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Original article

Building a better infarct: Modulation of collagen cross-linking to increase infarct stiffness and reduce left ventricular dilation post-myocardial infarction

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ABSTRACT

Matrix metalloproteinase-9 (MMP-9) deletion attenuates collagen accumulation and dilation of the left ventricle (LV) post-myocardial infarction (MI); however the biomechanical mechanisms underlying the improved outcome are poorly understood.

The aim of this study was to determine the mechanisms whereby MMP-9 deletion alters collagen network composition and assembly in the LV post-MI to modulate the mechanical properties of myocardial scar tissue. Adult C57BL/6J wild-type (WT; n = 88) and MMP-9 null (MMP-9$^{-/-}$; n = 92) mice of both sexes underwent permanent coronary artery ligation and were compared to day 0 controls (n = 42). At day 7 post-MI, WT LVs displayed a 3-fold increase in end-diastolic volume, while MMP-9$^{-/-}$ showed only a 2-fold increase ($p < 0.05$). Biaxial mechanical testing revealed that MMP-9$^{-/-}$ infarcts were stiffer than WT infarcts, as indicated by a 1.3-fold reduction in predicted in vivo circumferential stretch ($p < 0.05$). Paradoxically, MMP-9$^{-/-}$ infarcts had a 1.8-fold reduction in collagen deposition ($p < 0.05$). This apparent contradiction was explained by a 3.1-fold increase in lysyl oxidase ($p < 0.05$) in MMP-9$^{-/-}$ infarcts, indicating that MMP-9 deletion increased collagen cross-linking activity. Furthermore, MMP-9 deletion led to a 3.0-fold increase in bone morphogenetic protein-1, the metalloproteinase that cleaves pro-collagen and pro-lysyl oxidase ($p < 0.05$) and reduced fibronectin fragmentation by 49% ($p < 0.05$) to enhance lysyl oxidase activity. We conclude that MMP-9 deletion increases infarct stiffness and prevents LV dilation by reducing collagen degradation and facilitating collagen assembly and cross-linking through preservation of the fibronectin network and activation of lysyl oxidase.

1. Introduction

Myocardial infarction (MI) is a leading cause of death in the United States [1]. Post-MI, the left ventricle (LV) undergoes a dynamic cascade of remodeling events that result in increased LV size, decreased wall thickness, and reduced cardiac function [2]. LV dilation post-MI is the result of myocyte destruction and excessive degradation of the myocardial extracellular matrix (ECM) without adequate synthesis of new ECM components [3]. ECM remodeling is a key determinant of patient outcomes, as new ECM deposition in the infarct zone reduces infarct dilation and decreases the chance of cardiac rupture, while excessive ECM deposition can lead to stiffer scar tissue that hinders diastolic filling [4–6].

Myocardial scar tissue is mechanically a non-linear anisotropic material [3,7]. While the properties of the normal myocardium are dominated by the structure and orientation of the myocytes, the properties of the infarct region are primarily governed by the network of newly deposited collagen fibers [3,8]. Biaxial mechanical testing is the gold standard for testing planar anisotropic materials, making this method ideal for studying how variations in the properties of the collagen network alter scar mechanics in a mouse MI model [7,9,10]. Biomechanical

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analysis of infarct tissue, therefore, provides a critical understanding of the complex interactions between ECM structure, LV function, and patient outcome.

In order to vary the properties of the collagen network we utilized matrix metalloproteinase (MMP)-9 null (MMP-9$^{-/-}$) mice, a model that has been well studied [11,12]. MMPs are a class of proteins responsible for proteolysis of ECM proteins, and MMP-9, amongst its myriad targets, degrades both collagen and fibronectin [13,14]. Fragments of these proteins trigger inflammation and increase deposition of new ECM proteins including collagens I and III [15–17]. MMP-9 deletion has been shown to prevent LV dilation, lower collagen deposition, and increase angiogenesis in mice post-MI [12,18]. Despite these findings, the exact mechanisms by which MMP-9 deletion prevents adverse remodeling remain unclear; and given the cleavage targets of MMP-9, this is an excellent model for examining how variations in the collagen network alter infarct biomechanics.

Since MMP-9 degrades both collagen and the fibronectin network upon which collagen is assembled, we hypothesized that MMP-9 deletion would alter infarct stiffness. An increase in infarct stiffness could explain the reduction in LV dilation observed in MMP-9 null mice and could contribute to a reduction in LV remodeling through reduced myocardial wall stress. The aim of this study was to determine how MMP-9 deletion alters the mechanical properties of myocardial scar tissue and the composition and assembly of the collagen network post-MI.

2. Methods

2.1. Animals and surgery

All animal procedures were performed based on the "Guide for the Care and Use of Laboratory Animals" and have been approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and University of Mississippi Medical Center. Adult (4–10 month old) C57BL/6J mice (WT; n = 51 females and 59 males including day 0 controls), and MMP-9 null mice (MMP-9$^{-/-}$; n = 62 females and 50 males including day 0 controls) were used in this study. MI was induced through permanent ligation of the left coronary artery following a well-established method [19,20]. In summary, mice were anesthetized with 1–2% isoflurane in 100% oxygen, intubated, and placed on a standard rodent ventilator. An incision was made between the 3rd and 4th intercostal spaces, and a rib spreader was used to allow visualization of the heart. An 8-0 suture was used to ligate the left coronary artery at a location approximately 1–2 mm distal to the left atrium, and MI was confirmed by LV blanching and ST segment elevation on the electrocardiogram. Immediately before or after surgery, buprenorphine (0.1 mg/kg) was administered intraperitoneally to reduce pain. Animals were sacrificed at 1, 3, 5, 7, or 28 days post-MI. We have previously published survival, echocardiography, and collagen density analysis results for WT vs. MMP-9$^{-/-}$ at day 28 post-MI [21]. To avoid unnecessary duplication of animal use, those results have been integrated into the current study. All data have been re-analyzed in accordance with the methods used for this study. Day 0, no MI, served as controls. In an effort to minimize the number of animals, samples were shared across studies leading to varying sample sizes across groups.

2.2. Echocardiography

Echocardiography was performed using either a Vevo 770 system or a Vevo 2100 small animal imaging system (Visual Sonics). Imaging occurred serially at day 0 before surgery and at days 1, 3, 5, and 7 post-MI or up to the sacrifice time. Day 28 images, taken from a previous study, were also analyzed but were not measured serially. All images were taken with mice anesthetized with 1–2% isoflurane in an oxygen mix. Images were taken in both long and short axis views in both M-mode and B-mode. LV volumes and ejection fraction (EF, %) were calculated from long axis views, while LV dimensions were calculated from short axis views. All calculations were determined by averaging the values of three consecutive cardiac cycles. Dimensions for each mouse were normalized to its baseline image to evaluate relative changes.

Speckle tracking analysis was done using the VevoStrain analysis program (Visual Sonics). Longitudinal deformation was measured from long-axis views. Tracking data was exported to Matlab for further analysis. Stretch of the base region and mid-ventricle region was calculated from an average of three cardiac cycles. Measurements were made at the end-diastole, the end of isovolumic contraction, the end-systole, and the end of isovolumic relaxation.

2.3. Tissue collection

Prior to sacrifice, mice were anesthetized with 2–5% isoflurane. Cardioplegic solution was used to arrest the heart in diastole to ensure isolation of passive non-contracted tissue. The LV was separated from the right ventricle and sliced into the apex, middle, and base sections. The pieces were stained with 1% 2,3,5-triphenyltetrazolium chloride and photographed in order to determine the percentage of infarct area to total LV area. The mid-wall ring was sectioned into the infarct wall and septal wall and used for mechanical testing. The ratio of lung weight to tibia length was measured as an index for edema.

2.4. Mechanical testing

Mechanical testing was performed using a Biotester-5000 biaxial test system (Cellscale) following a previously described method [22]. The stresses and corresponding strains were fit to a four parameter Fung-type model where the strain energy density was calculated as:

$$W = \frac{1}{2}c(e^{Q}-1), \quad Q = b_1E_\theta^2 + b_2E_z^2 + 2b_4E_\theta E_z \tag{1}$$

to yield the Cauchy stress expressed as

$$\begin{aligned}\sigma_\theta &= (1+2E_\theta)(b_1E_\theta + b_4E_z)ce^{Q}\\ \sigma_z &= (1+2E_z)(b_2E_z + b_4E_\theta)ce^{Q}\end{aligned} \tag{2}$$

where c, b_1, b_2, and b_4 are the four material constants and E_θ and E_z are the circumferential and longitudinal Green strains [22,23]. Fitted material properties for each individual sample were calculated as well as group averaged material properties. Tissue stiffness was quantified as the slope of the Cauchy stress–stretch ratio curve between 5 and 15% equibiaxial stretch. In vivo wall stresses were estimated using the Law of Laplace, with the average wall thickness and dimension at the end diastole determined from echocardiography for each sample. An end-diastolic pressure of 10 mmHg was chosen based on our previously collected experimental data for the healthy mouse LV [12]. The estimated stresses and the fitted material properties for each sample were used to back-calculate the expected in vivo deformation.

2.5. Collagen histology

Histological analysis using picrosirius red (PSR) staining was used to examine collagen density and alignment. Collagen density was measured from ring sections of the LV taken from the midcavity wall. Slides for collagen alignment were taken from the samples used for mechanical testing and processed following a previously described method [22]. PSR stained LV rings were imaged with a brightfield microscope at 40× magnification with three representative images taken in the infarct region and two images taken in the remote region. Slides for collagen alignment were imaged at 10× magnification with three images taken near the middle of the infarct region for each slide and one additional image of the edge of the specimen taken so as to denote the circumferential direction. An in-house program written in Matlab

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