Controlled Release of Matrix Metalloproteinase-1 Plasmid DNA Prevents Left Ventricular Remodeling in Chronic Myocardial Infarction of Rats

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Background: The present study investigated whether administration of controlled release matrix metalloproteinase-1 (MMP-1) plasmid DNA prevents left ventricular (LV) remodeling in a rat chronic myocardial infarction (MI) model.

Methods and Results: Rats with a moderate-sized MI were randomized to 2 groups: injection of phosphate buffered saline (PBS) containing microspheres into the peri-infarct area (MI group, n=14) and injection of cationized gelatin microspheres incorporating MMP-1 plasmid DNA (MI+MMP-1 group, 50 µg MMP-1/20 µl; n=14). As a control group (n=14), rats received neither the coronary artery ligation nor the injection of PBS. Echocardiography, cardiac catheterization and histological studies were performed. At 2 and 4 weeks after the treatment, the MI+MMP-1 group had smaller LV end-diastolic and end-systolic dimensions, better fractional area change and smaller akinetic areas than the MI group. The LV end-systolic elastance and time constant of isovolumic relaxation were also better in the MI+MMP-1 group compared with the MI group 4 weeks after the treatment. Fibrosis evaluated with Masson's trichrome staining was less in the MI+MMP-1 group than the MI group.

Conclusions: Gelatin microspheres for the controlled release of MMP-1 plasmid DNA are promising for improving cardiac remodeling and function when they are administered during the chronic phase of MI. (Circ J 2009; 73: 2315–2321)

Key Words: Current (I_{NCX}) of Na/Ca exchange (NCX); Matrix metalloproteinase-1; Microspheres; Myocardial infarction; Single cardiomyocyte shortening

Survivors of myocardial infarction (MI) are at high risk of developing chronic heart failure.¹ Rapid recanalization of the occluded coronary artery is presently the best clinical treatment for acute MI, which, if carried out in time, enables considerable salvage of the ischemic myocardial cells. Unfortunately, not all patients have the chance for or respond to timely coronary reperfusion therapy because it must be performed within a few hours after the onset of infarction.² Indeed, patients with post-MI heart failure account for nearly half of the candidates for cardiac transplantation.³ Consequently, development of an effective therapy for post-MI heart failure is one of the most important issues.

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that denature and degrade collagens and other components of the extracellular matrix (ECM). MMPs are involved in many physiological processes, such as embryonic development, ovulation, bone remodeling and wound healing.⁴ Their enhanced activities have also been implicated in numerous disease processes, including arthritis,

tumor cell metastasis, periodontal disease, atherosclerosis and MI.^{5–7} In the progression of MI, MMPs are known to play an important role in several aspects of infarct healing, such as early ECM degradation, cell migration (inflammatory cells, fibroblasts), angiogenesis, remodeling of newly synthesized connective tissue and the regulation of growth factor activities.^{8,9} As a superfamily, MMPs consist of at least 16 members, including the collagenase group, gelatinase group and several membrane-type MMPs. The collagenase group consists of MMP-1, MMP-8 and MMP-13. But only MMP-1 is an interstitial collagenase and it degrades the structural collagens of type I, II and III with the characteristic cleavage site for type I collagen.¹⁰ The early rise of MMP-1 synthesis has been reported in the infarcted myocardium.⁹ The subsequent inactivation of MMP-1 synthesis in the infarcted myocardium leads to collagen accumulation,¹¹ and excessive accumulation may result in a stiff and non-compliant left ventricle (LV) and gradual heart dysfunction. Recently, Aoyama et al reported that local delivery of the MMP-1 gene prevents the onset of renal fibrosis in

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streptozotocin-induced diabetic mice.¹² We hypothesized that local delivery of MMP-1 decreases fibrosis in chronic post-MI hearts and improves cardiac function. The aim of the present study was to determine whether local controlled release of MMP-1 plasmid DNA prevents the progression of myocardial fibrosis and improves cardiac function in rats with chronic MI heart failure.

Methods

All experimental procedures were carried out in accordance with the guidelines for Animal Experiments in Kyoto University, Japan, and conformed with the "Care and Use of Laboratory Animals" published by the US National Institutes of Health.

Preparation of Cationized Gelatin

Gelatin samples with an isoelectric point of 9.0 (MW 100,000), prepared with pig skin using an acid process, were kindly supplied by Nitta Gelatin Inc, Osaka, Japan. The carboxyl groups of gelatin were chemically converted by introducing amino groups for cationization of gelatin.^{13,14} Briefly, ethylenediamine (Wako Pure Chemical, Ltd, Osaka, Japan) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt were added into 250 ml of 100 mmol/L phosphate buffered saline (PBS) containing 5 g of gelatin. The molar ratio of ethylenediamine to the carboxyl groups of gelatin was 50. The reaction mixture was agitated at 37°C for 18 h and then dialyzed against double-distilled water (DDW) for 48 h at room temperature. The dialyzed solution was freeze-dried to obtain a cationized gelatin. When determined using the conventional trinitrobenzene sulfonic acid method, the percentage of amino groups introduced into the gelatin was 50.9 mol percent per the carboxyl groups of gelatin.

Preparation of Cationized Gelatin Microspheres Incorporating MMP-1 Plasmid DNA

Cationized gelatin microspheres were prepared by chemical cross-linking of cationized gelatin in a water-in-oil emulsion state.¹³ Briefly, an aqueous solution of 10 W% cationized gelatin (10 ml) was preheated at 40°C and then added dropwise into 375 ml of olive oil preheated to 40°C, while an impeller stirred at 420 rpm for 10 min to yield a water-in-oil emulsion. The emulsion temperature was decreased to 4°C, followed by further stirring for 30 min for the natural gelation of the gelatin aqueous solution. Cold acetone (100 ml) was added to the emulsion and stirring was continued for 10 min. The resulting microspheres were washed 3 times with cold acetone, collected by centrifugation (5,000 rpm, 4°C, 5 min), fractionated in size by sieves with apertures of 70 and 100 μ m, and air-dried at 4°C. The average diameter of the microspheres was 75 μ m. The non-cross-linked and dried gelatin microspheres (50 mg) were placed in 25 ml of acetone/0.01 mol/L HCl solution (7/3, vol/vol) containing 60 μ l of 25 W% glutaraldehyde solution and stirred at 4°C for 24 h to allow the cationized gelatin to cross-link. After washing by centrifugation (5,000 rpm, 4°C, 5 min) with DDW, the microspheres were agitated in 25 ml of 100 mmol/L aqueous glycine solution at room temperature for 1 h to block the residual aldehyde groups of unreacted glutaraldehyde. The resulting microspheres were washed 3 times with DDW by centrifugation and freeze-dried.

Human MMP-1 genes were subcloned into mammalian transient expression vectors (pFLAG-CMV-5a; Sigma,

St Louis, MO, USA) as previously described.¹² To impregnate the MMP-1 plasmid DNA into the cationized gelatin microspheres, 20 μ l of 100 mmol/L PBS solution (pH 7.4) containing 50 μ g of MMP-1 plasmid DNA was dropped onto 2 mg of the freeze-dried cationized gelatin microspheres and left for 1 h to allow the plasmid DNA solution to impregnate the dried microspheres. The MMP-1 plasmid DNA was completely incorporated into the cationized gelatin microspheres by this procedure because the volume of MMP-1 plasmid DNA solution was small enough compared with that theoretically incorporated into the microspheres. A slow degradation of microspheres was expected to induce a gradual and long-lasting release of MMP-1 plasmid DNA.

Animal Model and Experimental Protocol

Male Lewis rats (10-week-old) were anesthetized with 1–1.5% isoflurane and ventilated through an endotracheal tube. MI was created by proximal ligation of the left anterior descending coronary artery (LAD) through left thoracotomy, as described previously.^{14,15} The rate of mortality by coronary artery ligation and the size of the MI created were similar to our previous reports.^{14,15} At 4 weeks following ligation, cardiac function was evaluated by echocardiography. Rats with moderate infarction (20–40% of endocardial circumference on echocardiography) were randomized to 2 groups. Through a left thoracotomy, 100 μ l of PBS containing the microspheres with MMP-1 (MI+MMP-1 group, n=14) or without MMP-1 (MI group, n=14) was injected subepicardially with a fine syringe into 5 points of the peri-infarct area of the LV free wall. Age- and sex-matched rats receiving neither the coronary artery ligation nor injection of PBS containing microspheres were used as controls (Normal group, n=14) and were also used for the single-cell study.

Evaluation of MMP-1 Plasmid DNA Release and Expression of MMP-1 In Vivo

MMP-1 plasmid DNA and/or microspheres were radioiodinated according to the method of Chan et al.¹⁶ The ¹²⁵I-labeled MMP-1 plasmid DNA was incorporated into gelatin microspheres that were later injected into rats 4 weeks after LAD ligation. To measure radioactivity, heart samples were obtained at 1, 3, 7, 14 and 21 days (n=3 at each sampling time). For the localization of MMP-1 protein, heart samples were obtained at 5 days after the injection and embedded in Tissue-Tek (Sakura Finetek USA, Torrance, CA, USA). Cryosections (5 μ m thick) were prepared from embedded samples and fixed with 4% paraformaldehyde for 20 min. Immunofluorescent examination was carried out using an M.O.M. immunodetection kit (Vector Laboratories, Burlingame, CA, USA). Each fixed section was incubated with mouse IgG blocking reagent for 1 h. Rabbit anti-MMP-1 antibody solution was diluted 100-fold with Tris buffered saline (TBS: 0.05 mol/L Tris, pH 7.4, with 0.15 mol/L NaCl). These sections were incubated with the anti-rabbit IgG-RITC conjugate for 1 h at 25°C. Finally, the stained sections were rinsed with PBS and examined for localization of MMP-1 protein derived from the injected solution using a fluorescence microscope equipped with a digital camera.¹²

Assessment of Cardiac Function

Echocardiographic assessment was carried out using the method described in our previous studies.^{14,15} In brief, LV dimensions and function were assessed at baseline, and 2 and 4 weeks after the treatment. Images were recorded using a 10–12-MHz phased array transducer (HP SONOS 5500,

Figure 1. In vivo release of MMP-1 plasmid DNA. (A) Time profile of remaining radioactivity in the myocardium of rats after the injection of cationized gelatin microspheres incorporation ^{125}I -labeled MMP-1 plasmid DNA. The rats were injected with the microspheres incorporating MMP-1 plasmid DNA (closed circles) and MMP-1 plasmid DNA solution (open circles). (B, C) Immunofluorescent image of MMP-1 protein visualized with RITC (red) and DAPI (blue) in the MI group (B) and MI+MMP-1 group (C). Original magnification, $\times 400$. The MMP-1 protein secreted de novo (colored in red) was detected in rat hearts in the MI+MMP-1 group, but not detected in the MI group. Abbreviations see in text.

Andover, MA, USA). The LV end-diastolic dimension (EDD) and end-systolic dimension (ESD) were measured by M-mode tracings from the short-axis view of the LV at the papillary muscle level.^{14,15,17} Fractional area change (FAC) and akinetic endocardial length to the whole LV endocardial circumference (AL) were also calculated from the same short-axis view of the 2-dimensional image. All measurements were performed in a blind fashion according to the American Society for Echocardiology and averaged over 3 consecutive cardiac cycles.^{14,15,17}

After the final echocardiographic examination, the rats underwent cardiac catheterization for a more precise assessment of global LV function, as described previously.^{14,15} In brief, under general anesthesia, a 2F micromanometer-tipped catheter (Millar Instruments Inc, Houston, TX, USA) was inserted via the right carotid artery into the LV, and a 3F occlusion balloon catheter was inserted through the right femoral vein into the inferior vena cava. LV pressure and its first time-derivative (dp/dt) were continuously monitored using a multiple recording system. LV end-systolic volume was calculated using M-mode echocardiograms by the cube formula. During inferior vena cava occlusion with the balloon, pressure waveforms and M-mode tracings were simultaneously recorded. Next, end-systolic elastance (E_{es}) and the time constant of isovolumic relaxation (τ) were derived from the recorded data. In calculating E_{es} , the end-systolic pressure–volume points obtained from echocardiography and cardiac catheterization were subjected to least-squared linear regression. All data were acquired under stable conditions.^{14,15}

Analysis of Fibrosis

After the final functional assessment, the rats were killed for the histological study. Masson's trichrome staining was

carried out to detect fibrosis. The infarct area was divided into 5 parts, and the central (central-infarct) and lateral areas (peri-infarct) were analyzed. The percentage of fibrotic area in the non-infarct, central-infarct and peri-infarct areas was calculated in each heart using image analysis software (Scion Image Beta 4.02 Win, Scion Corporation).

Electrophysiological Study of Single Myocytes

Cardiomyocytes were isolated from the LV as previously described.¹⁸ The myocytes were superfused with a control Tyrode solution containing (in mmol/L) 140 NaCl, 5.4 KCl, 1.8 CaCl_2 , 0.5 MgCl_2 , 0.3 NaH_2PO_4 , 5.5 glucose and 5.0 HEPES (pH=7.4). Contraction was induced by applying a depolarizing pulse of 2 ms duration through a patch pipette using a patch clamp amplifier (Axopatch 200B, Axon Instruments), and cell length was measured every 5 ms with a liner image sensor (Edge Detector Hamamatsu Photonics K.K., Hamamatsu, Japan) as described previously.¹⁹ Membrane current through Na^+ – Ca^{2+} exchange (I_{NCX}), a pivotal protein of cardiac excitation–contraction coupling, was measured in the whole-cell clamped cardiomyocytes as extracellular 1 mmol/L Ca^{2+} -induced current in the same manner as in our recent study.¹⁸

Northern Blot of Na^+ – Ca^{2+} Exchange (NCX 1)

Northern blots, prepared with 2 μg of poly(A)⁺ mRNA isolated from the indicated samples, were probed in hybridization solution (Life Technologies, Inc) and 50% formamide at 42°C with a radiolabeled DNA fragment derived from a coding region of the rat NCX1 (corresponding to amino acids 272–453), and washed with 0.2 $\times$ SSC and 0.1% SDS at 65°C for 1 h. Quantification of the bands was carried out using a bioimaging analyzer (model BAS3000; Fuji Photo Film Co, Tokyo, Japan).

Figure 2. Assessment of cardiac function by echocardiography and catheterization. (A) Echocardiography. Data are from the MI group (open circles) and MI+MMP-1 group (closed circles). * $P < 0.05$ vs baseline (labeled as pre) in the same group. † $P < 0.001$ vs group MI. Error bars show SEM. (B) Cardiac catheterization. * $P < 0.0001$ vs group MI. Error bars show SEM. Abbreviations see in text.

Data Analysis

All data are expressed as the mean $\pm$ SE. Statistical analysis was carried out with ANOVA using computer software (StatView for Windows version 5.0, SAS Institute Inc, Cary, NC, USA). Multiple comparisons were carried out according to Student–Newman–Keul’s method. A probability value (P) < 0.05 was considered significant.

Results

Evaluation of MMP-1 Plasmid DNA Release and Expression of MMP-1 In Vivo

As shown in **Figure 1A**, 125 I-labeled MMP-1 plasmid DNA was injected into the peri-infarct area of the LV free wall, and the remaining radioactivity was expressed as the percentage of radioactivity of initially injected solution containing MMP-1 plasmid DNA. MMP-1 plasmid DNA rapidly disappeared when it was injected without incorporation into the microspheres. However, MMP-1 plasmid DNA that was incorporated into the gelatin microspheres continued to exist in situ for 2–3 weeks. Furthermore, MMP-1 protein secreted de novo was detected in the MI+MMP-1 group (**Figure 1C**), but not in the MI group (**Figure 1B**), where de novo expression of MMP-1 was visualized with RITC (red).

Improved Cardiac Remodeling and Function by MMP-1 Plasmid DNA

At baseline, the heart rate was similar between the MI group and the MI+MMP-1 group (313 ± 19 vs 310 ± 18 beats/min, $P > 0.05$), as well as body weight (415 ± 12 vs 416 ± 10 g, $P > 0.05$). EDD, ESD, FAC and AL were also comparable between the 2 groups (**Figure 2A**). In the MI group, there was adverse cardiac remodeling and dysfunction, which progressively deteriorated over time, whereas in the MI+MMP-1 group there was favorable cardiac remodeling and increased wall motion in both the infarct and non-infarct remote areas. As

shown in **Figure 2A** (Upper panel), both the EDD and ESD in the MI+MMP-1 group were significantly smaller than those in the MI group at 2 and 4 weeks after the treatment. Moreover, in the MI+MMP-1 group both the EDD and ESD decreased gradually with time after the treatment; namely, both the EDD and ESD were significantly decreased at 4 weeks than at 2 weeks ($P = 0.0247$ for EDD, $P = 0.0081$ for ESD). The longer the time after the treatment, the larger the difference between the MI and MI+MMP-1 groups for either EDD or ESD. These data suggest that the injection of MMP-1 plasmid DNA into the peri-infarct area had a beneficial effect on post-MI cardiac remodeling.

The injection of MMP-1 plasmid DNA was also able to induce improved cardiac performance, as shown by an increased percentage of kinetic area and decreased akinetic area as well as increased FAC. As shown in **Figure 2A** (Lower panel), the FAC in the MI+MMP-1 group was significantly higher than that in the MI group at 2 and 4 weeks after MMP-1 treatment. Moreover, in the MI+MMP-1 group FAC increased gradually with time after treatment; namely, FAC was significantly higher at 4 weeks than at 2 weeks ($P = 0.008$). In the meantime, AL was significantly lower in the MI+MMP-1 group than in the MI group at 2 and 4 weeks after the treatment. Moreover, in the MI+MMP-1 group AL decreased gradually with time after treatment: AL was significantly lower at 4 weeks than at 2 weeks ($P = 0.0095$). These data demonstrate that the injection of MMP-1 plasmid DNA into the peri-infarct area improved cardiac performance.

The abovementioned effects induced by exogenous MMP-1 plasmid DNA were further confirmed by cardiac catheterization. As shown in **Figure 2B**, Ees in the MI+MMP-1 group was significantly higher than in the MI group ($P < 0.0001$), whereas Tau in the MI+MMP-1 group was significantly shorter than in the MI group ($P = 0.0021$). These data indicate that cardiac global function improved by the injection of MMP-1 plasmid DNA.

Figure 3. Assessment of myocardial fibrosis by immunohistochemistry with Masson's trichrome staining. (A) Fibrosis stained blue by Masson's trichrome in the peri-infarct area. Original magnification, $\times 400$. (B) Fibrosis as a percentage in the peri-infarct area ($n=10$). * $P=0.0003$ vs MI group. (C) Fibrosis as a percentage in the central-infarct area ($n=10$). * $P<0.0001$ vs MI group. (D) Fibrosis stained blue by Masson's trichrome in the non-infarct area. Original magnification, $\times 400$. (E) Fibrosis as a percentage in the non-infarct area ($n=10$). † $P=0.0007$ vs MI group. Abbreviations see in text.

Decreased Myocardial Fibrosis by MMP-1 Plasmid DNA

The fibrotic area evaluated with Masson's trichrome staining was significantly decreased in the MI+MMP-1 group compared with the MI group in both the peri-infarct ($P=0.0003$, **Figures 3A,B**) and central-infarct areas ($P<0.0001$, **Figure 3C**). The decrease was also found in the non-infarct regions ($P=0.0007$, **Figures 3D,E**) in which the percentage of fibrosis was comparable to that of the normal heart. The decrease in fibrotic area might be an explanation for the improved cardiac function.

Increased Myocyte Shortening by MMP-1 Plasmid DNA

To evaluate cardiomyocyte contractility, cardiomyocytes were isolated from the non-infarct regions of the LV free wall. The degree of cell shortening (% shortening), which was initiated by electrical stimuli, was assessed by monitoring the longitudinal cell length (**Figure 4**: summary of the pooled data at different beating frequencies from the Normal, MI and MI+MMP-1 groups). Cell shortening from the MI group was depressed when compared with that from the Normal group ($P=0.0389$). The MMP-1 treatment augmented the % cell shortening to almost the same degree as in the Normal group at 0.1 Hz, 1 Hz and 2.5 Hz, respectively.

Reduced $\text{Na}^+/\text{Ca}^{2+}$ Exchange Current (I_{NCX}) by MMP-1 Plasmid DNA

Up-regulation of $\text{Na}^+/\text{Ca}^{2+}$ exchange function has been recognized a characteristic biomarker in post-MI heart failure.²⁰ As shown in **Figure 5**, the expression of NCX1 protein and membrane current through the NCX1 (I_{NCX}) were enhanced in the MI group. In the MI+MMP-1 group, I_{NCX} was significantly reduced to a similar level as in the

Figure 4. Contractility of isolated cardiomyocyte. Comparison of cardiomyocyte contractility among Normal, MI and MI+MMP-1 groups. Contractility was expressed as a % shortening of cell length. Single cell contractility markedly improved in the MI+MMP-1 group over a wide range of beating rates. * $P<0.05$ vs MI group. Abbreviations see in text.

Normal group, but no significant decrease in mRNA encoding NCX1 was found.

Discussion

In this study, we used a new technique of cationizing gelatin microsphere incorporating MMP-1 plasmid DNA to express MMP-1 protein locally in the peri-MI area of chronic MI rat

Figure 5. $\text{Na}^+\text{-Ca}^{2+}$ exchange function. (A) I_{NCX} . I_{NCX} was normalized to cell capacitance and plotted at different membrane potentials. (B) Northern blot analysis of NCX1 mRNA from rat hearts. Each lane contains 2 μg poly (A)+ RNA extracted from the indicated sample. The size of the mRNA for NCX1 was estimated to be 7.2 kilobases (Left upper). Glyceraldehyde-3-phosphate dehydrogenase (Left lower) was used as a control. (Right) Quantification of band intensity obtained by northern blot. Bar diagram showing relative expression levels (mean $\pm$ SEM) of NCX1 in normals (n=4), MI (n=4) and MI+MMP-1 (n=4) rat ventricles. * $P < 0.01$ vs normal group. Abbreviations see in text.

hearts for a limited period of time. The release of MMP-1 plasmid DNA peaked within 24h after injection and continued to be released for 3 weeks. The released MMP-1 plasmid DNA directly permeated the myocardium in situ and resulted in the production of MMP-1 protein. The exogenously expressed MMP-1 then degraded collagen and attenuated myocardial fibrosis, thus improving myocyte contractility and leading to recovery of overall cardiac function. Our study demonstrated that inhibition of excessive accumulation of collagen could be a beneficial therapeutic strategy for chronic MI heart failure. Although MMP-1 does not express in rat hearts,^{21,22} it possesses a high substrate specificity for fibrillar collagen,⁸ which could be advantageous from a therapeutic viewpoint. However, its use as a therapeutic approach has been limited by its short bioavailability. Our method using cationized gelatin microspheres overcomes this shortcoming and enables MMP-1 to express its biological activity for an appropriate time when collagen fibrosis increases in heart tissue.

It has been demonstrated that after the development of a transmural MI in rats, adverse ventricular remodeling by fibrous tissue occurs at both the site of MI and in remote myocardium.^{23–26} Although the increase in reparative fibrosis and ECM serves to preserve the structural integrity of infarcted tissue in the early phase of MI, in the chronic phase, the accumulation of a large amount of fibrosis, especially interstitial fibrosis, leads to ventricular diastolic dysfunction rather than preservation of cardiac structure.^{27–30} In our rat model of MI, heart function continued to deteriorate 4–8 weeks after MI in the MI group, while the injection of MMP-1 plasmid DNA significantly improved heart function. These results imply that cardiac fibrosis continued to progress for 8 weeks after MI in our rat model. Although the detailed time course of fibrosis remains to be clarified, our study demonstrates that inhibition of excessive accumulation of collagen was indeed beneficial in this animal model. As demonstrated in **Figure 3**, fibrosis was

improved in the central and non-MI areas, as well as the peri-MI region where the MMP-1 plasmid was injected. Regarding the central MI area, it is likely that the released MMP-1 plasmid DNA diffused and was expressed in the central MI area because of the small size of the rat heart. The improved contractility of the peri-MI area might indirectly improve both the fibrosis and contractility of the non-MI areas that are connected with each other. The mechanism underlying this remote effect remains to be studied.

The underlying mechanism for the beneficial effects of controlled release of MMP-1 plasmid DNA needs to be further studied. The improvement in wall motion in the infarct area might be a result of degradation of interstitial collagen, which might decrease the stiffness of the ventricle. The increase in contractility of the remaining myocytes is likely to be 1 of the causes of improved systolic function. The cause-and-effect relationship of increased contractility of single cardiomyocytes is not totally clear in this study. However, because the function of NCX1, an important protein for cardiac Ca^{2+} handling, returned to normal levels after the MMP-1 plasmid treatment, an alteration in intracellular Ca^{2+} handling might be associated with this effect. The alteration might be caused indirectly by a decrease in the stiffness of the ventricle or by degradation of intracellular proteins by MMP-1, as demonstrated with MMP-2.³¹

Previous studies have demonstrated that overexpression of human MMP-1 within the normal myocardium of mice results in significant myocardial collagen disruption and LV dysfunction at 12 months of age,³² whereas it prevents LV dilation and preserves cardiac function in a mouse model of pressure overload.¹¹ Therefore, it is speculated that induction of MMP-1 is beneficial under pathophysiological conditions in which collagen accumulates in the heart, such as chronic MI (as in this study) and sustained hypertension.¹¹ Conversely, upregulation of MMP-1 in the normal heart may result in deterioration of cardiac function.

Study Limitations

First, the dose-dependent effect of MMP-1 needs to be further studied. We speculate that the expression level of MMP-1 is a key issue preventing MMP-1 from providing beneficial effects. A level of exogenous MMP-1 that is too high may even be detrimental to cardiac remodeling, leading to cardiac rupture. A level of MMP-1 that is too low may be inadequate to exert beneficial effects. The time-dependent response after the onset of infarction is also an important issue, not only in terms of efficacy but also safety. Second, further studies are needed to elucidate the underlying mechanisms by which the MMP-1 plasmid DNA microspheres improve cardiac function and the causative relationship between plasmid delivery and the observed effects. There is increasing evidence to suggest that alterations in the MMP/tissue inhibitor of MMP (TIMP) balance may contribute to myocardial fibrosis.^{8–11,13,21,22,24,31,32} Therefore, the induction of exogenous MMP-1 might affect other MMPs and TIMP, which are normally expressed in the heart. It is also possible that MMP-1 acts on non-ECM proteins both inside and outside the cell.³¹ Nevertheless, to the best of our knowledge the present study provides evidence for the first time that the local delivery of exogenous MMP-1 plasmid DNA with microspheres significantly improves cardiac remodeling and cardiac function.

Conclusion

The present study convincingly demonstrated that control-released administration of exogenous MMP-1 plasmid DNA is indeed able to effectively prevent the progression of adverse cardiac remodeling in a chronic MI heart failure model in rats.

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