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Phosphodiesterase-5 Inhibitors Improve Left Ventricular Function in Failing Hearts

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1. Introduction

Impaired systolic performance and/or diastolic function have long been detrimental consequences of acute myocardial infarction (AMI), which remains a major cause of morbidity and mortality worldwide. Despite considerable therapeutic improvements, left ventricular dysfunction secondary to infarction continues to pose serious health complications, including heart failure (HF), wherein the heart is unable to maintain a cardiac output appropriate for the requirements of the body. Several factors contribute to HF, including adverse ventricular remodeling, progressive hypertrophy and sustained cell death by apoptosis. Therefore, the search for a therapeutic strategy to overcome or mitigate the progression of HF is of paramount importance.

1.1 PDE-5 Inhibitors

Sildenafil citrate (Viagra™) is the first PDE-5 inhibitor approved for treatment of erectile dysfunction. The discovery of this drug in 1989 was the result of extensive research on chemical agents that hold potential promise in the treatment of coronary heart disease. Initial clinical studies on sildenafil in the early 1990s were not promising with respect to its anti-anginal potential. However, a remarkable side effect was reported by a number of volunteers participating in these investigations; sildenafil seemed to enhance penile erections, which soon thereafter became the main focus of further studies. More than 10 million men worldwide have been treated with sildenafil since its market debut in 1998. Sildenafil is highly specific for PDE-5 inhibition with relatively minor cross-reactivity with PDE-6 (Laties & Fraunfelder, 1999). It has a chemical structure similar to cGMP and inhibits PDE-5 by binding to the cGMP-catalytic sites (Corbin & Francis, 2002) thereby allowing the accumulation of cGMP in the erectile tissue. Two additional agents in this class (vardenafil [Levitra™] (Porst et al., 2001) and tadalafil [Cialis™]) have also been developed and approved by the FDA for treatment of erectile dysfunction and recently sildenafil and tadalafil were approved for treatment of pulmonary arterial hypertension (PAH) (Corbin & Francis, 2002). PDE-5 inhibitors are structurally similar to cGMP and therefore compete with cGMP for binding to PDE-5 at the catalytic site (reviewed in Kukreja et al., 2005). Interestingly, PDE expression has been reported to change in pathologic conditions. For instance, in patients with cardiovascular disease or diabetes, nitric oxide (NO) levels are

suboptimal due to endothelial dysfunction [damaged NO synthase (NOS)], and recently myocardial PDE-5 expression has been shown to increase in patients with heart failure (Pokreisz et al., 2009). In this regard, targeting PDE-5 is a promising therapeutic approach for treatment of cardiovascular disease and dysfunction.

2. PDE-5 Inhibitors preserve myocardial function following infarction

A number of pioneering investigations from our laboratory have demonstrated that PDE-5 inhibitors attenuate ischemic injury in animal and cell models (Ockaili et al., 2002; Salloum et al., 2003). In animal models, sildenafil and vardenafil exerted an infarct-sparing effect when given before ischemia (Ockaili et al., 2002; Salloum et al., 2003; Salloum et al., 2006) or at the time of reperfusion (Salloum et al., 2007). Furthermore, chronic treatment with sildenafil immediately after permanent occlusion of the left descending coronary artery (LAD) in mice attenuated ischemic cardiomyopathy (Salloum et al., 2008a). These cardioprotective effects are mediated by activation of protein kinase G (PKG), increased expression of endothelial and inducible nitric oxide synthase (eNOS & iNOS), and augmented Bcl-2/Bax ratio. Due to their powerful anti-ischemic effects, PDE-5 inhibitors became promising candidates for the preservation of cardiac function following AMI. In fact, several studies demonstrated that PDE-5 inhibition preserved left ventricular (LV) function in failing hearts as discussed in the following sections.

2.1 Sildenafil attenuates left ventricular dysfunction in ischemic heart failure

Salloum et al. showed that chronic treatment with sildenafil preserves cardiomyocytes post AMI through reduction of myocardial necrosis, apoptosis and hypertrophy thereby limiting the progression of HF (Salloum et al., 2008a). This study used a murine model of post-MI remodeling by permanent ligation of the left coronary artery. The experimental protocol is illustrated in Figure 1. LV function was assessed at 7 and 28 days post MI. Cardiac function

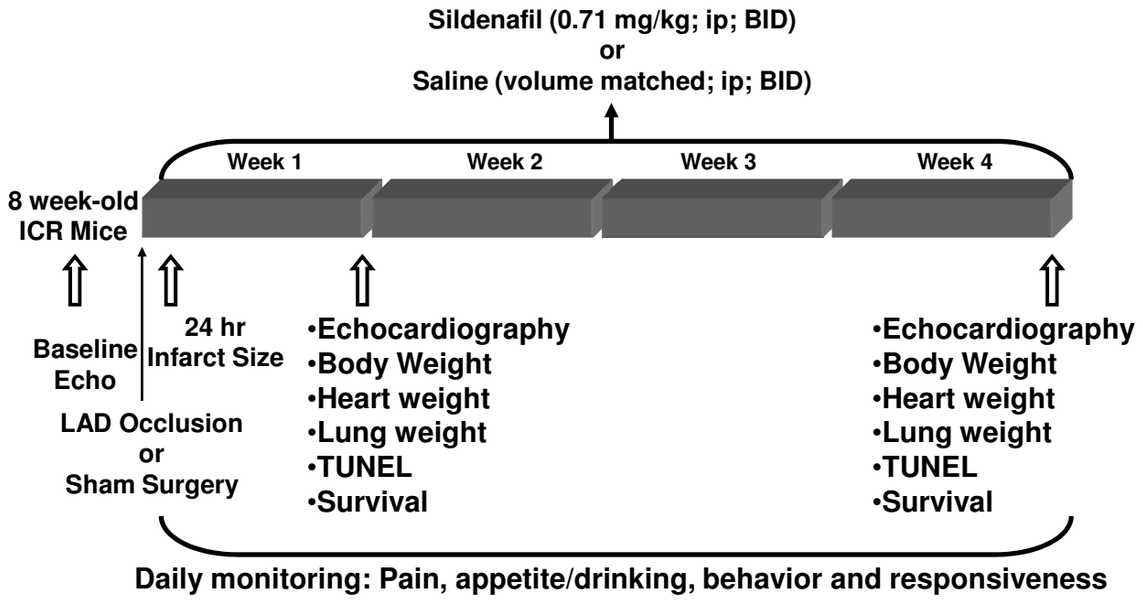

Fig. 1. Experimental protocol illustrating various parameters studied at different time points

was evaluated by echocardiography using the Vevo770™ imaging system (VisualSonics, Inc., Toronto, Canada). A 30-MHz probe was utilized 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 (Schiller et al., 1989). M-mode images of the LV were obtained and systolic and diastolic wall thickness (anterior and posterior) and LV end-systolic and end-diastolic diameters (LVESD and LVEDD, respectively) were measured.

Figure 2 is representative of M-mode images from mice on day 28 post MI. The hearts from sham and sildenafil-treated mice exhibited a smaller LV cavity and thicker infarct wall compared to the saline-treated mice. Increase in LVEDD, LVESD and a decrease in anterior wall diastolic thickness (AWDT), anterior wall systolic thickness (AWST) and fractional shortening (FS) in saline- and sildenafil-treated mice (vs. baseline and sham) were observed on day 7 and 28.

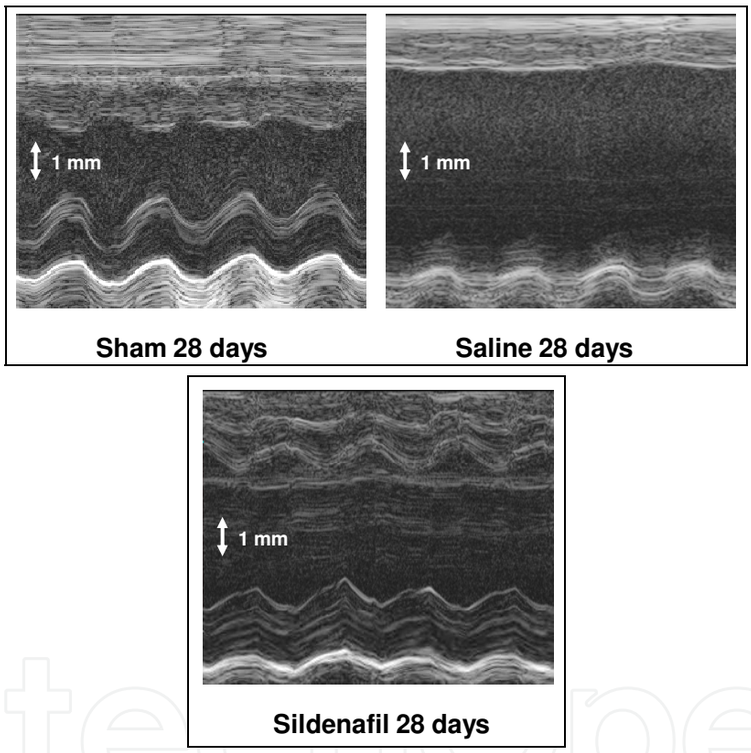

Fig. 2. M-mode images from mouse LV treated with sildenafil or vehicle 28 days after MI

Sildenafil-treated mice had smaller LVEDD, LVESD, greater FS, and lower Tei index (reflecting better myocardial performance) on day 7 and 28 as compared to saline-treated group ($P<0.05$, Figure 3). Sildenafil-treated animals also had a shorter isovolumetric relaxation time (reflective of lower LV end-diastolic pressure) 28 days after AMI when compared to saline-treated animals (11±3 vs. 27±7 ms, respectively, $P=0.03$), which was not different from sham operated animals (10±3, P=NS). AWDT and AWST were also greater in sildenafil-treated animals (vs. saline-treated animals, $P<0.05$) on day 7 and 28 post MI showing a protective effect in the peri-infarct region, while no differences in PWDT and PWST were seen. Aneurysmatic dilatation of the anterior wall and apex was observed on day 28 in 90% of saline-treated mice and 62% of sildenafil treated animals ($P>0.05$).

Moreover, the number of aneurysmatic segments [based on a 16-segment map (Schiller et al., 1989)] was 2.9 in saline-treated animals vs. 1.1 in sildenafil-treated animals (P<0.05).

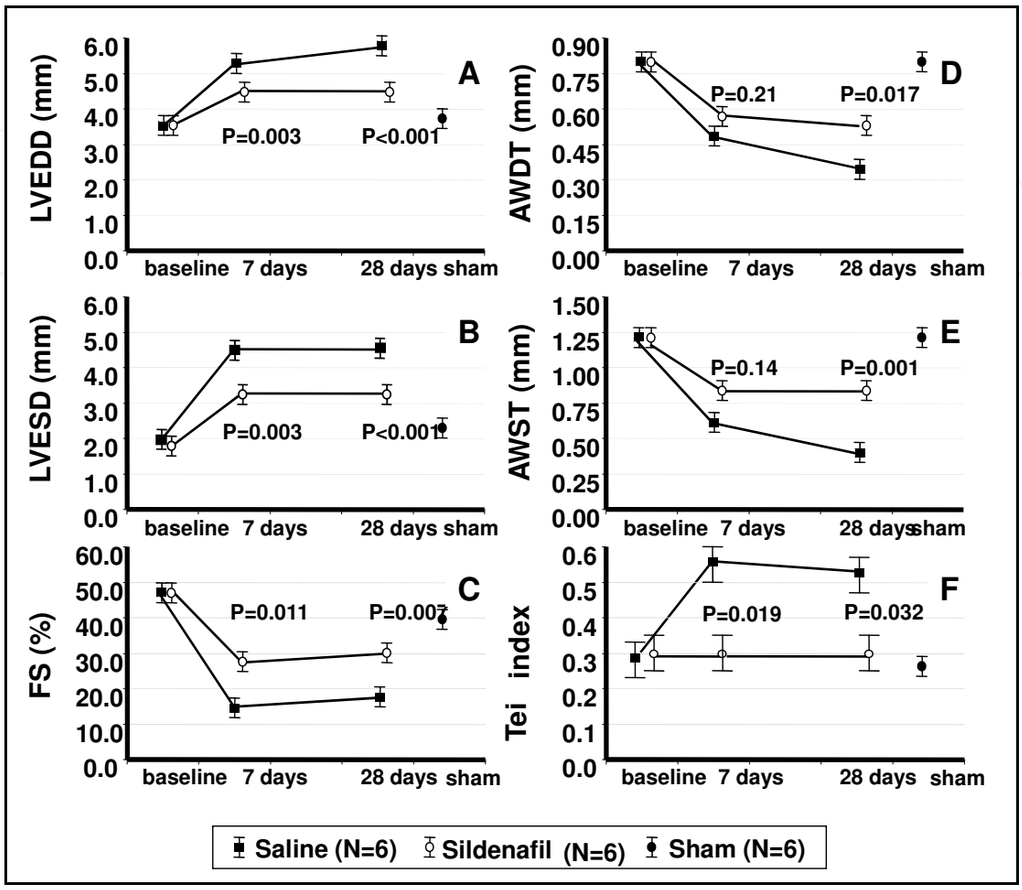

Fig. 3. Echocardiography results of LV function at 28 days post-MI in sildenafil- and saline-treated mice

2.2 Tadalafil preserves left ventricular function following MI through PKG-dependent generation of hydrogen sulfide

We also studied the effect of a longer acting PDE-5 inhibitor, tadalafil, on cardiac function in an acute model of myocardial infarction (Salloum et al., 2009). After baseline transthoracic echocardiography, adult male mice were injected i.p. with vehicle (10% DMSO) or tadalafil (1 mg/kg) with or without KT5823 (KT, PKG blocker, 1 mg/kg) or dl-propargylglycine [PAG, Cystathionine-γ-lyase (CSE, H2S-producing enzyme) blocker; 50 mg/kg] 1 h prior to coronary artery ligation for 30 min and reperfusion for 24 h, whereas C57BL-wild type and CSE-knockout mice were treated with either vehicle or tadalafil. After reperfusion, repeat echocardiography was performed. Similar to sildenafil, tadalafil preserved cardiac performance following MI as compared to vehicle. In this study, since ischemia was limited to 30 minutes, none of the groups presented with significant LV dilatation at 24 h post infarction, however, tadalafil preserved fractional shortening (FS: 31±1.5%) compared to control (FS: 22±4.8%, P<0.05, Figure 4). Baseline FS was 44±1.7%. KT and PAG abrogated the preservation of LV function with tadalafil by a decline in FS to 17±1% and 23±3%, respectively.

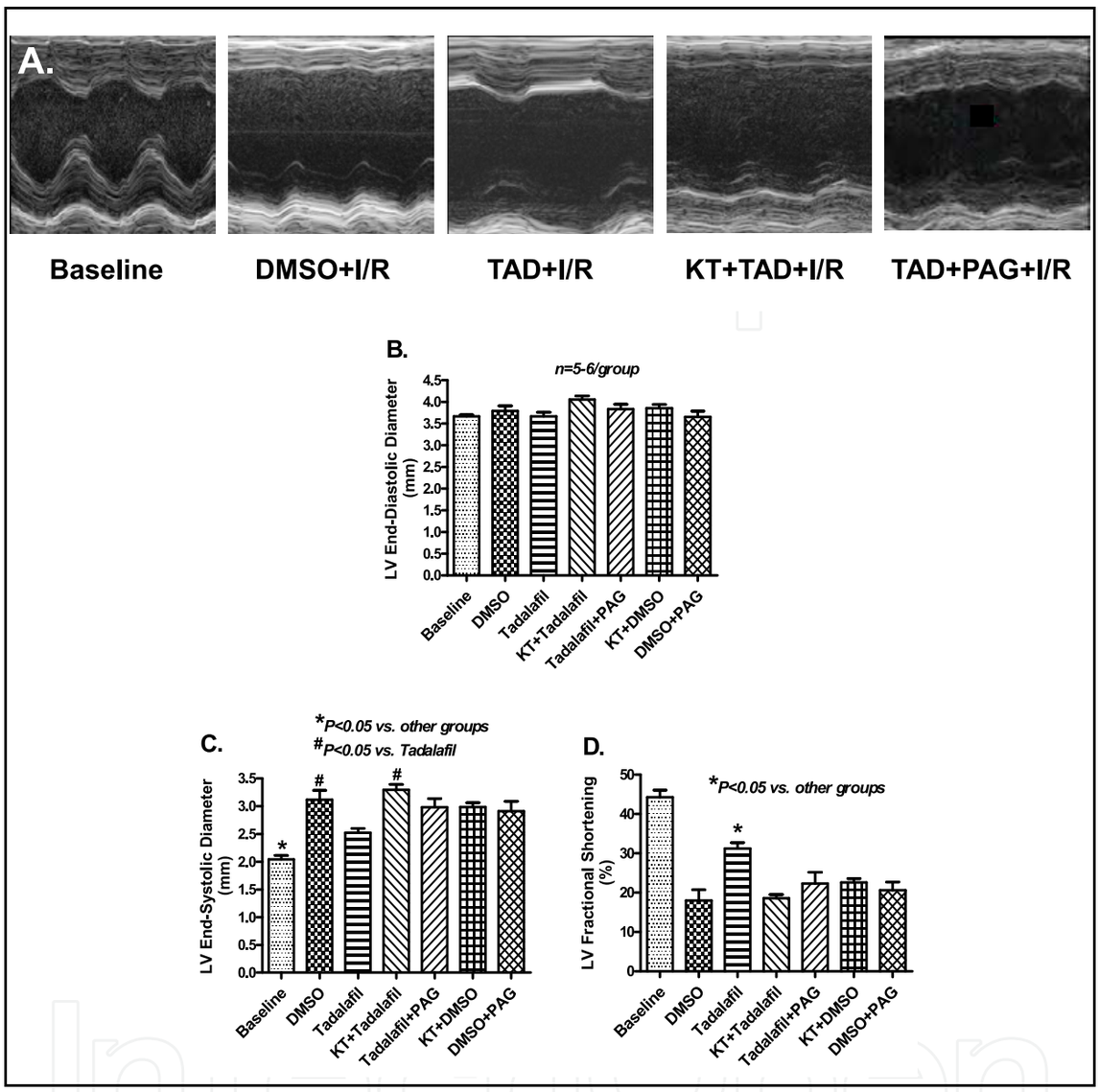

Fig. 4. Tadalafil preserves LV function at 24 hours following MI through PKG and H_2S signaling

2.3 Sildenafil treatment 3 days following MI mitigates the progression of heart failure

In the previous studies, PDE-5 inhibitors were administered either shortly prior to or immediately after infarction. This raises the question whether the preservation of cardiac function observed was a true phenomenon, or it was simply secondary to the anti-infarct effect of these drugs. Specially, little is known about the effects of PDE-5 inhibition on limiting adverse remodeling independent of its ability to modulate infarct size. This concept is clinically relevant, particularly in patients with advanced ischemic HF, because necrosis

has a negligible role in a post-infarct setting (Anversa et al., 1993). To address this question, we administered sildenafil 3 days following MI (Chau et al., 2011). Specifically, we sought to determine if sildenafil treatment following LV dysfunction, defined as FS less than 25% at day 3 post-MI, could prevent the progression of HF in a permanent LAD occlusion model. At 3 days post MI, mice receiving sildenafil or saline (control) treatment had similar FS (18±1% and 19±1%, respectively, P>0.05) as compared to baseline value of 47±1%. At days 7 and 28 post-MI, sildenafil-treated group had a significantly higher FS than saline-treated mice (P<0.05). Both LVEDD and LVESD were increased in saline-treated mice as compared to sildenafil-treated mice (P<0.05), indicating more dilatation. Moreover, AWDT was greater in sildenafil-treated animals versus saline-treated animals (P<0.05) on day 28 post-MI. Fractional shortening of sham-operated mice was 43±1.0% at 28 days post left thoracotomy. An increase in LVEDD from a baseline value of 3.5±0.1 mm and a decrease in FS in saline- and sildenafil-treated mice as compared to baseline and sham-operated mice (P<0.05) was observed on days 3 and 28, as shown in figure 5.

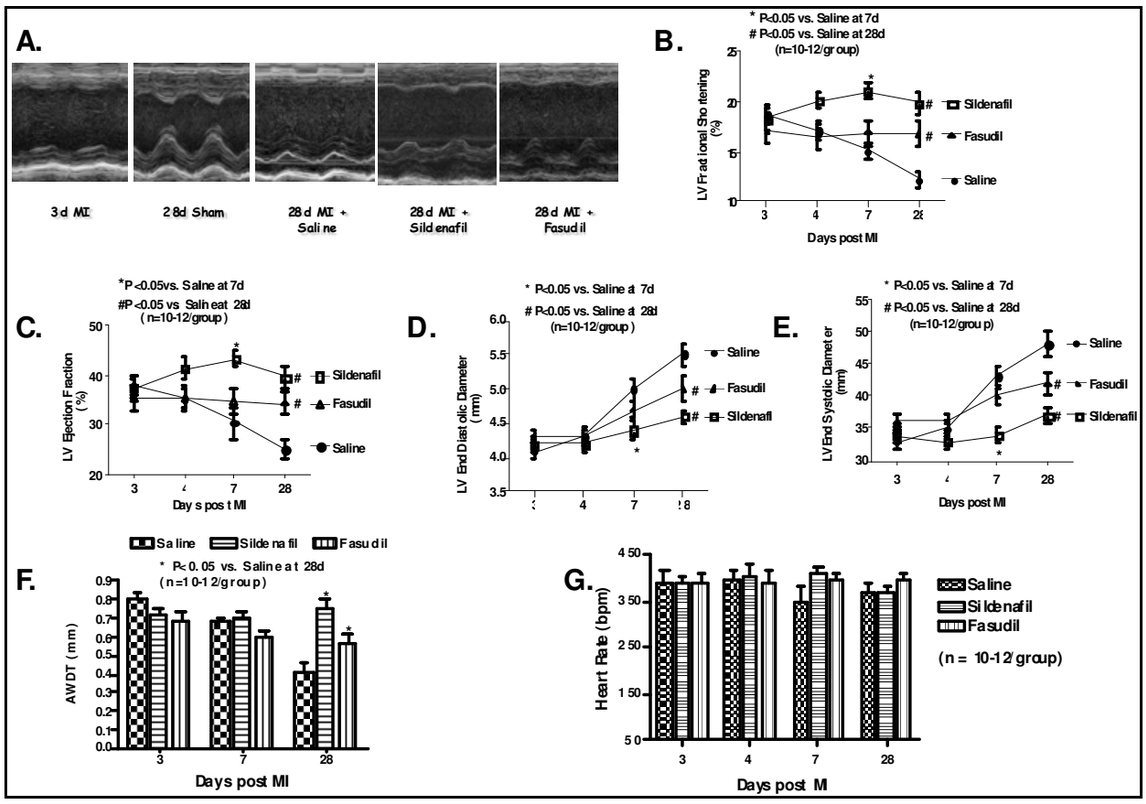

Fig. 5. Late sildenafil treatment preserves LV function and attenuates the progression of ischemic heart failure

2.4 Sildenafil preserves LV function in infant rabbits

Although we previously showed that PDE-5 inhibitors induce powerful preconditioning-like protective effects in the ischemic heart, it is not known whether sildenafil exerts similar

protective effects against ischemia/reperfusion injury in the infant rabbit hearts as well. In this study, we used the model of coronary artery occlusion and reperfusion in infant rabbits (Bremer et al., 2005), which is similar to our previously described adult rabbit model of myocardial infarction. The benefits of this work are immense since this model may be applicable in pediatrics, and especially in pediatric cardiovascular surgery where there may be periods of ischemia/reperfusion injury. Also, we used two-dimensional (2D) and Doppler trans-esophageal echocardiography (TEE) for the estimation of LV cardiac output (LVCO) and aortic velocity time integral (VTI) in this model. A 10-Fr AcuNav diagnostic ultrasound probe (Acuson Corp., Siemens, Iselin, NJ) was inserted into the esophagus, at baseline, after the 30 min period of ischemia, and after 3 h of reperfusion in both the control and sildenafil groups. Standardized 2D imaging in a long axis view of the LV to show LV inflow across the mitral valve and LV outflow tract (LVOT) was obtained. Aortic flow Doppler across the aortic valve was performed in a long axis view of the LV and LVOT to obtain LVCO. The standard equation: mean velocity (cm/s) × flow area (cm2) × 60 (s/min), where mean velocity (cm/s) = VTI (in cm/beat) ÷ RR interval (s/beat), was used to obtain LVCO, expressed in milliliters per minute. Laminar Doppler flow across the aortic valve confirmed the absence of aortic stenosis. Color Doppler assessment was made of both the aortic and mitral valves again in the long axis view at baseline, after the ischemic period, and after 3 h of reperfusion for the presence or absence of mitral or aortic regurgitation. Subjective functional assessment was also made after ischemia and reperfusion to demonstrate at least left ventricular apical diminished contractility to confirm infarction. In this study, we showed that both the control and sildenafil-treated groups had comparable LVCO and aortic VTI at baseline. The controls had a decline in LVCO and aortic VTI immediately after the 30-min period of ischemia (28% and 27% lower than baseline values, respectively, $p < 0.05$), whereas the LVCO and aortic VTI increased in the sildenafil group after ischemia (43% and 45% higher than baseline values, respectively, n = 6 per group, $p < 0.05$). Both groups, however, had significant decline in LVCO after 3 h of reperfusion (54% of baseline in the sildenafil group, $p < 0.05$, and 62% of baseline in the control group, $p < 0.05$), and were not statistically significantly different from each other (n = 4–6 per group). Both groups demonstrated a decrease in aortic VTI after 3 h of reperfusion. However, this decline was only statistically significant in the control group compared with baseline values. None of the rabbits had aortic stenosis or developed aortic regurgitation for the duration of the study. Moreover, both the control and sildenafil groups demonstrated a comparable amount of mitral regurgitation (no more than mild) after ischemia/reperfusion, and none of the rabbits had baseline mitral regurgitation.

2.5 Sildenafil and vardenafil preserve LV function in female mice

Since the impact of PDE-5 inhibitors on the female cardiovascular system following ischemia remains unknown, we interrogated the effect of sildenafil and vardenafil on ischemia/reperfusion injury in female mice. In this study, adult female mice were pretreated (ip, bid) with sildenafil (0.7 mg/kg), vardenafil (0.14 mg/kg) or saline one hour before left coronary artery ligation for 30 minutes and reperfusion for 24 hours (Salloum et al., 2008b). Cardiac function, evaluated using echocardiography, showed that LV end-diastolic and end-systolic diameters increased 7 days post myocardial infarction with saline

(3.5±0.1 mm and 2.4±0.2 mm, respectively). In contrast, no dilatation was detected in sildenafil (3.0±0. mm and 1.4±0.1 mm, respectively) and vardenafil (2.9±0.3 mm and 1.4±0.2 mm, respectively) groups. Fractional shortening decreased at 7 days post infarction with saline (30±4%; P<0.05), but was preserved with sildenafil (52±2%) and vardenafil (53±5%). These data clearly suggest that PDE-5 inhibitors induce powerful cardioprotection in female mice as well. For this reason, PDE-5 inhibition may be a novel therapeutic strategy against ischemia/reperfusion injury in women with coronary artery disease.

3. PDE-5 Inhibitors protect against doxorubicin-induced cardiac dysfunction

A number of pioneering investigations from our laboratory have demonstrated that PDE-5 inhibitors attenuate doxorubicin-induced cardiomyopathy in animal and cell models (Koka et al., 2010; Das et al., 2010). In a recent study, we tested whether sildenafil potentiates the antitumor efficacy of doxorubicin in prostate cancer. Our results show that doxorubicin and sildenafil induce a potent antitumor effect in prostate cancer while simultaneously providing a cardioprotective effect. This study was an elegant sequel to our previous work demonstrating that sildenafil attenuated doxorubicin-induced cardiomyopathy in mice (Fisher et al., 2005). In that study, we showed that treatment with sildenafil attenuates the decline in LV developed pressure caused by doxorubicin treatment. An important question, however, was whether sildenafil interferes with the anti-tumor effect of doxorubicin. Our recent study showed that sildenafil ameliorates doxorubicin-induced cardiac dysfunction without interfering with its chemotherapeutic benefits (Das et al., 2010). Cardiac function in nude mice with tumor xenografts was monitored by Doppler echocardiography using the Vevo770 imaging system (VisualSonics, Toronto, Canada) as previously reported (Salloum et al., 2008a). A slight increase in LVEDD and LVESD were observed with doxorubicin. LVFS and LVEF declined in doxorubicin-treated mice. Sildenafil co-treatment with doxorubicin improved LVFS and LVEF compared with the doxorubicin-treated group ($P < 0.05$). No differences in heart rate were observed between control, doxorubicin, and doxorubicin and sildenafil groups. Sildenafil-treated animals showed lower heart rates compared with other groups ($P < 0.01$; $n = 8$). These data suggest that changes in LVFS or LVEF were independent of heart rate.

In a separate study, tadalafil improved left ventricular function and prevented cardiomyocyte apoptosis in doxorubicin-induced cardiomyopathy through mechanisms involving upregulation of cGMP, PKG activity, and MnSOD level without interfering with the chemotherapeutic benefits of doxorubicin (Koka et al., 2010). In these studies, adult male CF-1 mice were randomized to receive saline (0.2 ml i.p.), doxorubicin (15 mg/kg i.p.), or doxorubicin + tadalafil (4 mg/kg p.o. daily) for 9 days starting 3 days before doxorubicin treatment. We chose to use a single dose of doxorubicin at 15 mg/kg i.p., which has been reported to be cardiotoxic. LV function was significantly impaired 5 days after doxorubicin treatment. However, mice treated with doxorubicin + tadalafil showed preserved fractional shortening and ejection fraction compared with those treated with doxorubicin as shown in Figure 6 ($n = 6$, $p < 0.05$). In addition, the LV systolic pressure decreased 36%, +dp/dtmax decreased 63%, −dp/dtmax decreased 57%, and heart rate decreased 30% as compared with the controls ($P < 0.05$). In contrast, mice treated with doxorubicin + tadalafil showed improved LV function (i.e., LV systolic pressure, 33%; +dp/dtmax, 35%; −dp/dtmax, 46%, and heart rate, 27%) as compared with the group treated with doxorubicin alone ($n = 6$, $p < 0.05$).

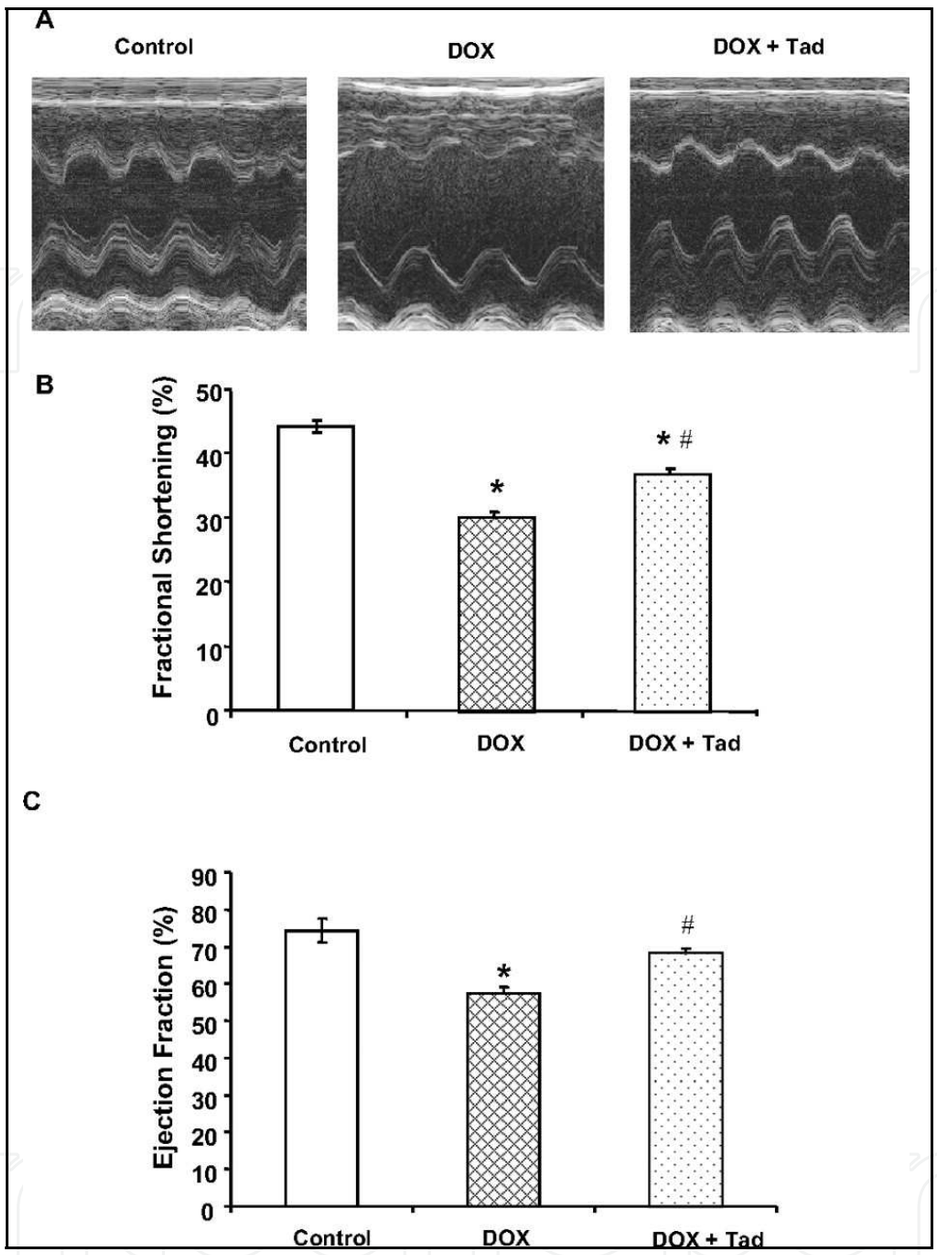

Fig. 6. Tadalafil attenuates doxorubicin-induced LV dysfunction

4. PDE-5 inhibitors protect against hypertrophy-induced cardiac dysfunction

Sustained pressure overload leads to cellular and molecular changes that are initially activated as compensatory mechanisms but later become maladaptive and contribute to progressive cardiac dysfunction and heart failure. This response involves a combination of complex signaling and transcription pathways that induce hypertrophic remodeling (Frey & Olson, 2003; Frey et al., 2004). The heart appears to have an intrinsic signaling system coupled to cGMP that can inhibit myocardial proliferative responses. Several studies using approaches that involve enhanced cGMP synthesis or prevention of its degradation have

been shown to blunt hypertrophy despite sustained pressure overload or neurohormonal stress. Interestingly, although cGMP synthesis is often increased by chronic exposure to such stresses, this increase appears to be ineffective to impede hypertrophy and remodeling progression, likely due to increase in PDE-5 expression and activity that accompany such stressors. For this reason, the use of PDE-5 inhibitors to reduce the catabolism may augment cGMP-dependent antihypertrophic effects. In the study by Takimoto et al., the authors show that PDE-5 inhibition with sildenafil prevents cardiac chamber, cellular and molecular remodeling induced by pressure overload (Takimoto et al., 2005). They next tested a more clinically relevant question of whether inhibition of PDE-5 can reverse pre-existing hypertrophy. Mice were exposed to transverse aortic constriction for 7–10 days, which increased heart mass by 63% ($P < 0.005$) without chamber dilatation. After hypertrophy was established, these mice were divided into 2 groups that received either sildenafil or vehicle for an additional 2 weeks. Cardiomyocyte hypertrophy and interstitial fibrosis were observed in mice exposed to 1 week of transverse aortic constriction, and both reversed to baseline with sildenafil treatment. Serial echocardiography also showed a gradual decline in LV mass and wall thickness, with preservation of systolic ejection in sildenafil-treated mice (Figure 7).

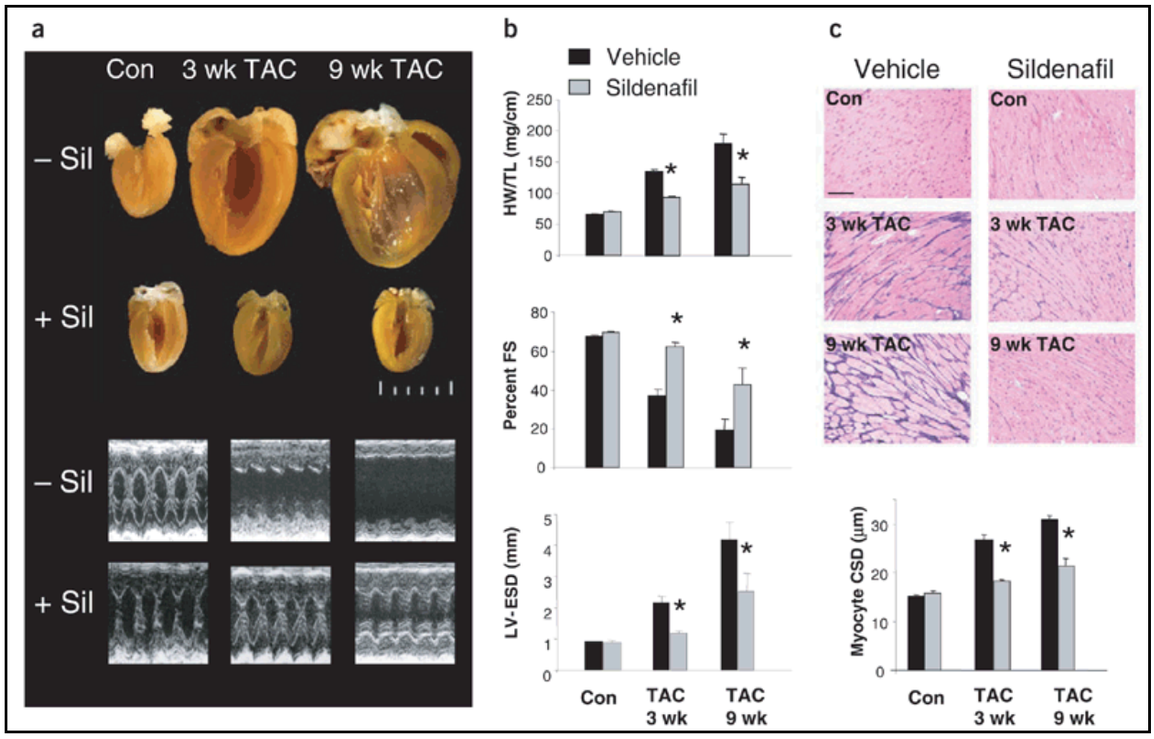

Fig. 7. Sildenafil attenuates and reverses hypertrophy-induced cardiac dysfunction

Another study by Nagayama et al. showed that delayed sildenafil treatment suppresses progressive cardiac dilatation, dysfunction, fibrosis, and hypertrophy in hearts subjected to sustained pressure-overload (Nagayama et al., 2009). In this study, following 3-week transverse aortic constriction, hearts had a +135% increase in LV mass, chamber end-systolic (+91%) and end-diastolic (+10%) dimensions, and reduced fractional shortening (−42%). Subsequent treatment with sildenafil fully arrested progressive remodeling, whereas control hearts further dilated and hypertrophied after 9-week transverse aortic constriction. Post-

mortem analysis confirmed that both heart and lung weights, normalized to tibia length, were lower with sildenafil treatment. Moreover, cardiomyocyte cross-sectional dimension and interstitial and perivascular fibrosis was also reduced in sildenafil-treated myocardium.

5. PDE-5 inhibitors reverse cardiac dysfunction in Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a degenerative, muscle-wasting disease caused by mutations in the dystrophin gene. The total loss of dystrophin mainly affects skeletal muscle and results in impaired respiratory function, primarily in older boys (Finsterer & Stöllberger, 2003; Adamo, 2010). Due to remarkable improvement of noninvasive respiratory support in the recent past, the lifespan of patients with DMD has increased. Unfortunately, this was also associated with an increase in the incidence of complications and eventual mortality from cardiomyopathy (McNally, 2008). Cardiomyopathy is a delayed symptom of the disease that usually develops by the second decade of life, with more than 90% of patients presenting clinical symptoms by 18 y of age (Finsterer & Stöllberger, 2003). Loss of cardiac dystrophin eventually leads to dilated cardiomyopathy, which manifests as congestive heart failure in at least 20% of patients (Finsterer & Stöllberger, 2003). Current treatment options for heart failure associated with DMD include angiotensin converting enzyme inhibitors and β-blockers. Despite the moderate benefits provided by these medications in patients with systolic heart failure, similar advantages have not been observed in dystrophic patients with features of systolic and diastolic dysfunction (Bushby, 2003). These findings highlight the need for treatments that slow the development of cardiomyopathy in DMD and improve cardiac function in older patients with established cardiomyopathy.

It has been shown that stimulation of cGMP synthesis by overexpression of cardiac-specific neuronal (n)NOS reduces impulse-conduction defects in dystrophin-deficient (mdx) mice (Wehling-Henricks et al., 2005; Wehling et al., 2001). Similarly, increased particulate guanylyl cyclase activity in young mdx mice has also been shown to decrease susceptibility to cardiac damage during sympathetic stress (Khairallah et al., 2008). These findings clearly implicate reduced NO-cGMP signaling as a key contributor to myocardial pathogenesis in patients with DMD. Therefore, it is plausible that restoration of NO signaling, particularly by preservation of cGMP, may provide therapeutic benefit to dystrophic hearts. In a recent study, Adamo et al. tested whether chronic inhibition of PDE-5 with sildenafil would reverse cardiac dysfunction in the mdx mouse model of DMD (Adamo et al., 2010)

Chronic sildenafil treatment prevented LV functional deficits in aging mdx mice. Furthermore, late sildenafil treatment, i.e. after developing cardiomyopathy, reversed the established symptoms.

Conventional echocardiography and tissue Doppler analysis were used to monitor the development of LV dysfunction in aging mdx mice. Both the myocardial performance index (MPI) and ratios of early diastolic velocity (Ea) to peak velocity with atrial contraction (Aa) were calculated. MPI is a sensitive measure of left ventricular systolic and diastolic performance, whereas the Ea/Aa largely reflects diastolic function. The majority of patients with DMD exhibit diastolic dysfunction and impaired myocardial performance, which can be identified by increased MPI (Bahler et al., 2005). This dysfunction usually precedes the

onset of systolic heart failure and dilated cardiomyopathy (Markham et al., 2006). Mdx mice show these same echocardiographic abnormalities (Jearawiriyapaisarn et al., 2010; Townsend et al., 2007).

Three different sildenafil treatment regimens were used: 1- long-term chronic sildenafil treatment starting at 1 month of age, 2- long-term treatment starting at 12 months with echocardiographic measurements taken 3 months later to assess whether established dysfunction could be reversed, and 3- a similar treatment starting at 12 months, but with multiple measurements to determine the time course of the reversal. The results showed impaired LV performance in mdx mice (increased MPI) by 11 to 13 months of age compared with treated and untreated WT controls. As mice approached 15 months of age, mdx mice continued to demonstrate impaired LV function whereas WT control mice began to show a slight age-related decline in cardiac performance. Although sildenafil did not have an effect on cardiac performance in WT mice, mdx mice that received chronic sildenafil treatment starting at 1 month of age retained a relatively normal MPI with age, indicating that sildenafil attenuated the cardiomyopathy in mdx mice. Furthermore, late sildenafil treatment following well-established cardiomyopathy at 12 months of age completely reversed LV dysfunction by age 15 months, as evidenced by normal MPI at that time point. Taken together, these results demonstrate that chronic treatment with sildenafil mitigates the progression of LV dysfunction and late treatment also reverses established LV dysfunction in mdx mice (Figure 8).

In order to better understand the underlying cause for the improvement in the MPI by sildenafil, which could be a result of effects on systolic or diastolic function, the authors measured the Ea/Aa using tissue Doppler imaging to more directly evaluate diastolic function in mdx mice. This parameter largely reflects the diastolic (chamber relaxation and filling) capacity of the LV. As shown in Figure 8B, diastolic dysfunction (indicated by Ea/Aa <1) was observed in mdx mice as early as 8 months of age. Moreover, chronic sildenafil treatment reduced the progression of diastolic dysfunction in mdx mice through 15 months of age. Even when sildenafil treatment was initiated after LV dysfunction was established at 12 months of age, it markedly reversed the diastolic dysfunction within 3 months. Based on this result, the authors suggest that diastolic dysfunction is a major component of the impaired MPI observed in 11- to 13-month-old mdx mice.

Cardiac remodeling after injury can result in hypertrophy, increased fibrosis and systolic dysfunction of the heart. However, cardiomyopathy in mdx mice is characterized by slow, progressive cell death, followed by compensatory hypertrophy of the surviving cardiomyocytes. In order to study the impact of sildenafil on cardiac dimensions and remodeling, the authors used M-mode echocardiography to determine LV dimensions in conscious mdx mice. By 12 months of age, the LV wall thickness of mdx mice was increased and the LV mass index (LVMI) was larger compared with sildenafil-treated mdx mice. Taken together, the anti-hypertrophic effect of sildenafil, coupled with the prevention of diastolic dysfunction, suggest that sildenafil may also have protective effects on some aspects of cardiac remodeling. However, the authors did not find any difference in the FS of 12-month-old, conscious mdx mice compared with WT controls or sildenafil-treated mdx mice, nor did they observe any effect on heart rate. This indicates a lack of major systolic dysfunction in these animals up to 12 month of age. Although systolic dysfunction may develop later in life, it appears that diastolic dysfunction plays a more prominent role in the

cardiomyopathy seen in the mdx mice used in this study.Overall, the findings of this study suggest that PDE-5 inhibitors may be an effective treatment for DMD-associated cardiomyopathy at early and late stages of the disease.

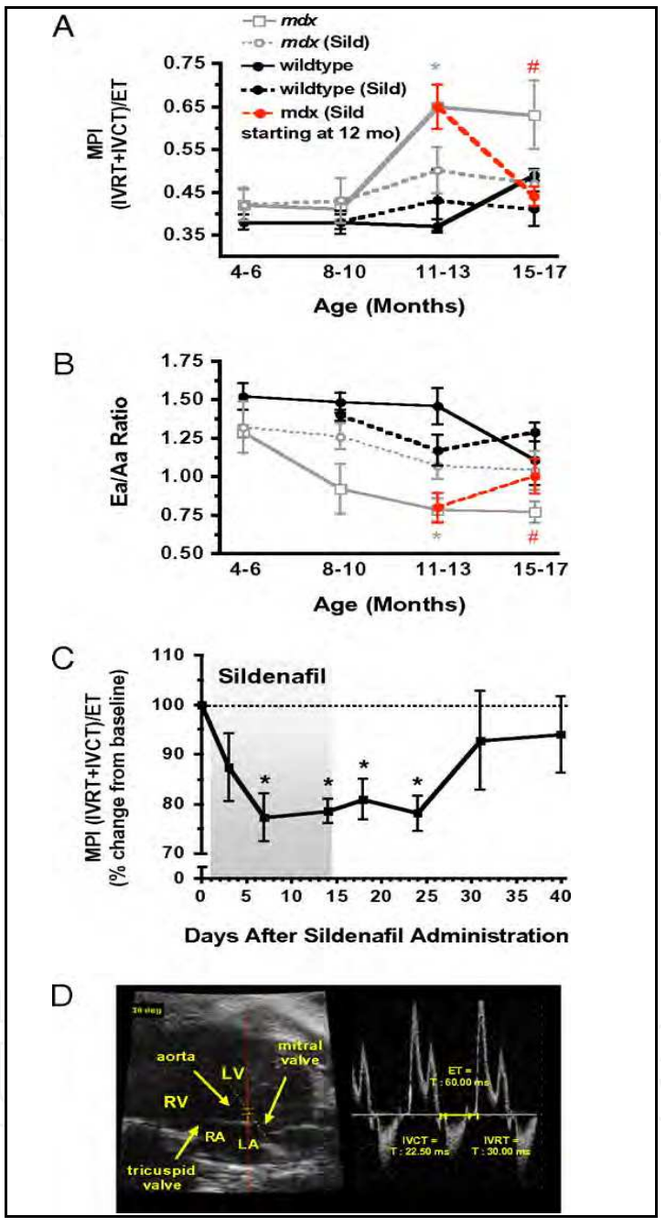

Fig. 8. Sildenafil reverses cardiac dysfunction in the mdx model of Duchenne Muscular Dystrophy

6. Clinical use of PDE-5 inhibitors in patients with heart failure

Following years of basic research examining the cardioprotective effects of PDE-5 inhibitors against ischemia, a recent study by Guazzi et al. demonstrated that sildenafil improves LV diastolic function, cardiac geometry, and clinical status in patients with stable systolic heart

failure (Guazzi et al., 2011). The study primarily focused on the effects of chronic PDE-5 inhibition on LV diastolic function and cardiac chamber remodeling, providing the first human evidence that PDE-5 inhibition can be beneficial for improving the diastolic and structural properties of the failing LV. Transthoracic echocardiography was performed using IE33, Philips ultrasound machine, equipped with a software for tissue Doppler (TD), using a 2.5- to 5.0-MHz probe (S5). Standard M-mode, 2D, and Doppler blood flow measurements were performed according to the current American Society of Echocardiography Guidelines (Quiñones et al., 2002). Chamber dimensions were obtained using standard procedures including left atrial volume index (LAVI) and LV mass index (LVMI) (Devereux & Reichek, 1977). Septal and posterior wall thickness, LA, and LV end-systolic and end-diastolic dimensions were obtained from the parasternal long-axis view. LVEF, end-diastolic volume index (LVEDVI), and end-systolic volume index were evaluated with the Simpson method.

Interestingly, E/E′, a variable repeatedly found related to LV filling pressures in a variety of left-sided cardiac disorders (Lester et al., 2008), significantly decreased at 6 months and 1 year of active treatment (Figure 9). Additional study findings that support the hypothesis that PDE-5 inhibition may represent a novel and viable therapeutic strategy for improving LV relaxation were the significant shortening in both lateral and septal T E-E′, a Doppler-derived index of LV relaxation performance validated against invasively measured negative dP/dT22, and the reverse remodeling effect on LV mass. Moreover, over 12 months, LAVI, LVEDV, and LVMI were unchanged in the placebo group and decreased in the active treatment group, which suggests reverse remodeling with sildenafil involving both the ventricle and the atrium. Over the same time period in the sildenafil group, there was a progressive increase in mean LVEF, from 29.5% at baseline to 34.9% and 36.3% at 6 and 12 months, respectively (P<0.01). Changes observed with sildenafil were significantly different

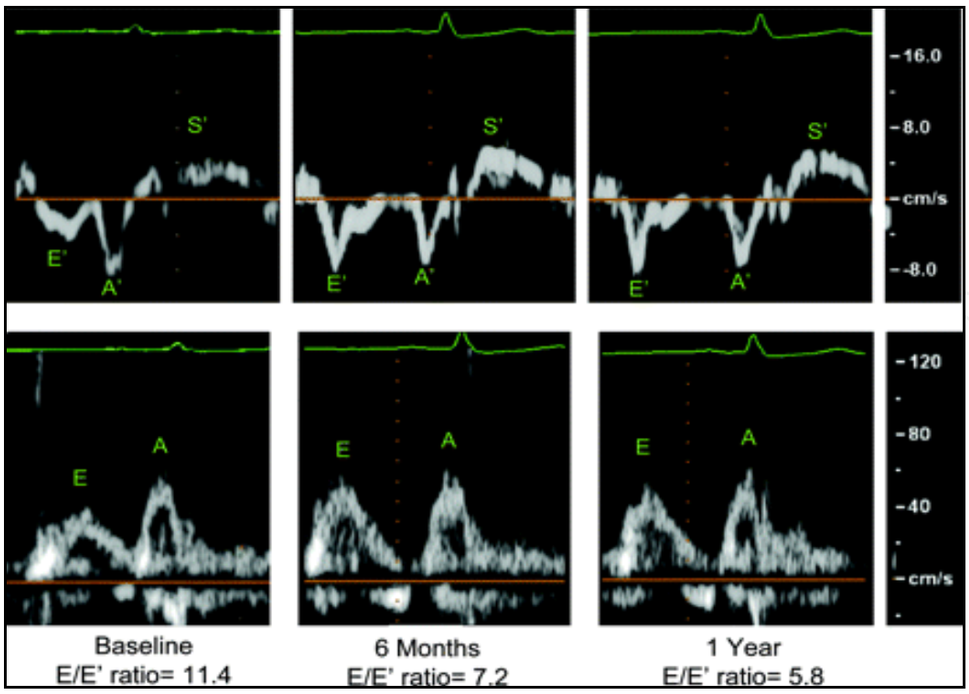

Fig. 9. Sildenafil significantly decreased E/E′ at 6 months and 1 year of active treatment in patients with stable systolic heart failure

compared with placebo (P<0.01). Additionally, diastolic measures of LV function demonstrated systematic and sustained improvement after both 6 months and 1 year of sildenafil treatment. The transmitral E/A ratio, isovolumic relaxation time, and both lateral and septal E/E′ decreased from baseline through 12 months (all P<0.01), which is indicative of an improvement in LV diastolic function and a decrease in LV filling pressure. Furthermore, septal T E-E′ was significantly reduced at 6 and 12 months of sildenafil treatment (P<0.01). All these changes were in agreement with the observed reverse remodeling on LAVI, which is viewed as morphological expression of LV end-diastolic pressure (Lester et al., 2008). Changes observed at 6 months and 1 year after sildenafil were significantly different compared with the placebo group (P<0.01).

7. Concluding remarks

With the advancement in the management of patients with cardiovascular disease and improvement in survival following cardiovascular events, the incidence of heart failure, especially in patients of age 65 and older is increasing. Using state-of-the-art echocardiography, we and others have demonstrated that treatment with PDE-5 inhibitors improve LV function in various models of myocardial dysfunction and heart failure. These studies suggest that PDE-5 inhibitors are immensely promising for further development as novel drug therapies for myocardial infarction, LV hypertrophy and dysfunction, doxorubicin-induced cardiotoxicity, and heart failure. Clinical studies of sildenafil on heart failure patients have reported improved exercise capacity, coupled with reduced pulmonary vascular resistance and better endothelial function (Lewis et al., 2007; Guazzi et al., 2007). Sildenafil also preserved LV function in patients with heart failure due to various etiologies (Guazzi et al., 2011). Several other studies indicated that PDE-5 inhibition with sildenafil has a therapeutic promise for stroke, neurodegenerative diseases and potentially other circulatory disorders (reviewed in Kukreja et al., 2007; Kukreja et al., 2011a; Kukreja et al. 2011b). These drugs may not only delay or reduce the pathological damage or defects in various vital organs, but also improve the overall well-being and quality of life in patients.

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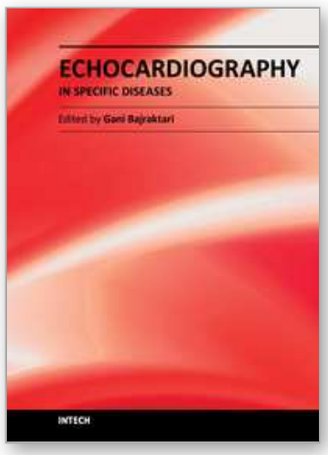

Echocardiography - In Specific Diseases

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The book "Echocardiography - In Specific Diseases" brings together contributions from well- known researchers from around the world, some of them specialized in imaging science in their clinical orientation, but also representatives from academic medical centers. Each chapter is structured and written to be accessible to those with a basic knowledge of echocardiography but also to be stimulating and informative to experts and researchers in the field of echocardiography. This book is primarily aimed at cardiology fellows during their basic echocardiography rotation, fellows of internal medicine, radiology and emergency medicine, but also experts in echocardiography. During the past few decades technological advancements in echocardiography have been developing rapidly, leading to improved echocardiographic imaging using new techniques. The authors of this book tried to explain the role of echocardiography in several special pathologies, which the readers may find in different chapters of the book.

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