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PRE-CLINICAL RESEARCH

Molecular Imaging of Interstitial Alterations in Remodeling Myocardium After Myocardial Infarction

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Objectives The purpose of this study was to evaluate interstitial alterations in myocardial remodeling using a radiolabeled Cy5.5-RGD imaging peptide (CRIP) that targets myofibroblasts.

Background Collagen deposition and interstitial fibrosis contribute to cardiac remodeling and heart failure after myocardial infarction (MI). Evaluation of myofibroblastic proliferation should provide indirect evidence of the extent of fibrosis.

Methods Of 46 Swiss-Webster mice, MI was induced in 41 by coronary artery occlusion, and 5 were unmanipulated. Of the 41 mice, 6, 6, and 5 received intravenous technitium-99m labeled CRIP for micro–single-photon emission computed tomography imaging 2, 4, and 12 weeks after MI, respectively; 8 received captopril or captopril with losartan up to 4 weeks after MI. Scrambled CRIP was used 4 weeks after MI in 6 mice; the remaining 10 of 46 mice received unradiolabeled CRIP for histologic characterization.

Results Maximum CRIP uptake was observed in the infarct area; quantitative uptake (percent injected dose/g) was highest at 2 weeks (2.75 ± 0.46%), followed by 4 (2.26 ± 0.09%) and 12 (1.74 ± 0.24%) weeks compared with that in unmanipulated mice (0.59 ± 0.19%). Uptake was higher at 12 weeks in the remote areas. CRIP uptake was histologically traced to myofibroblasts. Captopril alone (1.78 ± 0.31%) and with losartan (1.13 ± 0.28%) significantly reduced tracer uptake; scrambled CRIP uptake in infarct area (0.74 ± 0.17%) was similar to CRIP uptake in normal myocardium.

Conclusions Radiolabeled CRIP allows for noninvasive visualization of interstitial alterations during cardiac remodeling, and is responsive to antiangiotensin treatment. If proven clinically feasible, such a strategy would help identify post-MI patients likely to develop heart failure. (J Am Coll Cardiol 2008;52:2017–28) © 2008 by the American College of Cardiology Foundation

Heart failure (HF) is evolving as 1 of the most important cardiovascular health problems worldwide. In the U.S. alone, approximately 5 million people suffer from manifest HF and more than 500,000 new cases are diagnosed every year (1). The syndrome of HF post-myocardial infarction (MI) is characterized by a relentless course of myocardial remodeling and functional deterioration (2–4), which continues to occur even after the initial causative injury has abated (5,6). In addition to the replacement fibrosis in the region of MI, interstitial fibrosis in the noninfarcted myocardium significantly contributes to the adverse remodeling and HF (7). In fact, presence of fibrosis remote from the infarct zone accounts for two-thirds of the fibrous tissue in the cardiomyopathic heart (7–10). The magnitude of myo-

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Abbreviations and Acronyms

ASMA = alpha smooth muscle actin
CRIP = Cy5.5-RGD imaging peptide
CT = computed tomography
HF = heart failure
LV = left ventricle/ventricular
MI = myocardial infarction
PBS = phosphate-buffered saline
RGD = arginine-glycine-aspartate
SPECT = single-photon emission computed tomography
Tc = technetium

cardial fibrosis is correlated to the extent of ventricular dysfunction (11). It is conceivable that an ability to noninvasively detect the process of myocardial fibrosis would allow assessment of the likelihood of evolution of HF after MI.

Collagen production and fibrosis in the myocardium are associated with myofibroblastic proliferation (5). Myofibroblasts demonstrate up-regulation of angiotensin receptors and integrin moieties, which, in turn, may promote collagen genes and reduce metalloproteinase genes (12). Such effects of integrin up-regulation are prevented by abrogation of autocrine transforming growth factor-β signaling (12). The RGD peptide (containing the arginine-glycine-aspartate motif) that binds to integrins such as $\alpha_v\beta_3$ has been used to identify neovascularization in post-infarct animal models (13). Since integrin $\alpha_v\beta_3$ is associated with the supermature focal adhesions on the cell membrane of myofibroblasts, we hypothesized that appropriately labeled RGD probes should identify myofibroblasts in post-infarct myocardium (14). In addition, scrutiny of the pro-collagen I sequence revealed RGD binding domains, such as DDX, and could also constitute a target for RGD-based imaging. Therefore, uptake of RGD probe should indirectly represent the rate of fibrogenesis or collagen deposition.

In the present study, we used Cy5.5-RGD imaging peptide (CRIP) labeled with technetium (Tc)-99m, for feasibility of imaging the process of active myocardial fibrosis in a murine model of post-MI ventricular dysfunction. The noninvasive imaging ability of the radiolabeled probe was compared with echocardiographic parameters of left ventricular (LV) geometric changes and pathologic characterization of interstitial alterations. The fluorescent moiety of the targeting peptide allowed better histologic characterization of the probe localization. An antibody against the fluorescent moiety of CRIP was used to immunoelectron microscopically trace the localization of CRIP. In addition, in vitro experiments were performed for characterization of the CRIP binding to mature and pro-collagen.

Methods

Experimental myocardial infarction in mice. The experimental protocol was approved by the Institution Animal Care and Use Committee of the University of California, Irvine, School of Medicine. In 41 adult Swiss Webster male mice (age: 4 months; body weight: ~50 g), MI was induced under pentobarbital (75 mg/kg) and isoflurane gas anesthesia (2.0% to 3.0%) using a stereomicroscope (Leica MZ FL III, Leica, Switzerland). For this purpose, animals were placed on a heating pad in the supine position, endotracheal intubation was performed under direct laryngoscopy, and mechanical ventilation was maintained with a small animal respirator (Harvard Apparatus, Holliston, Massachusetts) (tidal volume = 1.0 ml, rate = 120 breaths/min). After thoracotomy, the lateral branch of the left coronary artery was ligated with a 6.0-silk suture 3 to 4 mm below the tip of the left atrium. Successful ligation was verified by visual inspection of the LV apex for myocardial blanching, indicating interruption in coronary flow. The chest cavity was closed in layers with 6.0-silk, and the skin closed with 4.0-silk sutures. Animals were gradually weaned from the respirator.

For evaluation of serial changes in collagen synthesis by radiolabeled CRIP imaging, animals were divided into groups at 2 weeks (n = 6), 4 weeks (n = 6), and 12 weeks (n = 5) after infarction (Table 1). Two groups of 4 animals each were treated with either captopril (60 mg/kg/day) alone or in combination with losartan (captopril 30 mg/kg/day, losartan 10 mg/kg/day) dissolved in the drinking water, to evaluate if molecular imaging with CRIP would allow determination of efficacy of therapeutic intervention. Five unmanipulated control mice were subjected to CRIP imaging for comparison with the infarcted mice after a 4-week wait. In addition, 6 animals, 4 weeks after MI, were imaged with Tc-labeled scrambled peptide (scrambled CRIP) to ensure the specificity of CRIP. In the remaining 10 mice, nonradiolabeled CRIP was used for pathologic characterization of the target using fluorescence microscopy and immunoelectron microscopy.

Echocardiography. All animals were subjected to extensive echocardiographic studies for the assessment of myocardial infarct size, LV cavity dimensions, and ventricular function.

Echocardiographic examination was performed under 2% isoflurane anesthesia for the assessment of infarct size, LV cavitary dimensions, and ventricular function before nuclear imaging. Echocardiograms were recorded with a commercially available ultrasound system (Sequoia, Siemens,

Table 1 Classification of Animals Used in the Study

						4 Weeks, n	
Probe	Label	Control, n	2 Weeks, n	4 Weeks, n	12 Weeks, n	Captopril	Captopril + Losartan
Cy5.5-RGD	Tc	5	6	6	5	4	4
Cy5.5-RGD	—	—	10	—	—	—	—
Cy5.5 scrambled	Tc	—	—	6	—	—	—

RGD = arginine-glycine-aspartate; Tc = technetium.