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SCIENCE DIRECT
Journal of Molecular and Cellular Cardiology 39 (2005) 259–268
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Time-dependent changes in myocardial structure following discrete injury in mice deficient of matrix metalloproteinase-3

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Received 5 November 2004; received in revised form 4 March 2005; accepted 23 March 2005

Available online 11 May 2005

Abstract

Myocardial scars from radiofrequency (RF) ablation can increase in size in the post-injury period, resulting in remodeling of the extracellular matrix (ECM). The matrix metalloproteinases (MMPs) contribute to adverse myocardial remodeling following injury. However, the role of specific MMP types in RF scar enlargement remains unclear. One MMP type, MMP-3, degrades a wide range of ECM substrates and can activate other MMPs. This project examined LV remodeling in wild type (WT) and MMP-3 deficient (*mmp-3*$^{-/-}$) mice following RF injury. RF lesions (0.5 mm probe, 80 °C, 30 s) were created on the LV epicardium of WT (C57/BL6) and *mmp-3*$^{-/-}$ mice and were terminally studied at 1 h, 3, 7, and 28 days post-RF ($n = 10$ each). Heart mass indexed to tibial length (mg/mm) was similar in the WT and *mmp-3*$^{-/-}$ mice at 1 h (8.1 ± 0.3 vs. 7.6 ± 0.3), but lower in the *mmp-3*$^{-/-}$ mice at 28 days post-RF (11.9 ± 0.4 vs. 10.5 ± 0.4, $P < 0.05$). Scar volumes were greater in the *mmp-3*$^{-/-}$ mice at 3 days, but similar in the two groups at 28 days. Immunohistochemical localization showed fewer macrophages and lymphocytes at the scar border at 3 days in the *mmp-3*$^{-/-}$ hearts, but similar staining for these cells in WT and *mmp-3*$^{-/-}$ hearts at 7 and 28 days post-RF. Post-RF, the early increase in scar volume was accelerated in *mmp-3*$^{-/-}$ mice and associated with abnormal inflammatory cell infiltration/migration to the area of injury. These findings define a mechanistic role for MMP-3 in RF scar expansion and provide a temporal window during which interruption of MMP-3 activation may impair post-RF myocardial wound healing.

Keywords: Myocardial remodeling; Matrix metalloproteinases; Injury; MMP-3

1. Introduction

Myocardial remodeling occurs following a pathologic injury and causes multifactorial changes in cellular and extracellular processes [1–5]. Changes in extracellular matrix (ECM) structure and composition are key components in post-injury myocardial remodeling [1,3,5–8]. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes implicated in ECM remodeling [1,5,8–10]. Increased myocardial MMP activation/expression occurs following myocardial infarction (MI) [1,3,5,8,11,12], and past studies have established a cause–effect relationship between post-injury myocardial remodeling and MMP activation utilizing pharmacologic MMP inhibition or genetic manipulation of certain MMP types [6,7,11,12]. To date, more than 20 unique MMP types have been identified and alterations in the abundance of certain MMPs occurs within the remodeling myocardium [3–5,7,8,13–15]. For example, increased myocardial MMP-3, or stromelysin-1, levels are associated with changes in myocardial structure [4,8,14–17]. MMP-3 has a wide range of biological activity, including cleavage of ECM substrates such as non-fibrillar collagens, denatured fibrillar collagen, fibronectin, laminin, and activation of other MMPs [7,10,18]. Therefore, MMP-3 may contribute to the amplification of ECM proteolysis and exacerbate adverse post-injury myocardial remodeling. However, a direct role of MMP-3 in the myocardial remodeling response following a

☆ This study was supported by National Heart, Lung, and Blood Institute Grants HL-66029, HL-45024, HL-97012, and PO1-48788
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doi:10.1016/j.yjmcc.2005.03.012

discrete injury remains unclear. A recent study has demonstrated that the application of radiofrequency (RF) energy to the myocardium caused discrete and reproducible lesions [6]. Moreover, the time-dependent changes in myocyte architecture and composition of the ECM that occurred post-injury were exacerbated in the absence of an endogenous tissue inhibitor of the MMPs [6]. Using this model of discrete injury induced by RF energy application, the present study tested the hypothesis that the myocardial remodeling response would be attenuated in mice deficient of MMP-3.

2. Materials and methods

This study used a model of myocardial injury, in which lesions were caused through the application of RF energy, as previously described [6]. To determine the role of MMP-3 on the post-injury myocardial remodeling process, mice deficient in the MMP-3 gene (*mmp-3*$^{-/-}$) and strain-matched wild type (WT) (C57/BL6) mice were used. The specific construct of the *mmp-3*$^{-/-}$ mice has been described previously and the original breeding pairs were a gift from Dr. Mudgett (Merck Laboratories) [19–21]. All animals were treated and cared for in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (National Research Council, Washington, 1996), and the protocol was approved by the Institutional Animal Care and Use Committee.

2.1. Surgery and RF lesion creation

WT ($n = 40$) and *mmp-3*$^{-/-}$ mice ($n = 40$) were of similar ages and weights (72 ± 3 vs. 76 ± 5 days, $P = 0.61$; and 22.8 ± 0.5 vs. 21.6 ± 0.6 g, $P = 0.78$, respectively), and of equal gender distribution. RF lesions were created on the left ventricular (LV) free wall as previously described [6]. Briefly, the mice were anesthetized by exposure to isoflurane vapors. The neck, chest, and back (2–3 cm^2) were shaved and laid supine over a stainless steel grounding plate, which was previously coated with an electroconductive gel. The mice were intubated (20 gauge Angiocath, Jelco) and connected to a ventilator (225–250 breaths per min, tidal volume: 250 μl; Hugo-Sachs, Germany). Anesthesia was maintained by delivering isoflurane (2%). A 1.0 cm left lateral thoracic incision was made over the third intercostal space and a pericardectomy was performed to provide access to the LV free wall. RF current (RFG-3C, Radionics) was delivered to the myocardium through a custom designed probe (0.5 mm diameter, Omega Engineering, Stamford, CT) using negative feedback temperature control to achieve a temperature of 80 °C for 30 s. RF lesion formation was visually confirmed as a discrete blanched region. All thoracic fluid was aspirated, the ribs were apposed with 5-0 silk sutures, and the overlying muscle layers and the skin incision were closed. The animals were rotated to the prone position, administered an analgesic (buprenorphine, 0.05 mg/kg s.c.), weaned from the ventilator, extubated, and recovered.

2.2. Time course

To examine the acute, mid-duration, and long-term post-injury myocardial remodeling, the mice were randomized to be studied at 1 h (acute), and at 3, 7 and 28 days ($n = 10$ for each timepoint for WT and *mmp-3*$^{-/-}$ mice).

At terminal study, the animals were deeply anesthetized by exposure to inhalation isoflurane, weighed, and the thoracic cavity was opened. The heart was arrested by injecting $CdCl_2$ (0.1 M in saline, 0.1 ml) through the LV apex, quickly extirpated, weighed, and fixed in 10% formalin. One tibia was isolated, immersed in saturated KOH solution, and tibial length was measured on the following day. To normalize for differences in body size, heart mass was normalized to tibial length.

2.3. Histology and determination of scar volume

The ventricles of the fixed hearts were sectioned parallel to the atrioventricular groove into basal, mid-ventricular, and apical sections and embedded in paraffin. Using a microtome, sequential slices were obtained at 100 μm intervals from each of the three sections. Following deparaffinization and standard histological preparation, the sections were stained using hematoxylin and eosin (H&E) and picrosirius red (PSR). Scar and myocardial volumes were determined as previously described [6]. Briefly, the H&E stained sections were digitized (600 dpi, Epson Expression 830XL, Japan) and the areas of the scar and LV myocardium were determined by digital planimetry (SigmaScan Pro, Jandel Scientific). Inter-section volume was computed as the multiplication product of the area and distance between the sections (100 μm) and the volumes were summated over all sections to determine total volume of the scar and myocardium. Scar volumes were normalized to indexed heart mass. LV wall thicknesses at the scar and diametrically opposite to the scar were determined as an average of measurements from three consecutive sections containing the scar/injury. Scars or areas of injury that extended from the epicardium to the endocardium were defined to be transmural. The myocardial regions at which histomorphometric measurements were performed are schematically depicted in Fig. 1A.

2.4. Measurement of cross-sectional areas

Myocyte cross-sectional areas (CSA) were determined from myocardial regions adjacent to the RF ablative scar and a remote region that was diametrically opposite to the scar. Briefly, H&E stained LV myocardial sections were imaged at a magnification of 400×. Myocytes in a cross-sectional orientation were digitized and analyzed with an image analysis system (NIH Image). CSA were determined from three consecutive sections that contained the scar. A minimum of 50 myocytes from LV myocardial regions adjacent to the scar and regions diametrically opposite to the scar were digitized.