Strain-related regional alterations of calcium-handling proteins in myocardial remodeling

Ahmet Kilic, MD,[a] Tieluo Li, MD,[a] Timothy D. C. Nolan, MS,[a] Jennifer R. Nash, MS,[a] Shuying Li, MD,[b] Deyanira J. Prastein, MD,[a] Gary Schwartzbauer, PhD,[a] Sina L. Moainie, MD,[a] G. Kwame Yankey, MD,[a] Christopher DeFilippi, MD,[b] Zhongjun Wu, PhD,[a] and Bartley P. Griffith, MD[a]

Background: Cardiac remodeling has been shown to have deleterious effects at both the global and local levels. The objective of this study is to investigate the role of strain in the initiation of structural and functional changes of myocardial tissue and its relation to alteration of calcium-handling proteins during cardiac remodeling after myocardial infarction.

Methods: Sixteen sonomicrometry transducers were placed in the left ventricular free wall of 9 sheep to measure the regional strain in the infarct, adjacent, and remote myocardial regions. Hemodynamic, echocardiographic, and sonomicrometry data were collected before myocardial infarction, after infarction, and 2, 6, and 8 weeks after infarction. Regional myocardial tissues were collected for calcium-handling proteins at the end study.

Results: At time of termination, end-systolic strains in 3 regionally distinct zones (remote, adjacent, and infarct) of myocardium were measured to be -14.65 ± 1.13, -5.11 ± 0.60 ($P \leq .05$), and 0.92 ± 0.56 ($P \leq .05$), respectively. The regional end-systolic strain correlated strongly with the abundance of 2 major calcium-handling proteins: sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase subtype 2a ($r^2 = 0.68$, $P \leq .05$) and phospholamban ($r^2 = 0.50$, $P \leq .05$). A lesser degree of correlation was observed between the systolic strain and the abundance of sodium/calcium exchanger type 1 protein ($r^2 = 0.17$, $P \leq .05$).

Conclusions: Regional strain differences can be defined in the different myocardial regions during postinfarction cardiac remodeling. These differences in regional strain drive regionally distinct alterations in calcium-handling protein expression.

From the Division of Cardiac Surgery,[a] Department of Surgery, and Division of Cardiology,[b] Department of Medicine, University of Maryland School of Medicine, Baltimore, Md.

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Address for reprints: Bartley P. Griffith, MD, University of Maryland School of Medicine, Department of Cardiothoracic Surgery, 22 S. Greene St, UMMS, N4W94, Baltimore, MD 21201 (E-mail: bgriffith@smail.umaryland.edu).

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After amyocardial infarction (MI), the heart undergoes alterations at the myocyte level that lead to changes in global function, known as remodeling.[1] The International Forum on Cardiac Remodeling defines the change as, "genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape, and function of the heart after cardiac injury."[2] Although initially an adaptive and compensatory mechanism, progressive cardiac remodeling has a deleterious and negative impact.

Gradients of electromechanical function and associated protein expression occur among 3 histologically different zones.[3-5] The 3 distinct zones include (1) the relatively normal healthy myocardium (remote zone), (2) the nonischemic but hypokinetic area near the MI (adjacent zone), and finally (3) the area of fibrosis and scar (infarct zone). The loss of mechanical function and its associated gene expression of calcium handling in the infarct zone is due to ischemic necrosis; however, the differences between the nonischemic adjacent and remote zones are less easily understood. We have been interested in the regional differences in post-MI strain that might explain alterations in regional function through mechanotransduction.

Abbreviations and Acronyms

LV = left ventricular
MI = myocardial infarction
NCX-1 = sodium/calcium exchanger type 1
PLB = phospholamban
SERCA2a = sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase subtype 2a

Methods

Surgical Protocol

Twelve Dorsett hybrid sheep between 50 and 70 kg and bred for laboratory use (Thomas Morris, Reisterstown, Md) were used in the study. Nine animals were instrumented with subsequent creation of an anterior MI. The sheep were allowed to recover and survived for 8 to 12 weeks after the initial MI. Three noninstrumented animals were used for healthy tissue controls. All the animals received treatment in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985). The surgical procedures and postoperative care were carried out according to the approved protocol by the Institutional Animal Care and Use Committee of the University of Maryland at Baltimore.

Anesthesia was induced by thiopental sodium (10 mg/kg) and maintained by 1% to 2% isoflurane (Draeger anesthesia monitor, North American Draeger, Telford, Pa). Surface electrocardiogram, arterial blood pressure, pulse oximeter, and esophageal temperature were continuously monitored for each animal during the operations described. The instrumented group underwent a left anterolateral thoracotomy with excision of the left fifth rib. Polypropylene snares were placed around the first and second diagonal coronary arteries of the left anterior descending artery and passed through pressure tubing. The snares were momentarily tightened (<30 seconds) to demarcate the border of the future infarct. Four specific transducers (2 mm; Sonometrics Corporation, London, Ontario, Canada) were placed at the superior, inferior, medial, and lateral aspects of the transiently ischemic myocardium with an additional transducer placed in the center of this ischemic region. An additional 11 transducers were sutured into the midmyocardium of the left ventricular (LV) free wall to create a final array of 3 short-axis aligned rows of 5 transducers with an additional transducer in the apex. The wires of the transducers were secured together with silk ties, tunneled subcutaneously, and their respective skin buttons exposed to allow for future data acquisition. The coronary snares were tunneled subcutaneously for subsequent permanent vessel occlusion. An ultrasonic flow probe (20 mm; Transonic Systems, Inc, Ithaca, NY) was placed around the main pulmonary artery for cardiac output monitoring. An atrial port silicone catheter (9F; Access Technologies, Stokie, Ill) was placed into the left atrium and placed in a subcutaneous pocket for future myocardial perfusion measurements.

Infarction

Seven to 10 days later, the sheep were reanesthetized and a catheter-tip mounted pressure transducer (SPC 350; Millar Instruments, Inc, Houston, Tex) was placed by fluoroscopy into the LV apex via the femoral artery. A midline laparotomy was made for subdiaphragmatic echocardiographic imaging. After all preinfarction baseline data (sonomicrometry, echocardiogram, hemodynamics) were recorded, the subcutaneous snares were permanently tightened to cause an anterior MI and the animal was supported with epinephrine infusion. The epinephrine infusion (240 μg/h) was started at the time of initial snare occlusion and lasted for 15 minutes, at which point the ionotrope was serially weaned in increments of 60 μg/h every 5 minutes until the animal was off epinephrine support. The induction of MI was seen as electrocardiographic changes initially and later confirmed by echocardiography. After an additional 15 minutes off epinephrine support, all immediate post-MI data were collected. The midline incision was closed and the animal was allowed to recover.

Data Collection

Transdiaphragmatic echocardiograms with sonomicrometry and hemodynamic data were collected at the time of infarction (before and after MI), 2 weeks and 6 weeks after MI, and at the time of terminal study. Echocardiograms were collected with a Sonos 5500 machine with a sterile covered transducer (Philips Medical, Andover, Mass). Sonomicrometry data were collected with a commercially available digital sonomicrometry system (Sonometrics Corporation). The pulmonary artery flow rate was measured with a transonic flowmeter (T401; Transonic Systems). Distance between all pairs of 16 transducers (120 unique distances) was measured at a sampling rate of 200 samples/sec, in real time, and synchronized with LV pressure and pulmonary artery flow.

Data Analysis

The LV short-axis views at the tips of the papillary muscles, at the base of the papillary muscles, and at the apex along with long-axis views were studied. LV volumes and infarct size expressed as percentage of endocardial circumference were measured and the ejection fraction was calculated by the Bullet formula.[6]

By use of the signal post-processing software and multidimensional scaling algorithm available from Sonometrics (Sonoview and Sonoxyz), the distances between the implanted 16 sonomicrometry transducers were first filtered to remove noises, and the instantaneous location of each transducer in a single 3-dimensional coordinate system was determined. The coordinate data were used to determine 3-dimensional motion and deformation of the LV free wall. The arrangement of the transducers consisted of 3 groups of 5 placed circumferentially along the LV free wall, with 1 transducer being placed into the LV apex.

The strain measure during an individual cardiac cycle is often referred to as the systolic strain whereas the strain measure used over time is referred to as the remodeling strain.[7,8] For the present study, an area strain measure was used and calculated by comparing the area change of the paired triangles between the reference frame and the deformed frames. Strain measurements were then calculated from the collected sonomicrometry transducer coordinate data to compile (1) an end-systolic regional strain and (2) an end-diastolic (remodeling) strain over the progression of myocardial remodeling.

The end-systolic regional strain was calculated with the LV free wall deformation during an individual cardiac cycle to