Regional remodeling strain and its association with myocardial apoptosis after myocardial infarction in an ovine model

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Supplemental material is available online.

Objective: Progressive left ventricular remodeling after myocardial infarction has been viewed as an important contributor to progressive heart failure. The objective of this study was to investigate the relationship between myocardial apoptosis and strain during progressive cardiac remodeling.

Methods: Before creation of an anterolateral left ventricular infarction by ligation of diagonal arteries, 16 sonomicrometry transducers were placed in the left ventricular free wall of 8 sheep to assess regional deformation in the infarct, adjacent, and normally perfused remote myocardial regions over 8 weeks' duration. Hemodynamic, echocardiographic and sonomicrometric data were collected before infarction and then 30 minutes and 2, 6, and 8 weeks after infarction. At the end of the study, regional myocardial tissues were collected for apoptotic signaling proteins.

Results: At terminal study, an increase in left ventricular end-diastolic pressure of 8.1 ± 0.1 mm Hg, a decrease in ejection fraction from 54.19% ± 5.68% to 30.55% ± 2.72%, and an end-diastolic volume increase of 46.08 ± 5.02 mL as compared with the preinfarct values were observed. The fractional contraction at terminal study correlated with the relative abundance of apoptotic protein expressions: cytochrome c ($r^2 = 0.02$, $P < .05$), mitochondrial Bax ($r^2 = 0.27$, $P < .05$), caspase-3 ($r^2 = 0.31$, $P < .05$), and poly (adenosine diphosphate–ribose) polymerase ($r^2 = 0.30$, $P < .05$). These myocardial apoptotic activities also correlated with remodeling strain: cytochrome c ($r^2 = 0.02$, $P < .05$), mitochondrial Bax ($r^2 = 0.28$, $P < .05$), caspase-3 ($r^2 = 0.43$, $P < .05$), and poly (adenosine diphosphate–ribose) polymerase ($r^2 = 0.37$, $P < .05$).

Conclusion: Increase in regional remodeling strain led to an increase in myocardial apoptosis and regional contractile dysfunction in heart failure.

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Cardiac remodeling after myocardial infarction (MI) is characterized by progressive global left ventricular (LV) dilatation and systolic dysfunction associated with an increased risk of congestive heart failure.[1,2] Post-MI remodeling has been described as a change in the structural properties between the ischemic zone and the nonischemic zone that leads to increases in regional wall stress, myocyte cell loss, side-to-side myocyte slippage, chronic lengthening, and hypertrophy of myocytes.[3,4] Increased mechanical stresses in the myocardium during cardiac

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Abbreviations and Acronyms

ANOVA = analysis of variance
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
LAD = left anterior descending coronary artery
LV = left ventricular
LVEDP = left ventricular end-diastolic pressure
MI = myocardial infarction
PARP = poly (adenosine diphosphate–ribose) polymerase
TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling
VDAC = mitochondrial porin

remodeling have been shown to activate stretch-activated signaling pathways and apoptotic molecular events.[5,6]

Studies have shown that myocyte apoptosis occurs in the infarct, peri-infarct (adjacent), and remote zone of myocardium after MI.[7] Apoptosis is programmed cell death characterized by cell shrinkage, membrane blebbing, and DNA fragmentation.[8,9] Both intrinsic and extrinsic pathways of apoptosis leading to cell death have been described. The intrinsic pathway relies on the release of mitochondrial cytochrome c into the cytosol whereas the extrinsic pathway relies on ligation of membrane-bound death receptors. Induction of either pathway leads to the activation of the aspartate-specific cysteine proteases such as caspase-3 and cleavage of poly (adenodiphosphate–ribose) polymerase (PARP).[10,11]

The objective of this study was to correlate changes in remodeling strain (end-diastolic dimensional stretch) and fractional contraction with molecular changes observed in the infarct, adjacent, and remote regions of the myocardium in a post-MI model of cardiac remodeling.

Methods

Study Design

Eight Dorsett hybrid sheep (male; weight, 60–70 kg) bred for laboratory use (Thomas Morris, Reisterstown, Md) were instrumented with subsequent creation of an anterolateral MI by coronary ligation. Four noninstrumented animals served as healthy tissue controls. LV geometry and function were measured with an echocardiogram. Sonomicrometry transducers were placed in the free wall of the LV to quantify the regional myocardial contractile function. At the time of terminal study, LV myocardial tissue samples from the excised heart from each animal were harvested in ice-cold solution and separated into the infarct region, adjacent region (which was defined as less than 2 cm from the edge of the infarct), and remote region for analysis of protein expression. 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 1996). 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.

Surgical Protocol

In each animal, anesthesia was induced with thiopental sodium (10 mg/kg) and maintained by mechanical ventilation with 1% to 2% isoflurane mixed with oxygen (Draeger anesthesia monitor; North American Draeger, Telford, Pa). Surface electrocardiogram, arterial blood pressure, pulse oximeter, and esophageal temperature were continuously monitored during the surgical operation. The instrumented group underwent a left anterolateral thoracotomy with polypropylene snares placed around the first and second diagonal coronary arteries of the left anterior descending artery (LAD) and tunneled subcutaneously. The snares were momentarily tightened (<30 seconds) to demarcate the border of the future infarct. Sixteen sonomicrometry transducers (2 mm; Sonometrics Corporation, London, Ontario, Canada) were placed in the free wall of the LV. The transducers were placed in 3 groups, each having 5 transducers placed circumferentially from anterior wall to posterior LV wall. One transducer was placed at the LV apex. An ultrasound flow probe (20 mm; Transonic Systems, Inc, Ithaca, NY) was placed around the main pulmonary artery for cardiac output monitoring.

Infarction

Ten days after the initial operation, the sheep were reanesthetized. The subcutaneous snares were permanently tightened to cause an anterolateral MI and the animal was supported with epinephrine infusion according to MI protocol described previously.[12] The pre-MI and post-MI data (sonomicrometry, echocardiogram, and hemodynamics) were collected.

Data Collection and Analysis

Hemodynamic, echocardiographic, and sonomicrometry data were collected in the pre-MI and post-MI periods and 2, 6, and 8 weeks after MI. The hemodynamic parameters were measured with a Millar pressure transducer (Millar Instruments, Inc, Houston, Tex) placed in the LV cavity through the aortic valve via the femoral artery with the aid of a fluoroscope. Parameters measured included heart rate, systolic and diastolic arterial pressure, mean arterial pressure, and LV end-diastolic pressure (LVEDP). Cardiac output was measured with the transonic flow probe placed around the main pulmonary artery with the flowmeter. Transdiaphragmatic echocardiographic data were collected with a Sonos 5500 machine with a sterile covered transducer (Philips Medical, Andover, Mass). LV end-systolic and end-diastolic volumes, wall motion abnormalities, and ejection fractions were measured as previously described.[13] Sonomicrometry data were collected with a commercially available digital sonomicrometry system (Sonometrics Corporation) to determine 3-dimensional motion and deformation of the LV free wall. Sonomicrometry data were used to calculate the fractional contraction (end-systolic strain) from end-diastole to end-systole over a cardiac cycle to quantify the regional contractile function of the myocardium. The end-diastolic and end-systolic time points were determined from the pressure and flow waveforms. Negative fractional contraction values as percent change over diastolic reference indicate functional contraction and positive values indicate dysfunctional dilatation during systole. The remodeling strain calculated between the geometries at end-diastole at one data collection time point and at end-diastole at the pre-MI measurement was used to quantify the progression of LV remodeling. We described the calculation of these strains previously.[12] In brief, an area strain measure