Myocardial remodeling after discrete radiofrequency injury: effects of tissue inhibitor of matrix metalloproteinase-1 gene deletion

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Mukherjee, Rupak, Andrea M. Parkhurst, Joseph T. Mingoia, Sarah E. Sweterlitsch, Jennifer S. Leiser, G. Patricia Escobar, Francis G. Spinale, and J. Philip Saul. Myocardial remodeling after discrete radiofrequency injury: effects of tissue inhibitor of matrix metalloproteinase-1 gene deletion. Am J Physiol Heart Circ Physiol 286: H1242–H1247, 2004. First published November 20, 2003; 10.1152/ajpheart.00437.2003.—Discrete myocardial lesions created through the delivery of radiofrequency (RF) energy can expand; however, the mechanisms have not been established. Matrix metalloproteinases (MMPs) play an important role in myocardial remodeling, and MMP activity can be regulated by the tissue inhibitors of the metalloproteinases (TIMPs). This study examined the role of TIMP-1 in postinjury myocardial remodeling. Lesions were created on the left ventricular (LV) epicardium of wild-type (WT, 8–12 wk, 129SVE) and age-matched TIMP-1 gene-deficient (timp-1−/−) mice through the delivery of RF current (80°C, 30 s). Heart mass, LV scar volumes, and collagen content were measured at 1 h and 3, 7, and 28 days postinjury (n = 10 each). Age-matched, nonablated mice were used as reference controls (n = 5). Heart mass indexed to tibial length increased in WT and timp-1−/− mice but was greater in the timp-1−/− mice by 7 days. Scar volumes increased in a time-dependent manner in both groups but were higher in the timp-1−/− mice than the WT mice at 7 days (1.48 ± 0.09 vs. 1.20 ± 0.11 mm3·mg−1·mm, P < 0.05) and remained higher at 28 days. In the remote myocardium, wall thickness was greater and relative collagen content was lower in the timp-1−/− mice at 28 days postinjury. Discrete myocardial RF lesions expand in a time-dependent manner associated with myocyte hypertrophy remote to the scar. Moreover, postinjury myocardial remodeling was more extensive with TIMP-1 gene deletion. Thus TIMP-1 either directly or through modulation of MMP activity may regulate myocardial remodeling following infliction of a discrete injury.

MATERIALS AND METHODS

This study was designed to address myocardial remodeling following RF ablation and to determine the potential role of TIMP-1 expression on this process. For these studies, mice deficient in the TIMP-1 gene (timp-1−/−, 8–12 wk old) and strain-matched wild-type (129SVE, WT) mice were used. The specific construct of the timp-1−/− mice has been described previously (7, 13), and the original breeding pairs were a kind gift from Dr. Paul D. Soloway (13). 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, DC, 1996), and the protocol was approved by the Institutional Animal Care and Use Committee.

Surgery and RF lesion creation. WT and timp-1−/− mice were of similar ages and weights (78 ± 3 vs. 73 ± 2 days, P = 0.36; and 23.2 ± 0.4 vs. 23.6 ± 0.6 g, P = 0.86, respectively) at the onset of the study. Anesthesia was induced by placing the mice in an induction chamber containing isoflurane vapors (~10%). After loss of consciousness, mice were weighed and the neck, chest, and back (2–3 cm2) were shaved. The shaved area on the back was coated with an electrically conductive gel (Redux paste for electrocardiography, providing a means to precisely target arrhythmogenic myocardium. Nevertheless, past clinical and experimental studies have provided evidence that RF ablative scars can expand with time and may result in deleterious consequences (5, 17). Whereas fibrosis and ultrastructural damage have been described to occur at and around the RF lesion site (12, 17), mechanisms that may contribute to the progressive expansion of RF scars remain unclear.

Matrix metalloproteinases (MMPs) are endogenous enzymes that contribute to myocardial remodeling (3, 4, 8–10, 15, 16, 18). MMPs degrade components of the extracellular matrix, including collagen, thereby facilitating tissue remodeling in normal and pathological states (14, 18). Alterations in the expression and activity of the MMPs and the tissue inhibitors of the metalloproteinases (TIMPs) have been implicated in myocardial remodeling in a number of cardiac disease states (8–10, 18). TIMP-1 has been shown to bind to a majority of the MMPs and to play a regulatory role by preventing MMP-mediated degradation of the extracellular matrix (3, 8, 16). Therefore, deletion of the TIMP-1 gene may be expected to cause an increase in the MMP activation state (3, 16). With the use of mice deficient in the TIMP-1 gene, this study tested the hypothesis that TIMP-1 deficiency would result in a more extensive myocardial remodeling response following infliction of a discrete injury.
Hewlett-Packard). The animals were placed supine on the operating table over a stainless steel grounding plate, and anesthesia was maintained by administration of 2% isoflurane in oxygen through a nose cone. A longitudinal incision (5 mm) was made along the anterior midline of the neck, the trachea was exposed, and an intravenous catheter (20 gauge, Angiocath, Jelco) was advanced through the mouth into the trachea under direct visualization using a dissecting microscope (Zeiss Opti 6). The endotracheal cannula was connected to a ventilator (Hugo-Sachs) set at a respiratory rate of 225–250 breaths/min with a tidal volume of 250 μL, delivering 2% isoflurane for the remainder of the procedure. A 1.0-cm left lateral thoracic incision was made over the third intercostal space. The muscle layers were identified individually and divided sharply until the thorax was entered. Care was taken to avoid injury to the lung upon division of the intercostal muscles. Appropriate exposure was obtained, and a small saline-soaked sponge was placed within the chest cavity to retract the lung away from the heart. A pericardectomy was performed to provide access to the left ventricular (LV) free wall.

RF current was delivered to the myocardium by using negative feedback temperature control. The RF probe was custom designed to deliver RF current and to simultaneously measure developed temperature using the same device. Specifically, the stainless steel sheath of an insulated thermocouple device (0.5 mm diameter, 15 cm long, T-type, Omega Engineering; Stamford, CT) was connected to the active output of a RF generator (RF-3G, Radironics), and a return current path was provided through the grounding plate. The RF probe was advanced to the LV free wall by using a micromanipulator (Narshige) and positioned on the anterolateral free wall carefully avoiding the placement of the probe tip over coronary vessels visible on the epicardial surface. After mechanical and electrical contact between the probe tip and the myocardium (contact impedance: 1.1 ± 0.1 kΩ) was confirmed, RF energy was applied through the probe to achieve a temperature of 80°C for 30 s. The power and current requirements necessary to achieve 80°C were 0.3 ± 0.1 W and 17 ± 3 mA, respectively, with no difference between the WT and timp-1−/− groups (P > 0.64). RF lesion formation was visually confirmed as a discrete blanched region on the epicardium. Any fluid that may have accumulated within the thoracic cavity was aspirated, and the lungs were reinfused by obstructing outflow on the ventilator for two breaths. The ribs were apposed with 5-0 silk sutures, and the overlying muscle layers were then closed (5-0 silk) in a single layer followed by skin closure with a running 5-0 prolene suture. The animals were rotated to the prone position, administered an analgesic (buprenorphine, 0.1 mg/kg ip), weaned from the ventilator, and extubated. The animal was then placed in an incubator for further recovery.

Time course. After the creation of the RF lesion, the mice were randomized to be studied at 1 h (acute), 3 days, 1 wk, and 1 mo (n = 10 for each time point for WT and timp-1−/− mice). The rationale for using these time points was to examine the acute, mid-duration, and long-term effects of RF ablation on LV remodeling. In a preliminary study (n = 3), application of RF current that resulted in a temperature of 50°C did not result in a visible myocardial lesion. Moreover, the postinjury recovery for these mice was unremarkable, and at 28 days postablation, body weights and heart masses were similar to those recorded in age-matched nonoperated mice (P = 0.51 and P = 0.64, respectively). Therefore, nonoperated mice were used in this protocol as reference controls. Age-matched, nonablated WT and timp-1−/− mice (n = 5 cumulatively for the 1 h and 3 day time points, and n = 5 each for the 1 wk and 1 mo time points) were included as reference controls. Equal numbers of male and female mice were used.

At terminal study, the animals were deeply anesthetized by exposure to inhalation isoflurane and weighed, and the thoracic cavity was opened. The heart was arrested by injecting CdCl2 (0.1 M, 0.1 ml) through the LV apex, which was extirpated, weighed, and fixed in 10% formalin. The tibia was isolated and 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.

Histology and determination of scar volume. The ventricles of the fixed hearts were dissected parallel to the atroventricular groove and embedded in paraffin. With the use of a microtome, sequential slices were obtained at 100-μm intervals from each of the three sections. After deparaffinization and standard histological preparation, the sections were stained with hematoxylin and eosin (HE) and picrosirius red (PSR). High-resolution digital images of the HE-stained sections and a 5 × 5 mm calibration grid were obtained by scanning on a flatbed scanner (600 dpi, Epson Expression 830XL). Areas of the scar and LV myocardium were determined by digital planimetry of each individual section (SigmaScan Pro, Jandel Scientific) and multiplied by the height of the section (100 μm) to compute volumes. Scar volumes were obtained as a summation of values obtained from each mouse and normalized to indexed heart mass. LV wall thicknesses at the RF scar and diametrically opposite to the scar were determined as an average of measurements from three consecutive sections containing the RF scar.

![Fig. 1](http://ajpheart.physiology.org/). A: heart mass indexed to tibial length increased in a time-dependent manner in the wild-type (WT) mice and mice deficient in the tissue inhibitor of the matrix metalloproteinase-1 gene (timp-1−/−). When compared with nonablated reference controls (dashed lines), heart mass was higher in both the WT and timp-1−/− groups at 7 and 28 days postinjury but to a greater degree in the timp-1−/− mice. B: scar volumes computed from the serial short-axis sections were normalized to indexed heart mass. Relative scar volume increased in a time-dependent fashion in the WT and timp-1−/− groups. By 7 days postinjury, scar volumes in the timp-1−/− group were higher than those in the WT group and remained higher at 28 days. *P < 0.05 vs. WT; †P < 0.05 vs. acute (time 0); §P < 0.05 vs. 3 days postinjury; ¶P < 0.05 vs. 7 days postinjury.
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, HE-stained LV myocardial sections were mounted on an inverted microscope (Axioskop-2, Zeiss), and cardiac myocytes were imaged at a magnification of 1,000. Myocytes in a cross-sectional orientation were digitized and analyzed with an image analysis system (NIH Image). Only those myocytes in which the nucleus was centrally located within the cell were digitized and analyzed so as to ensure that the short axis of the myocyte was perpendicular to the microscope objective. For sections obtained from the injured hearts, 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. For sections obtained from the reference control hearts, 3 consecutive sections from the mid-LV region were used to digitize cross-sectional profiles of a minimum of 50 myocytes per heart.

Myocardial collagen content. PSR-stained sections were analyzed for collagen content at the region of the scar as well as myocardium diametrically opposite the scar using methods described previously (3, 16, 20). Briefly, PSR-stained myocardial sections were imaged under high magnification (×200), illuminated by polarized light, and the resultant birefringence signals were digitized. The percent area of extracellular staining was computed from 15 random fields by normalizing the total area of birefringent illumination (NIH Image) to the total area of myocardium for the section.

Data analysis. Temporal changes in heart mass and scar volumes were compared among the nonablated, WT, and timp-1−/− mice by using two-way analysis of variance. A split-plot design was used to compare wall thickness, myocyte CSA, and collagen content between the groups by designating region and measurement time points as the main effects. Pairwise comparisons were performed by a Bonferroni adjusted t-test. Results are presented as means ± SE. Values of \( P < 0.05 \) were considered to be statistically significant.

RESULTS

The application of RF energy resulted in transmural myocardial lesions producing necrosis of 7 ± 1% of the LV wall (Fig. 2).

![Fig. 2](http://ajpheart.physiology.org/)

**Fig. 2.** A: postinjury left ventricular (LV) wall thickness at the scar decreased in a time-dependent manner in both the WT and the timp−/− groups. Compared with nonablated reference controls (dashed lines), LV wall thickness at the site of myocardial injury was lower by 7 days postinjury. B: LV wall thickness diametrically across from the site of injury increased in a time-dependent manner in the WT and timp−/− groups. At 28 days postinjury, LV wall thickness remote to the scar was higher in the timp−/− group compared with the WT group. \( *P < 0.05 \) vs. WT; \( +P < 0.05 \) vs. acute (time 0); \( §P < 0.05 \) vs. 3 days postinjury.

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, HE-stained LV myocardial sections were mounted on an inverted microscope (Axioskop-2, Zeiss), and cardiac myocytes were imaged at a magnification of ×1,000. Myocytes in a cross-sectional orientation were digitized and analyzed with an image analysis system (NIH Image). Only those myocytes in which the nucleus was centrally located within the cell were digitized and analyzed so as to ensure that the short axis of the myocyte was perpendicular to the microscope objective. For sections obtained from the injured hearts, 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. For sections obtained from the reference control hearts, 3 consecutive sections from the mid-LV region were used to digitize cross-sectional profiles of a minimum of 50 myocytes per heart.

Myocardial collagen content. PSR-stained sections were analyzed for collagen content at the region of the scar as well as myocardium diametrically opposite the scar using methods described previously (3, 16, 20). Briefly, PSR-stained myocardial sections were imaged under high magnification (×200), illuminated by polarized light, and the resultant birefringence signals were digitized. The percent area of extracellular staining was computed from 15 random fields by normalizing the total area of birefringent illumination (NIH Image) to the total area of myocardium for the section.

Data analysis. Temporal changes in heart mass and scar volumes were compared among the nonablated, WT, and timp−/− mice by using two-way analysis of variance. A split-plot design was used to compare wall thickness, myocyte CSA, and collagen content between the groups by designating region and measurement time points as the main effects. Pairwise comparisons were performed by a Bonferroni adjusted t-test. Results are presented as means ± SE. Values of \( P < 0.05 \) were considered to be statistically significant.

RESULTS

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![Fig. 3](http://ajpheart.physiology.org/)

**Fig. 3.** A: in the WT group, myocyte cross-sectional area adjacent to the scar remained similar to nonablated values (dashed lines) and acute values at all postinjury time points. In contrast, myocyte cross-sectional area was increased compared with acute and nonablated values by 7 days postinjury in the timp−/− mice. **B:** diametrically across from the scar, myocyte cross-sectional areas increased in a time-dependent manner in the both the WT and timp−/− groups. When compared with WT values, myocyte cross-sectional areas were higher in the timp−/− mice at 7 and 28 days postinjury. \( *P < 0.05 \) vs. WT; \( +P < 0.05 \) vs. acute (time 0); \( §P < 0.05 \) vs. 3 days postinjury; \( ¶P < 0.05 \) vs. 7 days postinjury.
myocardium when measured 1 h postinjury. Myocardial mass (Fig. 1A) and scar volumes (Fig. 1B) increased in a time-dependent manner in both the WT mice and mice deficient in the tissue inhibitor of matrix metalloproteinase-1 gene (timp-1). Whereas heart mass indexed to tibial length was greater than controls in both the WT and timp-1 mice by 7 days postinjury, this parameter was increased to a greater degree in the timp-1 mice at 7 and 28 days postinjury, scar volumes were larger in the WT and timp-1 mice than in the WT mice. Indexed scar volumes were larger than the acute values by 7 days postablation in the WT and mice. However, in the cohort of timp-1 mice studied at 28 days postinjury, collagen content was significantly lower than the corresponding WT mice.

In the uninjured reference control myocardium, total collagen content was lower in the timp-1 mice than WT values (0.78 ± 0.17 vs. 1.08 ± 0.22%, respectively; Fig. 4, A and D), but this difference did not reach statistical significance (P = 0.18). Collagen deposition was increased at the site of injury (Fig. 4, C and F) by 7 days postinjury in both the WT and timp-1 groups and remained higher at 28 days postinjury (Fig. 5A), with no difference observed between the groups. Diametrically across from the scar, collagen content postinjury was not different from acute values in the WT or timp-1 mice (Fig. 5B). However, in the cohort of timp-1 mice studied at 28 days postinjury, collagen content was significantly lower than the corresponding WT mice.

DISCUSSION

The ability to cause discrete and focal myocardial lesions through the application of RF current is commonly used for arrhythmia control (1, 2). Past clinical and experimental studies have provided evidence that postprocedural expansion of myocardial RF lesions can occur (5, 17). For example, in an experimental study using young lambs, myocardial scar dimensions were reported to increase in a time-dependent manner up to 9 mo following RF ablation (17). However, mechanisms that contribute to this remodeling process remained unclear. An imbalance between the MMPs and the endogenous TIMPs has been associated with pathophysiological myocardial remodel-
ing in a number of cardiac disease states (8, 18). The goal of the present study was to examine the myocardial remodeling response to the discrete injury inflicted by RF energy application and identify the role of TIMP-1 in this process through the use of a transgenic mouse line deficient in the TIMP-1 gene (i.e., \( \text{timp-1}^{-/-} \)). The important findings of this study were threefold. First, this study demonstrated that scars resulting from relatively small discrete myocardial lesions can expand in a time-dependent manner. Specifically, myocardial remodeling in response to injury occurred early, and scar volumes continued to increase at longer postinjury durations. Second, the postinjury remodeling process includes compensatory myocyte hypertrophy in the myocardium remote to the scar. Third, deletion of the TIMP-1 gene was associated with an extensive myocardial remodeling response at both the site of RF injury and in the remote myocardium. These unique findings demonstrate that TIMP-1 plays a regulatory role in postinjury myocardial remodeling following the creation of a discrete lesion.

Past in vitro and in vivo studies have demonstrated that myocardial cell death occurs when the tissue is heated to a temperature in excess of 50°C (11). With RF ablation, myocardial heating occurs primarily at the electrode-tissue interface with heat conduction to areas deeper in the myocardium (11, 12). Histological changes associated with the creation of a RF ablative lesion have been described as coagulation necrosis, with microvascular damage, fibrosis, and myocyte loss (12, 17). Whereas RF lesions have been described to be restricted to the site of energy application, past studies have demonstrated that ultrastructural damage can occur in adjacent tissue beyond the macroscopic edge of the RF lesion (12). The present study builds on the findings of these past studies by demonstrating that alterations in myocardial structure can occur in regions remote to the site of RF-induced injury. Specifically, myocyte CSAs and wall thicknesses in the region of the LV that was diametrically opposite the lesion were increased postinjury in both the WT and \( \text{timp-1}^{-/-} \) mice but to a greater degree in the \( \text{timp-1}^{-/-} \) mice. Potential mechanisms for this hypertrophic response include transduction of mechanical load to the remote myocardium and a superimposition of TIMP-1 deficiency on altered loading conditions may have differentially modulated myocyte growth in the remote myocardium of the \( \text{timp-1}^{-/-} \) mice. Whereas the results of a past in vitro study suggest that TIMP-1 promoted cell growth (6), it is possible that other in vivo systems (7) compensated for the loss of TIMP-1 in the present study and ameliorated the growthpromoting effects of TIMP-1. However, these issues remain speculative, and future studies that examine specific mechanical and biological stimuli operative in this postinjury model are warranted.

A commonly encountered phenotype of myocardial injury with subsequent remodeling is myocardial infarction (MI) (4, 10, 14, 15, 19). Past studies have clearly demonstrated that LV remodeling post-MI is associated with alterations in regional and global myocardial structure (10, 15). Accordingly, a number of investigations utilize transgenic mouse models to elucidate mechanisms of post-MI remodeling (4, 15). In murine MI models, infarct sizes can approach 40-50% of the LV, which may preclude examination of regional remodeling. In the present study, discrete foci of myocardial injury were targeted onto the LV free wall and resulted in the formation of reproducible lesions that were originally 6-7% of the LV. Importantly, results of the present study indicate that the remodeling response to an injury relatively smaller than that inflicted by MI induction progressed in a time-dependent manner. Therefore, this murine model of discrete myocardial injury may be potentially useful to determine mechanisms of regional myocardial remodeling.

In the present study, increased collagen deposition occurred at the site of myocardial injury consistent with a postinjury response (3, 4, 19). Specifically, collagen content at the site of injury was increased by a similar degree in the WT and \( \text{timp-1}^{-/-} \) mice in the late postinjury period, despite larger scar volumes in the \( \text{timp-1}^{-/-} \) mice. These findings suggest that the abundance of other components of the extracellular matrix, including glycoproteins and/or basement membrane components, may have been altered in the \( \text{timp-1}^{-/-} \) mice postinjury. An additional consideration for similar levels of collagen accumulation at the site of injury in the WT and \( \text{timp-1}^{-/-} \) mice is compensatory upregulation of the other TIMP species. For example, a past study examining renal fibrosis in the same \( \text{timp-1}^{-/-} \) mouse phenotype reported increased levels on TIMP-3 mRNA following urethral obstruction (7). However, it must be recognized that although relative collagen content within the site of injury was similar, the structure and function of the collagen matrix in the \( \text{timp-1}^{-/-} \) mice may have been altered, potentially resulting in a loss of myocardial structural support. In fact, past studies from this laboratory have demonstrated alterations in collagen structure occur in the \( \text{timp-1}^{-/-} \) mice (3, 16). In the myocardium remote to the site of injury,
collagen content was lower in the \textit{timp-1}{\textsuperscript{-/-}} mice than WT values at 28 days postinjury. A potential explanation for these findings may be that the loss of TIMP-1 resulted in a "gain of function" for the MMPs, shifting the stoichiometric balance between MMPs and TIMPs towards increased matrix degradation. However, whether changes in collagen accumulation that occurred in this postinjury model were due to "loss-of-TIMP-1" or "gain-of-MMP" functionality was not addressed in the present study and warrants further investigation. These issues notwithstanding, the results of the present study demonstrate that postinjury remodeling of the myocardium was more extensive in the absence of TIMP-1.

\textbf{Study limitations and conclusions.} There are several limitations of the present study that must be recognized. First, the wound healing response following myocardial injury has been documented to proceed in a time-dependent manner (14) leading to the formation and maturation of a discrete collagen scar (19). In addition, postinjury myocardial remodeling is associated with egress of a number of cell types into the area of injury, localizing an inflammatory response and resulting in a time-dependent elaboration of various MMP species (14). In the present study, the time course of collagen deposition at the site of injury was similar between the WT and \textit{timp-1}{\textsuperscript{-/-}} mice. Whereas not measured in the present study, a past study from this laboratory has reported that the egress of inflammatory cell types into the region of myocardial injury in the same \textit{timp-1}{\textsuperscript{-/-}} phenotype was similar to that observed in WT mice (3). From the findings of this past study, it is unlikely that TIMP-1 deficiency altered the inflammatory response at the site of injury and the subsequent elaboration of MMPs.

\textit{Future studies that directly examine MMP induction and evasion of various MMP species (14). In the present study, the time course of collagen deposition at the site of injury was similar between the WT and \textit{timp-1}{\textsuperscript{-/-}} mice. Whereas not measured in the present study, a past study from this laboratory has reported that the egress of inflammatory cell types into the region of myocardial injury in the same \textit{timp-1}{\textsuperscript{-/-}} phenotype was similar to that observed in WT mice (3). From the findings of this past study, it is unlikely that TIMP-1 deficiency altered the inflammatory response at the site of injury and the subsequent elaboration of MMPs.}

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