Stability of Hibernating Myocardium in Pigs With a Chronic Left Anterior Descending Coronary Artery Stenosis: Absence of Progressive Fibrosis in the Setting of Stable Reductions in Flow, Function and Coronary Flow Reserve

James A. Fallavollita, MD, FACC, Michael Logue, MD, John M. Canty, Jr., MD, FACC

Buffalo, New York

OBJECTIVES

This study was performed to determine whether hibernating myocardium is adaptive or is destined to undergo progressive irreversible injury.

BACKGROUND

Previous studies have suggested that hibernating myocardium eventually results in progressive dysfunction. Since serial studies cannot be performed in humans, the temporal progression of physiologic and structural adaptations was evaluated in pigs with hibernating myocardium.

METHODS

Pigs were instrumented with a left anterior descending coronary artery (LAD) stenosis (1.5 mm) and underwent physiologic studies three to five months later to quantify regional function, perfusion and 18F-2-deoxyglucose (FDG) uptake. Viability was confirmed by histology and contractile reserve.

RESULTS

Hibernating myocardium was characterized by severe regional dysfunction (centerline score, $-1.9 \pm 0.1$), reduced resting subendocardial flow (LAD: $0.85 \pm 0.03$ vs. normal: $1.02 \pm 0.03$ ml/min/g, $p < 0.01$), critically reduced subendocardial flow reserve (adenosine flow: $1.04 \pm 0.09$ ml/min/g, $p = $ NS vs. rest; epinephrine flow: $0.88 \pm 0.07$ ml/min/g, $p = $ NS vs. rest) and increased FDG uptake ($0.022 \pm 0.002$ vs. $0.014 \pm 0.001$ ml/g/min, $p < 0.01$). Physiologic parameters were not different among animals studied at three (93 ± 1 days, $n = 27$), four (118 ± 2 days, $n = 26$) or five months (150 ± 6 days, $n = 9$). Pathology revealed a small increase in LAD connective tissue ($6.4 \pm 0.4\%$ vs. $4.0 \pm 0.2\%$, $p < 0.001$), with no change over this time frame.

CONCLUSIONS

Thus, physiologic and structural features of hibernating myocardium remain constant for at least two months. The absence of functional deterioration or progressive fibrosis suggests that hibernation is adaptive rather than an unstable physiology destined to progress to irreversible injury. The stability of this model appears ideally suited for interventions targeted to improve flow and function in chronically dysfunctional myocardium. (J Am Coll Cardiol 2001;37: 1989–95) © 2001 by the American College of Cardiology

The natural history of patients with viable, chronically dysfunctional myocardium is controversial. While retrospective studies concur that revascularization improves prognosis, the early survival after diagnosis in the absence of revascularization is variable. Some studies have demonstrated an increase in mortality within the first several months of clinical presentation (1,2), whereas others found early stability followed by an increased mortality after two to three years (3). One of the hypotheses set forth to explain the poor prognosis is that repetitive subendocardial ischemia ultimately progresses to irreversible injury (4). Challenging this are studies of patients with single-vessel disease in which regional dysfunction occurs without reductions in global left ventricular function. In this setting, pathological evaluation has demonstrated limited fibrosis, a reversion to a fetal myocyte phenotype and an absence of degenerative changes (5–7).

Unfortunately, there are no longitudinal data regarding the temporal progression of physiologic or pathologic abnormalities in patients with chronically dysfunctional myocardium in which viability was confirmed by preserved metabolism, contractile reserve or recovery of function after revascularization. In addition, there is frequently no distinction made between viable, chronically dysfunctional myocardium with reduced resting flow (hibernating myocardium) (8,9) as compared with regions with normal resting perfusion (chronically stunned myocardium) (10). While both of these physiologic states develop in response to episodes of reversible ischemia and chronic stunning can eventually result in hibernating myocardium (10), the molecular mechanisms probably differ (11) and may differentially affect the natural history of the disease.

Since the relation among physiologic variables, myocardial fibrosis and survival cannot be studied in a prospective fashion in patients, we evaluated the temporal progression of flow, function, 18F-2-deoxyglucose (FDG) uptake and fibrosis in an established porcine model of hibernating...
myocardium (9). We have previously reported that this model gradually progresses to produce a critical reduction in coronary flow reserve that results in a temporal transition from viable dysfunctional myocardium with normal resting flow (chronic stunning) to viable dysfunctional myocardium with depressed resting flow (chronic hibernation) over a period of three months (12). Whether this remains stable, spontaneously improves or is associated with progressive functional and structural deterioration is unknown.

This study was designed to evaluate the temporal stability of hibernating myocardium with the following three objectives: first, we sought to determine whether endogenous angiogenesis of the collateral circulation resulted in spontaneous improvement in coronary flow reserve and function in hibernating myocardium; second, we quantified connective tissue in these animals to test the hypothesis that hibernating myocardium is unstable and destined to progress to structural fibrosis in the absence of revascularization; and finally, we retrospectively evaluated survival in pigs with viable, chronically dysfunctional myocardium to determine if chronically stunned and hibernating myocardium affect prognosis differently.

METHODS

All experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research. The initial instrumentation and experimental protocol have been previously published in detail (9,13). To specifically evaluate temporal changes in this model, 29 juvenile pigs were instrumented with a 1.5 to 2.25 mm Delran stenosis on the proximal left anterior descending artery (LAD) and studied 124 ± 28 days after instrumentation. The results of the studies of these animals were pooled with the results of studies of 41 animals previously reported (9,13,14). All animals were instrumented in an identical fashion and housed under similar conditions. Animals were excluded for myocardial infarction encompassing >1% of the left ventricular mass (n = 5) or if microsphere measurements of absolute regional perfusion were unavailable (n = 3). Thus, 62 animals were included in this study.

All studies were conducted in the closed-chest anesthetized state as previously described (9,13,14). After an overnight fast, anesthesia was induced with a Telazol (tiletamine 50 mg/ml and zolazepam 50 mg/ml)/xylazine (100 mg/ml) mixture (0.022 ml/kg intramuscularly) and maintained with halothane or isoflurane (1% to 3%) supplemented with additional Telazol/xylazine (0.011 ml/kg intramuscularly as needed). Catheters were placed into the left atrium (or left ventricle [LV]) for microsphere injections and the LV for contrast ventriculography. Pressure and reference withdrawal samples for microspheres were taken from a carotid or femoral artery. Pharmacologic agents were administered through a jugular vein. Animals were hepatized (100 U/kg intravascularly) and hemodynamics allowed to equilibrate for approximately 30 min.

Regional perfusion was assessed with colored microspheres (9,13,14). After resting flow measurements, myocardial function was assessed with contrast ventriculography (9,13,14), and anteroapical wall motion was quantified by centerline analysis (12). Flow and function were then evaluated during inotropic stimulation with a submaximal epinephrine infusion titrated to increase heart rate by approximately 40 beats/min (0.21 ± 0.02 μg/kg/min intravascularly). A final flow measurement was performed during adenosine vasodilatation (0.9 mg/kg/min intravascularly) with phenylephrine titrated to maintain arterial pressure (6.8 ± 0.4 μg/kg/min intravascularly). Caliper measurements of stenosis severity were obtained during coronary angiography (9,13). Angiography could not be performed in five pigs. In pigs with an occluded LAD, collateral circulation was semiquantitatively assessed using the following scoring system: 0: no collaterals, 1: faint opacification of the LAD, 2: delayed but complete opacification of the LAD, 3: rapid and complete filling of the LAD (9).

A subgroup of animals (n = 37) received FDG (1 to 3 mCi intravascularly) approximately 1 h after the last pharmacologic intervention, as previously described (9,14). After 45 min, the heart was arrested with intravenous KCl and rapidly excised. A midventricular ring was divided into 12 full-thickness wedges, then subdivided into subendocardial, midmyocardial and subepicardial layers. Samples were weighed and counted at 511 keV with a germanium well detector (Canberra Inc.) (9) or sodium iodide detector (Model 1470, Wallac Inc.) (14). The same samples were used for microsphere flow determinations as previously described (9,14). Additional samples from the LAD and normally perfused regions (n = 52 each) were trichrome-stained to quantify connective tissue by standard point counting techniques (9,13,14).

Data analysis. Data are presented as the mean ± SE. Flow and FDG in the LAD and normal regions represent weighted means for all samples within a given region (9,12–14). Measurements in LAD and normally perfused regions were compared using paired t tests. Differences between interventions or between groups were assessed using an analysis of variance and t tests with the Bonferroni correction for multiple comparisons. A p value of <0.05 was considered statistically significant.
RESULTS

Pigs were grouped according to the time after initial instrumentation (three months [75 to 104 days], mean 93 ± 6 days, n = 27; four months [105 to 134 days], mean 118 ± 6 days, n = 26; five months [>134 days], mean 150 ± 6, n = 9). Hematocrit and blood gas values were no different among groups (hematocrit: 0.33 ± 0.01, pH: 7.40 ± 0.00, pCO2: 41.9 ± 0.8, pO2: 490 ± 12). Left ventriculography demonstrated severe anterior hypokinesis (centerline score, 2.1 ± 0.1; normal 5.0) with an average ejection fraction (EF) of 49 ± 1%. The proximal LAD was severely stenotic with total occlusion and collateral-dependent myocardium in the majority of animals (68%). Resting hemodynamics, LAD stenosis severity, collateral score and ventricular function were no different over time (Table 1).

Paired samples from hibernating LAD and normal remote regions were evaluated to quantify temporal changes in myocardial connective tissue. Represented photomicrographs from animals studied at three, four and five months are shown in Fig. 1. There was a small increase in connective tissue staining in the hibernating LAD region as compared with remote, normal myocardium in each group (Table 2; average values: LAD: 6.4 ± 0.4% vs. normal: 4.0 ± 0.2%, p < 0.001). Most of this constituted an increase in the normal collagen network since replacement fibrosis was rare. Thus, there was no evidence of a time-dependent increase in fibrosis or development of necrosis in hibernating myocardium.

Like the pathologic and hemodynamic variables, regional perfusion remained remarkably constant over this period (Table 2). The flow distribution in the hibernating LAD and normally perfused remote regions are plotted in Fig. 2. There were no differences in resting flow or flow during submaximal epinephrine infusion over time. Resting subendocardial flow was significantly reduced in comparison to the corresponding normal region at each time point. During

| Table 1. Resting Hemodynamics, Stenosis Severity and Function |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Heart Rate (beats/min)          | Systolic Pressure (mm Hg) | EDP (mm Hg) | Stenosis Severity (%) | Collaterals | EF (%) | Centerline Score (Normal = 0) |
| 3 Months 83 ± 3                 | 127 ± 4         | 16.3 ± 1.0     | 94 ± 2            | 2.6 ± 0.1 | 1.9 ± 0.2 | 50 ± 2         | −1.9 ± 0.2     |
| 4 Months 83 ± 3                 | 129 ± 3         | 17.5 ± 1.0     | 92 ± 3            | 2.1 ± 0.2 | 2.5 ± 0.2 | 48 ± 2         | −1.9 ± 0.2     |
| 5 Months 82 ± 5                 | 144 ± 4         | 18.3 ± 1.5     | 98 ± 2            | 2.5 ± 0.5 | 2.3 ± 0.3 | 52 ± 4         | −1.7 ± 0.2     |

EDP = left ventricular end-diastolic pressure; EF = ejection fraction.

Figure 1. Represented trichrome images from pigs with hibernating myocardium. Images from representative animals studied three, four or five months after instrumentation were stained with Masson’s trichrome to facilitate quantification of connective tissue (blue). The left anterior descending coronary artery regions (upper row) illustrate the generalized and diffuse connective tissue staining pattern that was present in hibernating myocardium. Total connective tissue staining was slightly higher in hibernating myocardium as compared with the normally perfused region of the same animal (lower row). Magnification 600X.
submaximal epinephrine infusion, heart rate increased to 125 ± 3 beats/min (p < 0.01 vs. rest) with no change in LV systolic (131 ± 3 mm Hg) or end-diastolic pressure (EDP) (15.9 ± 0.9 mm Hg). This submaximal increase in demand resulted in an approximately 50% increase in flow in normally perfused myocardium. In contrast, subendocardial flow to the LAD region was unable to increase significantly above resting values (Fig. 2). Despite this, indexes of global function (EF: 49 ±% 1 to 56 ±% 2, p < 0.001) and anterior wall motion (centerline score: −1.9 ± 0.1 to −1.2 ± 0.1, p < 0.001) improved with inotropic stimulation confirming viability.

During adenosine infusion, heart rate (83 ± 2 to 88 ± 2 beats/min, p < 0.01) and LV EDP (17.0 ± 0.6 to 19.4 ± 0.8 mm Hg, p < 0.01) were slightly increased over control values with no change in systolic pressure (130 ± 2 to 133 ± 3 mm Hg, p = NS). Vasodilation resulted in five- to six-fold increases in flow to the normal region (Fig. 2). In contrast, subendocardial LAD flow did not increase over resting values (Table 2, Fig. 2). There were no differences in hemodynamics or adenosine flow among animals studied at the three time points. In addition, when only animals with an occluded LAD were evaluated, there were no temporal changes in regional flow at rest or during vasodilation. Thus, pigs with hibernating myocardium developed a critical impairment of subendocardial flow reserve that remained stable for at least two months.

Table 2. Subendocardial Perfusion (ml/min/g) and Connective Tissue Staining (%)

<table>
<thead>
<tr>
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<th>3 Months</th>
<th>4 Months</th>
<th>5 Months</th>
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<tr>
<td></td>
<td>LAD</td>
<td>Normal</td>
<td>LAD</td>
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<tr>
<td></td>
<td>n = 27</td>
<td>n = 27</td>
<td>n = 26</td>
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<tr>
<td>Rest</td>
<td>0.82 ± 0.05*</td>
<td>1.05 ± 0.06</td>
<td>0.90 ± 0.06*</td>
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<tr>
<td>Epinephrine</td>
<td>0.80 ± 0.08*</td>
<td>1.53 ± 0.09†</td>
<td>0.98 ± 0.12*</td>
</tr>
<tr>
<td>Adenosine</td>
<td>1.05 ± 0.14*</td>
<td>4.29 ± 0.22†</td>
<td>1.06 ± 0.16*</td>
</tr>
<tr>
<td>Connective Tissue</td>
<td>6.0 ± 0.6*</td>
<td>3.8 ± 0.3</td>
<td>6.9 ± 4.4*</td>
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*p < 0.05 vs. normal; †p < 0.05 vs. rest.
LAD = left anterior descending coronary artery.

There are three major new findings from this investigation. First, intrinsic angiogenesis was insufficient to result in spontaneous improvement in the physiologic abnormalities associated with hibernating myocardium, and regional flow reserve, function and FDG uptake remained unchanged over at least two months. Second, we found no evidence of progressive structural injury in hibernating myocardium. Connective tissue content remained low and did not increase over the period of study. Finally, while sudden death was common in this model, it frequently occurred before the development of hibernating myocardium. The stability of the physiologic and pathologic findings over this time frame indicate that the untoward short-term prognosis of patients with viable, chronically dysfunctional myocardium is not explained by progressive fibrosis or deterioration in function. On the contrary, they support the view that hibernating myocardium is an adaptive response that can be stable over at least the two-month time frame evaluated in our study.

**Lack of pathologic progression in hibernating myocardium.** Previous studies have demonstrated that increased connective tissue in chronically dysfunctional myocardium is a major determinant of reversibility (6,16). Schwarz et al. (4) attempted to estimate the duration of hibernation before revascularization in patients with globally reduced LV function. They concluded that a longer duration of clinical symptoms (>50 days) was associated with lower preoperative function and greater degrees of myocyte degeneration and fibrosis. Not surprisingly, they and others have found that patients with the most fibrosis had the worst recovery of function after revascularization (6,16). A similar, progressive increase in regional fibrosis and global dysfunction was also supported by a study of prolonged, moderate ischemia in pigs (17). While these data do not directly address the structural and functional stability of hibernating myocardium, they have supported the conclusion that hibernating myocardium is an adaptive response that can be stable over at least two months.
myocardium requires immediate revascularization since it is destined to undergo inexorable progression to fibrosis. Other studies in patients with collateral-dependent myocardium and single-vessel coronary artery disease have shown less pronounced increases in connective tissue in hibernating myocardium, and the structural changes suggest a reversion to a fetal myocyte phenotype in response to stress (5,6,16). These findings have led other investigators to hypothesize that hibernating myocardium is an adaptive mechanism to preserve myocyte viability in the setting of a chronic limitation in coronary flow reserve. Our data support this latter concept in that the regional increase in connective tissue in our porcine model of hibernating myocardium was similar to that reported in humans with collateral-dependent myocardium (7). Importantly, we found no progression in fibrosis despite a chronic limitation in subendocardial flow reserve over a two-month time frame. These observations indicate that myocardial fibrosis does not progress over the short term nor does progressive LV dysfunction over this time frame account for the poor survival attributed to viable, chronically dysfunctional myocardium. Thus, the discordant degree of reported fibrosis among clinical studies is likely related to evaluating different patient populations. Coexisting fibrosis from subendocardial infarction or progressive fibrosis from global LV dysfunction may be confounding factors in patients with ischemic cardiomyopathy and depressed EFs versus patients with regional dysfunction from single-vessel coronary artery disease.

Stability of physiologic abnormalities in hibernating myocardium. The stability of resting and vasodilated coronary flow in this model, characterized by the frequent presence of collateral-dependent myocardium, supports the notion that intrinsic stimuli to promote angiogenesis are insufficient to result in spontaneous functional improvement in pigs with hibernating myocardium. This lack of spontaneous improvement in coronary flow reserve contrasts with several other animal models that employed ameroid occlusion to stimulate coronary collateral growth. Canty and Klocke (18) produced a state of chronic stunning that was followed by relative reductions in resting flow and function consistent with hibernating myocardium in dogs with limited collateral vasodilator reserve. Nevertheless, in this model, spontaneous angiogenesis over a period of three weeks resulted in the normalization of function that reflected the rapid improvement in collateral vasodilator reserve. O’Konski et al. (19) evaluated flow during exercise and dipyridamole vasodilation 24 days after instrumenting pigs...
with a circumflex ameroid occluder. Flow during dipyridamole increased to over 2 ml/min/g as compared with 3 to 4 ml/min/g in remote myocardium. While reduced, vasodilated flow was much higher than what we found in pigs with hibernating myocardium in this study (approximately 1 ml/min/g). White et al. (20) evaluated temporal changes in adenosine flow reserve in pigs with a circumflex ameroid studied up to four months after instrumentation. Subepicardial flow during adenosine was not reduced as compared with normal remote regions or sham control pigs. Subepicardial adenosine flow increased from a nadir of 1.04 ml/min/g at three weeks to 1.42 ml/min/g 13 weeks after ameroid placement. While this was still reduced, maximum subendocardial flow in shams only increased to 1.64 ml/min/g because of adenosine-induced hypotension. In this study, both subendocardial and subepicardial flow were severely reduced in comparison with normal remote regions (Fig. 2). Differences in perfusion pressure make it impossible to directly compare the results of White et al. (20) with ours; however, estimates of vasodilated coronary vascular resistance in this study were two-fold higher than those in the circumflex ameroid model (approximately 90 vs. 45 mm Hg/ml/min/g). As summarized in Table 2, vasodilated flow remained constant in our model for at least two months.

This difference in collateral flow reserve in pigs with a fixed LAD stenosis versus circumflex ameroid occlusion likely reflects two factors. First, collateral flow per gram is partially determined by the size of the risk region (21), and the LAD perfusion territory is much larger than that of the circumflex (22). Second, the porcine circumflex ameroid model is associated with extracardiac collaterals from the descending thoracic aorta that may contribute to higher collateral flow reserve (20). These differences could explain why porcine circumflex ameroid models do not usually develop reductions in resting flow typical of hibernating myocardium (23). Thus, the chronic critical limitation in flow reserve in our model indicates that it will be useful to examine interventions intended to modulate regional flow and function in hibernating myocardium.

**Temporal progression of viable, chronically dysfunctional myocardium and survival.** We have previously demonstrated that there is a progression from chronically stunned to hibernating myocardium during progressive LAD stenosis in pigs. As early as one month after instrumentation, limited subendocardial flow reserve resulted in regional dysfunction associated with normal resting flow and normal FDG uptake (12). As coronary flow reserve fell further at two months, FDG uptake increased, but resting flow continued to be normal (12). Both time points demonstrated regional dysfunction with normal resting flow consistent with chronic stunning. Thereafter, the physiologic findings of hibernating myocardium developed and, as demonstrated in this study, remained stable for up to five months. This systematic temporal progression supports the view that impaired coronary flow reserve is the primary determinant of resting contractile dysfunction and the phenotypic changes of hibernating myocardium. Indeed, we have recently demonstrated that the temporal progression can be contracted from three months to two weeks by critically reducing flow reserve to values slightly greater than resting flow in chronically instrumented pigs (24).

As shown in Fig. 4, there was a risk of sudden death in this model that began approximately two weeks after instrumentation. Interestingly, much of the mortality occurred before the development of hibernating myocardium at three months. The transition period from chronic stunning to hibernation appears to be a particularly vulnerable time for spontaneous, ischemia-induced sudden death. Clinical studies do not typically characterize the time course of physiologic abnormalities nor do they distinguish between chronically stunned and hibernating myocardium. This could partially explain the differences in short-term prognosis of patients with viable, chronically dysfunctional myocardium (1,3). Unfortunately, we cannot comment on any benefits of therapy to reduce ischemia, such as the clinically held notion that revascularization may improve survival in this patient population.

**Methodologic limitations.** Although we found that the physiologic and pathologic findings of hibernating myocardium were stable between three and five months after instrumentation in this model, we cannot exclude the possibility that spontaneous improvement or progressive dysfunction might occur at a later time point. In addition, we are unable to comment on the stability of dysfunction in individual animals and to what extent acute stunning may be superimposed on chronic hibernation (25). Finally, this study does not specifically address the mechanism(s) for the stability of hibernating myocardium, and it is unclear whether some of the adaptations responsible for this involve signaling pathways that are similar to those of ischemic preconditioning.
Summary. Our results demonstrate remarkable stability of the physiologic and structural alterations in hibernating myocardium and, thus, are at odds with the hypothesis that hibernating myocardium is inherently destined to undergo progressive fibrosis and structural degeneration (4,17). Rather, our findings support the notion that hibernating myocardium is an intrinsic myocardial adaptation to prevent progressive irreversible injury in the setting of chronic repetitive ischemia (10,26). Like clinical studies, our data indicate that there is an increased risk for sudden cardiac death with viable, chronically dysfunctional myocardium. Further studies will be required to determine whether this is due to ventricular arrhythmias arising from transient ischemia or the result of cellular and structural remodeling that we have previously demonstrated in hibernating myocardium (13,15). Finally, in contrast with ameroid models in pigs and dogs that show an improvement in flow and function due to spontaneous angiogenesis over a period of several weeks, our findings show stable coronary flow reserve and function for at least two months. Thus, this model may be ideally suited to study pharmacologic and mechanical interventions intended to improve flow and function in viable, chronically dysfunctional myocardium.

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Reprint requests and correspondence: Dr. James A. Fallavollita, Biomedical Research Building, Room 347, Department of Medicine/Cardiology, University at Buffalo, 3435 Main Street, Buffalo, New York 14214. E-mail: jaf7@buffalo.edu.

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