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Revascularization of Chronic Hibernating Myocardium Stimulates Myocyte Proliferation and Partially Reverses Chronic Adaptations to Ischemia

Brian J. Page, MD,* Michael D. Banas, MD,* Gen Suzuki, MD, PhD,* Brian R. Weil, PhD,* Rebeccah F. Young, MS,* James A. Fallavollita, MD,*† Beth A. Palka, BS,* John M. Canty, Jr, MD*†‡

ABSTRACT

BACKGROUND The time course and extent of recovery after revascularization of viable dysfunctional myocardium are variable. Although fibrosis is a major determinant, myocyte structural and molecular remodeling may also play important roles.

OBJECTIVES This study sought to determine whether persistent myocyte loss and/or irreversibility of protein changes that develop in hibernating myocardium have an impact on functional recovery in the absence of infarction.

METHODS Swine implanted with a chronic left anterior descending artery (LAD) stenosis to produce hibernating myocardium underwent percutaneous revascularization, with serial functional recovery evaluated for 1 month (n = 12). Myocardial tissue was evaluated to assess myocyte size, nuclear density, and proliferation indexes in comparison with those of normal animals and nonrevascularized controls. Proteomic analysis by 2-dimensional differential in-gel electrophoresis was used to determine the reversibility of molecular adaptations of hibernating myocytes.

RESULTS At 3 months, physiological features of hibernating myocardium were confirmed, with depressed LAD wall thickening and no significant infarction. Revascularization normalized LAD flow reserve, with no immediate change in LAD wall thickening. Regional LAD wall thickening slowly improved but remained depressed 1 month post–percutaneous coronary intervention. Surprisingly, revascularization was associated with histological evidence of myocytes re-entering the growth phase of the cell cycle and increases in the number of c-Kit$^+$ cells. Myocyte nuclear density returned to normal, whereas regional myocyte hypertrophy regressed. Proteomic analysis demonstrated heterogeneous effects of revascularization. Up-regulated stress and cytoskeletal proteins normalized, whereas reduced contractile and metabolic proteins persisted.

CONCLUSIONS Delayed recovery of hibernating myocardium in the absence of scar may reflect persistent reductions in the amounts of contractile and metabolic proteins. Although revascularization appeared to stimulate myocyte proliferation, the persistence of small immature myocytes may have contributed to delayed functional recovery.
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Hibernating myocardium is characterized by viable, dysfunctional myocardium that develops as an adaptive response to chronic repetitive ischemia from a flow-limiting stenosis. It arises from a severe impairment in coronary flow reserve (1,2), which leads to myocyte apoptosis with regional myocyte loss, compensatory cellular hypertrophy (3), reduced myocardial oxygen consumption

From the *UB Clinical and Translational Research Center and Department of Medicine, University at Buffalo, Buffalo, New York; †VA Western New York Health Care System, Buffalo, New York; and the ‡Department of Physiology and Biophysics and Department of Biomedical Engineering, University at Buffalo, Buffalo, New York. This project was supported by National Heart, Lung, and Blood Institute grants HL-55324 and HL-61610 and by the Albert and Elizabeth Rekate Fund. All authors have reported that they have no relationships relevant to the contents of this paper to disclose.
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(4), regional down-regulation of enzymes involved in oxidative metabolism, and up-regulation of stress proteins that allow the heart to adapt and prevent infarction (5). In some patients, these adaptations are incomplete, and progressive fibrosis and myocyte loss develops. In others, these adaptations ultimately minimize stress-induced ischemia, which prevents further myocyte death, but at the expense of chronic regional contractile dysfunction.

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Although contractile dysfunction in hibernating myocardium can improve after revascularization, complete recovery is infrequent (<25% of patients) (6,7), and 1 in 4 dysfunctional segments without fibrosis (determined by magnetic resonance imaging) fail to improve (8). The time course of functional recovery is also variable. Different studies have demonstrated rapid recovery (9), recovery within several weeks (10–12), and delayed improvement requiring 1 year (13–15). This protracted course contrasts with the complete normalization of function within hours or days following acute stunning and short-term hibernation (16). The roles of myocyte loss (3) and myocardial protein changes (5,17) in the delayed response to revascularization are unclear.

We performed percutaneous revascularization of swine with chronic hibernating myocardium to determine the initial time course of functional recovery and the cellular mechanisms contributing to persistent dysfunction in the absence of scar. We demonstrated that revascularization of hibernating myocardium stimulated myocyte proliferation (**Central Illustration**). Nevertheless, functional improvement was delayed and incomplete, with persistent reductions in metabolic and contractile proteins 1 month after revascularization.

METHODS

Experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research and were approved by the University at Buffalo Institutional Animal Care and Use Committee.

CORONARY ARTERY INSTRUMENTATION. Juvenile farm-bred pigs (8 to 10 kg) were chronically implanted with a left anterior descending artery (LAD) stenosis to produce hibernating myocardium using a modification of previously published techniques (1). Fasted pigs pre-medicated with tiletamine 50 mg/ml and zolazepam 50 mg/ml (tiletamine)/ketamine 100 mg/ml (0.037 ml/kg intramuscular [IM]), cefazolin 0.5 g intravenous (IV), and gentamicin 40 mg IV were intubated and anesthetized with isoflurane (1% to 2%). A thoracotomy was performed in the fourth left intercostal space. The proximal LAD was implanted with a short piece of expandable 1.5-mm internal diameter silicone tubing with a longitudinal slit for vessel insertion (18). It was secured with circumferential sutures that could be expanded using standard angioplasty balloon inflation pressures. The chest was closed, intercostal nerves were infiltrated with 2% lidocaine, and the pneumothorax was evacuated. Postoperative antibiotics and analgesics (butorphanol 0.025 mg/kg) were administered as needed for pain.

SERIAL PHYSIOLOGICAL STUDIES AND PERCUTANEOUS INTERVENTION. Pigs with hibernating myocardium undergoing revascularization (n = 12) were compared with nonrevascularized animals (n = 12) and normal sham controls that underwent stent insertion (n = 10). Animals underwent baseline physiological studies at approximately 3 months post-instrumentation in the closed-chest sedated state (tiletamine/zolazepam/

ABBREVIATIONS AND ACRONYMS

CSC = cardiac stem cell

LAD = left anterior descending artery

LADΔWT = LAD wall thickening (end-systolic − end-diastolic wall thickness)

LV = left ventricular

PCI = percutaneous coronary intervention

TABLE 1 Hemodynamics in Revascularized and Nonrevascularized Animals With Hibernating Myocardium

	Heart Rate, beats/min	LV Systolic Pressure, mm Hg	LVEDP, mm Hg	LV dP/dt$_{Max}$, mm Hg/s	LV dP/dt$_{Min}$, mm Hg/s	Regional Wall Thickening			
						WT, %		ΔWT, mm	
						LAD	Remote	LAD	Remote
Revascularized									
Baseline	108 ± 4	108 ± 14	28 ± 3	2,549 ± 165	-2,467 ± 148	38.6 ± 4.0‡	92.2 ± 7.5	2.9 ± 0.3‡	5.8 ± 0.3
2 h post-PCI	105 ± 6	113 ± 6	30 ± 3	1,988 ± 89*	-2,247 ± 125	35.2 ± 4.6‡	86.6 ± 7.2	2.9 ± 0.4‡	5.7 ± 0.4
1 Month post-PCI	101 ± 2	128 ± 5	28 ± 3	2,439 ± 136†	-2,514 ± 96	58.6 ± 4.9*‡	86.9 ± 6.9	4.6 ± 0.4*‡	6.3 ± 0.3
Nonrevascularized									
Baseline	107 ± 5	121 ± 4	23 ± 2	2,284 ± 168	-2,225 ± 154	34.0 ± 2.1‡	80.6 ± 8.4	3.5 ± 0.3‡	6.1 ± 0.4
1 Month	103 ± 5	120 ± 4	23 ± 2	2,073 ± 69	-2,184 ± 139	26.5 ± 3.2‡	76.9 ± 5.4	2.8 ± 0.3‡	7.2 ± 0.5

Values are mean ± SEM. Baseline measurements were made 3 months after surgical placement of a LAD stenosis. *p < 0.05 versus baseline. †p < 0.05 versus 2 h post-PCI. ‡p < 0.05 LAD versus remote.
LAD = left anterior descending artery; LV = left ventricular; LVEDP = left ventricular end-diastolic pressure; PCI = percutaneous coronary intervention; WT = wall thickening.