Diminazene Acetate Enhances Angiotensin-Converting Enzyme 2 Activity and Attenuates Ischemia-Induced Cardiac Pathophysiology

YanFei Qi, Juan Zhang, Colleen T. Cole-Jeffrey, Vinayak Shenoy, Andrew Espejo, Mina Hanna, Chunjuan Song, Carl J. Pepine, Michael J. Katovich, Mohan K. Raizada

Abstract—Angiotensin-converting enzyme 2 (ACE2) plays a critical role against myocardial infarction (MI). We hypothesized that activation of intrinsic ACE2 would be protective against ischemia-induced cardiac pathophysiology. Diminazene acetate (DIZE), a small molecule ACE2 activator, has been used to evaluate this hypothesis. DIZE (15 mg/kg per day, s.c.) was injected 2 days before MI surgery and continued throughout the study period. MI rats showed a 62% decrease in fractional shortening (%; control, 51.1±3.2; DIZE alone, 52.1±3.2; MI, 19.1±3.0), a 55% decrease in contractility (dP/dt max mm Hg/s; control, 9480±245.3; DIZE alone, 9585±97.4; MI, 4251±657.7), and a 27% increase in ventricular hypertrophy (mg/mm; control, 26.5±1.5; DIZE alone, 26.9±1.4; MI, 33.4±1.1). DIZE attenuated the MI-induced decrease in fractional shortening by 89%, improved dP/dt max by 92%, and reversed ventricular hypertrophy by 18%. MI also significantly increased ACE and angiotensin type 1 receptor levels but decreased ACE2 activity by 40% (control, 246.2±25.1; DIZE alone, 254.2±20.6; MI, 148.9±29.2; RFU/min), which was reversed by DIZE treatment. Thus, DIZE treatment decreased the infarct area, attenuated LV remodeling post-MI, and restored normal balance of the cardiac renin-angiotensin system. In addition, DIZE treatment increased circulating endothelial progenitor cells, increased engraftment of cardiac progenitor cells, and decreased inflammatory cells in peri-infarct cardiac regions. All of the beneficial effects associated with DIZE treatment were abolished by C-16, an ACE2 inhibitor. Collectively, DIZE and DIZE-like small molecules may represent promising new therapeutic agents for MI. (Hypertension. 2013;62:746-752.)

Key Words: angiotensin-converting enzyme 2 ▪ diminazene ▪ macrophages ▪ myocardial infarction ▪ stem cells

The renin–angiotensin system (RAS) plays a critical role in maintaining cardiovascular homeostasis. Angiotensin-converting enzyme 2 (ACE2) metabolizes angiotensin II (AngII) into the heptapeptide angiotensin-(1–7) (Ang-[1–7]), thereby maintaining a balance between the deleterious axis (AngII) into the heptapeptide angiotensin-(1–7) (Ang-[1–7]),1,2 possessing the potential to activate ACE2, prevents cardiac injury and reduces myocellular damage to ischemia-induced cardiac dysfunction.3,4 It has been demonstrated that the use of these agents to inhibit the deleterious axis of the RAS leads to an upregulation of ACE2.5

ACE2 modulates cardiac function and could be a potential therapeutic target for cardiovascular diseases, on the basis of the following evidences: (1) ACE2 and Ang-(1–7) levels are increased as a compensatory mechanism in the heart after myocardial infarction (MI) in both humans and rats5-8; (2) we and others have demonstrated that cardiac overexpression of ACE2 renders protection against ischemia-induced cardiac pathophysiology9,10,11; (3) overexpression of Ang-(1–7) protects the heart from the ischemic-induced cardiac dysfunction12; (4) we have previously demonstrated that a small molecule, XNT (1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one), possessing the potential to activate ACE2, prevents cardiac remodeling in spontaneously hypertensive rats.13 Collectively, these findings have led several groups to propose that administration of recombinant ACE2 could be a potential therapeutic strategies for the treatment of heart failure.14,15

Recently, diminazene acetate (DIZE), another US Food and Drug Administration–listed small molecule used as an antitrypanosomal drug, has been shown to exert off-target effects of activating ACE2.16 We have recently demonstrated that DIZE treatment renders cardioprotective effects and improves angiogenic progenitor cell functions in rat model of pulmonary hypertension.17 This complied with the fact...
that current standard treatments for MI cannot efficiently improve cardiac function and prevent further damage to the myocardium. We decided to evaluate the effects of DIZE on ischemic-induced cardiac pathophysiology. Our observations demonstrate that DIZE treatment prevents MI-induced cardiac pathophysiology, thus providing us an opportunity to explore the therapeutic potential of this compound in ischemic heart disease and heart failure.

**Material and Methods**

All experimental protocols are presented in the Methods in the online-only Data Supplement.

**Results**

**DIZE Treatment Prevents the MI-Induced Left Ventricular Dysfunction, and C-16 Abolishes the Protective Effects of DIZE**

MI caused a 62% reduction in fractional shortening (Figure 1A) and a 27% increase in ventricular hypertrophy (Figure 1B), which were significantly prevented by DIZE treatment. Measurement of cardiac hemodynamic functions in MI animals revealed higher left ventricular end diastolic pressure and decreased dP/dt_{max} and dP/dt_{min} compared with normal and DIZE controls, which were restored by DIZE treatment (Figure 1C–1E). We had hypothesized that these beneficial effects of DIZE are associated with the activation of endogenous cardiac ACE2. Therefore, we coadministered C-16, a selective ACE2 inhibitor, along with DIZE. Four weeks of cotreatment with C-16 abolished the improvements in cardiac function produced by DIZE (Figure 1A–1E). In contrast to these beneficial effects on cardiac pathophysiology, DIZE treatment did not alter systolic blood pressure (Figure 1F).

**DIZE Treatment Alters Cardiac RAS Levels**

mRNA levels of ACE and ACE2 and protein levels of AT1R and MasR were measured to determine whether DIZE treatment shifts the balance from deleterious to vasoprotective axis of the RAS. Cardiac ACE mRNA increased by 2-fold, whereas ACE2 mRNA decreased 2-fold in MI animals (Figure 2A and 2B). DIZE treatment reduced ACE mRNA and significantly elevated ACE2 mRNA in the MI animals. As a result, the ratio of the mRNA levels of ACE2/ACE was 2-fold higher in DIZE-treated MI hearts (Figure 2C). MI induced 2-fold increase in ACE activity, although this activity in DIZE-treated MI hearts was not significantly different from the control and DIZE-treated hearts (Figure 2D). In contrast, ACE2 activity was modestly decreased in the peri-infarct area of the MI hearts, and DIZE treatment resulted in 2.5-fold increase in this activity (Figure 2E). Plasma ACE2 activity was slightly lowered in MI animals and significantly increased by DIZE treatment, which was abolished by C-16 (Figure 2F). Moreover, Western blot analysis demonstrated a 1.9-fold increase in AT1R protein levels (Figure 2G) and a moderate increase in MasR (Figure 2H) in the MI group. DIZE treatment significantly inhibited the increase in AT1R.

These changes induced by DIZE were blocked by treatment...
with C-16. However, DIZE treatment did not significantly alter the increase in MasR compared with the MI group.

**DIZE Treatment Increases Numbers of Cardiac Progenitor Cells and Circulating Endothelial Progenitor Cells**

Our next objective was to determine whether the reparative capacities of DIZE are because of its effects on progenitor cells. Thus, we determined the levels of circulating endothelial progenitor cells (EPCs) and Islet-1 (a marker for cardiac progenitor cells [CPCs]). We observed a 2-fold increase in the CPCs in the peri-infarct region of the DIZE-treated ischemic heart (Figure 3A and 3B). This increase in the number of CPCs suggests a higher regenerative potential in the hearts from DIZE-treated animals. Moreover, the number of circulating EPCs was also increased by 48% in the DIZE-treated animals over that observed in the MI group (Figure 3C). Cotreatment of DIZE animals with C-16 abolished both increases in the CPCs and in the EPCs.

**DIZE Treatment Decreases Infiltration of Macrophages in the Peri-Infarct Area of the Heart and Modulates Proinflammatory Cytokines**

Infiltration of macrophage in the peri-infarct area of the heart is associated with MI. Thus, we examined whether DIZE treatment would influence this process. DIZE treatment
significantly prevented the infiltration of CD68+ cells (a marker for macrophages; Figure 4, top panel). Western blot analysis showed that CD68 immunoreactivity in MI hearts was increased by 46%, which was completely attenuated by DIZE treatment (Figure 4A and 4B). Interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) mRNA levels were examined next to determine whether the beneficial effects of DIZE on cardiac hemodynamic were associated with changes in the proinflammatory cytokines. As expected, MI resulted in 2.5- and 3-fold increases in IL-1β and TNF-α, respectively (Figure 4C and 4D). DIZE treatment alone did not affect these cytokines in control animals. However, DIZE treatment in the MI animals prevented the increase in these cytokines, which further supported the anti-inflammatory effects of DIZE. C-16 blocked all the anti-inflammatory effects of DIZE (Figure 4).

**DIZE Treatment Decreases Infarct Area and MI-Induced Apoptosis**

DIZE treatment decreased the infarct size by 50% (Figure S1A in the online-only Data Supplement). Next, we examined whether DIZE exerts antiapoptotic effects in response to ischemia. Heart sections of the MI group showed 6-fold increase in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, which was prevented by DIZE treatment (Figure S1B). C-16 treatment abolished all the effects produced by DIZE (Figure S1).

**DIZE Treatment Prevents the MI-Induced Loss of Capillary Density**

A significant reduction (1-fold) in capillary density was observed in MI hearts. DIZE treatment alone did not alter capillary density; however, DIZE treatment in the MI animals significantly prevented the decrease in the capillary density of the MI group (Figure S2).

**Discussion**

Our study is significant in that it provides evidence that DIZE, an ACE2 activator offers impressive protection against MI by preserving fractional shortening, attenuating the increase in LVEDP, preventing the dysfunctional changes in dP/dt, and decreasing infarct size and apoptosis. This treatment also modulates the RAS in a positive manner, reduces inflammatory processes, and increases the engraftment of CPCs and circulating EPCs. All these beneficial effects were blocked by C-16.

The imbalance in the RAS as a result of an increase in the vasodeleterious (ACE/AngII/AT1R) axis and a decrease in the vasoprotective (ACE2/Ang-(1–7)/MasR) axis has been proposed to be critical in the development and establishment of cardiac pathophysiology.19,20 ACE2 expression is upregulated at the beginning of the MI as a compensatory mechanism8 and then significantly decreased at heart failure stage.21 Because DIZE is administered before the injury is induced, it protects heart function and thus prevents the ACE2 downregulation. It
is also possible that DIZE modulates ACE2 expression and acts in a positive feedback manner because DIZE has been found to be able to bind DNA. Thus, we hypothesize that restoring this imbalance by the activation of endogenous ACE2 could provide protection against cardiac damage. This study provides evidence in support of this concept and confirms the protective role of the vasoprotective axis because all the dysfunctional and pathological parameters were prevented by the ACE2 activator.

It has been shown that ACE2 overexpression in the human umbilical vein endothelial cells leads to an increase in tube formation and can promote endothelial cell migration in vitro, and ACE2 can promote capillary formation and neovessel maturation in vivo. DIZE, as an ACE2 activator, may also promote angiogenesis and exert its effects via Ang-(1–7). Ang-(1–7) also has been shown to directly act on hematopoietic progenitor cells and modulate their function. In the present study, DIZE causes an increase in CPCs homing to the peri-infarct area of the ischemic heart, increases circulating EPCs, and restores capillary density, all of which are correlated with an improvement of cardiac function. Our study and others indicate that the ACE2/Ang-(1–7)/MasR axis induces mechanisms that are pivotal for regenerative therapy, and further studies on the signaling pathways activated by DIZE in progenitor cells are warranted.

Elevated levels of proinflammatory cytokines, such as IL-1β and TNF-α, contribute to the development and progression of ischemic injury. The ACE2/Ang-(1–7)/MasR axis possesses an anti-inflammatory role. Our observation that DIZE prevents increases in proinflammatory cytokines, and macrophage cells further supports the importance of this compound in influencing many pathological events, directly and indirectly. This is supported by our previous observations that overexpression of Ang-(1–7) and DIZE treatment decreases pulmonary proinflammatory cytokines in models of lung disease.

In summary, our study illustrates that DIZE treatment preserves cardiac function and attenuates cardiac remodeling post-MI. The beneficial effects of DIZE may be a result of preventing the imbalance of the RAS observed post-MI, subsequently modulating various proinflammatory cytokines associated with MI injury, decreasing the infiltration of macrophages associated with cardiac ischemia, and increasing the homing of CPCs and mobilizing EPCs.

Finally, it is pertinent to point out that we have used DIZE as a compound to test this proof of concept. DIZE has been shown to exert toxic effects in some studies and may not be the pharmacodynamically ideal molecule for translational studies. Nonetheless, the study is relevant in that it provides evidence that the chemical structure resides in DIZE has the
potential to activate ACE2, provide beneficial outcomes in MI-induced cardiac pathophysiology, and form the basis to develop structure similar and safe compounds.

**Perspectives**

Recently, Oudit and Penninger have suggested that recombinant ACE2 can be used as a novel therapy for heart failure. However, the application of recombinant ACE2 protein for MI is limited by its peptide nature and possible metabolic degradation if administrated orally. The use of synthetic small molecules, such as DIZE, or other compounds that are able to activate ACE2, provide beneficial outcomes in MI-induced cardiac pathophysiology, and form the basis to identify small molecules, such as DIZE, or other compounds that are able to activate ACE2, provide beneficial outcomes in MI-induced cardiac pathophysiology, and form the basis to develop a novel therapeutic strategy in the treatment of MI and its associated complications.

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**Disclosures**

None.

**References**


What Is New?

• This study demonstrates, for the first time, that chronic administration of ACE2 activator (diminazene aceturate [DIZE]) attenuated the myocardial infarction–induced cardiac dysfunction.

• DIZE increased the number of circulating endothelial progenitor cells and cardiac progenitor cells and decreased the macrophage infiltration in the ischemic myocardium, which reveals a previously unidentified role of DIZE in modulating progenitor cells and the regulation of inflammation.

What Is Relevant?

• It is significant that chronic activation of endogenous ACE2 by DIZE or as such attenuated MI-induced cardiac pathophysiology, which provides a new therapeutic approach for ischemic heart diseases and related cardiovascular disorders.

Summary

DIZE attenuated MI-induced cardiac pathophysiology through modulating the gene expression of the RAS and inflammatory cytokines, increasing EPCs and CPCs, and suppressing macrophage infiltration.
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Diminazene aceturate enhances ACE2 activity and attenuates ischemia-induced cardiac pathophysiology

YanFei Qi a b, Juan Zhang b d, Colleen T Cole-Jeffrey a, Vinayak Shenoy a b, Andrew Espejo b, Mina Hanna b, Chunjuan Song a, Carl J Pepine c, Michael J Katovich b, and Mohan K Raizada a

Departments of a Physiology and Functional Genomics, b Pharmacodynamics, c Medicine, University of Florida; d Department of Anesthesiology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Short title: Diminazene attenuates cardiac pathophysiology

Corresponding Author: Mohan K. Raizada, Ph.D.

Department of Physiology & Functional Genomics

University of Florida

Gainesville, FL 32610

Email: mraizada@ufl.edu

Phone: 352-392-9299

Fax: 352-294-0191
Supplement Methods and Data

Material and Methods

Myocardial Infarction

Eight week old Sprague Dawley rats were divided into five experimental groups: 1) control (N=9); 2) DIZE (N=9); 3) MI (N=12); 4) MI+DIZE (N=12); and 5) MI+DIZE +C-16 (N=6). MI surgery was performed as described previously. Briefly, the proximal left anterior descending coronary artery was ligated (CAL) using a 7–0 polypropylene suture. Successful cessation of coronary blood flow was confirmed by elevation of the ST segment on electrocardiogram and cyanosis of anterior LV wall. DIZE (15mg/kg, s.c.) was initiated two days before CAL surgery and continued throughout the four-week period. For the ACE2 inhibition study, compound 16 (C-16), a selective ACE2 inhibitor, was given (25mg/kg, s.c.) at same time with DIZE treatment, which was selected based on our published report. C-16 was obtained from Millennium Pharmaceuticals Inc. (Cambridge, MA).

All animals were housed in a temperature-controlled room (25 ± 1°C) and were maintained on a 12:12-hour light:dark cycle with free access to water and food. All procedures involving experimental animals were approved by the Institutional Animal Care and Use Committee at the University of Florida and complied with National Institutes of Health guidelines.

Echocardiography

Cardiac function was assessed by echocardiography immediately before sacrifice, four weeks after the CAL surgery. Echocardiography was performed using a GE vivid7 ultrasound machine with a 12-Hz transducer (GE Healthcare, NJ, USA). Rats were anesthetized with the 2% isoflurane-oxygen mixture during the assessment. M-mode echocardiography was performed by using a parasternal short-axis view at the level of the papillary muscles. All measurements were based on the average of three consecutive cardiac cycles. LV internal diameter at end diastole (LVIDd) and end systole (LVIDs) were obtained. The fractional shortening was calculated according to the formulae: FS = [(LVIDd-LVIDs)/LVIDd] × 100

Hemodynamic Measurements

Cardiac function was also assessed by Millar catheterization four weeks after the CAL surgery. Rats were anesthetized with the 2% isoflurane-oxygen mixture, placed in supine position and the body temperature was maintained at 37°C using a heated pad. The left ventricular function was measured by Millar catheterization by cannulating the right carotid artery and threading the catheter into the left ventricle. The catheter was interfaced to a PowerLab (ADInstruments, Colorado Springs, CO, USA) signal transduction unit. Data were analyzed using the Chart program supplied along with the PowerLab system. The parameters measured included systolic blood pressure (SBP), heart rate (HR), left ventricular end diastolic pressure (LVEDP), and maximal positive and negative rate of rise of the left ventricular pressure (dP/dt\text{max} and dP/dt\text{min}).

Histological Analysis

Following the hemodynamic measurement, under deep anesthesia and laparotomy, hearts were collected. The ventricles were separated from atria and rinsed in 1x PBS, weighed, and cut into
three different sections made perpendicular to the long axis. The basal and apex section was snap frozen in liquid nitrogen and stored at –80°C for subsequent quantitative real-time PCR measurements. The middle section was used to measure the infarct size and cardiac remodeling. Ventricular sections were stained with Picro-Sirius Red to measure infarct size. The mid-ventricular section provides adequate estimation of total LV infarct size. Total infarct size was calculated as a fraction of the left ventricular circumference. Cardiac remodeling was assessed by determining ventricular hypertrophy, which was calculated by normalizing the wet weights of rat heart ventricles to the tibia length.

**Apoptosis Assay**

Apoptosis was determined using the Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay (TMR red In Situ Cell Death Detection Kit) according to the manufacturer’s protocol (Roche Diagnostics, Indianapolis, IN). Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI). For each slide, five different fields were evaluated at high magnification (×400). The number of the apoptotic nuclei (red fluorescence) was counted using ImageJ.

**Capillary vessel density**

Cross section of the heart ventricles were stained with Lectin GSI (Sigma-Aldrich Chemie©, Zwijndrecht, The Netherlands), as previously described. Sections of 5μm thickness were deparaffinized and rehydrated. The sections were incubated overnight with the biotinylated Lectin GSI (1:250) at room temperature. The next day sections were rinsed, incubated with Sterpt AV Alexa 594 conjugated secondary antibody at room temperature for 1 h, and mounted with anti-fade mounting medium (Vectashield, Vector Laboratories). Since Lectins stain not only capillaries but also other vessels, a size criterion of 10μm was used to exclude small arterioles and venules. Six randomly selected fields in the viable LV free wall were photographed and analyzed in Image J software. Capillary density was calculated as the number of capillaries per tissue area in the viable LV wall.

**ACE and ACE2 Activity in cardiac tissue and plasma**

Plasma was diluted in ACE/ACE2 buffer [1M NaCl, 75mM Tris-HCl, and 0.5μM ZnCl2, at pH 7.4] at 1:10 ratio, as well as cardiac tissue (from the peri-infarct region of the LV), were homogenized in ACE/ACE2 buffer. All assays were performed in triplicate in ACE/ACE2 buffer. Ten nM ACE or ACE2 enzyme was used as positive controls. Human recombinant ACE [catalog:929-ZN] and ACE2 [catalog:933-ZN-010] were obtained along with its fluorogenic substrates [ACE substrate: fluorogenic peptide V (FPS V), Mca-RPPGFSAFK(Dnp)-OH, catalog. ID: ES005; ACE2 substrate: fluorogenic peptide VI (FPS VI), Mca-YVADAPK(Dnp)-OH, catalog. ID: ES007] obtained from R&D Systems, Minneapolis, MN. Enzymatic activity was measured in a total volume of 100μL using a fluorescence plate reader (Synergy HT, Biotek, USA) at an excitation wavelength of 320nm and emission wavelength of 405nm as described previously. All assays were performed at least in triplicate and samples were read at 37°C for 4h immediately after the addition of fluorogenic peptide substrate.

**Western Blot**
Cardiac tissue (from the peri-infarct region of the LV) was homogenized in radioimmuno-precipitation assay buffer (RIPA buffer). Thirty μg total protein were separated on 12% SDS-PAGE gels, and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, USA). The membranes were blocked with 5% non-fat milk solution in Tris buffered saline (TBS) with 0.1% Tween 20 (TBS-T) for 1 hour and incubated either with AT1R rabbit polyclonal antibody (1:400, Santa Cruz Biotechnology Inc.), Mas R rabbit polyclonal antibody (1:1000, Alomone Labs.), Islet-1 rabbit polyclonal antibody (1:500, Abcam.), or CD68 mouse monoclonal antibody (1:1000, Abcam), overnight at 4°C. Polyclonal anti-GAPDH antibody (1:2000, Sigma Aldrich.) was used to confirm equal loading. After overnight incubation with the primary antibody, the membranes were washed three times for 5 minutes in TBS-T, and then incubated with secondary antibody conjugated with horseradish peroxidase (GE Healthcare, New Jersey, USA; anti-rabbit IgG 1:2500) for 1 hour. Finally, the membranes were subjected to a chemiluminescence detection system and exposed to a photographic film.

**RNA Isolation and Real-time PCR**

Cardiac tissue (from the peri-infarct region of the LV) was homogenized and total RNA was isolated using RNAqueous 4 polymerase chain reaction (PCR) kit (Ambion, Foster City, CA, USA) according to the manufacturer's instructions. 500ng RNA was reverse transcribed using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). The ACE2, ACE, IL-1β and TNF-α were analyzed by quantitative real-time PCR using Taqman probe (Applied Biosystems). Real-time PCR was run using ABI Prism 7600 sequence detection system. All cDNA samples were assayed in triplicate. Data were normalized to GAPDH.

**Cardiac progenitor cell and macrophage staining**

Cross section of the heart ventricles were incubated overnight with rabbit anti-rat Islet-1 antibody (1:100, Abcam) and mouse anti-rat CD68 monoclonal antibody (1:100, Abcam). The next day sections were rinsed, incubated with anti-rabbit Alexa Fluor 594 and anti-mouse Alexa Fluor 594 conjugated secondary antibody (Invitrogen) at room temperature for 1 h, and mounted with anti-fade mounting medium (Vectashield, vector Laboratories).

**Direct flow cytometry analysis**

To profile the levels of blood EPCs, the mononuclear cells from blood were prepared in a concentration of 1x10⁶ cells/100ul in PBS+2% FBS+1mM EDTA mixture media. CD4⁻/5⁻/8⁻/CD90⁺ cells were used as representative for EPCs, as these have previously shown angiogenic and endothelial reparative properties in the rat. Antibodies were purchased from AbD Serotec (Alexa647 conjugated CD4/5/8 and Percp-cy5.5 conjugated CD90), and used as recommended by the company. Cells were incubated with antibodies for 30 minutes at 4°C. Individual antibodies were prepared in each cell suspension and used as control. After spinning down and washing twice, cells were resuspended in 500μl PBS+2% FBS+1mM EDTA mixture media. All samples were read on an LSR-II (BD Biosystems) at the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR) and the data were analyzed with FACS Diva software, version 6.1.2.

**Statistical Analysis**
Data are expressed as mean ± SE and were analyzed by one way ANOVA with Bonferroni correction for multiple comparisons. Values of P<0.05 were considered statistically significant. All of the data were analyzed using GraphPad Prism 5 software (GraphPad Prism Institute Inc).

**Supplement figure legend:**

**S1:** Attenuation of infarct area and apoptosis. Top panel: representative Picro Sirius red–stained sections and quantitative analysis of infarct area (a). Bottom panel: representative heart sections and quantification of apoptosis (b). TUNEL-positive cells (apoptotic cells, purple color) were stained in red, merged with DAPI-stained nuclei (blue), and counted as the number of TUNEL-positive cells, which were indicated by yellow arrow head. &: M+D vs all other groups; **: M and M+D+C vs Con, D and M+D; p<0.05. Scale bar: 10µm.

**S2:** Capillary density of the left ventricle free wall is significantly reduced in the MI treated animals and this reduction is prevented with DIZE treatment. Data represent mean ± SEM. *: M vs all other groups; p<0.05. Scale bar: 20µm.

**References:**


