의학석사 학위논문

Long-Term Effects of Sildenafil in a Rat Model of Chronic Mitral Regurgitation;

Benefits on Ventricular Remodeling and Exercise Capacity

만성 승모판 폐쇄부전 설치류 모델에서 실데나필의 효과

좌심실 재형성억제와 운동능력 향상의 효과

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by

Kyung-Hee Kim

A thesis submitted to the Department of Clinical Medical Sciences, Graduate School in partial fulfillment of the requirements for the Degree of Master of Science in Clinical Medical Sciences at Seoul National University College of Medicine

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Approved by Thesis Committee:

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ABSTRACT

Background: Recent reports have noted the favorable pleiotropic effects of sildenafil in heart failure. We tested the hypothesis that chronic treatment with sildenafil attenuates left ventricular (LV) remodeling and prevents exercise intolerance in chronic mitral regurgitation (MR).

Methods: MR was created in Sprague Dawley rats by making a hole on the mitral leaflet. Two weeks after MR creation MR and LV dilatation were confirmed by echocardiography and rats were randomly assigned to sildenafil (SIL) treatment (MR+SIL group, 50mg/kg p.o. bid, N=16) or normal saline only group (MR group, N=16) and continued for 4 months. Sixteen sham rats were compared to MR rats.

Results: Three of 16 rats in MR group died during the study period. No deaths were noted in MR+SIL and sham group. After 4 months, LV size was smaller in MR+SIL compared to MR (LV end-systolic dimension, 4.7±0.3 for sham vs 5.9±0.3 for MR+SIL vs 7.4 ± 0.5 mm for MR $p<0.05$; LV end-diastolic dimension,8.3±0.4 vs 10.5±0.2 vs 11.7±0.61 mm, $p<0.05$). LV ejection fraction was greater in MR+SIL than MR. (70.2%±2.2 for sham vs 67.0±4.2 for MR+SIL vs 58.9±2.5 for MR,

p=0.01). In pressure-volume analysis, MR+SIL showed greater LV end-systolic pressure-volume relation than MR. Serial treadmill test revealed that exercise capacity was reduced in MR but not in MR+SIL. Transcriptional profiling of cardiac apical tissues revealed that gene sets related with inflammatory response, DNA damage response, cell cycle checkpoint, and cellular signaling pathways were significantly enriched by genes with reciprocal changes. Pathological analysis showed perivascular fibrosis was more prominent in MR than MR+SIL and the percentage of TUNEL-positive cells was two-fold greater in MR compared to MR+SIL.

Conclusion: Sildenafil significantly attenuates LV remodeling and prevents exercise intolerance in a rat model of chronic MR. This benefit might be associated with anti-apoptotic, anti-inflammatory effects of sildenafil.

Keywords: **Mitral regurgitation, Sildenafil, Left ventricular remodeling, Exercise capacity, Microarrays**

Student number: 2011-21966

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LIST OF ABBREVIATIONS

MR=mitral regurgitation

ACE=angiotension-converting enzyme

PDE5=phosphodiesterase-5

NO=nitric oxide

cGMP=cyclic guanosine monophosphate

LV=left ventricle

EF=ejection fraction

BP=blood pressure

IHC=immunohistochemistry

INTRODUCTION

Mitral regurgitation (MR) is a growing clinical problem as the mean age of the population advances [1, 2]. Currently, effective medical therapy is not available to prevent left ventricular (LV) remodeling and deterioration of exercise capacity, and thus delay the need for surgery. Although vasodilator is effective in acute MR [3], its benefit is not established in chronic MR [4, 5].

Sildenafil citrate (SIL) is a selective inhibitor of phosphodiesterase-5 (PDE5) that catalyzes the breakdown of cGMP, one of the primary factors causing smooth muscle relaxation. By its potent action of enhancing NO-driven cGMP accumulation and ensuing vasodilation, sildenafil is widely used for the treatment of erectile dysfunction. However, growing evidence supports that inhibition of PDE5 may have important antihypertrophic, anti-apoptotic, and ischemic preconditioning effects that may limit myocardial remodeling in response to stress and attenuate the substrate for heart failure development. In murine models of myocardial pressure overload, inhibition of PDE5 was associated with inhibition of myocyte hypertrophy and interstitial fibrosis, preservation of cardiac function, and deactivation of various hypertrophy signaling cascades [6-8]. PDE5 inhibition also promotes opening of mitochondrial KATP channels, a

putative target of reactive oxygen species generated in response to both ischemia/reperfusion and doxorubicin treatment, and reduces doxorubicin or ischemia/reperfusion-induced apoptosis [9, 10]. Additionally, clinical studies of sildenafil in heart failure patients have reported improved exercise capacity coupled to reduced pulmonary vascular resistance and better endothelial function [11, 12].

Accordingly, we hypothesized that sildenafil may have a beneficial effect in chronic MR because the process of LV remodeling shares some of aforementioned molecular mechanisms in chronic MR. In the present study, we evaluated long term effect of sildenafil on survival, myocardial function, remodeling and exercise capacity using a rat model of chronic MR. Additionally, we investigated potential mechanisms for the beneficial effects of sildenafil using cDNA microarray technology.

MATERIALS AND METHODS

Animal handling

Male Sprague Dawley (SD) rats (n=52) with an initial weight of 350-400g were used. The rats were given free access to tap water and standard rat chow and housed in a room with a 12:12-h light cycle, a temperature of 21℃, and a humidity of 55%. The experimental protocols were approved by Institutional Animal Care and Use Committee of Seoul National University Hospital.

Creation of MR model and study design

Surgical creation of MR was performed under the guidance of transesophageal echocardiography in 11-week-old rats [13, 14]. We used intracardiac echocardiographic catheters (Acuson/Siemens Corp, Mountain View, CA) for transesophageal studies. After the insertion of echocardiographic catheter into the esophagus, left thoracotomy was performed through the fourth or fifth intercostals space. A needle (0.5mm external diameter) was inserted into the LV via apical puncture. After we found the needle tip under the echocardiographic image, we advanced the needle toward the anterior mitral valve to puncture the leaflets and create MR. MR was considered significant if a regurgitant jet area larger than 45% of the left atrium[14]. Sham animals underwent

the same procedures except for mitral leaflet injury.

Two weeks after the surgery, we confirmed the development of MR with LV dilation and the rats were randomly assigned to sildenafil (SIL) treatment (MR+SIL group, 50mg/kg p.o. bid, N=16) or normal saline only group (MR group, N=16). An oral dose of 100mg/kg/day sildenafil (Viagra, Pfizer) were administered orally by gavage twice a day based on the previous studies showing the pleiotropic vascular effect in a heart failure model[15-17]. Sixteen sham rats were compared to MR rats.

We started the treatment 2 weeks after MR creation and continued for 14 weeks according to the results of our pilot study[13]; (1) ejection fraction (EF) remained in the normal range despite progressive increase of LV size and (2) no additional death occurred during this period. Cardiac function and exercise capacity was monitored every 2 or 4 weeks. At the end of the 14-week treatment, pressure-volume analysis was performed and the hearts and lungs were harvested for tissue determinations. The detailed experimental protocol is shown in Fig. 1.

Table 1. General and Echocardiographic Characteristics 2 Weeks after Mitral Regurgitation (13 weeks of age)

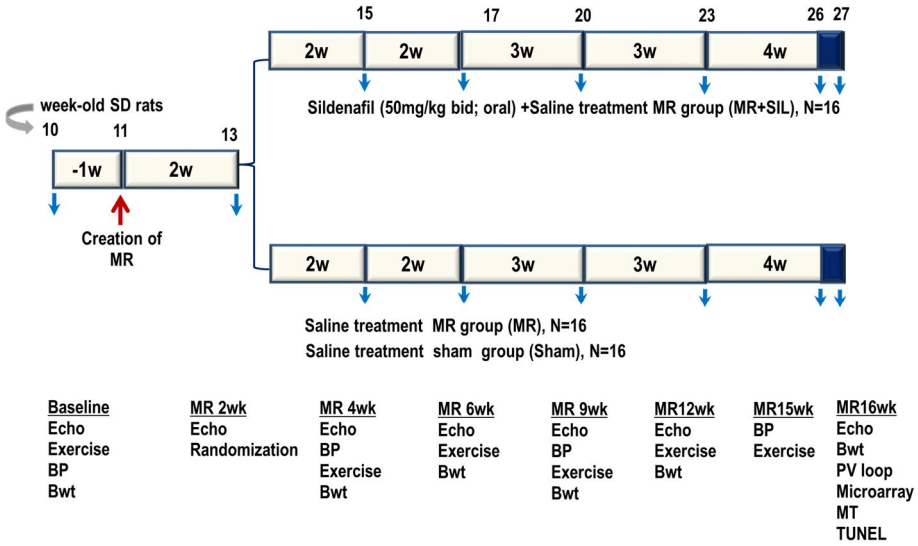
Variables	Sham Group (n= 16)	MR group	
		MR (n=16)	MR+SIL (n=16)
Body weight, g	437.0±8.8	422.8±8.8	423.8±10.4
Systolic BP, mmHg	130.5 ±12.9	133.2 ±6.5	128.4 ±8.2
Diastolic BP, mm Hg	93.6 ±15.1	95.7 ±14.4	90.1 ±10.1
HR, beats/min	350 ±18.1	354 ±14.9	347 ±22.4
HR, beats/min*	312.8 ± 17.3	318.3 ± 15.7	315.3 ± 18.7
EF,%	70.4±0.94	78.5 ± 1.0†	78.7 ± 1.54†
EDD, mm	7.76 ± 0.2	9.01 ± 0.44†	9.01 ± 0.48†
ESD, mm	4.21±0.19	4.15±0.19	4.11±0.22
SWT, mm	1.32±0.04	1.1±0.05†	1.09±0.06†
PWT, mm	1.35±0.01	1.07±0.06†	1.08±0.07†
E velocity	65.2 ± 7.1	106.1 ± 12.1	104.1 ± 16.9
LV mass, g	683±14.44	695±15.7	697.7±14.3
LV mass index, mg/g*	1.60±0.04	1.65±0.06	1.62±0.05

Data are means±S.E.M. MR, mitral regurgitation; SIL, sildenafil; HR, heart rate; EF, ejection fraction; SWT, end-diastolic wall thickness of interventricular septum; PWT, end diastolic wall thickness of posterior wall; EDD, end diastolic dimension; ESD, end systolic dimension; E velocity, mitral inflow E velocity cm/s

*Heart rate and LV mass were estimated in vivo by echocardiography.

†$p<0.05$ for difference from control.

Figure 1 - Experimental protocol, a schematic illustration. wk; week, MR; mitral regurgitation, Echo; Echocardiography, BP; blood pressure, Bwt; body weight, PV; pressure-volume



Survival Analysis

We examined the effects of sildenafil on the survival of MR rats. The day of oral administration of sildenafil was defined as day 0. This survival analysis covered the entire experimental period to day 100.

Physiologic study

Echocardiography

Transthoracic echocardiography was performed 1 week before and 2, 4, 6, 9, 12 and 16 weeks after the creation of MR on spontaneously breathing rats placed on their dorsal recumbency position. Rats were lightly sedated with inhalation of the lowest possible dose of isoflurane (initially 4%, then approximately 2-3%) mixed with oxygen. Images were acquired with a 9 MHz transducer connected to a Toshiba echocardiography machine (Nemio, Toshiba Co., Tokyo, Japan). LV septal and posterior wall thickness (SWT and PWT) and LV end diastolic/systolic dimension (EDD/ESD) were measured using M-mode echocardiography at the papillary muscle level. LV ejection fraction (EF) was calculated as $(LVEDD^2\text{-}LVESD^2)/LVEDD^2$. LV mass was estimated by a formula validated in small animals[18] and adjusted for body weight: $LV\text{-}mass=([SWT+PWT+LVEDD]^3\text{-}[LVEDD]^3)x1.04)$.

Color Doppler mapping of MR jets was used to semiquantitatively assess the severity of MR[19]. MR jet areas and LA areas were measured in the apical long-axis view and the ratio of MR jet area to the LA areas was calculated. Early diastolic transmitral flow (E velocity) was measured in apical 4-chamber view with a 1.5mm–sized sample volume placed at the tips of the mitral leaflets. All parameters were

evaluated on an average of 5 consecutive beats. A single echocardiographer experienced in rat echocardiography over 500 cases performed all data acquisition.

Exercise Test and BP Monitoring

Maximal exercise capacity was evaluated with Rota Rod Treadmill (Ugo Basile, Comerio, Italy). Rats run on a knurled drum as the drum rotates in order to avoid falling off. Animals were trained twice before the test to adjust the treadmill more familiarly. Treadmill speed was gradually increased from 3 rpm (revolutions per minute) to 15 rpm every 1 minute. Exercise time was recorded. The observer blinded to the study group recorded episodes of the immobility response due to exhaustion.

Systolic and diastolic blood pressures were measured in conscious rats via the tail-cuff method (Biopac System Inc) at 1 week before and 2, 4, 9, 15 weeks after the creation of MR. At least 1 day interval was given for the BP measurements after echocardiographic examination or exercise test to minimize the stress on the animals.

Invasive Hemodynamic Measurements

At 16 weeks after MR creation, invasive hemodynamic measurements were obtained using a 1.4-French high-fidelity Millar pressure catheter,

as described previously [20, 21] .

Histopathological analysis

After hemodynamic measurements were performed, the rats were euthanized and the hearts, lungs were harvested and weighed and apical scar excluded.

RNA isolation

Fresh LV apex, excluding apical scar was immediately stored in RNAlater (Ambion/Applied Biosciences, Streetsville, ON, Canada) at –80°C until use. The hearts were homogenized in TRIzol Reagent (Invitrogen, Burlington, ON, Canada), and total RNA was isolated according to the manufacturer's instructions. RNA was further purified to remove genomic DNA contamination and concentrated using an RNeasy Plus Mini kit (Qiagen, Mississauga, ON, Canada). Samples with optical density ratio 260/280 >1.8, 28S/18S >1.6 using a Bioanalyzer 2100 (Agilent, Santa Clara, CA) were selected for microarray processing.

Microarray Analysis

Apical heart RNA excluding scar tissue was labeled either with Cyanine 3-CTP (Cy3) or Cyanine 5-CTP (Cy5) (PerkinElmer, Boston,

MA, USA) by Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies) and hybridized onto Agilent whole rat genome array (G4131A). RNAs from three rats in each group were pooled and hybridized onto one chip. The arrays were scanned at two different intensities, and the images were analyzed for background correction. Both BMP4 and FGF2 samples were co-hybridized with RNA from the starting point and a dye-swap was performed. The arrays were normalized and the differential gene expression was analyzed using the R and bioconductor-based method.

We performed the Gene Set Enrichment Analysis to find molecular pathways or Gene Ontologies that are made up of differentially expressed genes in each biological condition [22]. Difference in mean log transformed intensity for each experimental group was used as a metric for sorting probes in GSEA. After sorting genes according to the metric, Enrichment Scores were assigned to 2,394 different gene sets curated in the mSigDB database. (http://www.broadinstitute.org/gsea/msigdb/) Gene sets with high absolute values of enrichment score are molecular pathways or Gene Ontologies that are made up of up- or down-regulated genes and are differentially regulated pathways. P-values for the Enrichment Scores were calculated after permuting class labels of each experimental condition and gene sets with p values less than 0.05 were extracted. Gene sets with absolute Enrichment Score more than

0.35 and p-value less than 0.05 were visualized as a network with nodes representing gene sets and edges connecting two nodes if hypergeometric p-valvue for testing the significance of gene overlap is within one percentile of the whole p- values[23].

Verification of Gene Expression With RT-PCR

Total RNA was isolated using RNeasyPlus Mini kit (Qiagen) and cDNA was synthesized using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara) with 1 μg of each total RNA sample according to the manufacturer's instruction. cDNA (1 μl) was amplified by PCR using TaKaRa Ex Taq™ kit (Takara) with specific primers (Table 2). GAPDH was chosen as an endogenous control. Quantification of band intensity was analyzed using TINA 2.0 (RayTest) and normalized to the intensity of GAPDH.

Table 2. Primer Sequences for Validating Microarray by Real-Time PCR

Gene name	Forward Primer	Reverse Primer	Genebank ID
IL6	TCTCGAGCCCACCAGGAA CGAAA	GTAGGGAAGGCAGTGGCTGTCA	NM_012589
IL18	ATCCTAGCTGCCTGCTCC AGCTG	CTGGTCTGGGATTCGTT GGCTGT	AY077842
CDKN2a	CTCTCCCGACCGGTG CACGA	TAGGCACCTGGGCGTGCTTG	NM_031550
NOS2	ACTCCATCGACCCGCCCACA	GCAGTGCTACAGCTCCGGGC	NM_012611
NOS3	TGCCTTTGCTCGAGCGGTGG	CTCCACTAGGCCAGGCCGGT	NM_012589
GAPDH	CAAATTCGTTGTCATACCAG	CGTGGAAGGACTCATGAC	NM_017008.3

Immunohistochemical Analysis

Mid ventricle were removed for histopathology and were preserved in 4% paraformaldehyde and embedded in paraffin. The tissue was

sectioned into 4 μm sections, stained with Masson's trichrome for evaluation of the degree of fibrosis and with picrosirius red for measurement of collagen deposition. Apoptosis was assessed using the Terminal deoxynucleotidyl transferase-mediated UTP nick-end labeling (TUNEL) technique (DNA fragmentation, Oncor, Gaithersburg, MD). The detailed protocol was previously published [24].

Statistical analysis

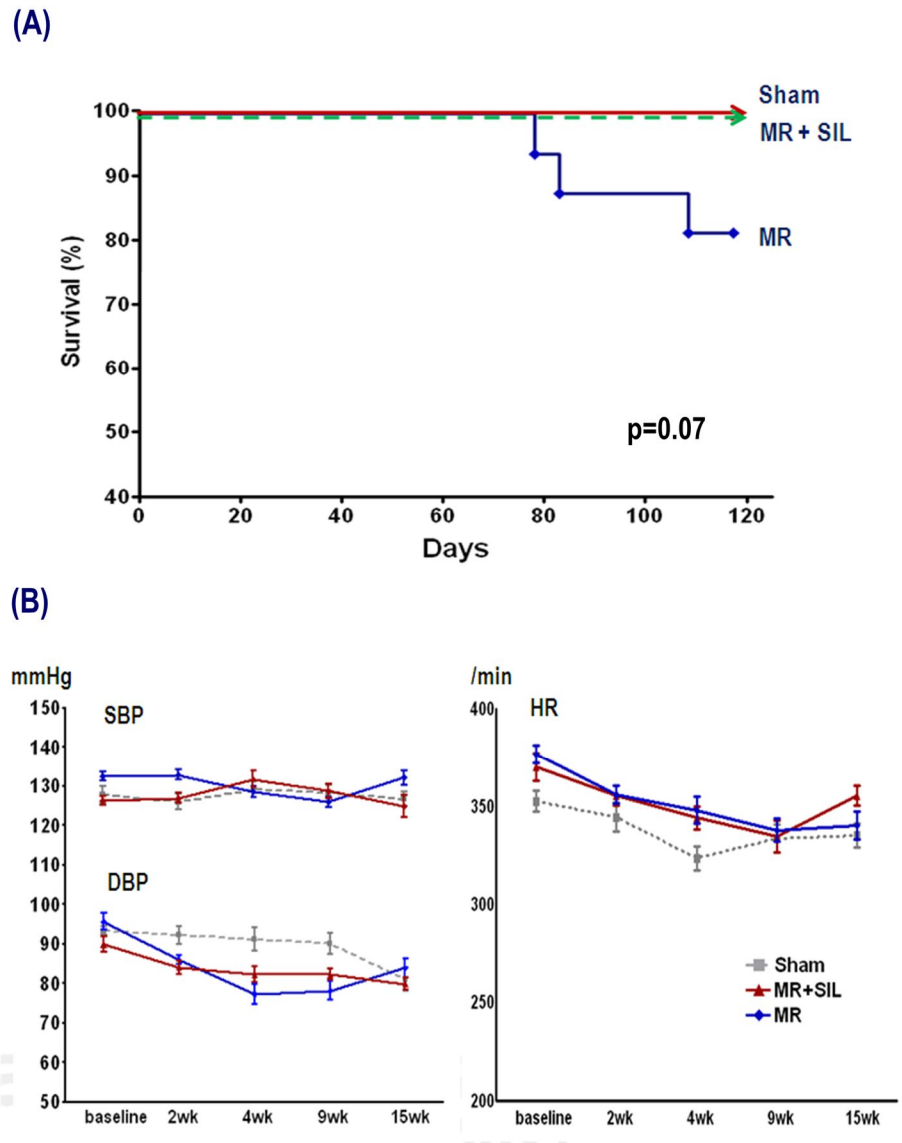
Data are presented as mean±S.E.M. The normality of all parameters was tested using the Kolmogorov-Smirnov test. The differences among groups were compared by the unpaired t-test or one-way analysis of variance, followed by the Bonferroni test. In cases in which normality was excluded, the non-parametric Kruskal-Wallis H test was performed, and the Mann-Whitney U test was used for post hoc analysis. Repeated measures ANOVA was performed to analyze the changes of several variables over time such as LV ejection fraction, LV dimension, LV mass index and exercise capacity. Survival data was evaluated by the Kaplan-Meier method with pairwise comparison conducted using the log-rank test. All statistical analyses were performed using SPSS 17.0 version (SPSS Inc., Chicago, US) and p-values of < 0.05 were considered statistically significant.

RESULTS

Survival Analysis

A total of 52 rats were used in this study. Upon surgery, two rats died of bleeding and two rats died in acute stage within 2weeks after MR operation due to pulmonary edema and unknown cause. Three of 16 rats in MR group died during the study period. However, no deaths were noted in MR+SIL and sham group. . Kaplan-Meier survival curve showed the comparison between MR + SIL rats and MR rats. ($p=0.07$, Fig. 2A)

Figure 2 - (A) Survival analysis. Kaplan-Meier survival curve showed the comparison between MR+ SIL rats and MR rats. (p=0.07) (B) Serial change in blood pressure and heart rate



Hemodynamic study

LV Remodeling after MR Creation

Mean MR jet area was 15±3 mm^2 and the mean ratio of MR jet area to the LA area was 56 ± 8 % at 2 weeks after operation. There was no MR in the sham group. Mitral E velocity increased at 2 week (65.2 ± 7.1 vs 105.1 ± 15.1 cm/s, $p < 0.05$) in MR groups whereas it did not change in the sham group (66.4 ± 6.4 vs 64.5 ± 5.5 cm/s, $p = 0.76$). Fig. 3 demonstrates the time-dependent changes in LV diameter, EF and LV mass index. Baseline examinations showed no difference of LV diameters among the three groups (LVESD; 4.0± 0.1 vs 4.1±0.1 vs 4.1±0.1 mm, LVEDD; 7.4±0.1 vs 7.5±0.1 vs 7.5±0.1 mm for sham, MR and MR+SIL groups, $p=0.82$). The LV started to dilate immediately after MR creation and showed progressive dilation until the 16^{th} week. There was significant difference in LVESD between MR and MR+SIL from the 6^{th} week, whereas LVEDD did not differ significantly until the 12^{th} week. Consequently LVEF started to decrease from the 6^{th} week in MR group whereas it was preserved until the 16^{th} week in MR+SIL group. With increasing LVESD, death started to occur as shown in the Kaplan-Meier curve (Fig. 2A). Weight gain tended to lag behind the sham rats in MR and MR+SIL rats without statistical significance. The LV mass index increased significantly in the MR group compared with

the sham group (1.15±0.04 vs 1.71±0.05 mg/g, $p < 0.05$). Sildenafil treatment attenuated the increase of LV mass index (11.7% reduction, $p = 0.01$). There were no significant changes in blood pressure and heart rate among the groups (Fig. 2B)

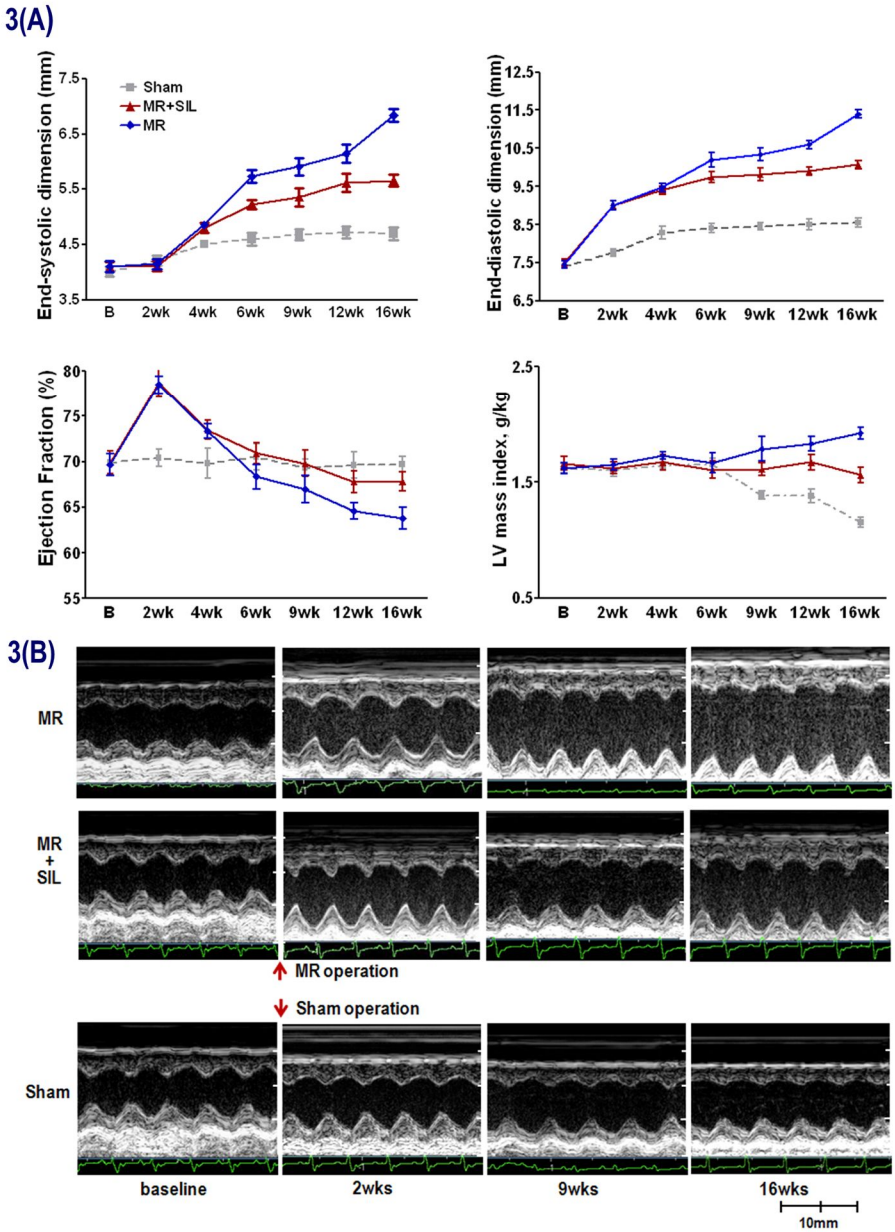


Figure 3 - (A) Serial changes in echocardiographic measurements. (B) Representative M-mode echocardiograms from the three groups. LV; left ventricle, MR; mitral regurgitation, B; baseline, wk; weeks after MR operation, SIL; sildenafil. *p < 0.05 for difference from sham group. † p <0.05 for difference from MR group

Exercise Capacity

There was no difference in exercise duration among the groups until the 6th week after MR when it became shorter in MR group than sham group (720±15 for sham vs 588±15 for MR, 617±21 seconds for MR+SIL, $p < 0.05$ for difference from sham vs MR, Fig.4). Thereafter, exercise capacity was impaired progressively in MR group compared with sham group (at the 15th week, 705± 18 for sham vs 462±21 seconds for MR, $p < 0.05$). However exercise duration was maintained in the sildenafil treated MR rats (641±14 seconds for MR+SIL, $p<0.05$ for difference from MR).

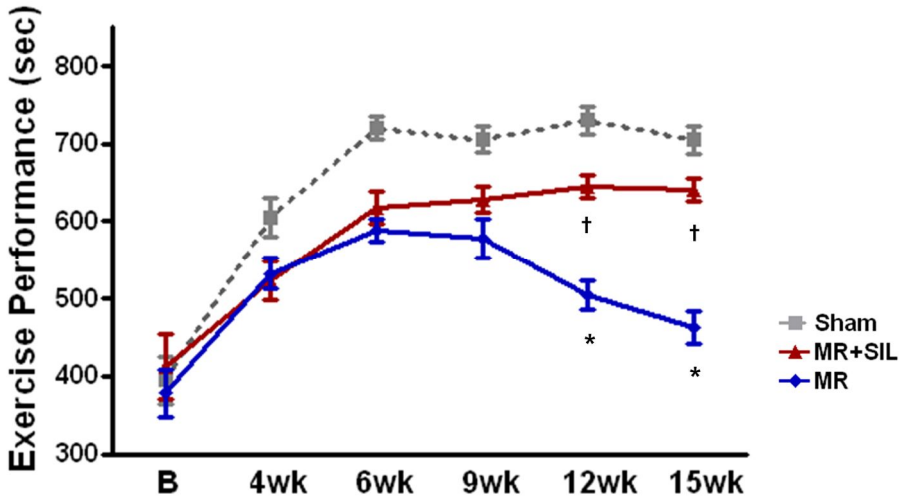


Figure 4 - Comparison of exercise duration among the three groups. * p < 0.05 for difference from sham group. † p <0.05 for difference from MR group. B; baseline, MR; mitral regurgitation, SIL; sildenafil.

Invasive hemodynamic measurement

At the 16th week, rats underwent invasive hemodynamic assessment and were sacrificed for the pathological analysis (Table 3, Figure 5).

Heart rate, LV end-diastolic pressure, -dP/dt, and the end-diastolic pressure-volume relation were not different among the groups. However, end-systolic volume, end-diastolic volume and stroke volume were greater in MR group compared with sham group. The end-systolic pressure-volume relation (ESPVR), +dp/dt, preload-recruitable stroke work (PRSW) and EF were significantly decreased in MR group, indicating contractile dysfunction. Sildenafil treatment prevented systolic functional impairment in the MR+SIL group compared with the MR group; EF (+28%, $p < 0.05$), ESPVR (+116%, $p<0.05$), PRSW(+30%, $p< 0.05$).

Table 3. Hemodynamic Parameters 16 Weeks after Mitral Regurgitation (27 weeks of age)

Variables	Sham Group (n= 16)	MR group	
		MR (n=13)	MR+SIL (n=16)
Body weight, mg	614.0±10.0	587.1±10.2	594.2±5.7
Lung (g)	1.08±0.15	1.46±0.20†	1.23±0.17†‡
Lung/BW (mg/g)	1.75±0.24	2.48±0.31†	2.07±0.27†‡
HR, beats/min	321.5±20.1	322.7±24.7	325.4±22.8
ESV, μl	127.2±5.9	443.5±25.4†	321.2±37.4†‡
EDV, μl	430.9±27.6	1113.6±61.9†	998.7±54.7†‡
EF, %	70.2±1.4	60.7±3.5†	67.2±3.7
SV, μl	303.1±21.0	670.4±35.8†	677.5±48.5†
LV ESP, mmHg	101.3±7.5	105.4±6.3	106.4±4.0
LV EDP, mmHg	10.5±3.5	12.3±2.3	11.2±2.1
+dP/dt, mmHg/s	5124±228	4367±315†	4527±204
-dP/dt, mmHg/s	-3781±366	-3543±312	-3431±311
ESPVR, mmHg/μl	0.324±0.102	0.121±0.010†	0.262±0.031‡
EDPVR, mmHg/μl	0.010±0.002	0.013±0.001	0.011±0.003
PRSW, mm Hg	111.1± 24.5	68.7±14.2†	89.3±10.1‡

Data are means±S.E.M.; MR, mitral regurgitation group; MR+Sil, sildenafil treated MR; HR, heart rate; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; LV ESP, LV end systolic

pressure; LV EDP, LV end diastolic pressure; ESPVR, end systolic pressure volume relationship; EDPVR, end diastolic pressure volume relationship; PRSW, preload recruitable stroke work.

†$p<0.05$ for difference from control, ‡ $p < 0.05$ for difference from MR

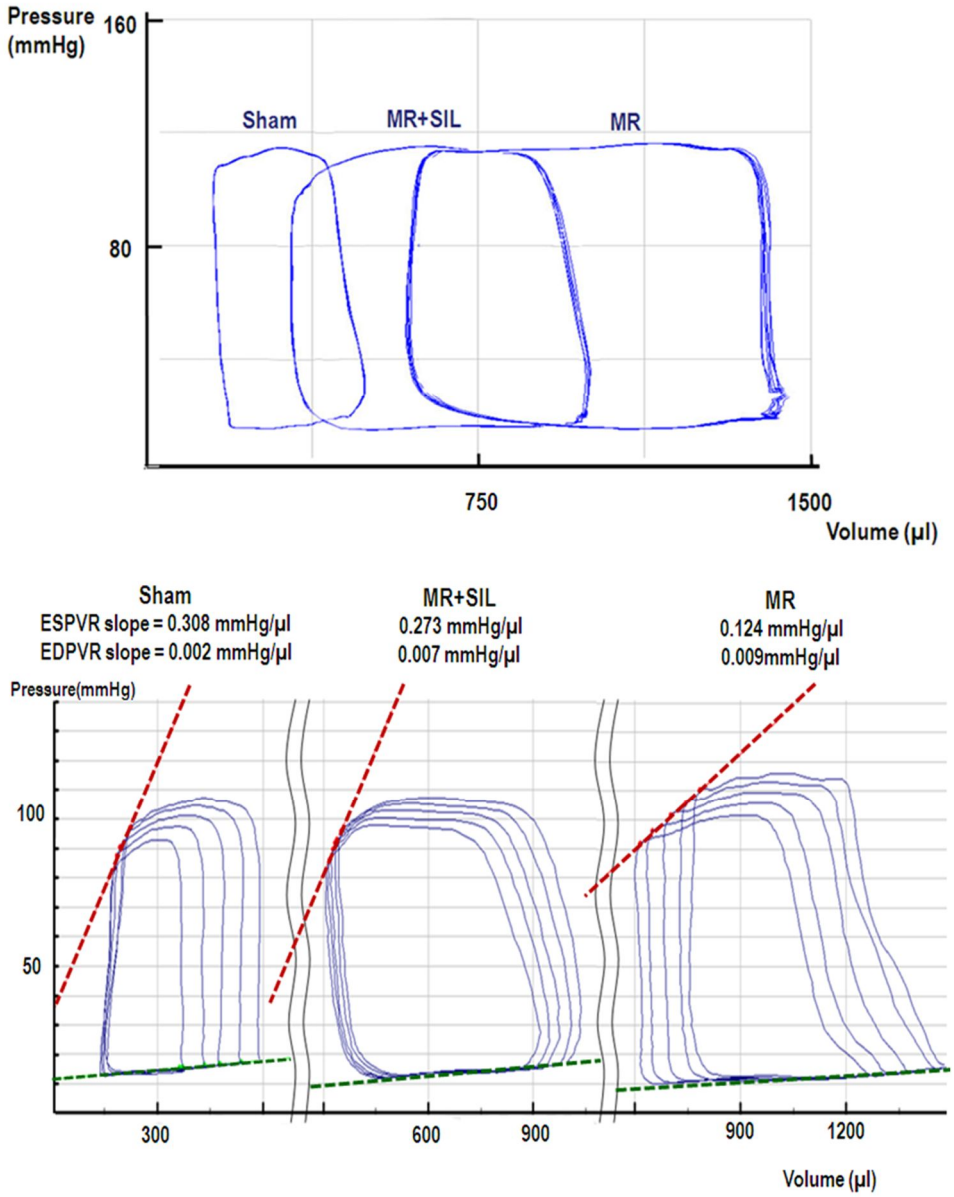


Figure 5 - Representative pressure-volume loops from the three groups. MR+SIL group showed smaller left ventricular volume and greater end-systolic pressure volume relation (ESPVR, dotted line) compared with MR group.

Microarray analysis

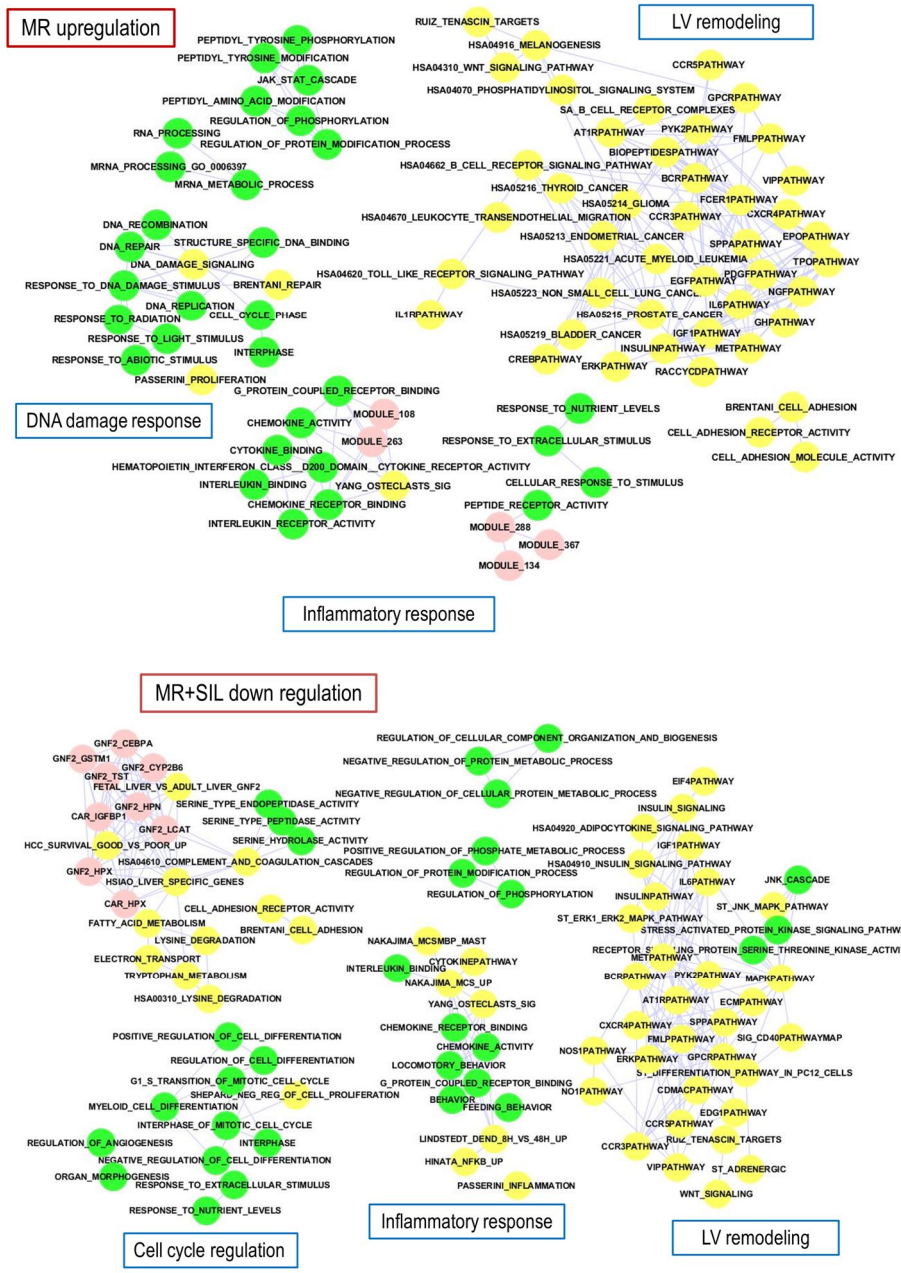
A total of 448 genes were differentially expressed by at least1.5-fold in MR rats ($P<0.05$), including 197 up-regulated and 251 down-regulated genes compared with sham or MR+SIL. The heat map in supplementary figure 3 demonstrates a consistent pattern of change of these genes in each condition. Supplementary table 1 lists genes well established in the pathophysiology of cardiovascular disease that were up-regulated>1.5-fold in MR and down-regulated in MR+SIL.

For biological interpretation, we performed Gene Set Enrichment Analysis (see methods section for details) to explore the transcriptional changes at the level of molecular pathways or Gene Ontologies. Figure 6 (A,B) is the network representation of gene sets that are enriched with genes up-regulated in MR and reciprocally down-regulated after treatment with sildenafil and presumed to be primarily associated with the pharmacological effect of sildenafil in MR. It is noticeable that gene sets form several tightly interconnected structures or clusters which we can annotate with inflammatory response, DNA damage response and cell cycle checkpoint, and cellular signaling pathways associated with LV remodeling.

To confirm the changes in transcription level, we selected 5 representative genes implicated in DNA damage response,

inflammatory response, endothelial function and vascular remodeling, CDKN2A, IL-6, IL-18, eNOS, and iNOS26, 27. RT-PCR demonstrated the validity of DNA microarray (Figure 6 C,D).

With the transcriptional profiling study, it is suggested that the chronic hemodynamic stress induced by mitral regurgitation might activate stress response pathways like inflammatory pathways, DNA damage response and cell cycle checkpoint pathways, leading to cell fate decisions like apoptosis and LV remodeling. With the attenuation of the hemodynamic stress by treatment with sildenafil, the activated stress response pathways are moderated, suggesting that the three clusters of pathways provide the basis for the molecular and cellular mechanism of the pathological changes in heart undergoing chronic volume and pressure overload from mitral regurgitation.


MR upregulation
PEPTIDYL_TYROSINE_PHOSPHORYLATION
PEPTIDYL_TYROSINE_MODIFICATION
JAK_STAT_CASCADE
PEPTIDYL_AMINO_ACID_MODIFICATION
REGULATION_OF_PHOSPHORYLATION
RNA_PROCESSING
REGULATION_OF_PROTEIN_MODIFICATION_PROCESS
MRNA_PROCESSING_GO_0006397
MRNA_METABOLIC_PROCESS
LV remodeling
RUIZ_TENASCIN_TARGETS
HSA04916_MELANOGENESIS
HSA04310_WNT_SIGNALING_PATHWAY
CCR5PATHWAY
HSA04070_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM
GPCRPATHWAY
SA_B_CELL_RECEPTOR_COMPLEXES
AT1RPATHWAY
PYK2PATHWAY
FMLPPATHWAY
BIOPEPTIDESPATHWAY
HSA04662_B_CELL_RECEPTOR_SIGNALING_PATHWAY
BCRPATHWAY
VIPPATHWAY
HSA05216_THYROID_CANCER
HSA05214_GLIOMA
FCER1PATHWAY
HSA04670_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
CCR3PATHWAY
CXCR4PATHWAY
HSA05213_ENDOMETRIAL_CANCER
EPOPATHWAY
SPPAPATHWAY
HSA05221_ACUTE_MYELOID_LEUKEMIA
TPOPATHWAY
EGFPATHWAY
PDGFPATHWAY
HSA04620_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY
HSA05223_NON_SMALL_CELL_LUNG_CANCL
NGFPATHWAY
IL6PATHWAY
HSA05215_PROSTATE_CANCER
GHPATHWAY
IL1RPATHWAY
IGF1PATHWAY
HSA05219_BLADDER_CANCER
INSULINPATHWAY
METPATHWAY
CREBPATHWAY
ERKPATHWAY
RACCYCDPATHWAY
DNA_RECOMBINATION
DNA_REPAIR
STRUCTURE_SPECIFIC_DNA_BINDING
DNA_DAMAGE_SIGNALING
RESPONSE_TO_DNA_DAMAGE_STIMULUS
BRENTANI_REPAIR
DNA_REPLICATION
RESPONSE_TO_RADIATION
CELL_CYCLE_PHASE
RESPONSE_TO_LIGHT_STIMULUS
INTERPHASE
RESPONSE_TO_ABIOTIC_STIMULUS
PASSERINI_PROLIFERATION
DNA damage response
G_PROTEIN_COUPLED_RECEPTOR_BINDING
CHEMOKINE_ACTIVITY
MODULE_108
MODULE_263
CYTOKINE_BINDING
HEMATOPOIETIN_INTERFERON_CLASS__D200_DOMAIN__CYTOKINE_RECEPTOR_ACTIVITY
INTERLEUKIN_BINDING
YANG_OSTECLASTS_SIG
CHEMOKINE_RECEPTOR_BINDING
INTERLEUKIN_RECEPTOR_ACTIVITY
RESPONSE_TO_NUTRIENT_LEVELS
RESPONSE_TO_EXTRACELLULAR_STIMULUS
CELLULAR_RESPONSE_TO_STIMULUS
PEPTIDE_RECEPTOR_ACTIVITY
MODULE_288
MODULE_367
MODULE_134
BRENTANI_CELL_ADHESION
CELL_ADHESION_RECEPTOR_ACTIVITY
CELL_ADHESION_MOLECULE_ACTIVITY
Inflammatory response
MR+SIL down regulation
GNF2_CEBPA
GNF2_GSTM1
GNF2_CYP2B6
GNF2_TST
FETAL_LIVER_VS_ADULT_LIVER_GNF2
GNF2_HPN
SERINE_TYPE_ENDOPEPTIDASE_ACTIVITY
CAR_IGFBP1
SERINE_TYPE_PEPTIDASE_ACTIVITY
GNF2_LCAT
HCC_SURVIVAL_GOOD_VS_POOR_UP
SERINE_HYDROLASE_ACTIVITY
HSA04610_COMPLEMENT_AND_COAGULATION_CASCADES
GNF2_HPX
HSIAO_LIVER_SPECIFIC_GENES
CAR_HPX
CELL_ADHESION_RECEPTOR_ACTIVITY
FATTY_ACID_METABOLISM
BRENTANI_CELL_ADHESION
LYSINE_DEGRADATION
ELECTRON_TRANSPORT
TRYPTOPHAN_METABOLISM
HSA00310_LYSINE_DEGRADATION
POSITIVE_REGULATION_OF_CELL_DIFFERENTIATION
REGULATION_OF_CELL_DIFFERENTIATION
G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE
SHEPARD_NEG_REG_OF_CELL_PROLIFERATION
MYELOID_CELL_DIFFERENTIATION
INTERPHASE_OF_MITOTIC_CELL_CYCLE
REGULATION_OF_ANGIOGENESIS
INTERPHASE
NEGATIVE_REGULATION_OF_CELL_DIFFERENTIATION
ORGAN_MORPHOGENESIS
RESPONSE_TO_EXTRACELLULAR_STIMULUS
RESPONSE_TO_NUTRIENT_LEVELS
Cell cycle regulation
REGULATION_OF_CELLULAR_COMPONENT_ORGANIZATION_AND_BIOGENESIS
NEGATIVE_REGULATION_OF_PROTEIN_METABOLIC_PROCESS
NEGATIVE_REGULATION_OF_CELLULAR_PROTEIN_METABOLIC_PROCESS
POSITIVE_REGULATION_OF_PHOSPHATE_METABOLIC_PROCESS
REGULATION_OF_PROTEIN_MODIFICATION_PROCESS
REGULATION_OF_PHOSPHORYLATION
NAKAJIMA_MCSMBP_MAST
CYTOKINEPATHWAY
INTERLEUKIN_BINDING
NAKAJIMA_MCS_UP
YANG_OSTECLASTS_SIG
CHEMOKINE_RECEPTOR_BINDING
CHEMOKINE_ACTIVITY
LOCOMOTORY_BEHAVIOR
G_PROTEIN_COUPLED_RECEPTOR_BINDING
BEHAVIOR
FEEDING_BEHAVIOR
LINDSTEDT_DEND_8H_VS_48H_UP
HINATA_NFKB_UP
PASSERINI_INFLAMMATION
Inflammatory response
EIF4PATHWAY
INSULIN_SIGNALING
HSA04920_ADIPOCYTOKINE_SIGNALING_PATHWAY
IGF1PATHWAY
HSA04910_INSULIN_SIGNALING_PATHWAY
IL6PATHWAY
JNK_CASCADE
INSULINPATHWAY
ST_JNK_MAPK_PATHWAY
ST_ERK1_ERK2_MAPK_PATHWAY
STRESS_ACTIVATED_PROTEIN_KINASE_SIGNALING_PATHWAY
METPATHWAY
BCRPATHWAY
PYK2PATHWAY
MAPKPATHWAY
AT1RPATHWAY
ECMPATHWAY
CXCR4PATHWAY
SPPAPATHWAY
FMLPPATHWAY
SIG_CD40PATHWAYMAP
NOS1PATHWAY
ERKPATHWAY
GPCRPATHWAY
CDMACPATHWAY
EDG1PATHWAY
CCR5PATHWAY
CCR3PATHWAY
RUIZ_TENASCIN_TARGETS
VIPPATHWAY
ST_ADRENERGIC
WNT_SIGNALING
LV remodeling

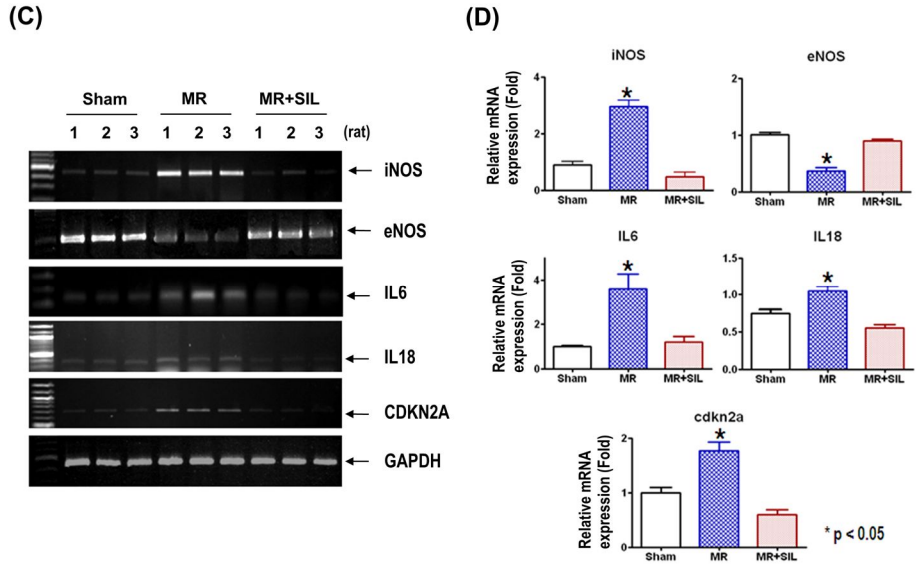
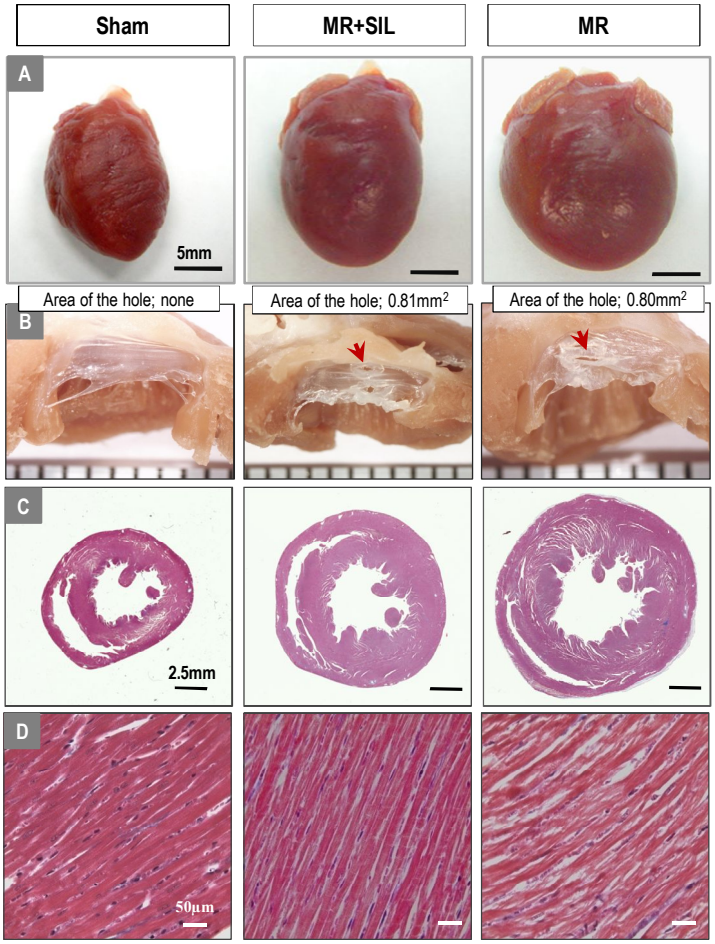


Figure 6 - (A and B) A network of gene sets with significant Enrichment Scores (P<0.05), made up of genes that are up-regulated in MR rat model. Nodes represent gene sets and edges connect two nodes if the two gene sets share a significant number of genes in common by hypergeometric test.(see methods) Isolated nodes are omitted. Yellow nodes are Gene Ontology gene sets (C5) and green nodes are curated gene sets from public pathway databases (C2) and pink nodes represent computational gene sets (C4) B: a network of gene sets with significant Enrichment Scores (P<0.05), made up of genes that are down-regulated after treatment with sildenafil in MR rat model. (C) mRNA regulation in MR with sildenafil group. RT-PCR of iNOS, eNOS, IL-6, IL-18 and CDKN2A (n=3 each). mRNA of factors for vascular remodeling, inflammation and DNA damage were increased in mitral regurgitation (MR) group but down-regulated in MR+sildenafil (MR+SIL) group. However, eNOS was down-regulated in MR group but increased in

MR+sildenafil group. (D) Quantitative graph (n=3). *p<0.05 for difference from MR+SIL group.

Histopathological analysis with cardiac fibrosis and apoptosis

Gross pathological examination showed that the heart was significantly enlarged with eccentric hypertrophy in MR group at the 16^{th} week (Fig 7A). However the hypertrophy was reduced in sildenafil treated MR rats. We found no difference in interstitial fibrosis among the groups. However, the extent of perivascular fibrosis was significantly larger in MR group compared to MR+SIL group (Figure 7C,D,E,F). TUNEL staining showed a significant increase of cardiac apoptosis in MR group (0.24±0.3%, p <0.05, figure 8). In contrast, a significant inhibition of apoptosis was evident in MR+SIL (0.05±0.02%), which was comparable to sham group (0.03±0.04%).


Sham
MR+SIL
MR
A
5mm
Area of the hole; none
Area of the hole; 0.81mm2
Area of the hole; 0.80mm2
B
C
2.5mm
D
50μm

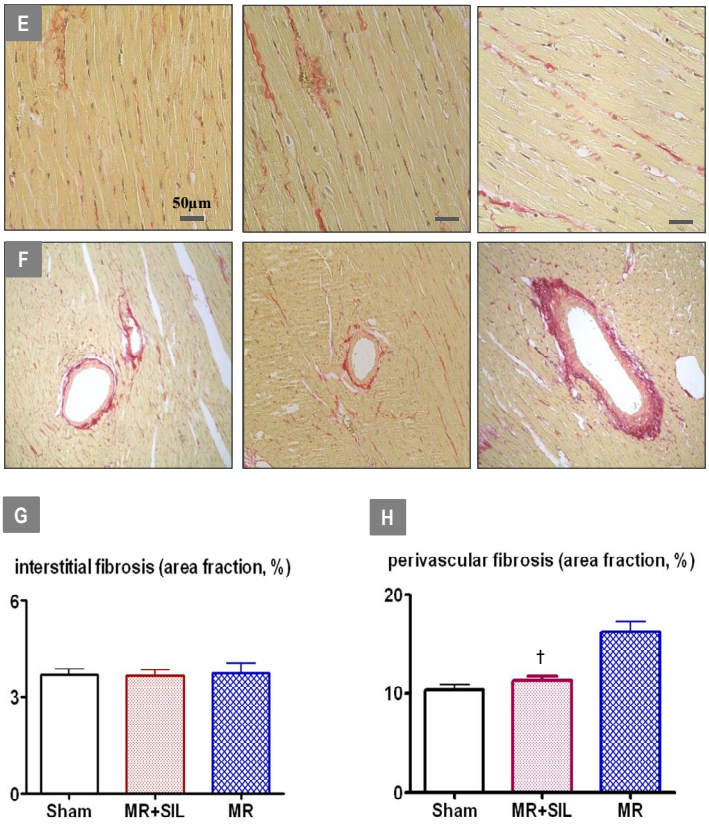


Figure 7 - Comparison of the pathologic results (A) Eccentric hypertrophy was developed in MR group. (B) The hole created in the anterior leaflet of mitral valve. (C,D,E,G) Masson trichrome and picrosirius red staining showed no difference of interstitial fibrosis (F,H) The extent of perivascular fibrosis was significantly greater in MR group compared to MR+SIL group († $p < 0.05$ for difference from MR group)

DISCUSSION

The purpose of this study was to evaluate the effect of chronic administration of sildenafil on cardiac remodeling, function, and exercise capacity in rats with chronic MR. The major finding of the present study was that sildenafil prevented LV remodeling and exercise intolerance caused by chronic experimental MR. To our knowledge this is the first study that shows sildenafil is efficacious in the treatment of MR-induced LV remodeling and cardiac dysfunction. Additionally we proposed the potential mechanisms related with the effect of sildenafil; inhibition of inflammation and apoptosis.

Medical management in chronic MR

MR induces chronic LV volume overload and leads LV contractile dysfunction, heart failure and, finally, death. Although surgical correction of MR, the only definitive way to cure, carries reasonably low mortality and morbidity, medical therapeutics would have a role in many clinical situations such as in patient population with greater surgical risk. It becomes more relevant as the main etiology of MR has been changed from rheumatic to degenerative

valve disease [25] and the prevalence of MR increases with age because operative mortality for elderly patients is still high[26]. However, there is

currently no recommended pharmacological therapy for chronic MR. Despite previous efforts, medical therapies for chronic MR have produced disappointing and conflicting results.

Small animal model of chronic MR

Little is known about molecular and cellular mechanisms of LV remodeling induced by volume overload partly due to the absence of small animal model. In their pioneering work, Pu et al. developed an MR rat model for the first time[14]. We successfully established a small animal model of chronic MR and verify the pathophysiologic features of this model. We described the detailed time course of LV remodeling and the relationship between progressive LV dilatation and LV dysfunction. Immediately after MR creation, we observed slight increase of LV diastolic dimension without change of systolic dimension and thus hyperdynamic LV. In this stage, LV wall thinning occurred as LV began to dilate. Chronic compensated stage could be defined from the 3rd week to around the 16th week. In this stage, LV diastolic and systolic volume increased progressively. Though remained in the normal range, LVEF became significantly lower in MR group from the 12th week. At the 16th week, load-independent contractile indices were significantly lower in MR group whereas diastolic parameters were not different. These results are consonant with several

previous studies that demonstrated that MR is one of the few cardiac diseases in which diastolic function is supernormal[27]. Chronic adaptation to volume overload tends to decrease LV chamber stiffness and increase diastolic filling rates and dimension lengthening irrespective of systolic function in chronic MR[28]. Additionally, we did not find any increase of the LV interstitial fibrosis in this stage of MR. Interstitial fibrosis is closely related with LV diastolic function.

Mechanism for LV remodeling in MR

MR causes progressive LV dilation, wall thinning, and cardiomyocyte elongation, which recapitulates eccentric remodeling with inflammatory cell infiltration and extracelluar matrix degradation. It was suggested that inflammation and energy metabolism initiate cardiac remodeling[29]. Nemoto et al. showed that administration of the PPAR-γ agonist rosiglitazone ameliorates MR-induced LV dysfunction accompanied by a decline in lipid content[30]. Similarly in volume overload model of aortocaval fistula, there was upregulation of genes related to inflammation, the extracellular matrix, the cell cycle, and apoptosis[31]. There was a 40-fold increase in the matricellular protein periostin, which inhibits connections between collagen and cells, thereby potentially mediating a side-to-side slippage of cardiomyocytes and LV dilatation. These findings are consistent with our results that

inflammation, the cell cycle and DNA damage pathways were upregulated in MR that were prevented by sildenafil treatment.

Increased matrix metalloproteinase activity is associated with progressive LV dilatation and extracelluar matrix degradation, contractile dysfunction, and neurohormonal activation in volume overload animal models. Zheng J. et al. recently reported that eccentric LV remodeling in isolated MR was associated with increased matrix metalloproteinase activity using a gene array analysis[32]. However, we could not find a significant difference in matrix metalloproteinase families among the groups. This may imply that the relative contributions of each of these mechanisms change at the different stage of the remodeling. Extracellular matrix loss, inflammation or bioenergetics dysfunction has been implicated at different time points in the pathophysiology of volume overload[33].

Sildenafil for treatment of MR

Emerging data indicate that PDE5 is present within cardiac myocytes and may play a role in modulating cGMP activity in various cardiac diseases[34]. Several observations from preclinical and short-term clinical studies support a role of sildenafil in the treatment of heart failure. In the present study, anti-inflammatory and anti-apoptotic effect of sildenafil seemed to play a key role to prevent LV remodeling.

The endocardial perivascular area may represent an early fibrotic change in MR model, probably due to relative endocardial ischemia due to the prolonged elevation of LV diastolic filling pressures. However the extent of perivascular fibrosis was significantly smaller in MR +SIL group compared to MR group. In recent years, there has been considerable interest in studying the effect of sildenafil on endothelial cell protection that may trigger a signaling cascade (through the action of kinases including protein kinase C and other mitogen-activated protein kinases) and generation of nitric oxide (NO) by phosphorylation of endothelial NO synthase (eNOS). An important property of sildenafil is its ability to increase eNOS and inducible NOS proteins in the heart and this has a direct cause and effect relationship in protection against myocardial infarction, as well as apoptosis in cardiomyocytes[35, 36]. Because of the ability of sildenafil to augment NOS and cGMP levels, it is logical to hypothesize that cardiovascular dysfunction induced by chronic NOS inhibition would be alleviated by concomitant treatment with sildenafil.

Clinical and experimental data support a link between endothelial dysfunction and inflammation. Proinflammatory cytokines are increased in the myocardium as a response to stretch in MR patients and correlated with the extent of LV dilatation[37]. Inflammatory response and cytokine elaboration are integral components of the host

response to tissue injury and play an active role for myocardial remodeling and thus inflammatory mediators play a crucial role in the development of heart failure. Accordingly, several strategies to counterbalance the inflammatory response have been suggested. Possible targets involve pro- and anti-inflammatory cytokines and their receptors, adhesion molecules, NO and NOS, reactive oxygen species, and different types of leucocytes. In this study, we demonstrated that inflammatory response was decreased in the sildenafil treated group compared with MR rats by microarray analysis.

"Cell loss" is another unifying feature found in nearly all forms of cardiomyopathies. Currently, the best understood form of cell loss or cell death in heart failure is apoptosis. DNA fragmentation and cellular and nuclear shrinkage are one of the biochemical and morphological changes that classically characterize apoptosis. Barouch et al. demonstrated cardiomyocyte apoptosis is associated with increased DNA damage and decreased survival in murine model of obesity[38]. In this study, we showed that DNA damage pathway was upregulated in MR rats compared with sham and MR+SIL rats and apoptotic cells were more prevalent in MR rats.

Nagayama et al. demonstrated that sildenafil treatment prevents further cardiac and myocyte dysfunction and progressive remodeling in animal model of established hypertrophy caused by pressure-overload.

In the present study, we found similar beneficial effect of sildenafil in animal model of volume-overload. The therapeutic effect of sildenafil has also been demonstrated in other animal models including ischemia-reperfusion model and anthracycline cardiomyopathy. These findings suggest that there is substantial overlap of molecular pathways in the development of different types of cardiac remodeling induced by various stimuli. In addition, they also suggest the multiple effects of sildenafil and cGMP.

Limitation of the study

MR was created by making a hole on the mitral leaflet and this MR model may not represent MR in humans, especially ischemic MR. This is an inherent limitation of animal model of valve disease. However, our model is still useful to evaluate the effect of medical treatment on LV remodeling induced by MR. In this regard, the beneficial effect of sildenafil on volume overload-induced LV remodeling might have clinical implication. Further research is needed to delineate the exact mechanisms involved to allow for translation into the clinical setting.

Conclusion

Sildenafil attenuates LV remodeling and prevents exercise intolerance in a rat model of chronic MR. This benefit might be associated with

anti-apoptotic, anti-inflammatory effects of sildenafil. Further research is needed to delineate the exact mechanisms involved and demonstrate the beneficial effects in patients with chronic MR.

REFERENCES

1. Nkomo V, Gardin J, Skelton T, Gottdiener J, Scott C, Enriquez-Sarano M. Burden of valvular heart diseases: A population-based study. *The Lancet*. 2006;368:1005-1011
2. Iung B, Baron G, Butchart E, Delahaye F, Gohlke-Barwolf C, Levang O, Tornos P, Vanoverschelde J, Vermeer F, Boersma E. A prospective survey of patients with valvular heart disease in europe: The euro heart survey on valvular heart disease. *European heart journal*. 2003;24:1231
3. Yoran C, Yellin E, Becker R, Gabbay S, Frater R, Sonnenblick E. Mechanism of reduction of mitral regurgitation with vasodilator therapy. *The American Journal of Cardiology*. 1979;43:773-777
4. Otto C. Evaluation and management of chronic mitral regurgitation. *The New England Journal of Medicine*. 2001;345:740
5. Perry G, Wei C, Hankes G, Dillon S, Rynders P, Mukherjee R, Spinale F, Dell'Italia L. Angiotensin ii receptor blockade does not improve left ventricular function andremodeling in subacute mitral regurgitation in the dog. *Journal of the American College of Cardiology*. 2002;39:1374
6. Takimoto E, Champion H, Li M, Belardi D, Ren S, Rodriguez E, Bedja D, Gabrielson K, Wang Y, Kass D. Chronic inhibition of cyclic gmp phosphodiesterase 5a prevents and reverses cardiac hypertrophy. *Nature medicine*. 2005;11:214-222
7. Nagayama T, Hsu S, Zhang M, Koitabashi N, Bedja D, Gabrielson K, Takimoto E, Kass D. Sildenafil stops progressive chamber, cellular, and molecular remodeling and improves calcium handling and function in hearts with pre-existing advanced hypertrophy caused by pressure overload. *Journal of the American College of Cardiology*. 2009;53:207
8. Nagayama T, Hsu S, Zhang M, Koitabashi N, Bedja D, Gabrielson K,

Takimoto E, Kass D. Pressure-overload magnitude-dependence of the anti-hypertrophic efficacy of pde5a inhibition. *Journal of molecular and cellular cardiology*. 2009;46:560-567

9. Fisher P, Salloum F, Das A, Hyder H, Kukreja R. Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity. *Circulation*. 2005;111:1601
10. Salloum F, Abbate A, Das A, Houser J, Mudrick C, Qureshi I, Hoke N, Roy S, Brown W, Prabhakar S. Sildenafil (viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice. *American Journal of Physiology- Heart and Circulatory Physiology*. 2008;294:H1398
11. Lewis G, Lachmann J, Camuso J, Lepore J, Shin J, Martinovic M, Systrom D, Bloch K, Semigran M. Sildenafil improves exercise hemodynamics and oxygen uptake in patients with systolic heart failure. *Circulation*. 2007;115:59
12. Guazzi M, Samaja M, Arena R, Vicenzi M, Guazzi M. Long-term use of sildenafil in the therapeutic management of heart failure. *Journal of the American College of Cardiology*. 2007;50:2136-2144
13. Kim K-H, Kim Y-J, Lee S-P, Kim H-K, Seo J-W, Sohn D-W, Oh B-H, Park Y-B. Survival, exercise capacity, and left ventricular remodeling in a rat model of chronic mitral regurgitation: Serial echocardiography and pressure-volume analysis. *Korean Circ J*. 2011;41:603-611
14. Pu M, Gao Z, Zhang X, Liao D, Pu DK, Brennan T, Davidson WR. Impact of mitral regurgitation on left ventricular anatomic and molecular remodeling and systolic function: Implication for outcome. *American Journal of Physiology-Heart and Circulatory Physiology*. 2009;296:H1727
15. Clozel M, Hess P, Rey M, Iglarz M, Binkert C, Qiu C. Bosentan, sildenafil, and their combination in the monocrotaline model of pulmonary hypertension in rats. *Experimental biology and medicine*. 2006;231:967-973
16. Hassan M, Ketat A. Sildenafil citrate increases myocardial cgmp

content in rat heart, decreases its hypertrophic response to isoproterenol and decreases myocardial leak of creatine kinase and troponin t. *BMC pharmacology*. 2005;5:10

17. Andersen A, Nielsen JM, Peters CD, Schou UK, Sloth E, Nielsen-Kudsk JE. Effects of phosphodiesterase-5 inhibition by sildenafil in the pressure overloaded right heart. *European journal of heart failure*. 2008;10:1158
18. Reffelmann T, Kloner RA. Transthoracic echocardiography in rats evalution of commonly used indices of left ventricular dimensions, contractile performance, and hypertrophy in a genetic model of hypertrophic heart failure (shhf-mcc-fa cp-rats) in comparison with wistar rats during aging. *Basic research in cardiology*. 2003;98:275-284
19. Helmcke F, Nanda N, Hsiung M, Soto B, Adey C, Goyal R, Gatewood Jr R. Color doppler assessment of mitral regurgitation with orthogonal planes. *Circulation*. 1987;75:175
20. Lips DJ, vd Nagel T, Steendijk P, Palmen M, Janssen BJ, v. Dantzig JM, de Windt LJ, Doevendans PA. Left ventricular pressure–volume measurements in mice: Comparison of closed–chest versus open–chest approach. *Basic research in cardiology*. 2004;99:351-359
21. Pacher P, Nagayama T, Mukhopadhyay P, Bátkai S, Kass DA. Measurement of cardiac function using pressure–volume conductance catheter technique in mice and rats. *Nature protocols*. 2008;3:1422-1434
22. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:15545
23. Rivals I, Personnaz L, Taing L, Potier MC. Enrichment or depletion of a go category within a class of genes: Which test? *Bioinformatics*. 2007;23:401-407
24. Loo DT. In situ detection of apoptosis by the tunel assay: An

overview of techniques. *Methods Mol Biol*. 2011;682

25. Ahmed MI, McGiffin DC, O'Rourke RA, Dell'Italia LJ. Mitral regurgitation. *Current Problems in Cardiology*. 2009;34:93-136
26. DiGregorio V, Zehr KJ, Orszulak TA, Mullany CJ, Daly RC, Dearani JA, Schaff HV. Results of mitral surgery in octogenarians with isolated nonrheumatic mitral regurgitation. *The Annals of thoracic surgery*. 2004;78:807-813
27. Zile MR, Tomita M, Nakano K, Mirsky I, Usher B, Lindroth J, Carabello B. Effects of left ventricular volume overload produced by mitral regurgitation on diastolic function. *American Journal of Physiology-Heart and Circulatory Physiology*. 1991;261:H1471-H1480
28. Corin WJ, Murakami T, Monrad ES, Hess OM, Krayenbuehl HP. Left ventricular passive diastolic properties in chronic mitral regurgitation. *Circulation*. 1991;83:797-807
29. Kai H, Kuwahara F, Tokuda K, Imaizumi T. Diastolic dysfunction in hypertensive hearts: Roles of perivascular inflammation and reactive myocardial fibrosis. *Hypertension research*. 2005;28:483-490
30. Nemoto S, Razeghi P, Ishiyama M, De Freitas G, Taegtmeyer H, Carabello BA. Ppar-γ agonist rosiglitazone ameliorates ventricular dysfunction in experimental chronic mitral regurgitation. *American Journal of Physiology-Heart and Circulatory Physiology*. 2005;288:H77
31. Chen Y, Pat B, Gladden JD, Zheng J, Powell P, Wei CC, Cui X, Husain A, Dell'Italia LJ. Dynamic molecular and histopathological changes in the extracellular matrix and inflammation in the transition to heart failure in isolated volume overload. *American Journal of Physiology-Heart and Circulatory Physiology*. 2011;300:H2251
32. Zheng J, Chen Y, Pat B, Dell'Italia LA, Tillson M, Dillon A, Powell PC, Shi K, Shah N, Denney T. Microarray identifies extensive downregulation of noncollagen extracellular matrix and profibrotic growth factor genes in chronic isolated mitral regurgitation in the dog. *Circulation*. 2009;119:2086-2095
33. Hutchinson KR, Stewart JA, Lucchesi PA. Extracellular matrix

remodeling during the progression of volume overload-induced heart failure. *Journal of molecular and cellular cardiology*. 2010;48:564-569

34. SENZAKI H, SMITH CJ, JUANG GJ, ISODA T, MAYER SP, OHLER A, PAOLOCCI N, TOMASELLI GF, HARE JM, KASS DA. Cardiac phosphodiesterase 5 (cgmp-specific) modulates ß-adrenergic signaling in vivo and is down-regulated in heart failure. *The FASEB Journal*. 2001;15:1718-1726
35. Das A, Xi L, Kukreja RC. Phosphodiesterase-5 inhibitor sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis. *Journal of Biological Chemistry*. 2005;280:12944-12955
36. Sam F, Sawyer DB, Xie Z, Chang DLF, Ngoy S, Brenner DA, Siwik DA, Singh K, Apstein CS, Colucci WS. Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction. *Circulation research*. 2001;89:351
37. Oral H, Sivasubramanian N, Dyke DB, Mehta RH, Grossman PM, Briesmiester K, Fay WP, Pagani FD, Bolling SF, Mann DL. Myocardial proinflammatory cytokine expression and left ventricular remodeling in patients with chronic mitral regurgitation. *Circulation*. 2003;107:831-837
38. Barouch LA, Gao D, Chen L, Miller KL, Xu W, Phan AC, Kittleson MM, Minhas KM, Berkowitz DE, Wei C. Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. *Circulation research*. 2006;98:119-124

국문 초록

서론: 최근의 연구들에서는 실데나필이 심부전에서의 효과를 입증하는 결과들이 발표되고 있다. 이에 본 저자는 실데나필이 만성 승모판 폐쇄부전 설치류에서 좌심실의 재형성을 억제하고 운동능력을 향상시킬 것이라는 가정하에 약물을 투여하는 실험을 진행하였다.

방법: 승모판 폐쇄부전은 설치류의 승모판 판막에 구멍을 만들어 형성하였다. 2 주가 지난후 심초음파를 이용하여 좌심실이 커져 있음을 확인하고 실데나필 그룹과 식염수를 투여한 그룹으로 각군당 16 마리씩 임의로 나누어 4 주간 약물을 투여하였다.

결과: 승모판 폐쇄부전 설치류 (MR)에서 16 마리중 3 마리가 사망하였으나 실데나필을 투여한 그룹에서는 사망한 쥐들이 없었다. 4 개월이 지난후 좌심실의 크기는 실데나필 그룹(MR+SIL)에서 줄어듬이 확인되었고 이에 따라 좌심실의 구혈률은 증가하였다. (좌심실 수축기 크기 4.7±0.3 sham vs 5.9±0.3 MR+SIL vs 7.4 ± 0.5 mm MR $p<0.05$; 좌심실 이완기 크기 8.3±0.4 vs 10.5±0.2 vs 11.7±0.61 mm, $p<0.05$. 좌심실 구혈률 70.2%±2.2 for sham vs 67.0±4.2 for MR+SIL vs 58.9±2.5 for MR, $p=0.01$). 압력-용적 곡선에서 실데나필 투여군에서 ESPVR 의 상승이 관찰되었다. 운동부하 검사를 시행하였을 때 승모판 폐쇄부전 쥐들의 운동 능력은 감소하였으나

실데나필 투여시 정상 쥐들과 동등한 정도로 유지되었다. 병리학적 소견상 혈관 주위의 섬유화가 실데나필 투여군에서 줄었으며 심근의 apoptosis 또한 감소하였다.

결론: 결론적으로 실데나필은 만성 승모판 폐쇄부전 설치류에서 좌심실의 재형성을 억제시키고 운동능력을 향상시킨다. 이것은 실데나필의 항 섬유화, 항 염증 작용에 기인하는 것으로 생각된다.

주요어 : 승모판 폐쇄부전, 실데나필, 좌심실 재형성, 운동 능력, 마이크로 어레이

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