—Original—

Preventive effect of sildenafil on right ventricular function in rats with monocrotaline-induced pulmonary arterial hypertension

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Abstract: The present study aimed to evaluate the preventive effect of sildenafil treatment on pulmonary hypertension (PH) induced by monocrotaline (MCT) in rats. Fifty-four 12-week-old male Sprague–Dawley rats were injected with MCT or saline solution (MCT-injected rats: n=36; saline: n=18). Serial echocardiography and right ventricular systolic pressure (RVSP) measurements via a cardiac catheter were performed at 2, 4 and 6 weeks after the injection. After injection of MCT, rats received oral sildenafil (MCT/sildenafil group: n=18) or no treatment (MCT group: n=18) until undergoing echocardiography and cardiac catheterization. RVSP in the MCT/sildenafil group was lower than that in the MCT group at 4 ($P<0.001$) and 6 weeks ($P<0.001$). The septal curvature was improved in the MCT/sildenafil group compared with the MCT group. This finding showed that sildenafil prevented flattening of the interventricular septum because of right ventricular pressure overload. The ratio of peak trans-tricuspid early diastolic wave velocity to active filling with atrial systolic velocity showed that sildenafil improved diastolic function. Tricuspid annular plane systolic excursion and tricuspid annular systolic velocity in the MCT/sildenafil group did not show preserved myocardial contraction after administration of sildenafil. Administration of sildenafil leads to a reduction in RVSP and improvement in cardiac function in rats with PH induced by MCT. The vasodilatory action of sildenafil improves right ventricular diastolic function, but the intrinsic, positive, inotropic effect of sildenafil is minimal.
Key words: monocrotaline, pulmonary hypertension, rat, sildenafil

Introduction

Pulmonary hypertension (PH) is a progressive disease, characterized by an increase in mean pulmonary arterial pressure to ≥ 25 mmHg at rest as assessed by right heart catheterization [11]. Although invasive hemodynamic assessment still remains important in evaluation of patients with PH, echocardiography is useful for noninvasive and easily repeatable assessment of right ventricular function in these patients [11].

Sildenafil citrate is a phosphodiesterase type V inhibitor. Sildenafil citrate causes vasodilation by increasing pulmonary vascular concentrations of cyclic guanosine monophosphate. A previous study reported decreased pulmonary arterial pressure, improvement in symptoms, and increased cardiac output with treatment of sildenafil for pulmonary arterial hypertension, and secondary pulmonary hypertension [10, 20, 21]. Despite updated guidelines and advances in treatment, the long-term prognosis for patients with pulmonary PH remains poor.

The previous study indicates that an early diagnosis of PH and early therapeutic intervention may result in an improvement in long-term outcomes [5, 7, 15, 16], and early phase treatment of sildenafil prolonged survival rate in the patients with PH [29]. Echocardiographic measurements of cardiac and hemodynamic parameters in the monocrotaline (MCT)-induced PH rat model have already been evaluated [3, 4, 6, 9, 17, 27]. Although the MCT-induced PH rat model has contributed to a better understanding of echocardiographic assessment of PH [3, 4, 9, 17, 27], the preventive effect of sildenafil on right ventricular function is not fully understood. Therefore, the present study aimed to evaluate the preventive effect of sildenafil on right ventricular function using echocardiography for the monitoring of PH. We investigated several echocardiographic parameters to investigate the cardiovascular effect of sildenafil on PH.

Materials and Methods

The present study was approved by Tokyo University of Agriculture and Technology (Approval number 24–53). Rats were managed and cared for in accordance with the standards established by Tokyo University of Agriculture and Technology, as described in its Guide for the Care and Use of Laboratory Animals.

Animals

A total of 54 male 12-week-old Sprague–Dawley rats were purchased from Charles River Laboratories (Kanagawa, Japan). They were kept in a constant-temperature room, with a daily constant 12/12-h light/dark ratio. Monocrotaline (MCT, Sigma-Aldrich, St. Louis, MO, USA), which was dissolved in saline, was randomly administered to 36 rats as a single subcutaneous injection (30 mg/kg) at a volume of 3 ml/kg, and an equal volume of saline was administered to another 18 rats (saline group).

MCT-induced PH rats (n=36) were randomly assigned to two groups: MCT group (n=18) and MCT/sildenafil group (n=18). The MCT-injected group (MCT group) was the placebo drug group. In the sildenafil-treated group (MCT/sildenafil group), sildenafil (10 mg/kg, Kamagra, Ajanta Pharma India Co., Ltd., Mumbai, India) was initiated immediately after MCT injection, for a maximum duration of 6 weeks. All of the rats in the MCT/sildenafil group were trained to eat margarine, which served as the vehicle for sildenafil. The entire sildenafil dosage was consumed.

Echocardiographic tests and hemodynamic examinations

In the present study, rats were evaluated at 2, 4, and 6 weeks after administration of either MCT (2 weeks, n=6; 4 weeks, n=6; 6 weeks, n=6; MCT group), vehicle (2 weeks, n=6; 4 weeks, n=6; 6 weeks, n=6; saline group), or MCT and sildenafil (2 weeks, n=6; 4 weeks, n=6; 6 weeks, n=6; MCT/sildenafil group). Echocardiographic tests and hemodynamic examinations were performed in all of the groups. Hemodynamic examinations were performed after the echocardiographic tests.

Echocardiography

After the rats were anesthetized (isoflurane 1.0% in 1:1 O_2 /air mix; DS Pharma Animal Health Co., Ltd., Osaka, Japan), their chests were shaved and transthoracic echocardiography was performed (Fig. 1). Two-dimensional echocardiography, pulsed Doppler, and tissue Doppler imaging were performed using the Pro-Sound SSD α-10 (Hitachi-Aloka Medical, Ltd., Tokyo, Japan) with a 10.0-MHz sector transducer. Sweep speed during the Doppler and M-mode recordings was set at 150–200 mm/s. An electrocardiogram was recorded by echocardiography measurement. All of the echocardiographic recordings were stored on an internal hard drive of the echocardiography unit and transmitted to the DICOM server online (Image ONE Co., Ltd., Tokyo, Japan). The following echocardiographic parameters were measured. The eccentricity index (EI), defined as the ratio of the length of two perpendicular minor-axis diameters, one of which bisects and is perpendicular to the interventricular septum, was obtained at end-systole [22]. Using the Doppler signals of tricuspid inflow, peak trans-tricuspid early diastolic wave (E wave) velocity and active filling with atrial systolic (A wave) velocity were measured, and E/A was calculated [31, 37]. The same tricuspid inflow tract view on the four-chamber view was used to evaluate lateral tricuspid annular velocity (Em) with tissue Doppler imaging, using the same sample volume. E/Em was also calculated. Tricuspid annular plane systolic excursion (TAPSE) was measured by M-mode. An M-mode cursor was oriented to the junction of the tricuspid valve plane and the right ventricular free wall using the apical four-chamber view to measure TAPSE [13]. Tricuspid annular systolic velocity (Sm) was acquired in apical four-chamber views at the right ventricular free wall using tissue Doppler imaging [30].
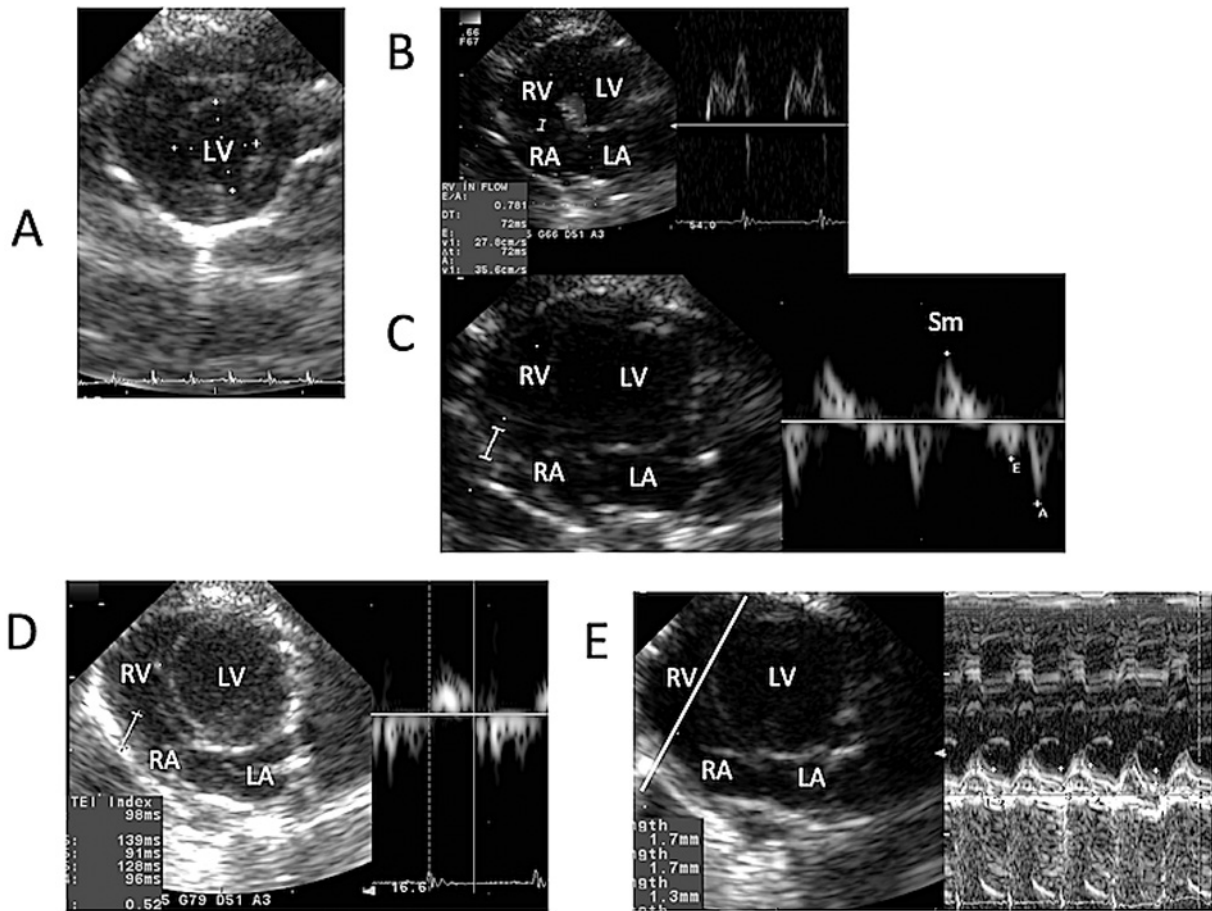
Fig. 1. A picture of echocardiography. A. the eccentricity index (EI), defined as the ratio of the length of two perpendicular minor-axis diameters, one of which bisects and is perpendicular to the interventricular septum, was obtained at end-systole. B. Using the Doppler signals of tricuspid inflow, peak trans-tricuspid early diastolic wave (E wave) velocity and active filling with atrial systolic (A wave) velocity were measured, and E/A was calculated. C. The tricuspid inflow tract view on the four-chamber view was used to evaluate lateral tricuspid annular velocity (Em) with tissue Doppler imaging. E/Em was calculated using E wave from the Doppler signals of tricuspid inflow. Tricuspid annular systolic velocity (Sm) was acquired in apical four-chamber views at the right ventricular free wall using tissue Doppler imaging. D. The Tei index is expressed by the following formula: (myocardial isovolumic contraction time + myocardial isovolumic relaxation time)/ ejection time. Ejection time was measured from the velocity time integral of Sm. The Tei index was measured in the right ventricular free wall using the apical four-chamber view. In the present study, the Tei index was determined by tissue Doppler imaging. E. Tricuspid annular plane systolic excursion (TAPSE) was measured by M-mode. An M-mode cursor was oriented to the junction of the tricuspid valve plane and the right ventricular free wall using the apical four-chamber view to measure TAPSE.

The Tei index is expressed by the following formula: (myocardial isovolumic contraction time + myocardial isovolumic relaxation time)/ ejection time. Ejection time was measured from the velocity time integral of Sm [1, 30]. The Tei index was measured in the right ventricular free wall using the apical four-chamber view. In the present study, the Tei index was determined by tissue Doppler imaging.

Hemodynamic examinations using a cardiac catheter

After echocardiographic tests, a pressure-sensing cath-eter (Codman MicroSensor; Codman & Shurtleff, Inc., Raynham, MA, USA) was inserted from the right jugu-lar vein into the right ventricle under anesthesia for measurement of right ventricular systolic pressure (RVSP).

Statistical analysis

The results are expressed as mean values ± SD. The statistical significance of differences in echocardio-graphic parameters, heart rate, and body weight were estimated using two-way analysis of variance for facto-

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Table 1. Summary of right ventricular systolic pressure and echocardiographic parameters

	2 week			4 week			6 week		
	saline	MCT[a]	MCT/sildenafil	saline	MCT	MCT/sildenafil	saline	MCT	MCT/sildenafil
RVSP[b] mmHg	30.7 ± 4.8	33.2 ± 2.5	31.8 ± 1.6	28.0 ± 4.7	60.2 ± 14.9***	28.7 ± 1.8 †††	29.5 ± 2.7	60.7 ± 16.5***	29.8 ± 6.6†††
HR[c] /min	357 ± 34	339 ± 48	300 ± 18***†††	338 ± 42	325 ± 34	344 ± 60	334 ± 41	341 ± 28	281 ± 27***†††
BW[d] g	309 ± 68	285 ± 77	253 ± 28	323 ± 33	329 ± 79	248 ± 8	358 ± 74	357 ± 97	248 ± 13**†
EI[e]	0.88 ± 0.09	0.95 ± 0.14	0.98 ± 0.15	0.88 ± 0.1	1.28 ± 0.19***	0.99 ± 0.10†††	0.91 ± 0.15	1.34 ± 0.43***	1.0 ± 0.09†††
E/A[f]	0.99 ± 0.31	1.06 ± 0.33	0.88 ± 0.27	0.91 ± 0.32	0.95 ± 0.34	0.73 ± 0.26†	0.76 ± 0.39	0.95 ± 0.28*	0.71 ± 0.14††
E/Em[g]	6.76 ± 3.63	11.86 ± 4.59***	7.75 ± 1.53†††	5.86 ± 1.53	8.09 ± 3.05	6.86 ± 2.25	6.53 ± 3.81	12.22 ± 7.73***	7.23 ± 1.52†††
TAPSE[h] mm	2.6 ± 0.7	2.4 ± 0.6	1.5 ± 0.4*** †††	2.0 ± 0.8	1.4 ± 0.5**	1.6 ± 0.3	2.7 ± 1.0	1.4 ± 0.4***	1.8 ± 0.5***†
Sm[i] cm/s	6.6 ± 1.2	5.4 ± 1.1**	6.0 ± 1.8	7.3 ± 2.2	6.0 ± 1.1**	6.5 ± 0.8	7.5 ± 1.6	5.0 ± 1.7***	6.0 ± 1.0**†
TDI[j] Tei index	0.65 ± 0.21	0.82 ± 0.24**	0.42 ± 0.05***†††	0.43 ± 0.19	0.64 ± 0.11***	0.43 ± 0.09†††	0.62 ± 0.28	0.78 ± 0.36**	0.56 ± 0.11†††

[a]MCT, Monocrotaline. [b]RVSP, right ventricular systolic pressure. [c]HR, heart rate. [d]BW, body weight. [e]EI, eccentricity index. [f]E/A, the ratio of peak velocity of the early tricuspid inflow wave (E) to peak velocity of the late diastolic wave. [g]E/Em, the ratio of trans-tricuspid early diastolic wave velocity to early diastolic myocardial velocity. [h]TAPSE, tricuspid annular plane systolic excursion. [i]Sm, systolic wave. [j]TDI, tissue Doppler imaging. The data represent means ± SD; *$P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. the saline group. †$P<.05$, ††$P<0.01$, †††$P<0.001$ vs. the MCT group.

rial measures with Bonferroni multiple comparisons for post hoc analysis. A significant difference was defined as $P<0.05$. Statistical analyses were performed using statistical software (Prism 6.0v; GraphPad Software, Inc., La Jolla, CA, USA).

Results

Hemodynamic effects

Hemodynamic effects are shown in Table 1. RVSP in the MCT group was higher than that in the saline group and the MCT/sildenafil group at 4 ($P<0.001$) and 6 weeks ($P<0.001$). The results of RVSP in the MCT/sildenafil group showed that continuous administration of sildenafil prevented elevated RVSP, with progressive PH 4 weeks later.

Heart rate results

Heart rate results are shown in Table 1. Heart rate in the MCT/sildenafil group was lower than that in the saline group and the MCT group at 2 ($P<0.001$) and 6 weeks ($P<0.001$).

Effects of sildenafil therapy on right ventricular function as evaluated by echocardiography

The serial change in the EI was similar to the time course of RVSP. The EI in the MCT group was higher than that in the saline group ($P<0.001$) and the MCT/sildenafil group ($P<0.001$) at 4 and 6 weeks ($P<0.001$). The EI results showed that sildenafil prevented flattening of the interventricular septum because of right ventricular pressure overload after elevated RVSP (Table 1). E/A in the MCT group was higher than that in the saline group ($P<0.05$) and the MCT/sildenafil group

($P<0.01$) at 6 weeks. This finding indicated that administration of sildenafil improved diastolic function in the MCT/sildenafil group in the progressive stage of PH after elevated RVSP. E/Em in the MCT group showed deterioration of diastolic function with PH. E/Em in the MCT/sildenafil group showed improvement of diastolic function with administration of sildenafil compared with the MCT group at 2 and 6 weeks ($P<0.001$). Sildenafil treatment significantly improved right ventricular diastolic function according to E/A and E/Em.

The sequence of TAPSE measurements showed a similar change to the serial measurements of Sm. TAPSE and Sm, representing systolic function in the MCT and MCT/sildenafil groups, were significantly lower than those in the saline group at the following time points. TAPSE was significantly lower in the MCT and MCT/sildenafil groups at 2 and 6 weeks ($P<0.001$), and it was significantly lower in the MCT group at 4 weeks compared with the saline group ($P<0.01$). Sm was significantly lower in the MCT group at 2, 4, and 6 weeks compared with the saline group ($P<0.01$, $P<0.01$, $P<0.001$, respectively). Sm was significantly lower in the MCT/sildenafil group at 6 weeks compared with the saline group ($P<0.01$). The results of TAPSE and Sm in the MCT group showed that right ventricular systolic function had deteriorated before elevated RVSP (Table 1). Although TAPSE and Sm in the MCT/sildenafil group showed improvement of systolic function compared with the MCT group at 6 weeks ($P<0.05$), these parameters of systolic function in the MCT/sildenafil group were lower than those in the saline group (TAPSE at 2 weeks, $P<0.001$; TAPSE at 6 weeks, $P<0.001$; Sm at 6 weeks, $P<0.01$). These findings indicated that myocardial contraction in the MCT/sildenafil group was not improved

during the examination period. Sildenafil treatment did not improve right ventricular contractility according to TAPSE and Sm.

Throughout the examination period, the Tei index in the MCT group showed deterioration of right ventricular myocardial function with PH (2 weeks, $P<0.01$; 4 weeks, $P<0.001$; 6 weeks, $P<0.01$ vs. the saline group). The Tei index in the MCT/sildenafil group showed that sildenafil treatment improved right ventricular myocardial function (2, 4, and 6 weeks, $P<0.001$ vs. the MCT group). The Tei index was an early indicator of right ventricular myocardial dysfunction with PH and improvement of right ventricular myocardial function after administration of sildenafil compared with the EI and E/A (Table 1).

Discussion

The present study aimed to investigate the preventive effect of sildenafil on right ventricular function. Our study showed that echocardiography was useful for assessment of right ventricular function for monitoring PH and the effect of sildenafil as a therapeutic agent. The Tei index indicated improvement of cardiac function in the MCT/sildenafil group. Additionally, an improvement in diastolic function according to E/A and E/Em was observed in the MCT/sildenafil group, and the EI was decreased in accordance with the reduction in RVSP.

Echocardiography plays an important role in the diagnosis of PH, which indicates increased right ventricular pressure [22, 30], systolic function [19, 35], and diastolic function [32]. Echocardiography might be useful in assessment of the therapeutic effect of sildenafil on right ventricular function in PH. However, the serial changes in parameters of echocardiography after administration of sildenafil are not fully understood. Therefore, we evaluated serial changes in RVSP combined with serial changes in echocardiographic parameters. Use of MCT to induce PH in rats is a well-established model, and use of echocardiography for monitoring PH has been evaluated in this model [6, 23].

In the present study, E/A was improved in the MCT/sildenafil group in the progressive stage of PH after elevated RVSP. The vasodilatory effect of sildenafil reduces afterload because of prevention of right ventricular pressure overload [12] and improved diastolic function [2, 8]. Right ventricular E/A is a predictor of right ventricular diastolic function [30]. The results of E/A in the MCT/sildenafil group indicated that the va-

sodilatory effect of sildenafil led to improvement of right ventricular diastolic function. The results of the EI in the MCT/sildenafil group showed that the vasodilatory effect of sildenafil reduced afterload and prevented flattening of the interventricular septum because of right ventricular pressure overload. Serial changes in the EI were similar to the time course of RVSP in the present study. The EI was well correlated with RVSP in a previous study [22]. Reduction of RVSP with sildenafil treatment might affect improvement of interventricular septal curvature.

The results of TAPSE and Sm in the MCT/sildenafil group indicated that the therapeutic effect of sildenafil did not prevent systolic dysfunction with progressive PH. Sildenafil does not increase cyclic AMP, and lacks inotropic effects [28]. Additionally, the pulmonary vasodilator effect of sildenafil reduces afterload and improves diastolic function. However the lack of inotropic effects of sildenafil did not have indirect effects on myocardial contractility [28].

The Tei index in the MCT group indicated that cardiac function was deteriorated at the early phase of disease. This finding showed that deterioration of right ventricular function with progressive PH occurred before elevated RVSP. The Tei index is a useful diagnostic parameter in patients with right ventricular dysfunction [30]. Echocardiography was useful for early detection of right ventricular dysfunction in rats with PH in a previous study [14], and tissue Doppler imaging has been shown to be useful for early detection of myocardial abnormality [25]. The Tei index in the MCT/sildenafil group indicated that the reduction of afterload following sildenafil treatment improved cardiac function in the early phase of treatment. This finding suggested that sildenafil improved right ventricular myocardial function in the early phase of treatment.

In early phase of PH (2-week), the increase of E/Em ratio and the decrease of Sm were observed in MCT group before the increase of EI, a marker of right ventricular remodeling. The previous study reported that TDI was early detection of right ventricular dysfunction [18, 34]. EI was well correlated with interventricular septal curvature caused by elevated RVSP after progressive PH. The present study suggested that right ventricular function might be deteriorated before elevated RVSP.

The increase in RVSP in the MCT group suggested progressive PH in the present study. The reduction in

RVSP with sildenafil treatment indicated the vasodilatory effect of sildenafil. The serial changes in echocardiographic parameters showed that sildenafil improved right ventricular myocardial function. The serial change in echocardiography was compared with RVSP to evaluate the therapeutic effects of sildenafil on cardiac function for monitoring PH.

In the present study, echocardiography could detect cardiac dysfunction in the early phase of PH before increased RVSP. Echocardiography indicated not only cardiac dysfunction after injection of MCT, but also improvement of cardiac function after administration of sildenafil in the early phase of PH. The previous study reported about therapeutic effect of sildenafil in progressive PH after increased RVSP [3, 4, 6, 9, 17, 27]. However it had not been fully understood about preventive effect of sildenafil. Sildenafil treatment prevented right ventricular dysfunction in early phase of PH and this effect contributed in progressive PH after elevated RVSP (6-week). This result suggested that right ventricular function deteriorated before elevated RVSP, and supported beneficial effect in early phase treatment of sildenafil [29]. Assessment of right ventricular function using echocardiography showed that sildenafil improved right ventricular function with progressive PH.

The present study has some limitations. First, tricuspid regurgitation velocity was not measured. Some of the rats in the MCT group had tricuspid regurgitation, but it was difficult to obtain an accurate measurement because of the small size of the hearts. However, the present study did not focus on the utility of tricuspid regurgitation to estimate RVSP, but focused on the utility of combinations of parameters for evaluating right ventricular function for monitoring PH with sildenafil treatment. Additional studies are required to evaluate echocardiography for monitoring PH and the effect of various therapeutic agents. Heart rate of the MCT/sildenafil group was lower than that in the saline group and the MCT group. The previous study reported that sildenafil reduced heart rate by the opening of mitochondrial K_{ATP} channels [26]. Sildenafil has beneficial effect for cardioprotective effects [24, 33, 36]. However the present study did not investigated about increased mitochondrial K_{ATP} channels activity and cardioprotective effects, but the preventive effect of sildenafil on right ventricular function by echocardiography.

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