Sildenafil (Viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice


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Sildenafil (Viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice. Am J Physiol Heart Circ Physiol 294: H1398–H1406, 2008. First published January 25, 2008; doi:10.1152/ajpheart.91438.2007.—We tested the hypothesis that chronic treatment with sildenafil attenuates myocardial infarction (MI)-induced heart failure. Sildenafil has potent protective effects against necrosis and apoptosis following ischemia-reperfusion in the intact heart and cardiomyocytes. ICR mice underwent MI by left anterior descending coronary artery ligation and were treated with sildenafil (0.71 mg/kg bid) or saline for 4 wk. Infarct size (IS) was measured 24 h postinfarction, and apoptosis was measured by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling. Left ventricular end-diastolic diameter (LVEDD) and fractional shortening (FS) were measured by echocardiography. Sildenafil reduced IS (40.0 ± 4.6%) compared with that in saline (69.6 ± 4.1%, P < 0.05). Nω-nitro-l-arginine methyl ester, a nitric oxide synthase (NOS) inhibitor (15 mg/kg bid), blocked the protective effect of sildenafil (IS, 60.2 ± 1.6%, P < 0.05 vs. sildenafil). Western blot analysis showed a significant increase in endothelial NOS/inducible NOS proteins 24 h post-MI after treatment with sildenafil versus saline. Apoptosis decreased from 2.4 ± 0.3% with saline to 1.2 ± 0.1% with sildenafil (P < 0.05) on day 7 and from 2.0 ± 0.2% with saline to 1.2 ± 0.1% with sildenafil on day 28 (P < 0.05), which was associated with an early increase in the Bcl-2-to-Bax ratio. LVEDD increased from baseline value of 3.6 ± 0.1 mm on days 7 and 28, respectively, with saline (P < 0.05) but was attenuated to 4.4 ± 0.2 mm on day 7 and 4.4 ± 0.1 mm following sildenafil treatment on days 28, respectively (P > 0.05 vs. baseline). FS significantly improved post-MI with sildenafil. A marked decline in cardiac hypertrophy was observed with sildenafil, which paralleled a reduction in pulmonary edema. Survival rate was lower with saline (36%), compared with sildenafil (93%, P < 0.05). Sildenafil attenuates ischemic cardiomyopathy in mice by limiting necrosis and apoptosis and by preserving left ventricular function possibly through a nitric oxide-dependent pathway.

Animals. Adult male outbred ICR mice were supplied by Harlan Sprague-Dawley (Indianapolis, IN). The mean body weight was 34.4 ± 0.4 g. All experimental preparations and protocols involving animals were reviewed and approved by the Animal Care and Use Committee of Virginia Commonwealth University. The studies conformed to the American Physiological Society guidelines and principles for research involving animals.

Drugs and chemicals. Nω-nitro-l-arginine methyl ester (l-NNAME) and triphenyltetrazolium chloride (TTC) were purchased from Sigma-Aldrich (St. Louis, MO). Sildenafil powder was kindly provided by Pfizer.

Myocardial infarction protocol. A total of 126 mice were used. The animals were anesthetized with the injection of pentobarbital sodium (70 mg/kg ip), intubated orotracheally, and ventilated on a positive-pressure ventilator. The tidal volume was set at 0.2 ml, and the

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respiratory rate was adjusted to 133 cycles/min. All surgical procedures were carried out under sterile conditions. A left thoracotomy was performed at the fourth intercostal space, and the heart was exposed by stripping the pericardium. The left anterior descending coronary artery (LAD) was then identified and permanently occluded by a 7-0 silk ligature that was placed around it. After coronary artery occlusion, the air was expelled from the chest. The animals were exsanguinated and then received intramuscular doses of analgesia (Buprenex; 0.02 mg/kg) and antibiotic (gentamicin; 0.7 mg/kg for 3 days).

**Experimental groups.** Five groups were used: 1) saline—each mouse received 0.2 ml saline (ip, bid), starting post-MI until explanation of heart; 2) sildenafil—mice received injections of 0.71 mg/kg ip (in 0.2 ml saline) bid, starting immediately after coronary artery ligation until the collection of the heart; 3) NO synthase inhibitor—L-NAME (15 mg/kg ip) + sildenafil-L-NAME was given 1 h before sildenafil, which was administered at a dose of 0.71 mg/kg (in 0.2 ml saline) bid, starting immediately after coronary artery ligation until collection of the heart; 4) L-NAME + saline controls—L-NAME (15 mg/kg ip) given 1 h before saline; and 5) sham—mice were subjected to a left thoracotomy without ligation of the coronary artery as a control for the surgical procedure. The animals in this group received no treatment until the sampling of the heart. In groups 1, 2, 3, and 4, the infarct size was measured 24 h after coronary artery occlusion. Another group of hearts was collected at 7 days for assessment of apoptosis following functional analysis using echocardiography. The last set of mice was euthanized at 28 days following infarction after echocardiography, and the hearts were explanted for evaluation of apoptosis. Six mice in each group were used for infarct size assessment; three to six mice per group for TUNEL, and 12–16 mice per group for functional measurements using echocardiography. The detailed experimental protocol is shown in Fig. 1.

**Survival.** The survival rate was determined based on the animals that survived the experimental protocol starting at recovery following surgery until 28 days after infarction. Animals that died during surgical recovery were excluded.

**Infarct size assessment.** After completion of the infarction protocol, the heart was quickly removed and mounted on a Langendorff apparatus. The coronary arteries were perfused with 0.9% NaCl containing 2.5 mM CaCl2. After the blood was washed out, ~2 ml of 10% Evans blue dye were injected as a bolus into the aorta until most of the heart turned blue. The heart was perfused with saline to wash out the excess Evans blue. Finally, the heart was removed, frozen, and cut into 8–10 transverse slices from apex to base of equal thickness (~1 mm). The slices were then incubated in a 10% TTC in isotonic phosphate buffer (pH 7.4) at room temperature for 30 min. The areas of infarcted tissue, the risk zone, and the whole LV were determined by computer morphometry using a Bioquant imaging software.

**Western blot analysis.** Western blot analysis was used to measure inducible NO synthase (iNOS), endothelial NO synthase (eNOS), and Bcl-2 and Bax proteins as described previously (45). In brief, triplicate heart samples were collected 2 and 24 h after MI and saline or sildenafil injection and were homogenized in ice-cold radioimmuno-precipitation assay buffer (Upstate Biotechnology). The homogenate was centrifuged at 10 000 g for 10 min at 4°C, and the supernatant was recovered as the total cellular protein. Total protein (60 μg) from each sample was separated by SDS-PAGE on 10% acrylamide gels, transferred to a nitrocellulose membrane, and then blocked with 5% nonfat dry milk in Tris-buffered saline. The membrane was subsequently incubated with a rabbit polyclonal antibody (dilution 1:500, Santa Cruz) of iNOS, eNOS, Bcl-2, Bax or goat polyclonal antibody of actin. The secondary antibody was a horseradish peroxidase-conjugated anti-rabbit IgG (1:1,000 dilution, Amersham) or anti-goat IgG (1:1,000 dilution, Santa Cruz). The membranes were developed using enhanced chemiluminescence and exposed to X-ray film.

**Doppler echocardiography.** Doppler echocardiography was performed using the Vevo770 imaging system (VisualSonics, Toronto, Canada) before surgery (baseline) on days 7 and 28 after surgery before the animal was euthanized. Light anesthesia was used during the exam with the injection of pentobarbital sodium (30 mg/kg ip). The mice were placed in the supine position, and ECG limb electrodes were attached. The chest was carefully shaved, and ultrasound gel was used on the thorax to optimize visibility during the exam. A 30-MHz probe was used to obtain two-dimensional, M-mode and Doppler imaging from parasternal short-axis view at the level of the papillary muscles and the apical four-chamber view (35). M-mode images of the LV were obtained, and systolic and diastolic wall thickness (anterior and posterior) and LV end-diastolic and end-diastolic diameters (LVESD and LVEDD, respectively) were measured. LV fractional shortening (FS) was calculated as (LVEDD – LVESD)/LVEDD×100. Ejection fraction was calculated using the Teichholz formula (41). The LV mass was calculated using the following formula (41). The LV mass was calculated using the following

$$LV mass (g) = \frac{1.04 (LV volume cm^3) + 0.66}{LV mass (g) = \frac{1.04 (LV volume cm^3) + 0.66}{0.84} \times 10$$

**Daily monitoring:** Pain, appetite/drinking, behavior and responsiveness

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**Fig. 1.** Experimental protocol: 28-day heart failure experimental protocol for studies of sildenafil therapy following myocardial infarction (MI). Sildenafil (0.71 mg/kg) was administered intraperitoneally immediately after left anterior descending coronary artery (LAD) ligation. Additional doses (0.71 mg/kg) were administered twice daily (bid) for 28 days. Arrows indicate the time points for saline/sildenafil treatment, performance of surgical procedure(s), and measurement of various parameters (listed under each arrow). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.
formula \((LVEDD + AWDT + PWDT)^3 - (LVEDD)^3 \times 0.8 \times 1.04 + 0.6/1,000\), where AWDT and PWDT are anterior and posterior wall diastolic thickness, respectively (10). Transmitral and LV outflow tract pulsed-Doppler flow spectra were obtained from the apical view. Measurement of the outflow tract flow was performed. Isovolumetric contraction (ICT) and relaxation (IRT) times and ejection time (ET) were measured. LV outflow tract (LVOT) flow and aortic velocity-time integral (AoVTI) were also measured. These data were used to calculate the Tei index (Tei Index = ICT + IRT/ET) (40) and cardiac output [cardiac output = AoVTI \(\times\) (LVOT diameter/2)^2 \(\times\) heart rate], where LVOT was measured as the cross-sectional area at the parasternal long-axis view. The Tei index provides prognostic information in a variety of cardiac conditions, including heart failure (14) and MI (24, 16), and elevated IRT 4–6 wk after AMI is reflective of elevated LV end-diastolic pressure (16). The stroke volume was calculated as AoVTI \(\times\) LVOT area. The allocation to different treatments was random, and the investigators performing and reading the ECG were blinded to the treatment.

**Evaluation of apoptosis.** The heart was quickly removed and mounted on a Langendorff apparatus. The coronary arteries were perfused with 0.9% NaCl containing 2.5 mM CaCl_2_. After the blood was washed out, the coronary arteries were then perfused with 10% formalin for 10 min. The heart was then stored in a 10% formalin solution until paraffin embedding. Transverse sections of the median third of the LV were taken and immediately fixed in paraformaldehyde as previously specified (4). Apoptosis was assessed using the TUNEL technique (DNA fragmentation, Oncor, Gaithersburg, MD). The detailed protocol was previously published (3, 4). The peri-infarct area was defined as the zone bordering the infarct where viable myocardium was prevalent and reparative fibrosis was only marginal as identified by standard hematoxylin-eosin staining (3). The apoptotic rate was expressed as the number of apoptotic cells of all cardiomyocytes per field. The apoptotic rate in the peri-infarct regions was calculated using 10 random fields, which virtually cover the entire peri-infarct area. The allocation to different treatments was random, and the pathologist was blinded to the treatment.

**Evaluation of cardiac hypertrophy and pulmonary edema.** On day 28 post-MI, the mice were weighed before death. The hearts and lungs were also weighed immediately after collection, and cardiac hypertrophy was calculated as the ratio of heart weight to body weight. Pulmonary edema was also calculated as the ratio of lung weight to body weight.

**Statistics.** All measurements of infarct size and risk areas are expressed as group means \(\pm\) SE. Changes in echocardiography, infarct size, TUNEL, and hypertrophy index variables were analyzed using two-way repeated-measures ANOVA to determine the main effect of time, group, and time-by-group interaction. If the global tests showed major interactions, post hoc contrasts between different time points within the same group or between different groups were performed using \(t\)-test. Statistical differences were considered significant if the \(P\) value was <0.05. Discrete variables were presented as absolute and percent value. The \(\chi^2\)-square test (or the Fisher exact test when appropriate) was used to compare discrete variable in different groups. The Bonferroni correction for post hoc analysis was used when comparing two groups from three or more groups. Kaplan-Meyer analysis was used to test for differences in survival.

**RESULTS**

**Survival.** A total of 126 mice were used in this study. Some 28 out of 30 mice survived in the sildenafil-treated group (93%) compared with 14 out of 39 in the saline control group (36%, \(P < 0.001\), Fig. 2). The survival rate was 100% in sham-operated mice.

**Expression eNOS/iNOS.** eNOS and iNOS expression was measured in myocardial samples 24 h after LAD occlusion in saline- and sildenafil-treated mice. Protein was extracted, and eNOS, iNOS, and \(\beta\)-actin were identified by Western blot analysis, as we have described previously (9, 33). The eNOS/iNOS-to-\(\beta\)-actin ratio was used to express the results. As shown in the representative blots and quantitative bar graphs in Fig. 3, myocardial levels of both eNOS and iNOS were increased in the sildenafil-treated mice compared with saline controls (\(P < 0.05\)).

**Infarct size.** The infarct size (% of risk area) was reduced from 69.6 \(\pm\) 4.1 in the saline-treated group to 40.9 \(\pm\) 4.6 in the sildenafil-treated mice 24 h after infarction (\(P < 0.05\), Table 2). The infarct-sparing effect of sildenafil was abolished with L-NAME as shown by an increase in infarct size to 60.2 \(\pm\) 1.6 (\(P < 0.05\)). Control animals treated with L-NAME alone had an infarct size of 66.7 \(\pm\) 2.3, which was not different from the saline group (\(P > 0.05\)). The risk areas (%LV) were not statistically different between the groups (Table 2).

**LV remodeling and function.** Figure 4 shows representative M-mode images from sham-operated, saline (vehicle)-treated, and sildenafil-treated mice on day 28 post-MI. The hearts from sham-operated and sildenafil-treated mice exhibited a smaller LV cavity and thicker infarct wall compared with the saline-treated mice. An increase in LVEDD and LVESD and a decrease in AWDT, AWST, and FS in saline- and sildenafil-treated mice (vs. baseline and sham-operated) were observed on days 7 and 28 (Fig. 5). Sildenafil-treated mice had smaller LVEDD and LVESD, greater FS, and lower Tei index (better LV remodeling and function) compared with saline-treated mice on days 7 and 28 compared with the saline-treated group (\(P < 0.05\), Fig. 5). Sildenafil-treated animals also had shorter IRT values (reflective of lower LV end-diastolic pressure) 28 days after AMI compared with saline-treated animals (11 \(\pm\) 3 vs. 27 \(\pm\) 7 ms, respectively, \(P = 0.030\)), which was not different from sham-operated animals (10 \(\pm\) 3, \(P = 0.46\)). AWDT and AWST were also greater in sildenafil-treated animals (vs. saline-treated animals, \(P < 0.05\)) on days 7 and 28 post-MI, showing a protective effect in the peri-infarct region, whereas no differences in PWDT and PWST were seen. Aneurysmatic dilatation of the anterior wall and apex was observed on day 28 in 90%
stroke volume, calculated as AoVTI, was significantly higher in sildenafil-treated mice treated animals (395 beats/min) at 7 days after infarction compared with saline-treated animals (279 beats/min; baseline (290 beats/min)) and at 7 days after surgery. No differences in heart rate at baseline (290 ± 18 beats/min for each sample. *Significant difference vs. saline group.

Expression bcl-2/bax. Bcl-2 and Bax expression was measured in myocardial samples 2 h after LAD occlusion in saline- and sildenafil-treated mice. Protein was extracted, and Bcl-2, Bax, and β-actin were identified by Western blot analysis as described previously (9, 33). The expression of Bcl-2 and Bax was normalized with β-actin. Moreover, the ratio of Bcl-2 to Bax was calculated as an index of apoptotic signaling. As shown in the representative blots and quantitative bar graphs in Fig. 6, myocardial levels of Bcl-2 was increased in the sildenafil-treated mice compared with saline controls (P < 0.05, n = 3 hearts), whereas Bax levels did not change significantly (P > 0.05). The Bcl-2-to-Bax ratio was also increased in the sildenafil-treated hearts compared with the saline-treated hearts (P < 0.05).

Apoptosis. The TUNEL-positive apoptotic cells in salinetreated hearts were 2.4 ± 0.3% cells compared with 1.2 ± 0.1% in the sildenafil group on day 7 post-MI in the peri-infarct area (P < 0.05, n = 4 hearts). Similarly, the TUNEL-positive cells decreased from 2.0 ± 0.2% in the saline group to 1.2 ± 0.1% in the sildenafil group on day 28 post-MI (P < 0.05, n = 6 hearts) (Fig. 7).

Cardiac hypertrophy and pulmonary edema. The heart weight-to-body weight ratio (a measure of cardiac hypertrophy) was 3.6 ± 0.3 mg/g in sham-operated animals. It increased to 5.9 ± 0.4 mg/g in the saline-treated hearts (P < 0.05 vs. sham). Sildenafil treatment decreased hypertrophy to 4.8 ± 0.1 mg/g (P < 0.05 vs. saline). Pulmonary edema (the ratio of lung weight to body weight) was 5.2 ± 0.5 mg/g in the sham-operated animals, which increased to 6.9 ± 0.3 mg/g in the saline-treated group (P < 0.05). Pulmonary edema decreased to 5.6 ± 0.2 mg/g in the sildenafil-treated group (P < 0.05 vs. saline group). These data demonstrate a marked decline in the development of cardiac hypertrophy with sildenafil which paralleled the decrease in pulmonary edema (Table 1).

DISCUSSION

We have shown that sildenafil limits infarct size following ischemia and reperfusion (28, 3). In addition, both sildenafil and vardenafil reduced infarct size following ischemia when infused at the onset of reperfusion in rabbits (32). Based on these compelling data, we further hypothesized that chronic treatment with sildenafil would limit the progression to heart failure post-MI. Our results showed that infarct size was smaller in the sildenafil-treated group compared with saline-
treated controls 24 h after LAD ligation, and functional parameters were significantly improved on days 7 and 28 post-MI. Correspondingly, myocardial apoptosis was also significantly lower in sildenafil-treated mice compared with saline-treated groups. In addition, we observed reduced cardiac hypertrophy and pulmonary edema post-MI in sildenafil-treated mice. Interestingly, the survival of mice at 28 days was significantly improved by sildenafil (93% vs. 36% in saline-treated group). Taken together, these studies suggest that sildenafil attenuates ischemic cardiomyopathy in mice by limiting necrosis and apoptosis and by preserving LV function.

PDE-5 inhibitors prevent the breakdown of NO-driven cGMP, primarily in vascular smooth muscle cells, and therefore are potent vasodilators. PDE-5 enzyme is found in high abundance in the corpus cavernosum and in pulmonary artery smooth muscle, where its inhibition produces an increase in penile blood flow and a reduction in pulmonary vascular resistance, respectively (36). The PDE-5 gene is present in the human heart (36), although protein expression and enzyme activity were questioned by Ito et al. (15) and Wallis et al. (43). However, PDE-5 has been demonstrated in mouse cardiomyocytes and intact heart (10) as well as in dogs (37). To explain the cardioprotective effect, we hypothesized that PDE-5 inhibitors could potentially release endogenous mediators of cardioprotection, including adenosine or bradykinin from endothelial cells, that may trigger a signaling cascade (through the action of kinases, including protein kinase C and other MAPKs) and generation of NO by phosphorylation of eNOS. NO activates guanylate cyclase, resulting in enhanced formation of cGMP, which activates protein kinase G (PKG). PKG can subsequently open mitochondrial ATP-sensitive K⁺ (mitoK<sub>ATP</sub>) channels, resulting in cardioprotective effects against I/R injury (17, 18). In the present study, there is no clear evidence that PKG activation by NO is involved in sildenafil-induced protection. However, recently, we demonstrated that direct adenoviral overexpression of PKG-I<sub>i</sub> (the isozyme expressed in the heart) in cardiomyocytes induced both iNOS and eNOS and protected these cells against simulated ischemia/reoxygenation injury (8). Interestingly the protective effect of PKG-I<sub>i</sub> overexpression was blocked by L-NAME in this model, suggesting that enhanced expression of iNOS/eNOS with resulting NO generation may be downstream of PKG-I<sub>i</sub>. On the other hand, a recent study by Elrod et al. (11) showed that the reduction in infarct size with sildenafil was independent of NO/cGMP pathway in a model of 30 min ischemia and 24 h reperfusion. In this study, however, the authors administered sildenafil at a low dose of 0.06 mg/kg in the LV lumen 5 min before reperfusion. The differences in dosage, route of administration, and experimental model (reperfusion vs. permanent ischemia) could explain the NO/cGMP-independent cardioprotective effect of sildenafil. Further studies are needed to clarify these issues.

A number of studies have shown that apoptosis in cardiomyocytes contributes to the progression of heart failure after MI (22), and chronic cardiac remodeling with chamber dilation and impaired systolic function is associated with increased myocyte apoptosis in the infarct border zone after MI (34). In this study, we observed a significant increase in Bcl-2-to-Bax...
ratio in the sildenafil-treated hearts compared with the saline-treated hearts, which indicates the antiapoptotic capacity of sildenafil after coronary artery occlusion (Fig. 6). Furthermore, the extent of apoptosis was significantly lower in the hearts from sildenafil-treated mice compared with the saline controls at 7 and 28 days post-MI. These results are consistent with the limitation of necrosis and apoptosis in the adult cardiomyocytes subjected to simulated ischemia and reoxygenation (9) and doxorubicin-induced cardiotoxicity in mice (12). We assessed infarct size at 24 h when cell viability (from necrosis) is best demonstrated by TTC staining and apoptosis at 7 and 28 days when apoptosis represents the prevalent modality of cell death (2). However, making distinctions between the effects of sildenafil on necrosis and apoptosis separately may be difficult because the terminal events in necrosis and apoptosis are significantly different. The initial events may be indistinguishable, and necrosis may occur in cells already committed to apoptosis (secondary necrosis) (1).

To explain how sildenafil is causing protection against necrosis or apoptosis in the ischemic zone, we speculate that the upregulation of eNOS and iNOS after sildenafil treatment (9, 33) would induce NO generation in the adjacent nonischemic tissue, which would diffuse into the ischemic region and preserve cardiomyocytes possibly through the opening of mitoKATP channels and preventing Ca²⁺ overload. A similar conclusion was drawn by Li et al. (20) where iNOS gene transfer reduced infarct size by an average of 67% despite the fact that only 18% of the risk region exhibited transgene expression. In the current study, for the first time, we have shown that the sildenafil reduced infarct size 24 h after permanent coronary artery occlusion. Our results demonstrate complete blockade of the infarct-sparing effect of sildenafil with 1-NAM, thereby supporting the notion that NO plays an important role in protection of the heart (Table 2). Previous studies in the nonischemic hearts also showed that levels of iNOS/eNOS transcripts increased transiently, peaking at 45 min (eNOS) and 2 h (iNOS) after sildenafil treatment, and returned to baseline levels several hours later (33). In addition, a significant increase in protein of these enzymes was detected 24 h after sildenafil treatment. The present data further suggest that sildenafil-induced increase in NO is involved in infarct size reduction after 24 h, even in a model of permanent LAD occlusion (Fig. 3). Decreasing oxygen demand is another possible mechanism for reducing infarct size after permanent LAD occlusion. In fact, it has been reported that sildenafil does increase oxygen uptake in heart failure patients and hence decreases myocardial oxygen demand (13, 19). Since sildenafil-treated mice demonstrated higher levels of both eNOS and iNOS, the potential role of NO in decreasing oxygen demand in this model cannot be ruled out. In support of this notion, it has been shown eNOS-derived NO markedly suppresses in vivo oxygen consumption in the postischemic heart through modulation of mitochondrial respiration based on alterations in enzyme activity and mRNA expression of NADH dehydrogenase and cytochrome c oxidase (46).

Fig. 6. Cardiac expression of Bcl-2 and Bax protein 2 h after MI and sildenafil treatment. A: representative Western blots showing Bcl-2 and Bax protein expression. B: densitometric quantification of Bcl-2-to-Bax ratio averaged from 3 individual hearts for each group. *Significant difference vs. saline group.

Fig. 7. Myocardial apoptosis: representative pictures showing TUNEL-positive nuclei at 28 days (28D) in saline (A) and sildenafil-treated (B) mice. Representative pictures for TUNEL staining on day 7 (7D) are not shown. Arrows indicate TUNEL-positive nuclei. C: apoptotic index as percentage of TUNEL-positive nuclei compared with total nuclei. N, number of mice.
It has been shown that NO is overproduced in the failing myocardium as a result of the increased expression and activity of iNOS (23). There is a correlation between myocardium-specific, chronic overexpression of iNOS and peroxynitrite generation and cardiac enlargement, conduction defects, sudden cardiac death, and, less commonly, heart failure in mice (26). In contrast, reduced eNOS activity in cardiomyocytes has been shown to contribute to the onset of myocardial hypertrophy and increased cytokine expression, both of which are involved in the transition to heart failure (44). Our results suggest that an early increase in NO following sildenafil treatment at least during 24 h post-MI is involved in the infarct-limiting effect, which apparently reduces the severity of LV dysfunction and improves survival in sildenafil-treated mice.

We observed a significantly high rate of survival in the sildenafil-treated compared with saline-treated mice (Fig. 2). This might be related to the reduced incidence of severe pump failure as well as ventricular arrhythmias. Previous studies by Nagy et al. (27) showed that oral sildenafil decreased the incidence and severity of ventricular arrhythmias during coronary artery occlusion. Our data also showed that sildenafil improved cardiac function post-MI. We used a 30-MHz probe to obtain two-dimensional, M-mode and Doppler imaging from parasternal short-axis view at the level of the papillary muscles and the apical four-chamber view. As shown by M-mode measurements, LVEDD and LVESD dilatation was significantly less pronounced in the sildenafil-treated group compared with the saline controls. FS was preserved with sildenafil, showing a better LV function compared with that in saline-treated animals. We also observed greater anterior wall thinning (indicative of increased cardiomyocyte death) in the area at risk, which was significantly less pronounced in the sildenafil group compared with the saline group. The Tei index, which is an echocardiographic index of combined systolic and diastolic function [calculated as (IRT + ICT)/ET], was significantly lower in sildenafil-treated mice (Fig. 5F).

These data are consistent with the observations that a greater Tei index at the onset of AMI is associated with a higher incidence of subsequent cardiac death, congestive heart failure, and progressive LV remodeling in patients (42). The improvement in LV function was paralleled by a decrease in pulmonary edema in the sildenafil-treated group. Similar results were reported by Takimoto et al. (39), where sildenafil was shown to ameliorate pressure overload-induced cardiac hypertrophy, prevention of chamber dilation, and improved cardiac function despite sustained overload. Our data further support the findings that sildenafil may be an effective treatment strategy for cardiac hypertrophy induced by chronic MI.

Our results also showed that there is no difference in heart rate at baseline (290 ± 12 for saline vs. 288 ± 18 beats/min for sildenafil; P = 0.8) and a significantly lower heart rate in sildenafil-treated animals (279 ± 20 beats/min) at 7 days after infarction compared with saline-treated animals (395 ± 30 beats/min; P = 0.02). Moreover, the stroke volume at 7 days post-MI was significantly higher in sildenafil-treated mice (0.041 ± 0.003 ml) versus saline-treated animals (0.029 ± 0.007 ml, P = 0.048) and not different from control animals (0.046 ± 0.008 ml, P = 0.45). This indicates that the significantly lower heart rates in sildenafil-treated animals may possibly reflect better systolic function and greater stroke volumes.

In the present study, we administered two doses of sildenafil (0.71 mg·kg⁻¹·day⁻¹), which translate to a total of one 100-mg Viagra pill used for management of erectile dysfunction in men. Sildenafil has a rapid onset of action and a plasma half-life of 4 h (6, 29). The duration of action was originally estimated at 4–6 h based on its plasma half-life, but empirical testing shows that sildenafil can have effects for up to 12 h (25). We have previously shown that a single dose of sildenafil induces cardioprotective effect up to 24 h which is well beyond the 4 h half life of the drug in plasma (28, 33). Therefore, it appears that it is not essential to sustain a high plasma level of sildenafil to induce the protective effect. It is rather the triggering of a signaling cascade involving activation of cGMP-dependent kinases, MAP kinase, PKC, and induction of NOS, which are essential for the long-lasting effects of the drug. Our data (Fig. 3) further support this notion since eNOS/iNOS protein levels were significantly elevated 24 h post-MI following treatment with sildenafil.

In summary, we have demonstrated that acute and prolonged treatment with sildenafil during MI is associated with myocardial salvage from necrosis within the first 24 h, reduction of apoptosis at 7 and 28 days, prevention of adverse cardiac remodeling and heart failure, and improved survival. We propose that sildenafil and potentially other PDE-5 inhibitors can be promising drugs for the prevention of heart failure in patients with MI.

Our data further support the findings that sildenafil may be an effective treatment strategy for cardiac hypertrophy induced by chronic MI.

### Table 1. Cardiac hypertrophy and pulmonary edema

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<th>Sham</th>
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<th>Sildenafil</th>
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<td>14</td>
<td>16</td>
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<td>Heart/body wt, mg/g</td>
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<td>5.90±0.38*‡</td>
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<td>Lung/body wt, mg/g</td>
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<td>6.85±0.29‡</td>
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Values are means ± SE; N, number of mice. Cardiac hypertrophy was determined by the heart-to-body weight ratio, and pulmonary edema was measured by lung-to-body weight ratio in the sham-operated, saline-treated, and sildenafil-treated animals. An increase in these indexes indicates cardiac hypertrophy and pulmonary edema. *P < 0.05 vs. sham; †P < 0.05 vs. saline; ‡P < 0.05 vs. sildenafil.

### Table 2. Myocardial infarct size and area at risk

<table>
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<tr>
<th>Group</th>
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<th>Sildenafil</th>
<th>L-NAME + Saline</th>
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</tbody>
</table>

Values are means ± SE. Myocardial infarct size (% risk area) and area at risk (% of left ventricle) measured at 24 h after coronary artery occlusion in saline-, sildenafil-, N⁰-nitro-L-arginine methyl ester (L-NAME) + sildenafil-, and L-NAME + saline-treated mice (N = 6/group). Note that infarct size was significantly reduced by sildenafil treatment, which was blocked by L-NAME. The area at risk was similar in all 4 study groups (P > 0.05). *Significant difference vs. all other groups.
Sildenafil-induced protection in the failing heart

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