Sildenafil ameliorates left ventricular T-tubule remodeling in a pressure overload-induced murine heart failure model

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Aim: Sildenafil, a phosphodiesterase 5 (PDE5) inhibitor, has been shown to exert beneficial effects in heart failure. The purpose of this study was to test whether sildenafil suppressed transverse-tubule (T-tubule) remodeling in left ventricular (LV) failure and thereby providing the therapeutic benefits.

Methods: A pressure overload-induced murine heart failure model was established in mice by thoracic aortic banding (TAB). One day after TAB, the mice received sildenafil (100 mg·kg\(^{-1}\)·d\(^{-1}\), sc) or saline for 5 weeks. At the end of treatment, echocardiography was used to examine LV function. Then the intact hearts were dissected out and placed in Langendorff-perfusion chamber for \textit{in situ} confocal imaging of T-tubule ultrastructure from epicardial myocytes.

Results: TAB surgery resulted in heart failure accompanied by remarkable T-tubule remodeling. Sildenafil treatment significantly attenuated TAB-induced cardiac hypertrophy and congestive heart failure, improved LV contractile function, and preserved T-tubule integrity in LV cardiomyocytes. But sildenafil treatment did not significantly affect the chamber dilation. The integrity of LV T-tubule structure was correlated with cardiac hypertrophy (\(R^2=0.74, P<0.01\)) and global LV function (\(R^2=0.47, P<0.01\)).

Conclusion: Sildenafil effectively ameliorates LV T-tubule remodeling in TAB mice, revealing a novel mechanism underlying the therapeutic benefits of sildenafil in heart failure.

Keywords: sildenafil; PDE5 inhibitor; T-tubule; pressure overload; left ventricular remodeling; left heart failure

Original Article

Introduction

Heart failure is a common end stage of different cardiovascular diseases and remains a source of considerable morbidity and mortality despite significant advances in its prevention, diagnosis, and treatment[1]. In recent years, there has been a tremendous increase in the number of experimental and clinical studies on the use of sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor, for heart failure. Numerous studies have provided evidence that sildenafil delays the progression of heart failure and reverses cardiac remodeling in animal models of heart failure, such as cardiac hypertrophy induced by pressure overload[2-4] and ischemic cardiomyopathy evoked by myocardial infarction[5,6]. In clinical studies, sildenafil therapy enhanced exercise tolerance and improved clinical status in patients with heart failure and reduced ejection fraction (HFrEF)[7-9]; however, unfortunately, sildenafil therapy did not exhibit such beneficial effects in heart failure patients with preserved ejection fraction (HFrEF) or diastolic heart failure[10,11]. A meta-analysis of randomized controlled trials suggests that PDE5 inhibitors have anti-remodeling properties and improve cardiac inotropism independent of afterload changes and with a good safety profile[12]. Despite the beneficial effects of sildenafil in heart failure, as demonstrated by basic and clinical research, the mechanism by which sildenafil protects the heart and improves LV pump function remains to be fully defined.

Cardiac transverse-tubules (T-tubules) are regularly spaced invaginations of the surface sarcolemma that occur along the Z-line regions in mammalian ventricular myocytes and branch within the cell to form a highly organized membrane network with predominantly transverse elements but also longitudinal components running from one Z-line to the next[13]. T-tubule remodeling has been documented in failing ventricular myo-
cytes from animal heart failure models of different species and etiologies\cite{14-19}, as well as in human heart failure patients with different underlying diseases\cite{26, 29}. The T-tubule system plays a significant role in cardiac excitation-contraction coupling (E-C coupling) and guarantees the instantaneous excitation and synchronous activation of nearly all Ca\textsuperscript{2+} release channels/ryanodine receptors (RyRs) on the sarcoplasmic reticulum (SR)\cite{21}. T-tubule remodeling leads to dyssynchronous SR Ca\textsuperscript{2+} release and defective E-C coupling in heart failure. This is characterized by Ca\textsuperscript{2+} handling dysfunction, specifically a reduction in Ca\textsuperscript{2+} release amplitude and slowed kinetics including both time to peak and decay rate of Ca\textsuperscript{2+} transients, and thus impaired contractility\cite{19, 21}. Moreover, in failing cardiomyocytes, the structurally remodeled T-tubule system exhibits abnormal electrical activity, i.e., failure of action potential propagation and presence of local spontaneous depolarizations\cite{22}. Such T-tubule electrical remodeling is a major determinant of altered kinetics, amplitude, and homogeneity of Ca\textsuperscript{2+} release. Additionally, spontaneous depolarization in failing T-tubules can trigger local Ca\textsuperscript{2+} release, resulting in Ca\textsuperscript{2+} sparks\cite{22, 24}. Sildenafil treatment applied to well-established heart failure may include effects at the ultrastructural level, i.e., on T-tubule remodeling.

In the present study, using a thoracic aortic banding (TAB) murine heart failure model, we used in situ confocal imaging of the T-tubule network from Langendorff-perfused intact hearts to test the hypothesis that sildenafil ameliorates T-tubule remodeling following TAB. Here, we demonstrate that early sildenafil administration prevented LV T-tubule remodeling, attenuated cardiac hypertrophy and heart failure progression, and improved LV systolic function under TAB stress. Therefore, our findings suggest that restoration of T-tubule integrity is one novel mechanism by which sildenafil improves LV function following pressure overload stress.

Materials and methods

Thoracic aortic banding (TAB) model and sildenafil treatment

Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85-23, revised 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Iowa. Male C57BL/6 mice (9-10 weeks of age) were subjected to sham or pressure overload by TAB surgery as described\cite{23}. Briefly, mice were anesthetized with ketamine/xylazine (100 mg kg\textsuperscript{-1} /5 mg kg\textsuperscript{-1}, ip). The mice were then intubated with a 20-gauge tube and ventilated using a small rodent ventilator. A thoracotomy was created between the second and third intercostal space, and the aortic arch was visualized. Aortic constriction was performed by tying a 7-0 nylon suture ligature against a 27-gauge needle to yield a narrowing of 0.4 mm in diameter when the needle was removed and a reproducible TAB of 65%-70%. In sham mice, the aortic arch was visualized but not banded. Then, the chest wall was closed and the pneumothorax was evacuated. Sildenafil (Pfizer Inc, 100 mg/kg, daily, sc) was administered beginning 1 day after TAB and was continued until 5 weeks after TAB. Control mice were injected with 0.9% saline. Five weeks after surgery, the numbers of surviving mice for each group were Sham (n=8), TAB (n=10), and TAB+sildenafil (SIL) (n=8). LV function was examined by echocardiography at the end of 5 weeks after sham or TAB. In situ confocal imaging of T-tubule structure from epicardial myocytes of intact hearts was performed on the day after echocardiography.

Echocardiography

Prior to sacrificing the mice, transthoracic echocardiograms were performed at the University of Iowa Cardiology Animal Phenotyping Core Laboratory using a Vevo 2100 Imager (VisualSonics, Toronto, ON, Canada). Light general anesthesia was achieved with midazolam (0.2-0.3 mg, sc). The anterior chest was shaved, and prewarmed acoustic coupling gel was applied. The 2D images were acquired in the LV short- and long-axis planes with a 30-MHz sector-array probe at a frame rate of 100 frames per second. LV short-axis images were taken at the papillary muscle level and were deemed acceptable when the LV epicardial silhouette best approximated the arc of a circle. LV long-axis views were acquired perpendicular to the short axis and were deemed appropriate when the LV length was longest and both the mitral and aortic valves were contained in the image. The apex was visualized in all mice. Images were stored digitally without analog conversion and were replayed in single-frame mode. End diastole was defined as the frame in which ventricular volume was largest, and end systole was defined as the frame in which ventricular volume was lowest. In the short-axis view, the endocardial and epicardial silhouettes were manually traced at end diastole and end systole by use of the “leading edge-to-leading edge” convention. In the long-axis projection, LV length was measured for both endocardial and epicardial surfaces by measuring from each respective location at the cardiac apex to the center of the mitral valve plane. LV volumes and ejection fraction (EF) were calculated using the biplane area-length method, which has been validated in rodents\cite{26} and humans\cite{27}. The 2D-guided LV M-mode images at the level of the papillary muscle were recorded from either the short-axis view or the long-axis view. After completion of 2D imaging, the animals were euthanized, and both the heart and lungs were dissected and weighed.

In situ confocal imaging of myocyte T-tubule structure in intact hearts

Studies were performed as previously described\cite{27}. Briefly, intact mouse hearts were Langendorff-perfused at room tem-
perature with Ca\(^{2+}\)-free Tyrode’s solution (137 mmol/L NaCl, 5.4 mmol/L KCl, 10 mmol/L HEPES, 10 mmol/L glucose, 1 mmol/L MgCl\(_2\), and 0.33 mmol/L NaH\(_2\)PO\(_4\); pH adjusted to 7.35–7.45 with NaOH and oxygenated with 95% O\(_2\) and 5% CO\(_2\) during the experiments) containing 2.5 µmol/L MM 4–64 (AAT Bioquest Inc, Sunnyvale, CA, USA), a lipophilic fluorescence indicator of membrane structure, for 30 min. The hearts were then placed in a perfusion chamber attached to the stage of a confocal microscope and perfused with indicator free/Ca\(^{2+}\) free solution (with continuous oxygenation). The membrane structure of epicardial myocytes was visualized in situ with confocal microscopy (LSM510, Carl Zeiss MicroImaging Inc, Germany). The microscope was equipped with a 63× (NA=1.4) oil-immersion lens. The optical pinhole was set to 1 Airy disc (axial resolution<1 µm). Ten to twenty T-tubule images were acquired from different locations of LV free walls of each heart. Quantitative analysis of T-tubule integrity was processed offline with custom routines generated using the IDL image analysis program (ITT VIS Inc, Boulder, CO, USA), as previously reported\(^{17}\). Power values (TT power) from each heart in one group were averaged to represent and reflect the strength of the regularity of the global T-tubule structure of that group.

Criteria for distinguishing murine heart failure

LV heart failure is characterized by decreased EF and lung edema. Mice with both a significant decrease in EF and an increase in lung weight/body weight (mg/g) ratio were considered to have heart failure\(^{29}\). Specifically, in the unband control group, values within the range of two standard deviations (2×SD) from mean values were considered normal. For EF, the value of 2×SD below the unband control mean EF (mean −2×SD =~48%) was considered abnormal. For lung weight/body weight ratio, the measure of 2×SD above the unband control mean lung weight/body weight ratio (mean +2×SD=7) was considered abnormal. TAB mice with both an EF value <48% and a lung weight/body weight ratio >7 mg/g were classified as having heart failure.

Statistical analysis

Data were expressed as mean±SEM. One-way ANOVA and Student’s t tests were used when appropriate. One-way ANOVA was used for multiple group comparisons of in vivo animal experiments. Student’s t-test was used for two group comparisons. For T-tubule analysis, as multiple images were acquired from each heart in each group, a mixed effects model test was performed to compare the variance found across different hearts to that found within a single heart. The regression/correlation analysis was performed using Winks 4.62 statistics software (TexasSoft, Cedar Hill, Texas, USA). A P value <0.05 was considered statistically significant.

Results

Sildenafil attenuates pressure overload-induced myocardial hypertrophy

We first examined the ability of sildenafil to protect hearts from developing hypertrophy and heart failure under LV pressure overload. The ratios of heart weight to body weight (HW/BW) and lung weight to body weight (LW/BW) for sham-operated mice, TAB mice and TAB mice administered sildenafil beginning 1 day after TAB surgery (TAB+SIL) are illustrated in Figure 1A, 1B. The HW/BW ratio significantly increased from 5.00±0.14 in sham-operated mice to 11.81±0.91 in TAB mice (P<0.01) at 5 weeks after surgery, which is indicative of cardiac hypertrophy caused by pressure overload. The LW/BW ratio also increased more than 2-fold in the TAB mice compared with the sham-operated mice, suggesting increased fluid volume in the pulmonary vasculature. However, sildenafil significantly attenuated TAB-induced cardiac hypertrophy as shown by changes in the HW/BW ratio from 11.81±0.91 to 8.81±0.66 (P<0.05, Figure 1A). The LW/BW ratio was also significantly reduced with sildenafil interven-
tion (P<0.05, Figure 1B), suggesting that sildenafil prevented pulmonary edema and the development of congestive heart failure in TAB mice.

We also analyzed the proportion of surviving TAB+SIL mice and TAB mice with either hypertrophy or heart failure. We previously reported that pulmonary edema was apparent in heart failure but not in hypertrophy and that the LW/BW ratio was significantly increased in failing hearts [17]. Therefore, surviving mice with lung weight/body weight ratio >7 (mg/g) and EF value <48% were considered to have developed heart failure (see Methods for details on the definition of heart failure), whereas the other mice were considered to have cardiac hypertrophy. For the TAB group, 8 of 10 surviving mice were classified as having heart failure. Sildenafil decreased the fraction of mice with heart failure to 5 of 8 (Figure 1C). These morphometric data indicate that early sildenafil intervention prevents TAB-induced hypertrophy and the development of heart failure.

Sildenafil improves cardiac function after TAB

Echocardiography was used to evaluate cardiac function and chamber size to examine how sildenafil affects LV function following TAB. Typical long-axis and short-axis echocardiographic images at end systole and LV M-mode recordings are shown in Figure 2. The baseline long-axis and short-axis views of the sham-operated mice demonstrated normal LV muscle thickness during systole, with typical nearly complete LV emptying in end systole (Figure 2A, upper left). In contrast, the long-axis and short-axis images of the TAB mice revealed a dilated left ventricular chamber (Figure 2B, middle left). Furthermore, the TAB mice exhibited depressed LV systolic function with a decrease in the reduction in distance between the anterior wall (AW) and the posterior wall (PW) of the LV at end systole (Figure 2B, long-axis view, indicated by arrowheads). Sildenafil failed to significantly reverse the LV dilation induced by pressure overload (Figure 2C, lower left, long-axis and short-axis images). However, the increase in the reduction in distance between the LV anterior and the posterior wall at end systole, compared with that of the TAB group, predicted that sildenafil rescued LV ejection performance impaired by pressure overload (Figure 2C, long-axis view, indicated by arrowheads). The 2D-guided LV M-mode at the papillary muscle level was recorded from either the long-axis view or the short-axis view. Representative LV M-mode echocardiographic recordings showed the dilated heart in the TAB group with significantly increased LV internal dimensions (LVID) at end diastole and systole that was insignificantly rescued in the TAB+SIL mice, consistent with the results of the long-axis and short-axis echocardiographic image data.

Figure 2D–F summarizes the quantitative echocardiographic data from each group. We measured end diastolic volume (EDV), end systolic volume (ESV), and left ventricle ejection fraction (LVEF) following TAB. After 5 weeks of banding, pressure overload damaged LV systolic function and caused ventricular dilatation, consistent with findings from other murine TAB models[4, 17]. TAB mice displayed dilated LV cavities and enlarged hearts, as demonstrated by massive increases in end systolic and diastolic volumes. Both EDV and ESV were significantly increased in the TAB mice compared with the sham control mice (99.85±9.02 μL vs 57.46±4.13 μL; 68.86±7.68 μL vs 21.40±2.75 μL, respectively; P<0.01; Figure 2D, 2E), paralleling the changes observed in the image data. In addition, a clear decrease in LV contraction was observed in the TAB mice. There was a drastic decline in EF from 63.54±2.70% in the sham-operated mice to 32.01±2.87% in the TAB group (P<0.01, Figure 2F), and these findings are suggestive of heart failure.

Sildenafil failed to significantly reverse LV dilation but maintained LV systolic function compared with age-matched TAB mice (Figure 2D–2F). The quantitative echocardiographic measurements showed that, compared to the TAB group, sildenafil moderately improved EDV and ESV (TAB+SIL vs TAB, 93.74±11.04 μL vs 99.85±9.02 μL; 52.61±10.51 μL vs 68.86±7.68 μL, respectively; P>0.05), which indicated no rescue of LV cavity dilation. In contrast, TAB+SIL mice showed significantly better LV contractile function compared with TAB mice, as shown by increased LVEF (P<0.05). A total of 50% (4 of 8) of the mice in the sildenafil group had improved LV systolic function (LVEF>40%) after the 5-week sildenafil treatment. Average EF was significantly improved in the TAB+SIL mice compared to that of the control mice in response to TAB stress (47.67%±6.95% vs 32.01%±2.87%, respectively, P<0.05, Figure 2F). These echocardiographic data provide evidence that sildenafil improves LV function following TAB.

Sildenafil protects against LV T-tubule remodeling after TAB

The therapeutic benefit of sildenafil with regard to LV systolic function led us to hypothesize that sildenafil prevents the extensive ultrastructural remodeling in LV myocytes following TAB. T-tubules are one of the key components of cardiac E–C coupling, and disruptive T-tubule remodeling has been documented in many forms of cardiac disease that share the common end-stage of heart failure. To further investigate the subcellular mechanism underlying the beneficial effects of sildenafil on gross cardiac function, we next examined myocyte T-tubule structure using in situ T-tubule imaging. While sham-operated animals displayed a highly organized LV T-tubule network, LV pressure overload caused drastic T-tubule remodeling (Figure 3A, 3B) that corresponded to a severe decrease in TTpower, an index of T-tubule integrity (Figure 3D). Specifically, at 5 weeks after TAB, the overall LV TTpower was 1.49±0.04 in the TAB mice compared to 2.11±0.029 in the sham-operated mice (P<0.01, Figure 3D).

As shown in the representative images and quantitated TTpower data, LV myocytes from the TAB+SIL group had improved T-tubule organization compared to the TAB group (Figure 3C, 3D), suggesting that this ultrastructural alteration is preventable with sildenafil therapy. This improvement of LV T-tubule structure with administration of sildenafil was shown by a significant increase in LV TTpower from 1.49±0.04 in the TAB group to 1.74±0.06 in the TAB+SIL group (P<0.01, Figure 3D).
We next analyzed the histogram distribution of TT$_{power}$ from individual LV images (Figure 3E). As multiple images were acquired from each heart in each group, we performed a mixed effects model test to compare the variance found across different hearts to that found within a single heart, and we determined that the results were significantly different ($P<0.01$). Under sham conditions, TT$_{power}$ in LV myocyte populations exhibited a single-mode, bell-shaped distribution (centered at 2.2), suggesting that TT$_{power}$ from all LV myocytes belongs to one single population in the unbanded hearts. After TAB stress, the entire histogram of LV TT$_{power}$ shifted leftward (centered at 1.6). However, the histogram retained its single-mode configuration, suggesting that the entire population of myocytes suffers from T-tubule remodeling in a similar
way. In particular, it is noteworthy that the highest LV TT-power (≥2.2) was decreased from 33% in the hearts of sham-operated mice to 0% in the hearts of TAB mice (Figure 3E, middle). These data strongly suggest that, in addition to overt subcellular loss of T-tubules in a fraction of myocytes, the entire population of LV epicardial myocytes examined undergoes global changes.

Figure 3. In situ confocal imaging of the LV myocyte T-tubule system from Langendorff-perfused intact hearts. (A–C) Representative LV T-tubule images from the sham, TAB, and TAB+SIL (100 mg/kg sc per day, given 1 day after TAB, for 5 weeks) groups, respectively. (B) TAB-treated mice exhibited severe T-tubule loss and disorganization in LV myocytes. (C) Treatment of sildenafil prevented TAB-induced T-tubule remodeling in LV myocytes. (D) Average data of LV TT-power (T-tubule power), an index of the strength of T-tubule regularity (for analysis, see Reference 17). LV TT-power of the TAB+SIL group was significantly higher than that of the TAB group but lower than that of the sham group. (E) Histogram analysis of TT-power of all T-tubule images acquired from different regions of LV free wall (10–20 images from each LV). The count numbers at each bin size were normalized by the total images of each group and are represented as frequencies. Each graph represents >1000 myocytes. TAB mice displayed a leftward shift in LV TT-power (mode shifted from 2.2 of sham to 1.6). The histogram of the TAB+SIL group was shifted to the right in comparison to that of the TAB group (with a mode shift from 1.6 to 1.9), indicating improvement of LV T-tubule ultrastructure by sildenafil treatment. *P<0.01, mixed effects model test. P<0.01 among groups, one-way ANOVA. ⅠP<0.01 vs sham. ⅡP<0.01 vs TAB. n=8, 10, and 8 hearts per group.
T-tubule remodeling in response to TAB stress.

For the TAB+SIL group, we found that sildenafil shifted the histograms of LV $T_T^{power}$ back to the right in response to TAB, while retaining a single mode of distribution in the LV (centered at 1.9; Figure 3E, bottom), indicating an improvement of LV T-tubule structure. The highest LV $T_T^{power}$ (≥2.2) was increased from 0% in the hearts of TAB mice to 7% in the hearts of TAB+SIL mice. Thus, histogram analysis of $T_T^{power}$ distribution further confirmed the beneficial effects of sildenafil on TAB-induced LV myocyte T-tubule remodeling.

T-tubule remodeling correlates with LV function

The role of the T-tubule in cardiac E–C coupling and the consequence of T-tubule remodeling in failing myocytes (ie, SR Ca$^{2+}$ handling dysfunction) are well recognized [14–16, 19, 29]. Our early study demonstrated that T-tubule integrity is highly correlated with cardiac EF in diseased hearts, indicating that T-tubule integrity is a crucial determinant of cardiac function [17].

Therefore, we examined the correlation between LV $T_T^{power}$ which represents the overall T-tubule status of myocardium, and cardiac morphometrics and performance (Figure 4). LV $T_T^{power}$ displayed a strong negative correlation ($R^2=0.74$, $P<0.01$) with HW/BW ratio (Figure 4A), a significant index of myocardial hypertrophy. LV $T_T^{power}$ also exhibited a positive, linear correlation with LVEF ($R^2=0.47$, $P<0.01$; Figure 4B). These results indicate a structure-function correlation between subcellular T-tubule remodeling and whole-animal cardiac performance in heart failure, further supporting our previous conclusions from a rat pressure overload heart failure model [17].

Discussion

Myocardial remodeling and attendant cardiac dysfunction remain major causes of morbidity and mortality in heart failure, and new approaches to combat this pathophysiological process are needed. The therapeutic benefits of sildenafil in heart failure were reported previously, yet the mechanism is still not completely understood. In the present study, using a TAB mouse heart failure model in combination with in situ confocal imaging of myocyte T-tubule structure in intact hearts, we examined the effect of sildenafil therapy on T-tubule remodeling and cardiac function following pressure overload. The major findings of our study are as follows. First, TAB-induced heart failure manifested with marked myocardial hypertrophy, LV dilation, systolic dysfunction, severe T-tubule loss and disorganization in LV myocytes. Second, sildenafil administered at the onset of the TAB stress attenuated the development of cardiac hypertrophy and heart failure, partially restored LV contractile function, and preserved normal T-tubule ultrastructure in LV myocytes. Third, T-tubule structural remodeling was strongly correlated with the degree of hypertrophy and global LV function in this TAB mouse model. These data provide insight into the mechanism by which sildenafil improves cardiac function following TAB.

Compelling experimental and clinical evidence indicates that sildenafil exerts beneficial cardiovascular effects [2–9, 12]. It has been demonstrated that, at the structural (organ) level, sildenafil prevents and reverses ventricular remodeling in chronic heart failure following pressure overload [2–4]. In a mouse model of left ventricular overload by TAB, sildenafil was reported to prevent myocardial hypertrophy, fibrosis, and chamber dilation and to improve in vivo cardiac function [2–4]. Additionally, sildenafil reverses pre-established hypertrophy induced by pressure overload while restoring chamber function [4]. Further study provided evidence that delayed sildenafil treatment also suppresses progressive cardiac dilation, dysfunction, fibrosis, and hypertrophy in hearts subjected to sustained pressure overload [3]. In our experiments, we found that early treatment with sildenafil after TAB prevented afterload increase-induced structural hypertrophy, improved LV contractile function, and thus attenuated the development of heart failure, consistent with previously published literature [4]. However, our findings showed that sildenafil treatment concurrent with TAB had minimal impact on LV chamber dilation. Others have suggested that PDE5 inhibition by chronic sildenafil treatment appears to increase the propensity of pressure overload-induced chamber dilation and cardiac failure [30]. The mechanism accounting for these mixed results is presently unknown. Chamber dilation in advanced heart failure signifies that ventricular remodeling has reached a point where hypertrophy is no longer adequate to maintain ejection performance. We speculate that sildenafil may lose its protective effects at advanced stages of heart failure and is unable to maintain normal chamber size.

Our data presented in this study extend these observa-
molecules have been shown to be involved in T-tubule remodeling and likely be caused by direct cardiac effects. To date, several mechanisms as well. For example, T-tubule remodeling constitutes a key mechanism underlying the transition from compensated hypertrophy to heart failure, indicating that preventing T-tubule remodeling during the hypertrophy stage may be clinically important for delaying the progression to heart failure. Our current data also revealed that LV T-tubule integrity is closely associated with LV contractile function, consistent with our previous results. Therefore, we can attribute the improvement in LVEF with sildenafil therapy, at least partly, to the preservation of the T-tubule network. However, many factors contribute to cardiac dysfunction in the progression of heart failure in the TAB model. Accordingly, sildenafil could improve cardiac performance by other mechanisms as well. For example, T-tubule remodeling leads to an increase in orphaned RyRs, a reduction or redistribution in Ca$^{2+}$ channels on the T-tubule membrane, impaired coupling efficacy between Ca$^{2+}$ channels on T-tubule and RyRs on the SR, and therefore asynchronous Ca$^{2+}$ release and a reduction in Ca$^{2+}$ transient amplitude and kinetics. In line with these findings, it has been reported that isolated myocytes from TAB+sildenafil hearts show improved calcium cycling. It remains unclear how inhibition of PDE5 with sildenafil mechanistically prevents LV T-tubule remodeling following TAB stress. Though PDE5 is more abundantly expressed in vascular smooth muscle, its expression and activity are increased in human and mouse cardiac hypertrophy and heart failure. We postulate that the anti-T-tubule remodeling effect from selective inhibition of PDE5 by sildenafil may likely be caused by direct cardiac effects. To date, several molecules have been shown to be involved in T-tubule formation and maintenance following cardiac stress, including amphiphysin-2 (Bridging Integrator 1, BIN1) and junctophilin-2 (JP2). BIN1 is downregulated in failing human cardiomyocytes, which impairs Cav1.2 trafficking to T-tubules, calcium transients, and contractility. However, no studies have examined the role of BIN1 in T-tubule remodeling in heart failure. Early studies demonstrated an essential role for the Gαq/phospholipase Cβ (PLCβ) pathway in the development of compensatory hypertrophy following pressure overload. PDE5 inhibition via sildenafil was found to blunt hypertrophy and enhance cardiac function early after TAB by suppressing Gαq/PLCβ signaling. Recent work from our group using a cardiac-targeted Gαq-overexpressing transgenic mouse model revealed that Gαq/PLCβ-dependent activation of the Ca$^{2+}$-dependent protease calpain and subsequent downregulation of JP2, a structural protein that bridges the T-tubule and SR membranes, is one molecular mechanism that leads to T-tubule remodeling and heart failure. Taken together, these data suggest that sildenafil may effectively prevent T-tubule remodeling through inhibition of this pathway. Future studies using the cardiac-specific Gαq overexpression mouse model are necessary to completely understand the mechanism.

In summary, we elucidated that early intervention with sildenafil preserves LV structure and function at the ultrastructural and whole-organ levels and uncovered a new mechanism by which sildenafil provides therapeutic benefits in heart failure. Specifically, the prevention of T-tubule remodeling appears to be an important benefit of sildenafil in LV pressure overload-induced heart failure. Our study provides new insights into the understanding of sildenafil in heart failure pathogenesis. Further mechanistic investigation would help in reaping additional benefits of sildenafil for heart failure.

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Author contribution
Long-sheng SONG and Jiang HONG designed and supervised the research; Chun-kai HUANG, Bi-yi CHEN, and Ang GUO performed the research and analyzed the data; William KUTSCHKE, Rong CHEN, and Yan-qi ZHU assisted with the research; and Chun-kai HUANG, Jiang HONG, and Long-sheng SONG wrote the paper.

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