventricular function was serially evaluated using transthoracic echocardiograms performed at approximately 1 week (n = 14), 1 month (n = 15) and 2 months (n = 19) after initial instrumentation. Echocardiographic parameters in animals that developed arrhythmic death were compared to those that survived to a terminal study. The results in animals instrumented with two coronary stenoses were compared to eight sham animals that underwent thoracotomy and dissection of the LAD and circumflex arteries without placement of a stenosis. Echocardiograms were performed with a 2.5 MHz (GE Vingmed System V, GE Medical Systems) or a 2.25 MHz (Ultramark 9, ATL Ultrasound) phased-array transducer, as we have previously published (18, 31). Sedated animals (Telazol/xylazine) were studied on their left side through a right parasternal window. Standard M-mode was used to obtain wall thickness measurements of the anteroseptum and posterior walls from a mid-ventricular short axis view. As recommended by the American Society of Echocardiography, end-diastole was defined as the onset of the QRS complex and end-systole was the point of minimum chamber
diameter(36). Regional function was assessed with myocardial wall thickening (ΔWT, end-systolic thickness – end-diastolic thickness). Fractional shortening, an assessment of global function, was defined as 

$$\frac{100 \cdot (LV \text{ end-diastolic dimension}) - (LV \text{ end-systolic dimension})}{(LV \text{ end-diastolic dimension})}.$$

Left ventricular ejection fraction was quantified from the estimated left ventricular volumes(18), and defined as 

$$100 \cdot \frac{(LV \text{ end-diastolic volume}) - (LV \text{ end-systolic volume})}{(LV \text{ end-diastolic volume})}.$$

Left ventricular mass was calculated as recommended by the American Society of Echocardiography (LV mass in grams = 0.8 • [1.04 (LV end-diastolic dimension + posterior wall + anteroseptal wall)³ – (LV end-diastolic dimension)³] + 0.6)(23), and reported relative to body weight (g/kg).

**Serial Programmed Ventricular Stimulation from the Right Ventricular Apex (n=9)**

Since previous studies documented a low risk of sudden death within the first month following coronary stenosis placement(18); programmed stimulation was delayed to allow sufficient growth that would facilitate placement of a chronic RV pacing lead for serial studies. Nine pigs were studied starting 35 ± 2 days after initial instrumentation. Fasted animals were pre-medicated with Telazol/xylazine, and given a dose of cefazolin (1.0 gm IV) and gentamicin (60 mg IV). Pigs were prophylactically intubated (and provided with oxygen at 3L/min) and sedation was maintained with propofol (5 - 10 mg/kg/hr IV continuous infusion). Using sterile technique, a 6 cm midline neck incision was made and the right external jugular vein was identified. A single 6F active fixation pacemaker lead was inserted through the jugular vein, advanced under fluoroscopy to the right ventricular apex, and secured in a stable position. The lead was connected to the ventricular port of a dual chamber Medtronic pacemaker (atrial port closed with a sterile plug). Excess pacing lead was coiled in the right atrium.

Programmed ventricular stimulation was delivered at ~3 times threshold voltage (programmer model 9790, Medtronic Inc.). Following an 8-beat train at a basic cycle length of 400 ms, a single extrastimulus was introduced late in diastole (starting at 300 ms after the preceding beat) and decremented in 15 ms steps to a lower limit of 200 ms (programmer limit). Subsequently, double and then triple extrastimuli were introduced with S2 (or S2 and S3) delivered at 200 ms (or 15 ms above the refractory period) and S3 (or S4) initially at 300 ms and then decremented by 15 ms steps to a lower limit
of 200 ms. If no sustained ventricular arrhythmias were induced, we evaluated burst pacing with the S1 train decremented by 15 ms steps to a lower limit of 200 ms. Upon completion of the programmed stimulation protocol, the pulse generator was secured in the subcutaneous tissue, the incision was closed, and a dressing applied. Additional doses of cefazolin and gentamicin were administered after completion of the study. Programmed stimulation was repeated transcutaneously every two weeks until animals died or were terminated (2.6 ± 0.6 studies per animal, range 1 - 6).

**Survival and Statistical Analyses**

Kaplan-Meier survival analysis was used to determine the frequency and temporal occurrence of sudden death. This analysis was conducted using all similarly instrumented animals studied in our laboratory since 1998 (n = 72), including those animals in which physiological studies were previously reported(18). Animals that were terminated or died in conjunction with a study were censored at that point and were not included as sudden deaths. The eight sham-instrumented animals served as controls.

The reported values are mean ± SEM. An analysis of variance was used to compare echocardiographic parameters between groups (Sudden Death, Survivor, and Sham) at each time point. The Holm-Sidak test was used for all pair-wise post-hoc comparisons. Kaplan-Meier survival curves were compared using the Log-Rank test. Significance for all statistics was defined as \( p \leq 0.05 \).

**RESULTS**

**Survival Analysis of Animals Chronically-Instrumented with LAD and Circumflex Stenoses**

A total of 72 animals underwent surgical implantation of stenoses on the proximal LAD and circumflex arteries; and 8 control animals had a similar surgical procedure with dissection of both arteries, but without placement of stenoses. One animal developed clinical signs of congestive heart failure and was electively terminated. **Figure 1** shows the Kaplan-Meier survival curve for all animals. There were only three deaths within the first week of surgery (4.2%), but beginning ~3 weeks after instrumentation sudden death occurred at a rate of ~30% per month. A total of 40 animals had spontaneous sudden death, with cumulative survival of 83% at one month, 56% at two months and 29%
three months after instrumentation. Sudden death was occasionally witnessed in association with feeding, transfer between cages, or sedation for procedures. There were no deaths in the sham-instrumented control group (p < 0.01 vs. chronically instrumented).

**Mechanism of Sudden Death in the 2-Vessel Model of Hibernating Myocardium**

Eight of the 13 animals with an implanted loop recorder developed spontaneous sudden death an average of 52 ± 7 days after instrumentation (range 25 - 82 days). All deaths occurred during the day, between the hours of 7 a.m. and 7 p.m. All recorded rhythms associated with sudden death are shown in **Figure 2**. Ventricular fibrillation occurred in 6 animals. Since the Reveal Plus rejects rates >300 bpm as artifact (Medtronic Inc., personal communication), loop recorders frequently triggered when low amplitude ventricular fibrillation developed. Therefore, the transition to ventricular fibrillation was only recorded in four animals. In 3 of these 4, ventricular fibrillation was preceded by ventricular tachycardia at 340 - 450 bpm (mean 403 ± 33 bpm). The fourth animal had a junctional tachycardia with a very closely-coupled ventricular ectopic beat triggering ventricular fibrillation. The other two rhythms associated with sudden death were brady-asystole.

**Progression of Myocardial Dysfunction in Relation to SCD in Pigs with Ischemic Cardiomyopathy**

Echocardiographic measurements at each evaluation are shown in the **Table**. There were no differences in age or animal weights between groups at any time-point. Spontaneous sudden death (n = 13) occurred 35 ± 7 days after the 1 Month echocardiogram and 5 ± 1 days after the 2 Month echocardiogram. In the Survivor group (n = 11), elective termination was performed 59 ± 26 days after the 1 Month echocardiogram, and 12 ± 9 days after the 2 Month echocardiogram.

Serial changes in regional function over time are illustrated in **Figure 3**. All three groups of animals had similar anteroseptal wall thickening (ΔWT) immediately after surgery (1 week). Wall thickening initially increased with growth of the animals, with no significant differences among groups 1 month after instrumentation. However, after 2 months anteroseptal dysfunction was evident in the chronically-instrumented animals (1.8 ± 0.2 mm vs. 4.2 ± 0.2 mm in shams, p<0.001). There were no significant differences in regional wall thickening between the animals that later developed SCD as
compared to those that survived (p = 0.45 at 2 months). Wall thickening in the posterior region supplied by the non-stenotic right coronary artery remained similar among groups over time.

Changes in global left ventricular function (Figure 4) paralleled the results of anteroseptal wall thickening distal to the chronic stenoses. Both left ventricular fractional shortening and estimated ejection fraction were similar among the groups of animals at 1 week and 1 month after instrumentation. However, after 2 months global function was reduced in both groups of instrumented animals in comparison to the sham controls. Fractional shortening in the instrumented animals averaged 21 ± 2% vs. 31 ± 1% in Shams (p = 0.01), and the average ejection fraction was 42 ± 3 % vs. 59 ± 2 % in Shams (p = 0.01). There were no significant differences in fractional shortening (p = 0.59) or ejection fraction (p = 0.53) between those that died suddenly and those that survived.

There were less consistent trends in left ventricular dimensions and estimated left ventricular volumes. At the 1 Week and 1 Month studies, there were no differences in end-diastolic or end-systolic dimensions (Table) or estimated end-diastolic or end-systolic volume indexes (Figure 5) between groups. The chronically-instrumented groups tended to have larger ventricular dimensions and indexed volumes at the 2 Month study; however, only the end-systolic parameters (end-systolic dimension, p = 0.04, Table; estimated end-systolic volume index, p = 0.04, Figure 5) of the Sudden Death group were significantly increased as compared to the sham controls. There were no significant differences between survivors and animals developing SCD.

Serial changes in left ventricular mass estimated by echocardiography and normalized to body weight are shown in Figure 6. There were no differences among the three groups at 1 week after initial surgery (p = 0.93). By one month, there was a trend toward left ventricular hypertrophy among animals with 2 vessel stenoses as compared to controls but the difference was not statistically significant (p = 0.17). After two months however, left ventricular hypertrophy was evident in both groups of instrumented animals as compared to Sham controls (p < 0.05 for each comparison), and LV mass in the Sudden Death group was greater than that in the Survivors (p = 0.04).
Serial Programmed Right Ventricular Stimulation

Spontaneous sudden death occurred in 5 of the 9 pigs undergoing serial programmed stimulation 63 ± 7 days after initial instrumentation (range 40 - 82 days). Despite the fact that ventricular tachyarrhythmias were the mechanism of sudden death in the majority of animals in this study, sustained ventricular tachycardia was not induced during right ventricular apical programmed stimulation (n = 21). The only induced arrhythmia was a single 6-beat run of ventricular tachycardia in an animal 82 days after initial instrumentation.

Post-Mortem Evaluation of Animals with Sudden Death versus Survivors

The direct measurement of LV mass at post-mortem (n = 49) confirmed the presence hypertrophy in the animals that experienced sudden death (Figure 7). LV mass to body weight ratios in the sudden death animals were 30% greater than survivors (p<0.001) and 52% greater than sham-operated controls (p < 0.001). The LV mass to body weight ratio was also significantly greater among survivors than sham controls (p = 0.04). Myocardial necrosis was present by triphenyltetrazolium chloride staining in 9 animals (4 animals with sudden death and 5 survivors), with an average infarct volume of 2.3 ± 0.9% of LV mass. Areas of negative staining were typically associated with gross fibrosis consistent with chronic infarction. There were no significant differences in the frequency or extent of necrosis between the Sudden Death and Survivor groups. As we have previously reported(18, 22), connective tissue staining was increased in regions subtended by a chronic coronary stenosis (Figure 8), but due to the variability among animals the regional differences did not achieve statistical significance (ANOVA p = 0.14). The values for regional connective tissue staining were nearly identical in the animals that experienced sudden death as compared to survivors (p = 0.99).

DISCUSSION

There are several new and important findings from our studies. First, increasing the area at risk of developing viable dysfunctional myocardium with critical stenoses on the LAD and circumflex coronary arteries increases the frequency of arrhythmic death in the absence of significant myocardial
necrosis. In contrast to pigs with a single LAD stenosis where SCD was uniformly due to ventricular fibrillation(12), brady-asystolic events appear to account for ~25% of arrhythmic events with VT/VF in the remainder. This spectrum of arrhythmias is similar to that described in patients with ischemic cardiomyopathy(24). Sustained ventricular arrhythmias were not inducible prior to sudden death and is consonant with findings in patients with ischemic cardiomyopathy where more than half of arrhythmic deaths occur in patients that are not inducible(3, 15). Finally, regional and global dysfunction preceded the development of SCD, yet this was not associated with advanced heart failure. While the degree of regional and global left ventricular dysfunction in animals developing SCD was similar to animals that survived, there was greater LV hypertrophy. Collectively, these findings lend further support to the hypothesis that viable dysfunctional myocardium increases the risk of sudden death independently of infarcted myocardium(1, 16, 35), and the risk of SCD is amplified by reductions in global ejection fraction in the absence of advanced heart failure.

**Sudden Death in Pigs as Compared to Patients with Ischemic Cardiomyopathy**

We have previously reported that sudden death occurs in the absence of infarction in pigs with a single LAD stenosis(12). In these studies, hibernating myocardium was associated with preserved LV function and coronary occlusion with collateral–dependent myocardium frequently preceded SCD. As shown in Figure 9, the rate of sudden death in pigs increased significantly with the addition of a second chronic stenosis (p<0.01). Although this could be due to the increase in the mass of myocardium at risk of ischemia from ~45% to ~75% of the LV(43, 44), these observations are also consistent with the well-established inverse relation between arrhythmic death and ejection fraction. The importance of this relation is underscored by the fact that ejection fraction is currently the major clinical variable used to risk stratify patients with ischemic cardiomyopathy for placement of an implantable defibrillator for the primary prevention of SCD(5, 33).

These two porcine models of chronic ischemic heart disease also show some differences in the arrhythmic mechanism of sudden death. In animals with a single LAD stenosis with normal LV function, spontaneous sudden death (n = 10) was always associated with ventricular fibrillation, and when
recorded, preceded by ventricular tachycardia(12). In contrast, two of the eight animals with ischemic cardiomyopathy had terminal brady-asystole. This distribution of arrhythmias is similar to the experience in patients with ischemic cardiomyopathy. Although there is very limited data, the available evidence suggests that the majority are due to ventricular tachycardia degenerating into ventricular fibrillation, but a substantial minority are due to bradyarrhythmias(24).

The mechanisms leading to brady-asystolic arrest are likely complex but similar to the diversity of mechanisms reported in dogs with acute ischemia in a two-vessel coronary artery disease model(25). It is unlikely that the development of brady-asystole reflects advanced heart failure based on clinical observations immediately preceding the event. It is also unlikely that this arises as a terminal event following a large infarction since pathological evidence of significant infarction in this model is absent at post-mortem. Furthermore animals that are subsequently resuscitated form SCD using an implantable cardiac defibrillator do not evolve a myocardial infarct (unpublished observations). A plausible explanation is that the mechanism of death when the circumflex artery is the “culprit vessel” may be brady-asystole and differ from VT/VF which arises when the LAD is involved. This may reflect regional differences in ischemia, size of the area at risk of ischemia, or the relation between risk region and collateral flow.

**Regional and Global Myocardial Dysfunction Precedes the Development of Sudden Death**

The serial echocardiographic evaluation of animals that subsequently developed lethal arrhythmias clearly shows that regional and global left ventricular dysfunction was present prior to sudden death. This suggests that recurrent ischemia led to regional myocyte apoptosis(20, 28) and subsequent cellular remodeling and hypertrophy(19), which was a vulnerable substrate. This contrasts with the development of acute coronary occlusion and transmural myocardial infarction which would have been associated with normal myocardial function before the event. It is also consistent with the observation in animals with a single LAD stenosis where the development of hibernating myocardium with total coronary occlusion and collateral-dependent myocardium was present prior to sudden death(12). Nevertheless, it is important to emphasize that any comparison between the Sudden Death and
Survivor groups is limited by the undefined duration of survival after elective termination of some of the animals. The shape of the survival curve (Figures 1 and 9) does not suggest any lessening of the risk of sudden death over time and thus, it is impossible to definitively identify a time when the animals are no longer at risk of spontaneous sudden death. Nevertheless, we found no significant differences in regional or global left ventricular function during the development of hibernating myocardium and ischemic cardiomyopathy between animals destined to die as compared to survivors.

Although systolic function was similar among instrumented animals, we found a higher LV mass among the animals that develop SCD. This is consistent with a large body of clinical data showing that left ventricular hypertrophy is a poor prognostic sign in patients with established heart disease. It is also compatible with the observation that left ventricular hypertrophy is a frequent pathological finding among victims of spontaneous sudden death as an initial manifestation of heart disease(10, 45). In this model, the development of hypertrophy is unrelated to increases in afterload. Rather, compensatory hypertrophy appears to develop secondary to global LV dysfunction and the reduction in ejection fraction. While not statistically significant, the trend toward larger left ventricular dimensions and volumes in animals developing SCD lends support to the clinical association between the risk of sudden death and levels B-type natriuretic peptide(8, 41). Further studies evaluating serial biomarker levels in pigs will be required to address this hypothesis directly.

**Inability of Serial Programmed Stimulation to Predict Sudden Death**

Programmed ventricular stimulation has been used to stratify patients with depressed LV function and myocardial scar to assess subsequent risk of SCD. While this is a risk factor for SCD when the ejection fraction is depressed, over half of the arrhythmic deaths arise in the subset of patients that are not inducible. The programmed ventricular stimulation protocol used in our study was limited by a single right ventricular apical site and the restrictive programming capabilities of a pacemaker generator. Nevertheless, we were unable to induce sustained VT or VF in any of the animals. While the protocol was similar to that used in humans, it is possible that induction of VT requires more aggressive pacing
protocols in the pig; and additional studies evaluating shorter cycle lengths, greater repetitive ventricular stimuli, or multiple pacing sites including the LV will be required.

Despite these limitations the absence of inducibility is consistent with the lack of significant myocardial necrosis in this model and suggests that other mechanisms may be operative in inducing VT/VF. These may be particularly relevant to the subset of patients considered candidates for primary prevention of SCD using an implantable defibrillator. For example a recent sub-study of the Multicenter Automatic Defibrillator Implantation Trial II (MADIT II) trial demonstrated that the two-year incidence of VT/VF was the same in patients with inducible VT/VF (31%, p-ns) as in patients who were non-inducible (27%)(15). In fact, since 64% of the patients were not inducible, most VT/VF events occurred in the non-inducible group. Interestingly, defibrillator therapy for VF was more common in patients who were non-inducible (8.1 vs. 2.2%, p = 0.08)(15). Dynamic alterations in cardiac action potential duration (APD) restitution rather than reentry associated with scar could be responsible for the development of VT/VF under these circumstances(14). In support of this, we have recently demonstrated an increase in the slope of the APD restitution curve in pigs with hibernating myocardium and a single LAD stenosis(4). These changes were limited to hibernating myocardium and resulted in inhomogeneity in APD restitution throughout the LV, which may lead to a particularly vulnerable substrate. Further studies will be needed to evaluate the spatial distribution of APD restitution as a substrate for SCD in this model.

Relation to other Large Animal Models of Sudden Cardiac Death

Advances in understanding substrate factors in SCD have partly been hampered by the inability to model human disease experimentally. While considerable insight has been made regarding mechanisms of arrhythmias arising during acute ischemia, there are very few animal models exhibiting a high rate of SCD, and documentation of the arrhythmic mechanism responsible for SCD has been limited. For example, models of inherited arrhythmias exist in dogs and include the German Shepherd model of polymorphic VT(32) and the Boxer model of arrhythmogenic right ventricular dysplasia(6). While spontaneous sustained VT has been recorded in these animals, the arrhythmic mechanism responsible for SCD has not been documented and spontaneous SCD only occurs in a minority of the dogs. Healed
myocardial infarction results in a low rate of SCD in dogs, but the incidence of VF can be increased by transient transmural ischemia during the intense sympathetic activation of peak exercise(37).

Tachycardia-induced heart failure models have demonstrated substantial cellular and molecular remodeling in association with spontaneous VT(34, 39). Nevertheless, the few documented rhythms at the time of death indicate brady-asystole occurring in the setting of advanced biventricular failure rather than VT degenerating into VF(27). Finally, chronic atrioventricular block and right ventricular pacing leads to hypertrophy and cellular remodeling with a 10 - 15% rate of spontaneous SCD(42). It can be increased in the setting of myocardial infarction and stimulation of sympathetic nerve sprouting with nerve growth factor(13) or sub-threshold electrical stimulation of the stellate ganglion(40).

The present model of ischemic cardiomyopathy differs from these models in several respects. First, the incidence of sudden death is ~70%, is entirely arrhythmic, and occurs in the absence of advanced heart failure. Second, the arrhythmic events occur spontaneously and like humans, appear to be related to transient sympathetic activation. Finally, they occur in the absence of healed infarction or an inherited genetic predisposition to SCD. While the specific physiological triggers and substrate factors responsible for SCD remain to be established, the model is characterized by LV hypertrophy, chronic coronary artery disease and LV dysfunction which are the pathological factors identified in patients with SCD. Other pathological substrates include cellular hypertrophy(28), altered calcium handling(19), inhomogeneity in myocardial repolarization(4), and a critical reduction in flow reserve(21, 22) that would result in subendocardial ischemia during sympathetic activation.

**Methodological Limitations**

Post-mortem coronary angiography was not performed in the animals following sudden death, and TTC staining may be insensitive for detecting acute myocardial infarction since it relies on the loss of dehydrogenase activity(38). Therefore we are unable to exclude acute coronary occlusion as a potential mechanism of sudden death. However, there are two lines of evidence that suggest that this was not the major mechanism of sudden death in this model. First, serial echocardiography clearly showed that regional and global left ventricular dysfunction was present prior to sudden death, and the degree of
dysfunction was similar to animals that survived. Coronary angiography in 9 of these surviving animals showed severe stenoses of both the LAD and circumflex coronary arteries, with complete occlusion and collateral-dependent myocardium associated with 6 of the 18 instrumented vessels(18). Second, in a chronic single vessel model of hibernating myocardium we previously documented complete coronary occlusion and collateral-dependent myocardium prior to sudden death in 5 of 7 animals(12). Therefore, in a similar chronic stenosis model, a majority of the episodes of sudden death could not be due to acute vessel occlusion. Additional studies will be required to clarify the role of acute ischemia in this model using telemetric physiological monitoring and the physiological investigation of animals resuscitated from sudden death.

**Clinical Implications**

The clinical relevance of our model is underscored by the chronic nature of ischemic heart disease and documentation of the spectrum of arrhythmic mechanisms of sudden death similar to those described in patients with cardiomyopathy(24). Thus, our model may offer potential insights into SCD in patients with ischemic heart disease. Similar to clinical studies, we found that LV mass was greater among animals destined to die(10, 45), but LV function and programmed ventricular stimulation were poor predictors(3, 15, 41). Perhaps most importantly, our findings support the hypothesis that viable myocardium in the setting of ischemic cardiomyopathy is associated with a worse prognosis and an increased risk of sudden death(1, 16, 35). Whether this is due to the risk of developing new acute coronary syndromes, inhomogeneity in action potential duration restitution properties(4), or heterogeneity in sympathetic nerve function(29, 30) will require further study.
ACKNOWLEDGEMENTS

We appreciate the technical expertise provided by Elaine Granica, Deana Gretka, Amy Johnson, Maureen Krieg, and Robin Testa in the conduct of these studies; and Anne Coe for her assistance with manuscript preparation.
**FIGURE LEGENDS**

**Figure 1.** Kaplan-Meier survival curves for animals with stenoses on the LAD and circumflex arteries (2 Vessel) versus Sham-operated controls. There was a high rate of SCD among animals with chronic coronary stenoses with 44% mortality at 2 months and 71% mortality three months after instrumentation. There were no deaths in sham-instrumented controls where the coronary arteries were dissected free but not instrumented with a stenosis (p < 0.01).

**Figure 2.** Spectrum of arrhythmic mechanisms of sudden death in pigs with hibernating myocardium and ischemic cardiomyopathy. The arrhythmic mechanism of sudden death was recorded with an implantable loop recorder in 8 animals. Ventricular fibrillation was present in 6 cases, and in 3 of the 4 animals in which the transition was documented (arrows) ventricular tachycardia was the pre-terminal rhythm. Brady-asystole was mechanism of death in the remaining two animals.

**Figure 3.** Serial changes in regional function during the development of ischemic cardiomyopathy. Wall thickening (\(\Delta WT\)) in the anteroseptum (upper graph) was similar in each group of animals one week and one month after initial surgery. However by two months, regional function was significantly depressed in the instrumented animals in comparison to Sham controls (p < 0.001), but there was no difference between those that would experience sudden death and survivors. Wall thickening in the normally-perfused posterior wall (lower graph) was similar among the groups at each time-point.

**Figure 4.** Serial changes in global left ventricular function during the development of ischemic cardiomyopathy. Both fractional shortening (upper graph) and ejection fraction (lower graph) were similar among the three groups of animals at 1 week and 1 month after surgery. However, at 2 months both estimates of global function were significantly reduced in the instrumented animals as compared to Sham controls, with no differences between Sudden Death and Survivor groups.

**Figure 5.** Serial changes in indexed left ventricular volumes during the development of ischemic cardiomyopathy. Both end-diastolic volume index (EDVI, upper graph) and end-systolic volume index (ESVI, lower graph) were similar among each of the groups of animals at 1 week and 1 month after surgery. At two months after instrumentation, the EDVI was higher in the Sudden Death group, but the
difference was not statistically significant \((p = 0.15)\). The ESVI was also higher in the Sudden Death group at two months, and was significantly different from the Sham controls \((p = 0.04)\).

**Figure 6.** Serial changes in echocardiographically-determined LV Mass during the development of ischemic cardiomyopathy. Left ventricular mass normalized to body weight was similar among the three groups 1 week after initial surgery, and there was no statistically significant difference at one month \((p = 0.17)\). However, at two months after instrumentation estimated LV mass was significantly greater in the Sudden Death group as compared to both the Survivors \((p = 0.04)\) and Sham controls \((p < 0.001)\). There was also ventricular hypertrophy in the Survivors as compared to Sham controls \((p = 0.01)\).

**Figure 7.** Post-mortem left ventricular mass to body weight ratio. The post-mortem assessment of LV mass confirmed the echocardiographic estimates (Figure 6). The LV mass to body weight ratio in the animals that experienced sudden death was 30% greater than survivors \((p < 0.001)\) and 52% higher than sham-operated controls \((p < 0.001)\). In addition, LV mass was significantly greater in the survivors than Sham controls \((p = 0.04)\).

**Figure 8.** Regional connective tissue staining in animals with sudden death as compared to survivors. Regional connective tissue was quantified by point counting of trichrome-stained histology specimens from territories supplied by the three major coronary arteries (LAD, LC – left circumflex artery, RCA – right coronary artery). The average values in each region were almost identical in the Sudden Death and Survivor groups. Connective tissue was higher in the myocardial territories subjected to stenosis placement, but the differences between regions were not statistically significant \((ANOVA p = 0.14)\).

**Figure 9.** Kaplan-Meier survival curves for pigs with chronic 2 Vessel versus 1 Vessel stenoses. The survival curve for animals with both LAD and circumflex stenoses (2 Vessel) is shown in comparison to previously published data from animals with a single LAD stenosis (1 Vessel)(12). Although the physiological features of hibernating myocardium developed in both models, the greater volume of myocardium at risk of ischemia of ischemia with a second stenosis resulted in global LV dysfunction and a significantly greater risk of spontaneous sudden death.
REFERENCES


27. **Lacroix D, Gluais P, Marquie C, D'Hoinne C, Adamantidis M, and Bastide M.**


Table. Serial Echocardiographic Parameters.

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<th>Interventricular Septum (mm)</th>
<th>Posterolateral Wall (mm)</th>
<th>Left Ventricular Dimensions (mm)</th>
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* p < 0.05 vs. Sham control
Figure 1

Survival Time (Days)

Survival (%)

Sham

2 Vessel
Ventricular Tachycardia – Ventricular Fibrillation

Brady-asystole

VF Only

Figure 2
Anteroseptal ΔWT (mm)

* p<0.05 vs. Sham

Posterior ΔWT (mm)

Figure 3
Figure 4
Figure 5

End-Diastolic

End-Systolic

* p<0.05 vs. Sham
LV Mass/Body Weight (g/kg)

Figure 6

*p<0.05 vs. Sham
†p<0.05 vs. Survivor
Figure 7

LV Mass/Body weight (g/kg)

** p<0.05 vs. Sham
† † p<0.05 vs. Survivor

Sudden Death
Survivor
Sham
Connective Tissue Staining (%) vs. Coronary Artery:

- **LAD**
  - Sudden Death: [Value]
  - Survivor: [Value]

- **LC**
  - Sudden Death: [Value]
  - Survivor: [Value]

- **RCA**
  - Sudden Death: [Value]
  - Survivor: [Value]

*Figure 8*
Figure 9

Survival (%)

Days Post Instrumentation

1 Vessel Model

2 Vessel Model

p<0.01

Figure 9