Treatment With Dimethylthiourea Prevents Left Ventricular Remodeling and Failure After Experimental Myocardial Infarction in Mice

Role of Oxidative Stress

Shintaro Kinugawa, Hiroyuki Tsutsui, Shunji Hayashidani, Tomomi Ide, Nobuhiro Suematsu, Shinji Satoh, Hideo Utsumi, Akira Takeshita

Abstract—Oxidative stress might play an important role in the progression of left ventricular (LV) remodeling and failure that occur after myocardial infarction (MI). We determined whether reactive oxygen species (ROS) are increased in the LV remodeling and failure in experimental MI with the use of electron spin resonance spectroscopy and whether the long-term administration of dimethylthiourea (DMTU), hydroxyl radical (‘OH) scavenger, could attenuate these changes. We studied 3 groups of mice: sham-operated (sham), MI, and MI animals that received DMTU (MI+DMTU). Drugs were administered to the animals daily via intraperitoneal injection for 4 weeks. ‘OH was increased in the noninfarcted myocardium from MI animals, which was abolished in MI+DMTU. Fractional shortening was depressed by 65%, LV chamber diameter was increased by 53%, and the thickness of noninfarcted myocardium was increased by 37% in MI. MI+DMTU animals had significantly better LV contractile function and smaller increases in LV chamber size and hypertrophy than MI animals. Changes in myocyte cross-sectional area determined with LV mid–free wall specimens were concordant with the wall thickness data. Collagen volume fraction of the noninfarcted myocardium showed significant increases in the MI, which were also attenuated with DMTU. Myocardial matrix metalloproteinase-2 activity, measured with gelatin zymography, was increased with MI after 7 and 28 days, which was attenuated in MI+DMTU. Thus, the attenuation of increased myocardial ROS and metalloproteinase activity with DMTU may contribute, at least in part, to its beneficial effects on LV remodeling and failure. Therapies designed to interfere with oxidative stress might be beneficial to prevent myocardial failure. (Circ Res. 2000;87:392-398.)

Key Words: antioxidant radicals heart failure myocardial infarction remodeling

Myocardial infarction (MI) frequently produces left ventricular (LV) dilatation and hypertrophy of the noninfarcted myocardium.1 These changes in LV geometry, referred to as remodeling, contribute to the development of depressed cardiac performance.2 Thus, surviving patients with MI are at an increased risk for occurrence of heart failure (HF), reinfarction, arrhythmia, and sudden cardiac death.2 LV remodeling is caused by the side-to-side slippage of cardiac myocytes at the infarcted region and the hypertrophic response of the noninfarcted myocytes, resulting in a progressive increase in the chamber diameter.3 The underlying mechanisms responsible for these processes have been attributed to hemodynamic stress as well as the activation of neurohumoral factors, including the renin-angiotensin system. However, the details of contributing factors in LV remodeling remain to be elucidated.

Reactive oxygen species (ROS) can produce myocardial contractile dysfunction and structural damage.4 There is growing evidence that ROS are increased in HF and may contribute to disease progression.5,6 Hill and Singal7 showed that antioxidant enzyme activities are decreased and that thiobarbituric acid reactive substances (TBARS) are increased in the failing myocardium due to MI. Further, recent study in isolated cardiac myocytes has shown that a subtle increase in ROS results in a phenotype characterized by hypertrophy and apoptosis,8 which play an important role in myocardial remodeling and failure.9 We recently demonstrated that ‘OH is increased with the development of rapid pacing-induced HF with the use of electron spin resonance (ESR) spectroscopy with 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (hydroxy-TEMPO).6 In our studies, ‘OH is generated from superoxide anion (‘O2-) and H2O2 via metal-catalyzed Harber-Weiss reaction and Fenton reaction within the myocardial tissue. These observations have prompted the thought that oxidative stress can contribute to LV remodeling and HF after MI.
Several important questions remain to be answered. First, no direct evidence for the increased production of ROS has been obtained in post-MI hearts. Therefore, it should be determined whether our previous observations in rapid pacing–induced HF and post-MI, as well as the modulatory effects of MMP activity in experimental models, in which the changes of ROS production have not been rigorously examined concurrently with myocardial response. It is critically important to examine whether the administration of ROS scavengers can attenuate both ROS production and HF.

Previous studies have reported increased myocardial metalloprotease (MMP) activity in experimental models, including rapid pacing–induced HF and post-MI, as well as in human end-stage HF. Recently, an MMP inhibitor was shown to limit early LV dilatation in a murine model of MI. Because MMP can be activated by ROS in vivo, one proposed mechanism of LV remodeling is the activation of MMP secondary to increased ROS production. We hypothesized that ROS production and myocardial elaboration of MMP activation are interdependent and that the effects of ROS scavengers on LV remodeling are related, at least in part, to the modulation of this axis.

Accordingly, the first goal of the present study was to examine whether the production of ROS is increased in the remodeled LV after MI; the second goal was to determine whether chronic inhibition of 'OH production could inhibit the progression of LV remodeling and failure. A pharmacological intervention that can be used to prevent 'OH-mediated injury should ideally be capable of entering myocardial cells rapidly to encounter the generation of reactive oxygen metabolites. It should also maintain sufficient levels of tissue concentrations to afford protection against low levels of 'OH. However, most ROS scavengers have serum half-lives on the order of minutes and do not readily cross cell membranes. Dimethylthiourea (DMTU) is an agent that is highly diffusible, has a long half-life, and is effective in scavenging hydrogen peroxide (H2O2) and 'OH. Therefore, DMTU is expected to be an effective antioxidant, especially when administered in vivo.

In the present study, we created MI in mice by ligating the left anterior descending coronary artery, and we assessed the production of 'OH by measuring the rate of reduction of hydroxy-TEMPO in the myocardial tissue by using ESR spectroscopy. Further, we examined whether chronic in vivo administration of DMTU into MI animals can attenuate the LV remodeling and HF. We also examined myocardial MMP activity by using gelatin zymography.

Materials and Methods

Animal Models

The study was approved by our institutional animal research committee and conforms to the animal care guidelines of the American Physiological Society. MI was created in male CD-1 (Charles River) mice (6 to 8 weeks old, weight 30 to 40 g) by ligating the coronary artery according to the methods described by Michael et al. 1

Experimental Protocol

MI mice were randomly grouped to receive either saline (MI group) or DMTU (MI+DMTU group). DMTU (50 mg/kg in sterile saline) was administered daily via intraperitoneal injection beginning 6 hours after the creation of MI and throughout the study (4 weeks). This dose was chosen based on the previous studies of its efficacy.

Echocardiography and Hemodynamic Evaluation

Serial echocardiographic measurements at baseline and 3, 7, 14, and 28 days after surgery were made in all groups of animals. After the echocardiographic measurements, LV pressure was measured according to the methods described by Williams et al. One subset of investigators (S.H. and N.S.), who were not informed of the experimental groups, performed in vivo LV function studies that included echocardiography and LV pressure measurements.

Experimental Protocol 1

Quantification of Myocardial 'OH by ESR Spectroscopy

We quantified 'OH in the noninfarcted LV myocardium according to the methods described previously.

Experimental Protocol 2

LV Morphology and Morphometry

A separate group of animals, treated identically as in protocol 1, was used to evaluate the effects of chronic DMTU administration on LV chamber diameters. Infarct size in these hearts was determined according to the method described by Pfeffer et al.

Myocyte Size and Collagen Volume Fraction

Myocyte cross-sectional area and collagen volume fraction were measured according to the methods described previously.

Experimental Protocol 3

Myocardial MMP Activity

MMP activity in the noninfarcted LV was measured with gelatin zymography according to the methods described previously.

Results

Animal Characteristics

Coronary artery ligation and a sham operation were performed in 84 and 32 mice, respectively. Eighty-one mice that survived 6 hours after coronary artery ligation were randomized to active treatment with DMTU (MI+DMTU; n = 33) or no treatment (MI; n = 48). During the follow-up period, 24 (30%) deaths occurred (6 mice receiving DMTU and 18 mice receiving no drugs; P = NS). All mice that died were confirmed to have MI on postmortem examination. All sham-operated animals survived until the end of the study period.

Echocardiography

Serial 2-dimensional and M-mode echocardiography was performed in a group of sham-operated (n = 10), MI (n = 10), and MI+DMTU (n = 8) animals. Figure 1 demonstrates marked LV dilatation and contractile impairment in the MI mouse. These changes were attenuated in MI+DMTU mice. Figure 2 shows that LV end-diastolic diameter increased and percent fractional shortening (FS) decreased by day 3 after ligation of coronary artery. They were significantly different.
from control values by days 3 to 28 of the control. DMTU significantly inhibited this LV diameter increase and percent FS decrease in MI as early as 3 days, which was maintained throughout the study period, indicating the persistent attenuation of LV dilatation and failure with DMTU from the early phase after MI.

The summarized data for echocardiographic measurements at baseline and after 4 weeks are presented in Table 1 online (data supplement available at http://www.circresaha.org). In comparison with sham-operated animals, MI animals showed a 38% decrease (P<0.01) in the thickness of the infarcted region and a 37% (P<0.05) increase in the thickness of the noninfarcted region. DMTU significantly attenuated the hypertrophy of the noninfarcted myocardium (P<0.01) but did not affect the thickness of the infarcted portion.

Hemodynamics and Organ Weights
Hemodynamic measurements could be obtained in a group of MI (n=8), MI+DMTU (n=8), and sham-operated (n=7) animals (Table 2 online; data supplement available at http://www.circresaha.org). They had similar body weights (P=NS). The MI mice tended to exhibit lower aortic blood pressures than the sham group, which, however, did not reach statistical significance (P=0.06). LV end-diastolic pressure was significantly elevated and LV +dP/dt was depressed in the MI group (P<0.01 for both), which was attenuated with DMTU. Coincident with an increased LV end-diastolic pressure, the ratio of lung weight to body weight was significantly increased in the MI group versus the sham group (9.1±1.5 versus 4.6±0.1 g/kg, P<0.01), which was also attenuated with DMTU treatment (5.7±0.4 g/kg, P<0.01 versus MI).

ROS in the Noninfarcted LV
ESR signals of hydroxy-TEMPO reduced more rapidly in the presence of homogenates from post-MI hearts compared with sham (Figure 3A). There was a linear relation in the semilogarithmic plot of peak signal intensity versus time, indicating the first-order kinetics of the signal decay (Figure 3B). The rate constant of signal decay was significantly (P<0.01) larger in MI than that in sham (Figure 3C) animals. DMTU (50 mmol/L) added to the reaction mixture completely abolished an increase of signal decay in MI, indicating that ROS indeed contributed to the increase of signal decay rate in MI. Catalase (50 U/mL) plus superoxide dismutase (SOD; 50 U/mL) also attenuated an increase in signal decay rate, which implies the contribution of O_{2}^{-} to the production of OH.

In the MI+DMTU group, the “DMTU-inhibitable” rate of signal decay was normalized, which provided evidence that the chronic in vivo administration of OH scavenger DMTU into MI animals completely prevented the production of OH (Figure 4).

LV Morphology and Morphometry
Figure 5 shows the transverse LV sections (midcavity) stained with Masson’s trichrome. The sections obtained from the MI mouse revealed an anteroapical infarct that extends into the anterolateral wall (Figure 5B). The interventricular septum was generally spared. Infarct size was estimated to establish whether the scarred myocardium was comparable between MI and MI+DMTU and to provide a basis of comparison. Two MI and 2 MI+DMTU mice with an infarct size of <40% were excluded from the analysis of LV...
morphology. Infarct size was identical between MI (59±3%, range 42% to 70%; n=12) and MI + DMTU (56±2%, range 49% to 66%; n=11) animals. Figure 6 illustrates MI size and LV chamber diameter in all groups. Consistent with echocardiographic data (Table 1 online; data supplement available at http://www.circresaha.org), MI + DMTU animals had significantly smaller LV chamber diameters and volume than MI animals.

Myocyte cross-sectional area was increased in MI, which was significantly attenuated with DMTU treatment (Figure 7). These results are concordant with LV wall thickness data obtained from echocardiography (Table 1 online; data supplement available at http://www.circresaha.org). Collagen volume fraction was also increased in MI, which was inhibited with DMTU treatment (Figure 7).

Myocardial MMP Activity

Figure 8 shows representative gelatin zymography of the LV from sham, MI, and MI + DMTU mice. There was minimal MMP activity in the sham group. MI after 7 days markedly enhanced MMP-2 (62- and 58-kDa gelatinases) activity in the noninfarcted LV. The increase in MMP-2 activity persisted at day 28 after MI.12,13 There was a modest but significant increase in MMP-1 activity (54 kDa) in MI at both days 7 and 28.12,13 With DMTU, there was significant attenuation of MMP activities at 7 and 28 days after the induction of MI (Figure 8).

Discussion

The present study demonstrates an increase of ROS in the noninfarcted myocardium, in association with LV remodeling (ie, the chamber dilatation and hypertrophy of the noninfarcted region). Despite the equal infarct size, chronic administration of DMTU into the MI animals results in a tendency toward normalization of LV size and contractile function. The changes in LV structure after DMTU treatment are associated with reduced myocyte hypertrophy and interstitial fibrosis, suggesting that they are responsible, at least in part, for post-MI LV remodeling and reverse remodeling with DMTU.

Increased ROS in the Post-MI Heart

The ESR method for the measurement of ROS in the myocardial tissue used in the present study has been well validated in our previous studies.5,6 This can provide a direct method to quantify the generation of ROS within biological tissue.6 We extended our earlier observations in rapid ventricular pacing–induced HF by showing that the production of 'OH was increased in the myocardium with remodeling and dysfunction after MI. These results also confirm and extend
Changes between C and hypertrophy of noninfarcted LV in association with HF. We observed that DMTU prevented LV dilatation and improvement in contractile function. The changes in LV structure were associated with reduced myocyte hypertrophy and interstitial fibrosis, suggesting that they are responsible, at least in part, for post-MI LV remodeling and reverse remodeling with DMTU.

The present results suggest that increased myocardial ROS could contribute to the activation of MMP and thus to the development of LV remodeling after MI. It has been reported that MMPs are increased in the noninfarcted myocardium obtained from a rat model of MI.13 Further, an MMP inhibitor has been shown to limit the chamber dilatation in a murine model of MI.15 Sustained MMP activation might therefore influence the structural properties of the myocardium by providing an abnormal extracellular environment with which the myocytes interact.16 Importantly, the present study has demonstrated that DMTU inhibits the activation of MMP in association with the development of LV remodeling. These data raise the interesting possibility that increased ROS after MI can be a stimulus for myocardial MMP activation, which might play an important role in the development of HF. Although this concept warrants further exploration, there is recent evidence for the activation of vascular MMP by ROS in vivo.16 In addition, the beneficial effects of DMTU on LV failure may be related to its antiedema action because small
increases in interstitial water content can greatly increase LV chamber stiffness.32

Moreover, ROS have direct effects on cellular structure and function and may be integral signaling intermediates in myocardial remodeling.33 Higher levels of ROS are known to cause direct damage to proteins and lipids, leading to myocyte death through necrosis or apoptosis.4 Further, more powerful pro-oxidant peroxynitrite can be produced, particularly when both \( \text{O}_2^- \) and nitric oxide are present.34 A recent study by Siwik et al8 demonstrated that a subtle increase in ROS caused by partial inhibition of SOD results in a phenotype characterized by hypertrophy and apoptosis in isolated cardiac myocytes, which appears to be present in the noninfarcted myocardium. ROS of mitochondrial origin may be particularly prone to trigger apoptosis.35

The demonstration of a preventive effect of DMTU on HF implies that antioxidants could be of clinical relevance. Recently, carvedilol, a \( \beta \)-blocker with antioxidant activity, has been shown to be beneficial in the treatment of patients with congestive HF.36 One must take into account that this drug possesses additional properties, including \( \alpha \)-adrenoceptor–blocking action and antiproliferative activities. However, as in the settings of ischemia-reperfusion, one might assume that the beneficial effects of carvedilol on HF are also mediated, at least in part, through the prevention of ROS-induced damage.

Study Limitations

There are several limitations that should be acknowledged in this study. First, because the hemodynamic profile of DMTU is incompletely described, we could not exclude the possibility that the beneficial effects of DMTU might be due to hypothetical favorable hemodynamic effects on systemic and coronary circulation. However, this possibility might be less likely because arterial pressure and heart rate were not influenced with DMTU (Table 2 online; data supplement available at http://www.circresaha.org). Second, we administered DMTU into the animals for 4 weeks to allow sufficient time to identify changes in LV morphology, because this study was designed to address the hypothesis that ROS inhibition could attenuate long-term LV remodeling. Thus, the present study does not preclude an additional effect if DMTU was initiated at the time of coronary ligation or the effects on coronary occlusion with reperfusion. Further studies in genetically altered mice and other models will also improve understanding of the role of oxidative stress in LV remodeling. Third, in the present study, we examined the amount of ROS at the time point after the development of LV remodeling. Significant changes in ROS may also occur at the onset and during the development of remodeling. Further studies that focus in the temporal changes of oxidative stress may be necessary.

Conclusions

\( \cdot \text{OH} \) radicals were increased within the post-MI hearts, which might be involved in myocardial remodeling and failure. Therapies designed to interfere with oxidative stress could be beneficial to prevent the progression of HF.


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Methods

Echocardiography

On the day of terminal study, 4 week after surgery, echocardiographic studies were performed under light anesthesia and spontaneous respiration.\textsuperscript{1} A commercially available echocardiography system (SSD-5500, Aloka, Tokyo) was utilized with a dynamically focused 10-MHz linear array transducer using a depth setting of 2.0 cm. Two-dimensional images and M-mode tracings were recorded from the short-axis view at the papillary muscle level. Care was taken not to apply too much pressure to the chest wall. The M-mode tracings were printed on glossy paper by using a digital color printer (SSZ-307, Aloka). LV end-diastolic diameter (EDD), end-systolic diameter (ESD), and wall thickness were measured, averaging three to five cardiac cycles. Fractional shortening (\% FS) was calculated using the following equation; \% FS = (LV EDD) - (LV ESD) / (LV EDD) \times 100

Hemodynamic Evaluation

After the echocardiographic measurements, the polyethylene tube (0.61 mm OD) connected to a pressure transducer (Nihon-Kohden) was inserted into the right carotid artery and secured in place. After at least 10 min for stabilization, systemic arterial pressure was recorded using a multichannel chart recorder (Nihon-Kohden). The tube was
further advanced into the LV cavity for the measurements of LV pressures and its first
derivative (dP/dt) according to the methods described by Williams et al.\textsuperscript{2}

After the physiological studies, with the animals under anesthesia, the heart was
arrested in diastole by injection of 0.15 mL of KCl (1 mol/L) through the aortic catheter.
The heart and lungs were excised, blotted dry, and immediately weighed.

**Experimental Protocol 1**

**ESR experiments**

·OH was quantified in the noninfarcted LV myocardium according to the methods
described previously.\textsuperscript{3} In brief, ESR spectra of hydroxy-TEMPO were recorded in the
presence of myocardial homogenates. The solutions of hydroxy-TEMPO (Sigma) were
prepared in phosphate-buffered saline (PBS; 150 mmol/L NaCl, 3 mmol/L KCl, and 5
mmol/L phosphate; pH 7.4) and stored at -80 °C. The ESR measurements were performed
at room temperature using X-band (9.43 GHz) ESR spectrometer (JES-RE-1X; JEOL).
The ESR settings were as follows; a microwave power of 10 milliwatt, a range of external
magnetic field of 20 mT, and a scan rate of 10 mT/min.

The amount of ROS was assessed by monitoring the time-dependent decay of the
amplitude of low field component of ESR spectra elicited by hydroxy-TEMPO. The rate
of decay was determined by the kinetics of ESR signal attenuation. Our recent studies
have confirmed that hydroxy-TEMPO is reduced in the presence of ·OH, but not of ·O2-,
indicating that the rate of signal decay is specific for the presence of ·OH.\textsuperscript{3} Further, the
rate of signal decay is proportional to the amount of ·OH.

**Quantitation of myocardial ·OH by ESR spectroscopy**

Freeze-clamped LV myocardial samples (100 mg) were homogenized in 50 mmol/L
sodium phosphate buffer (pH 7.4) containing protease inhibitors (leupeptin 10 µg/mL,
phenylmethylsulfonyl fluoride 100 µg/mL, dithiothreitol 1 mmol/L, and trypsin inhibitor
10 g/mL). The homogenate was immediately reacted with hydroxy-TEMPO (0.1 mmol/L)
in PBS and its ESR spectra were recorded up to 10 min at the intervals of 10 to 15 sec. In
the presence of ·OH within biological tissue, hydroxy-TEMPO is reduced to hydrogenamines, which leads to an attenuation of its ESR signals. To exclude the contribution of reductants known to be present in tissue homogenates, all measurements were performed in two parallel runs, in the presence and absence of ·OH scavenger DMTU (50 mmol/L). The amount of "DMTU-inhibitable" rate of signal decay was used as an index of ·OH amount in the myocardial tissue. Further, to determine the contribution of ·O2- and H2O2, the measurements were also performed in the presence of catalase (50 U/mL) plus superoxide dismutase (SOD; 50 U/mL) as competitive reagents.

**Experimental Protocol 2**

A separate group of animals, treated identically to protocol 1, was employed to evaluate the effects of chronic DMTU administration on LV pump function and remodeling after MI. The thoracic aorta was cannulated with a polyethylene catheter and filled with PBS (pH 7.4) and heparin (100 IU/mL). The heart was perfused with PBS containing formalin (6 %) and the right atrium was cut to allow drainage. Perfusion pressure was adjusted to the mean arterial pressure for each animal. A 26-gauge needle was inserted into the LV cavity through the apex and the LV chamber was filled with fixative from a pressure reservoir set at a height equivalent to the *in vivo*-measured LV end-diastolic pressure for 20 min.

**LV morphology and morphometry**

At the end of the procedures, the LV including the septum and the right ventricle were dissected. After measuring the major long-axis intracavitary diameter, each LV was serial-sectioned into three rings perpendicular to the major axis of the heart, after which the short-axis diameter was measured. At the mid region, the minimal and maximal chamber diameters were used with the long axis diameter to compute LV chamber volume.

Infarct size in these hearts was determined by the method described by Pfeffer et al. Briefly, serial 5-μm sections were prepared, mounted, and stained with Masson's
trichrome. Infarct length was measured along the endo- and epicardial surfaces from each of the three LV sections, and values from all three sections were summed. Total LV circumference was calculated as the sum of endo- and epicardial segment lengths from all three LV sections. Infarct size (in percent) was calculated as total infarct circumference divided by total LV circumference times 100.

**Myocyte size and collagen volume fraction**

To measure myocyte cross-sectional area, the coronal sections from mid-LV were fixed in 6% formaldehyde and 5 μm-thick paraffin-embedded sections were stained with hematoxylin and eosin and Masson's trichrome according to the methods described previously. Collagen volume fraction was measured at approximately 5 to 7 fields for each heart according to the methods described previously.

**Experimental Protocol 3**

**Myocardial MMP Activity**

MMP activity in the myocardium was examined by gelatin zymography according to the methods described previously. The LV myocardial samples were homogenized (~30-second bursts) in 1 mL of an ice-cold extraction buffer containing cacodylic acid (10 mmol/L), NaCl (0.15 mol/L), ZnCl₂ (20 mmol/L), NaN₃ (1.5 mmol/L), and 0.01% Triton X-100 (pH 5.0). The homogenate was then centrifuged (4 °C, 10 min, 10,000 g) and the supernatant decanted and saved on ice. The pH of the samples were adjusted to 7.5 using Tris (1 mol/L). Final protein concentration of the myocardial extracts was determined with a standardized colorimetric assay. The extracted samples were then aliquoted and stored at -80 °C until the time of assay.

The myocardial extracts were then directly loaded onto electrophoretic gels (SDS-PAGE) containing 1 mg/mL of gelatin under non-reducing conditions. The myocardial extracts at a final protein content of 5 μg were loaded onto the gels using a 3:1 sample buffer (10% SDS, 4% sucrose, 0.25 mol/L Tris-Cl, and 0.1% bromophenol blue, pH 6.8). The gels were run at 15 mA/gel through the stacking phase (4 %) and at 20 mA/gel for the
separating phase (10%), maintaining a running buffer temperature of 4 °C. After SDS-PAGE, the gels were washed twice in 2.5% Triton X-100 for 30 minutes each, rinsed in water, and incubated for 24 hours in a substrate buffer at 37 °C (50 mmol/L Tris-HCl, 5 mmol/L, CaCl₂, and 0.02% NaN₃, pH 7.5). After incubation, the gels were stained with Coomassie brilliant blue R-250. The zymograms were digitized, and the size-fractionated bands, which indicated MMP proteolytic activity, were measured by the integrated optical density in a rectangular region of interest.

**Statistical Analysis**

Values are expressed as means ± SEM. For multiple-group comparisons, one-way ANOVA followed by Scheffe's test was performed. P < 0.05 was considered statistically significant.
### Echocardiographic data

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 16)</th>
<th>MI (n = 16)</th>
<th>MI+DMTU (n = 13)</th>
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</thead>
<tbody>
<tr>
<td>LV EDD, mm</td>
<td>3.8 ± 0.1</td>
<td>5.8 ± 0.1*</td>
<td>4.1 ± 0.1†</td>
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<td>LV ESD, mm</td>
<td>2.6 ± 0.1</td>
<td>5.1 ± 0.1*</td>
<td>3.1 ± 0.1*†</td>
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<td>% Fractional shortening</td>
<td>33.8 ± 1.4</td>
<td>11.9 ± 0.9*</td>
<td>25.9 ± 1.3*†</td>
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<td>LV wall thickness</td>
<td></td>
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<tr>
<td>Infarcted region, mm</td>
<td>0.71 ± 0.03</td>
<td>0.44 ± 0.02*</td>
<td>0.41 ± 0.01*</td>
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<tr>
<td>Non-infarcted region, mm</td>
<td>0.79 ± 0.04</td>
<td>1.08 ± 0.02*</td>
<td>0.85 ± 0.04†</td>
</tr>
</tbody>
</table>

Data are the means ± SE. n indicated the number of animals studied.

LV, left ventricular. EDD, end-diastolic diameter. ESD, end-systolic diameter.

*P < 0.01 vs. Sham, †P < 0.01 vs. MI.
### Hemodynamic data

<table>
<thead>
<tr>
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<th>Sham (n = 8)</th>
<th>MI (n = 8)</th>
<th>MI+DMTU (n = 7)</th>
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<td>Body weight, g</td>
<td>36.2 ± 0.7</td>
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<td>Heart rate, / min</td>
<td>430 ± 14</td>
<td>383 ± 35</td>
<td>407 ± 16</td>
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<td>Mean aortic pressure, mmHg</td>
<td>97 ± 8</td>
<td>77 ± 6</td>
<td>90 ± 6</td>
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<td>LVEDP, mmHg</td>
<td>3.4 ± 0.8</td>
<td>14.4 ± 4.3**</td>
<td>6.2 ± 0.6†</td>
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<tr>
<td>LV +dP/dt, mmHg/s</td>
<td>4840 ± 210</td>
<td>2761 ± 238**</td>
<td>3896 ± 268**††</td>
</tr>
</tbody>
</table>

Data are the means ± SE. n indicated the number of animals studied.

LVEDP indicated the left ventricular end-diastolic pressure.

**p < 0.01 vs. Sham, †p < 0.05 vs. MI, ††p < 0.01 vs. MI
References


