Research Article

Pulmonary Circulation

DDCI-01, a novel long acting phospdiesterase-5 inhibitor, attenuated monocrotaline-induced pulmonary hypertension in rats

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Abstract

Pulmonary arterial hypertension is a progressive, malignant heart disease, characterized by pulmonary arteriole remodeling and increased pulmonary vascular resistance, which eventually leads to right heart failure. This study sought to evaluate the effects of a novel long-acting phospdiesterase-5 inhibitor, namely DDCI-01, as an early intervention for monocrotaline-induced pulmonary hypertensive rats. To establish this model, 50 mg/kg of monocrotaline was intraperitoneally injected into rats. At Day 7 after monocrotaline injection, two doses of DDCI-01 (3 or 9 mg/kg/day) or tadalafil (at 3 or 9 mg/kg/day) were intragastrically administered. The rats were anesthetized with pentobarbital for hemodynamic and echocardiographic measurements, at Day 21 after monocrotaline injection. Compared to the monocrotaline group, DDCI-01 at 3 and 9 mg/kg/day (P) reduced the mean pulmonary arterial pressure (mPAP), right ventricular systolic pressure, right ventricular transverse diameter, pulmonary arterial medial wall thickness (WT%), and right ventricle hypertrophy. However, no significant difference in the indices mentioned as above was found between DDCI-01 (3 mg/kg/day) and tadalafil (3 mg/kg/day). In addition, DDCI-01 at 9 mg/kg/day resulted in lower mPAP and WT%, as well as higher cyclic guanosine monophosphate levels in the lung and plasma compared with the same dose of tadalafil (9 mg/kg/day) (all $P < 0.05$). These findings suggested that DDCI-01 improved monocrotaline-induced pulmonary hypertension in rats, and a dose of DDCI-01 of 9 mg/kg/day might be more effective than the same dose of tadalafil in monocrotaline-induced pulmonary hypertension in rats.

Keywords

DDCI-01, phospdiesterase-5 inhibitor, pulmonary arterial hypertension, tadalafil

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Introduction

Pulmonary arterial hypertension (PAH) is a progressive and fatal cardiovascular disease. It is characterized by a mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg, as assessed by right heart catheterization at rest.[1] Elucidation PAH's pathological mechanisms of PAH may lead to the developing effective therapies to target specific aberrant pathways, especially given that PAH is not yet curable. Agents that modulate abnormalities in the prostacyclin,[2–4] endothelin[5] and nitric oxide pathways[6] improve functional status and pulmonary hemodynamics and possibly even slow disease progression.[7]

PAH pathogenesis likely involves an imbalance in the normal relationships between vasodilators and vasoconstrictors.[8] Phospdiesterase-5 (PDE-5) inhibitor could

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improve the vasoconstrictive-predominant condition in patients with PH by increasing cyclic guanosine monophosphate levels in pulmonary vessels[9]; this treatment for PAH has been approved by the United States Food and Drug Administration (FDA).[10] DDCI-01 is a novel, long-acting, PDE-5 inhibitor that highly and selectively inhibits PDE-5, effectively reducing the concentration of PDE-5 kinases. DDCI-01 has been approved by FDA (Investigational New Drug Application No. 135832) for erectile dysfunction clinic trails. Tadalafil (TDF), a PDE-5 long-acting inhibitor, improves exercise capacity, quality of life and delays the clinical worsening of patients with PAH.[11] Different PDE-5 inhibitors exert a variety of effects on PH because of the variation in pharmacokinetics and the selectivity relative to PDEs.[12–14] However, the effect of DDCI-01 on PH remains unknown. The present study aimed to evaluate the effects of DDCI-01 and compare with TDF in a rat model of MCT-induced PH.

Materials and methods

Animal model

Male Sprague–Dawley rats (aged six to eight weeks, weighing 200–250 g) were obtained from the Laboratory Animal Center of Chongqing Medical University. All animal procedures were performed in accordance with the recommendations listed in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The Ethics Committee of Chongqing Medical University (Chongqing, China) approved the study protocol.

The rats were blindly randomized to receive intraperitoneal MCT at 50 mg/kg ($n = 55$) or an equal volume of saline (control, $n = 10$). Rats with intraperitoneal MCT (Day 1) were blindly randomized into five groups to receive the following treatments: DDCI-01 at 3 mg/kg/day (MCT + 3DDCI-01, $n = 10$); TDF at 3 mg/kg/day (MCT + 3TDF, $n = 10$); DDCI-01 at 9 mg/kg/day (MCT + 9DDCI-01, $n = 10$); TDF at 9 mg/kg/day (MCT + 9TDF, $n = 10$); or vehicle (MCT + 0.5% carboxymethylcellulose sodium salt, $n = 15$). Treatments were given orally, once daily, from Days 7 to 21 after MCT injection.[15] All rats were given free access to food and water and were maintained in a room with controlled temperature ($22 \pm 2^{\circ}$C) and lighting under 12-h light/ dark exposure cycles. After the measurement of hemodynamic and echocardiography parameters, all rats were sacrificed and blood, heart, and lung tissue were collected.

Echocardiographic assessment

Two-dimensional images of the RVTD, aortic artery diameter (AO), and pulmonary artery (PA) transverse diameter were measured on apical four-chamber and parasternal short-axis views (IE33; Philips, Holland). The left ventricular internal diameter during systole (LVIDs) and left ventricular internal diameter during diastole (LVIDd) were measured on M-mode echocardiography parasternal long-axis views, and cardiac output (CO) was calculated by computer algorithms. Tricuspid annular plane systolic excursion (TAPSE) was measured from M-mode apical four-chamber views.[16] All data represented the mean of five uninterrupted cardiac cycles.

Hemodynamic and right ventricular hypertrophy index measurements

A polyethylene catheter (intramedical PE-50, inner diameter: 0.5 mm, outside diameter: 0.9 mm) was filled with heparin and connected to a multi-lead physiological recorder (MP150; BIOPAC Systems, Goleta, CA, USA). The catheter was inserted into the RV through the right jugular vein and introduced into the pulmonary artery (PA) guided by a pressure curve.[17,18] After the measurement of pulmonary arterial pressure, the catheter was inserted into the carotid artery to measure systemic arterial pressure (mSAP). The heart rate (HR) was also measured. The atria were removed to evaluate RV hypertrophy (RVH). The heart was dissected into the RV, left ventricle (LV) and septal wall and then weighed. The weight ratio of the RV to the LV plus septum RV/ (LV + S) was used as an RVH index.[19]

Histology of the heart tissues

After hemodynamic measurement, the hearts were removed, washed in phosphate-buffered saline, and fixed in 4% paraformaldehyde for 24 h. The fixed hearts were cut in the short-axis direction at the middle level from the apex to the base and then embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed on the hearts sections.[20–22] The images were scanned using the Pannoramic SCAN system (3DHISTECH, Budapest, Hungary) and analyzed with the Case Viewer software program.

Histology and immunohistochemistry of the lung tissues

Lung tissues were obtained from the rats and immersed in 4% paraformaldehyde overnight for fixation. The fixed tissues were dehydrated, cleared, embedded in paraffin wax, and cut into 5 -μm thick sections. H&E, Elastic–Van Gieson, and α-smooth muscle actin (α-SMA) staining were performed on the lung tissue sections. Immunohistochemical staining was performed as previously described.[23–25] The developed tissue sections were imaged under a microscope (DFC550; Leica Microsystems, Wetzlar, Germany). The wall thickness (WT) and external diameter (ED) of the pulmonary arteries were measured using IPP 6.0 image analysis software (Media Cybernetics, Rockville, MD, USA). Pulmonary arteriole remodeling was quantified as follows: $WT\% = (2 \times WT)/ ED \times 100$.[26]

Measurement of cGMP levels in plasma and lungs

Blood was collected, placed in EDTA, and centrifuged for 15 min at $4000 \times g$ at 4°C. The plasma was removed and stored in aliquot at −80°C for later use. The lung tissues were rinsed in ice-cold phosphate-buffered saline to thoroughly remove excess blood and were weighed before homogenization. The lung tissues were minced to small pieces and homogenized in fresh lysis buffer with a glass homogenizer on ice. The grinding solution was centrifuged at 4000 r/min for 10 min at 4°C, and the supernatant was stored at −80°C. The samples were thawed on ice, and cGMP concentration was measured using a cGMP enzyme-linked immunosorbent assay kit according to the manufacturer's instructions.

Statistical analysis

All experiments were repeated at least three times. Data were reported as mean ± standard deviation (SD). SPSS 22.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analyses. Differences among the groups were determined by Student's *t*-tests and one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Mortality and hemodynamics

Four rats died (27%) in the MCT group 21 days after MCT injection. No rats died in the other groups. There was no significant difference in mSAP or HR among the control, MCT, and the treatment groups ($P > 0.05$), (Table 1). Hence, the results suggested that MCT, TDF, and DDCI-01 had no effect on systemic arterial pressure.

RV systolic pressure (RVSP) and mPAP in the MCT group significantly increased (RVSP: 51.03 ± 3.90 mmHg vs. 30.92 ± 3.49 mmHg; $P < 0.01$; mPAP: 30.76 ± 2.44 mmHg vs. 15.81 ± 2.14 mmHg; $P < 0.01$), (Fig. 1a and b) when compared with the control group 21 days after MCT injection. This finding suggests the successful establishment of the PH model. Meanwhile, treatment with DDCI-01 and TDF significantly decreased the mPAP and RVSP. Hence, DDCI-01 and TDF can attenuate the MCT-induced PH. There was no significant difference in RVSP between groups treated with TDF or DDCI-01 at the same dose of 3 or 9 mg/kg/day ($P > 0.05$) (Table 1). High dose

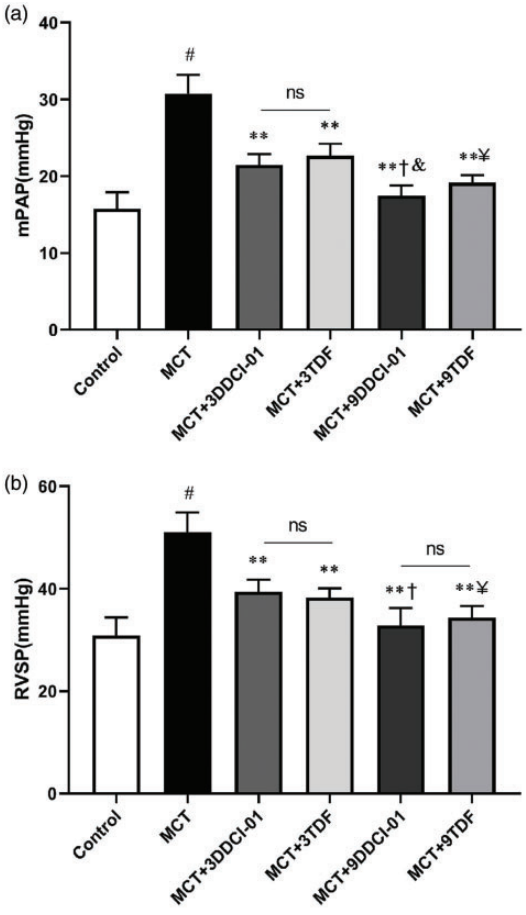

Fig. 1. (a) Effects of DDCI-01 and tadalafil on mean pulmonary arterial pressure (mPAP). (b) Right ventricular systolic pressure (RVSP) in monocrotaline (MCT)-induced pulmonary hypertension (PH) rat model. $^{\#}P < 0.01$ vs. Control group; $^{**}P < 0.01$ vs. MCT group; $^{\dagger}P < 0.05$ vs. MCT + 3DDCI-01 group; $^{¥}P < 0.05$ vs. MCT + 3TDF group; $^{\&}P < 0.05$ vs. MCT + 9TDF group; NS: not significant.

Table 1. Comparison of the hemodynamic parameters and RV function in monocrotaline (MCT)-induced pulmonary hypertension.

	Control	MCT	MCT + 3DDCI-01	MCT + 3TDF	MCT + 9DDCI-01	MCT + 9TDF
HR (beats/min)	414 ± 63	410 ± 43	419 ± 38	411 ± 48	417 ± 33	409 ± 54
mSAP (mmHg)	122 ± 8	118 ± 11	114 ± 11	116 ± 10	114 ± 13	116 ± 11
mPAP (mmHg)	15.81 ± 2.14	30.76 ± 2.44$^{\#}$	21.47 ± 1.43**	22.68 ± 1.57**	17.46 ± 1.37**,$^{\dagger,\&}$	19.16 ± 1.01**,$^{¥}$
TAPSE (mm)	1.91 ± 0.27	1.36 ± 0.16$^{\#}$	1.59 ± 0.23*	1.60 ± 0.16**	1.72 ± 0.18**	1.77 ± 0.21**
CO (ml/min)	194.90 ± 14.72	170.64 ± 16.90$^{\#}$	184.20 ± 12.15*	183.30 ± 10.16*	193.30 ± 15.1**	190.80 ± 12.63**
PA/AO	1.12 ± 0.09	1.35 ± 0.09$^{\#}$	1.15 ± 0.07**	1.14 ± 0.05**	1.12 ± 0.06**	1.13 ± 0.05**

$^{\#}P < 0.01$ vs. control group; $^{*}P < 0.05$ vs. MCT group; $^{**}P < 0.01$ vs. MCT group; $^{\dagger}P < 0.05$ vs. MCT + 3DDCI-01 group; $^{¥}P < 0.05$ vs. MCT + 3TDF group; $^{\&}P < 0.05$ vs. MCT + 9TDF group.

CO: cardiac output; HR: heart rate; mSAP: mean systemic arterial pressure; mPAP: mean pulmonary arterial pressure; PA/AO: ratio of the pulmonary artery to aortic artery diameters; TAPSE: tricuspid annular plane systolic excursion; TDF: tadalafil.

(9 mg/kg/day) of DDCI-01 decreased mPAP and RVSP more than low dose of (3 mg/kg/day) DDCI-01($P < 0.01$), so did TDF ($P < 0.05$). The MCT + 9TDF group (19.16 ± 1.01 mmHg) showed higher mPAP than the MCT +9DDCI-01 group (17.46 ± 1.37 mmHg) ($P < 0.05$, Fig. 1b and Table 1). Therefore, DDCI-01 might prompt a greater decrease in mPAP than TDF when the higher dose of 9 mg/kg/day is used.

Effects on echocardiographic parameters

RVTD was increased in the MCT group, compared with the control group at day 21 (4.35 ± 0.29 mm vs. 3.69 ± 0.36 mm, $P < 0.01$, Fig. 2a). At Day 21, RVTD was reduced in the DDCI-01 group when compared with the MCT group, including DDCI-01 at 3 mg/kg/day (4.02 ± 0.33 mm vs. 4.35 ± 0.29 mm; $P < 0.05$) or DDCI-01at 9 mg/kg/day (3.75 ±0.32 mm vs. 4.35 ± 0.29 mm, $P < 0.01$). Similarly, Fig. 2a shows that RVTD was attenuated in TDF groups, as compared with the MCT group, including TDF at 3 mg/kg/day (4.04 ± 0.36 mm vs. 4.35 ± 0.29 mm; $P < 0.05$) or TDF at 9 mg/kg/day (3.84 ± 0.81 mm vs. 4.35 ± 0.29 mm; $P < 0.01$). In addition, as compared with the MCT group, TAPSE and CO were increased in the groups treated with DDCI-01 or TDF at 3 mg/kg/day or 9 mg/kg/day ($P < 0.01$ and $P < 0.05$, respectively), (Fig. 2b and c), demonstrating the improvement in the cardiac function of the RV. The groups treated with DDCI-01 or TDF at 3 and 9 mg/kg/day showed a decrease in PA/AO ($P < 0.01$) as compared with the MCT group (Fig. 2d). There was no significant difference in echocardiographic parameters between groups treated with the same drug at different dose and different drugs at the same dose.

Improvement of RVH

RVH reflected RV adaptation to PH by calculating the weight ratio of the RV to the LV plus septum RV/(LV + S) in MCT-induced RVH. Compared with the

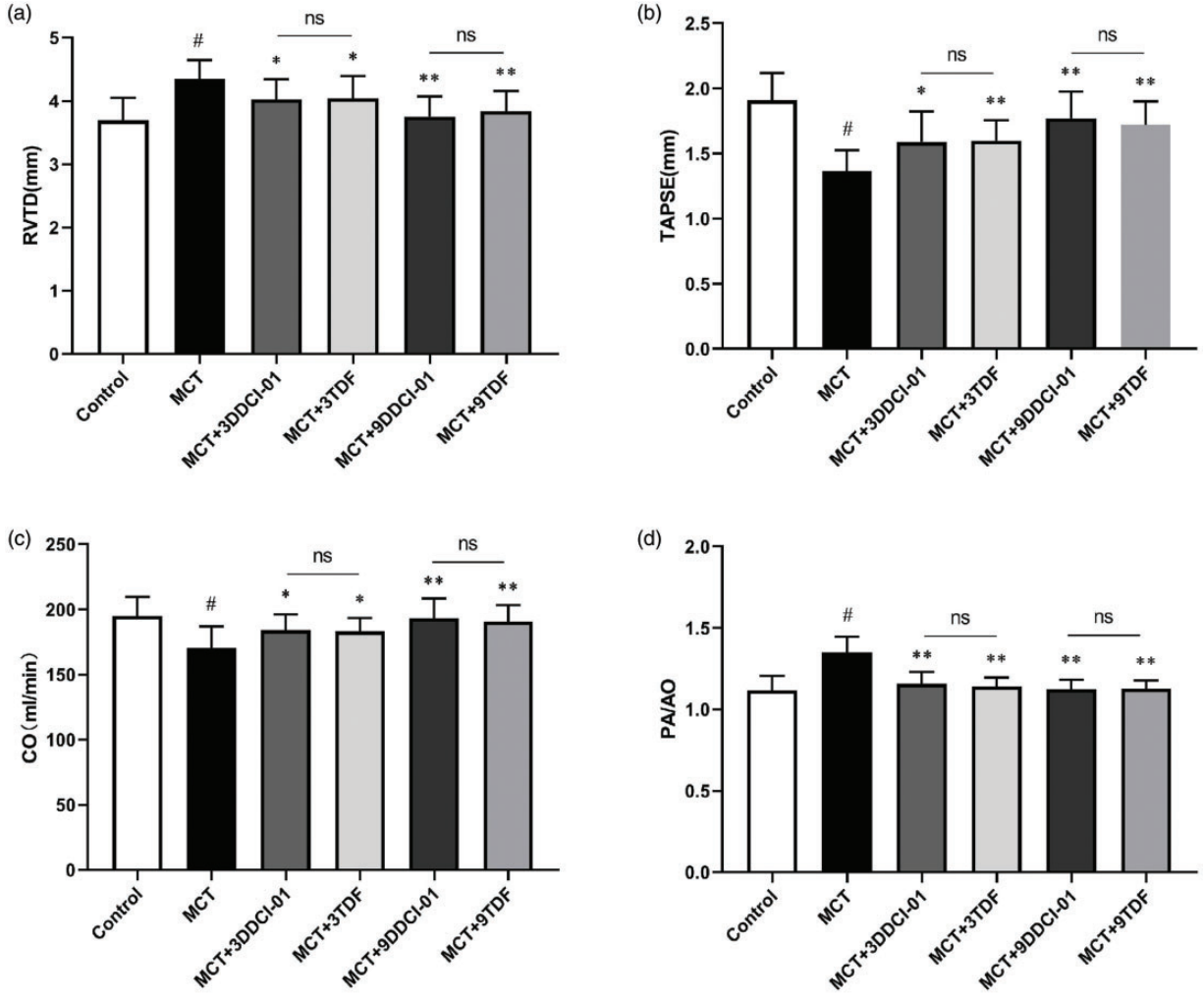

Fig. 2. (a) Right ventricular transverse diameter (RVTD) analysis. (b) Quantitative analysis of tricuspid annular plane systolic excursion (TAPSE). (c) Transverse diameter of the pulmonary artery (PA) to diameter of aortic artery (AO) (PA/AO). (d) Cardiac output (CO) measured by echocardiography. $^{\#}P < 0.01$ vs. Control group; $^{**}P < 0.01$ vs. MCT group; $^{*}P < 0.05$ vs. MCT group; NS: not significant.

MCT group, DDCI-01 at 3 mg/kg/day early intervention alleviates the RVH index (0.31 ± 0.03 vs. 0.41 ± 0.05, $P < 0.01$), and DDCI-01 at 9 mg/kg/day also decreased (RV/LV + S) (0.26 ± 0.06 vs. 0.41 ± 0.05, $P < 0.01$). RVH was confirmed further by heart cross-section H&E-staining images (Fig. 3a). Compared with the control group (1.18 ± 0.03 mm), the RV free wall (RVFWT) was thickened (1.95 ± 0.06 mm; $P < 0.01$); the DDCI-01 treatment alleviated this condition (3 mg/kg/day: 1.73 ± 0.03 mm; $P < 0.01$; 9 mg/kg/day: 1.48 ± 0.03 mm; $P < 0.01$). The RV myocardium was observed at high magnification ($\times 400$) (Fig. 3b), and the findings showed that, compared with the control group ($205.1 \pm 16.43\ \mu m^2$), the mean cross-sectional area (CSA) of RV cardiomyocytes obviously increased in MCT rats ($482.0 \pm 48.33\ \mu m^2$, $P < 0.01$). In contrast, compared with the MCT group, the administration of DDCI-01 at 3 mg/kg/day ($378.2 \pm 15.70\ \mu m^2$ vs. $482.0 \pm 48.33\ \mu m^2$, $P < 0.01$) and at 9 mg/kg/day ($278.9 \pm 23.74\ \mu m^2$ vs. $482.0 \pm 48.33\ \mu m^2$, $P < 0.01$) both attenuated the CSA. Furthermore, DDCI-01 at 9 mg/kg/day attenuated RVH more effectively than DDCI-01 at 3 mg/kg/day ($P < 0.05$), as well as TDF ($P < 0.05$). There was no significant difference in RVH between TDF and DDCI-01 at the same dose (Fig. 3).

Improvement of PA remodeling

H&E, Elastic–Van Gieson, and immunohistochemical staining of α-SMA were performed (Fig. 4a–c, respectively) to evaluate whether DDCI-01 treatment influenced pulmonary vascular remodeling in the MCT-induced PH model. Fig. 4d shows that PA WT% was increased in the MCT group compared with the control group ($69.25\% \pm 12.54\%$ vs.$17.20\% \pm 5.60\%$; $P < 0.01$). The WT% was significantly lower in the DDCI-01 (3 mg/kg/day: $41.78\% \pm 10.76\%$; 9 mg/kg/day: $27.73\% \pm 8.41\%$) and TDF (3 mg/kg/day: $45.46\% \pm 5.84\%$; 9 mg/kg/day: $35.96\% \pm 6.45\%$) group compared with the MCT group ($69.25\% \pm 12.54\%$) (All $P < 0.01$, Fig. 4d). There was no significant difference in WT% between groups treated with DDCI-01 and TDF at 3 mg/kg/day. Additionally, the WT% was significantly lower in the DDCI-01 (9 mg/kg/day) group than in the TDF (9 mg/kg/day) group ($P < 0.05$, Fig. 4d).

cGMP levels in lung tissue and plasma

There was no significant difference in cGMP levels in the lung between control and MCT groups (3.55 ± 0.76 pmol/mg protein vs. 3.30 ± 0.85 pmol/mg protein, $P > 0.05$). However, the cGMP levels in lung increased in the group

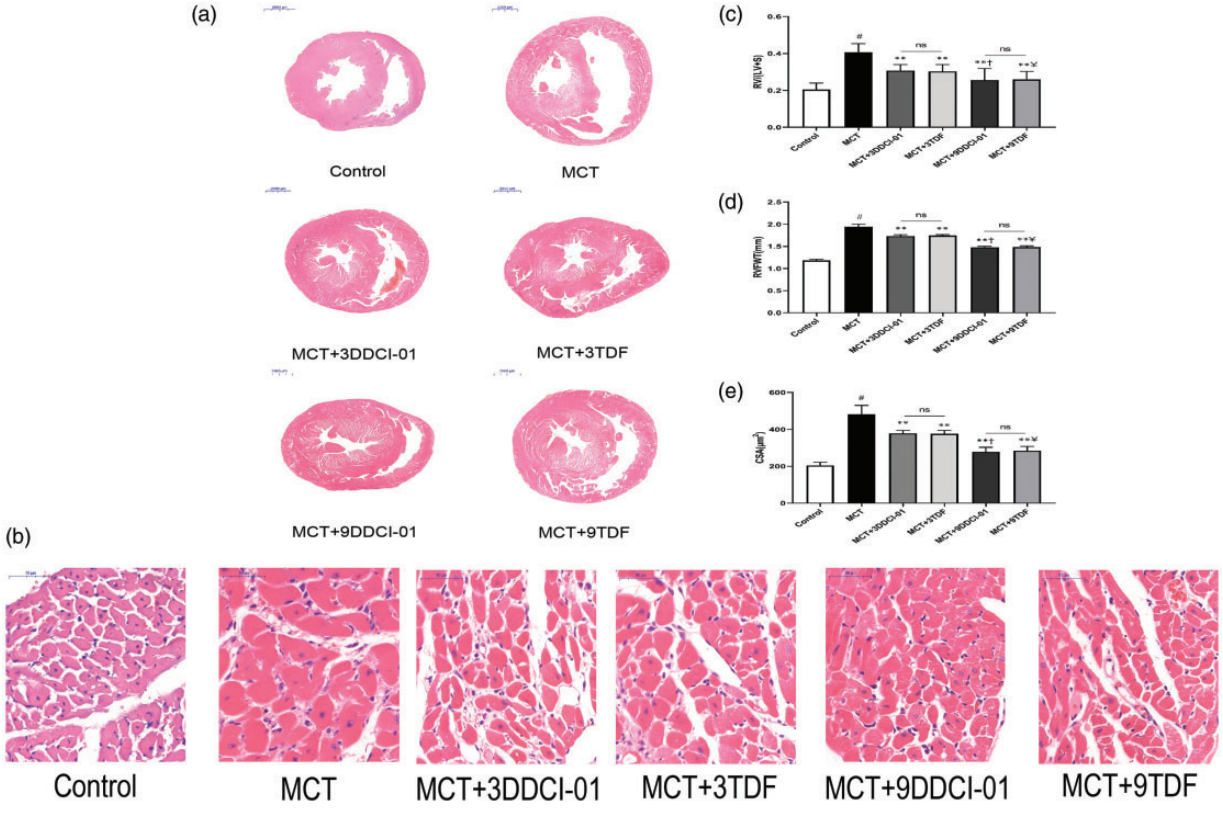

Fig. 3. (a) Representative heart cross-section staining (scale bar = 2,000 μm). (b) Representative H&E stained myocardium of right ventricle (scale bar = 50 μm). (c) Improved of the index of right ventricle hypertrophy: RV/(LV + S). (d) Analysis of right ventricle free wall thickness (RVFWT). (e) Quantitative analysis of mean cross-sectional area (CSA) of right ventricle (RV) cardiomyocytes in rats. $^{\#}P < 0.01$ vs. control group; $^{**}P < 0.01$ vs. MCT group; $^{\dagger}P < 0.05$ vs. MCT + 3DDCI-01 group; $^{¥}P < 0.05$ vs. MCT + 3TDF group; NS: not significant.

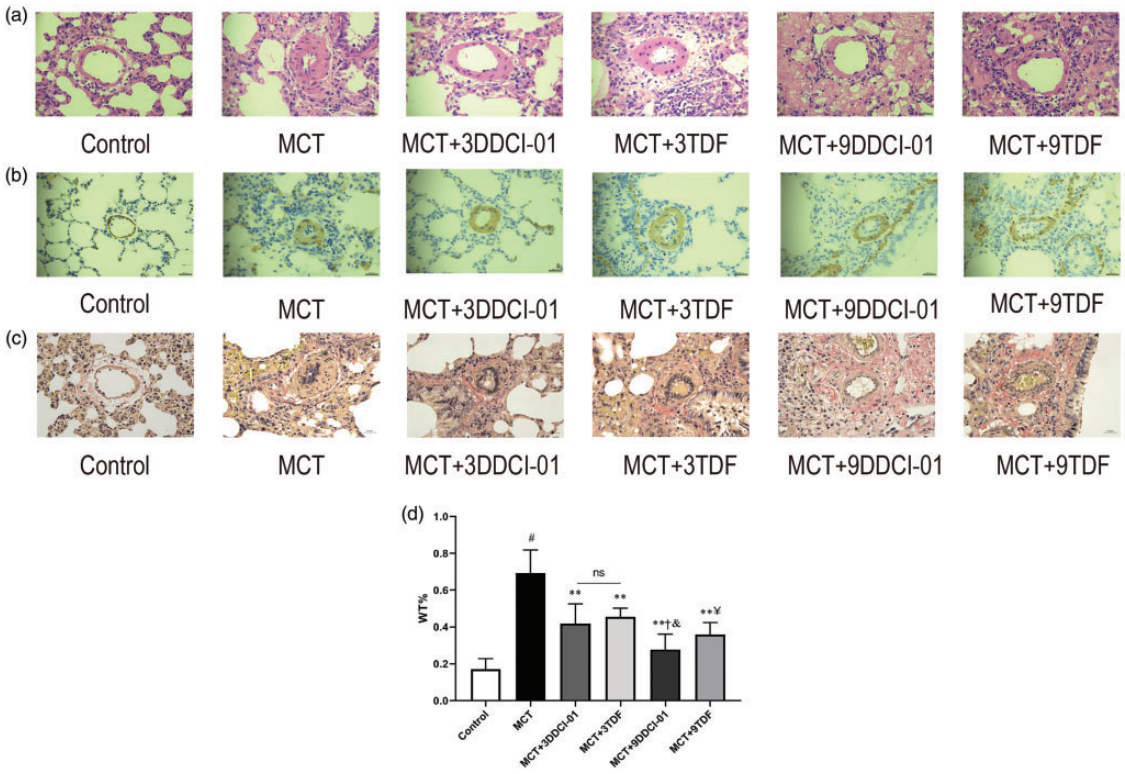

Fig. 4. (a) Representative H & E stained pulmonary sections from each group of rats (scale bar = 25 μm). (b) Representative micrographs of the pulmonary arterial vessels with α-SMA stain (scale bar = 25μm). (c) Representative Elastin-Van Gieson (EVG) staining photomicrographs of the pulmonary artery (scale bar = 25 μm). (d) DDCI-01 attenuated the ratio of pulmonary artery medial wall thickness to vessel radius (WT%). $^{\#}P < 0.01$ vs. control group; $^{**}P < 0.01$ vs. MCT group; $^{\dagger}P < 0.05$ vs. MCT + 3DDCI-01 group; $^{¥}P < 0.05$ vs. MCT + 3TDF group; $^{\&}P < 0.05$ vs. MCT + 9TDF group; NS: not significant.

treated with DDCI-01 at 3 mg/kg/day compared with the MCT group (4.54 ± 0.81 pmol/mg protein vs. 3.30 ± 0.85 pmol/mg protein, $P < 0.01$). The group treated with DDCI-01 at 9 mg/kg/day showed amplified lung cGMP level compared with the MCT group (6.03 ± 0.71 pmol/mg protein vs. 3.30 ± 0.85 pmol/mg protein, $P < 0.01$, Fig. 5a). Compared with the MCT group, the plasma cGMP levels increased in the group treated with DDCI-01 at 3 mg/kg/day (58.75 ± 9.20 pmol/mL vs. 19.05 ± 3.52 pmol/mL, $P < 0.01$), and also increased in the group treated with DDCI-01 at 9 mg/kg/day group (80.91 ± 6.57 pmol/mL vs. 19.05 ± 3.52 pmol/mL, $P < 0.01$, Fig. 4b). TDF also enhanced lung and plasma cGMP levels compared with MCT group ($P < 0.01$, Fig. 5). There was no significant difference in lung or plasma cGMP between groups treated with DDCI-01 or TDF at 3 mg/kg/day. lung or plasmaIn addition, both lung and plasma cGMP levels increased more in the group treated with DDCI-01(9 mg/kg/day) group than that in the group treated with TDF (9 mg/kg/day) ($P < 0.05$) (Fig. 5).

Discussion

This study demonstrated that DDCI-01 improved the survival rate and decreased mPAP, RVH, and medial wall thickening of pulmonary arterioles in a rat model of MCT-induced PH. A high dose of DDCI-01 (9 mg/kg/day) might be more effective than TDF at high dose because of significantly lower mPAP and WT% and higher cGMP levels in lung and plasma under the DDCI-01 treatment.

The present study showed the effects of DDCI-01 on PH and employed the TDF groups as a positive control. Treatment with TDF at 10 mg/kg in rats is considered the applied clinic dose of 40 mg/day in patients with PAH, with an expected plasma exposure levels in humans of 12,193 ng·h/mL. Separately, treatment with DDCI-01 at 3 mg/kg/day in rats corresponds to the approved clinic dose of 10 mg/day in humans, and the plasma exposure was 566 ng·h/mL. The efficacy of DDCI-01 indicates that it is a potential novel PDE5 inhibitor for use in the patients with PAH.

PDE-5 mainly affects cGMP's physiological function in regulating the muscle tension of vascular smooth muscle, especially the PA.[27] Endogenous vasodilators, such as atrial natriuretic peptides and nitric oxide (NO), activate soluble guanylate cyclase (GC), while the subsequent activation of protein kinase G (PKG) increases the cGMP concentration and relaxes vascular smooth muscle.[28] PDE activity determines GC activity and cGMP's intracellular concentration due to rapidly inactivated cGMP to GMP.

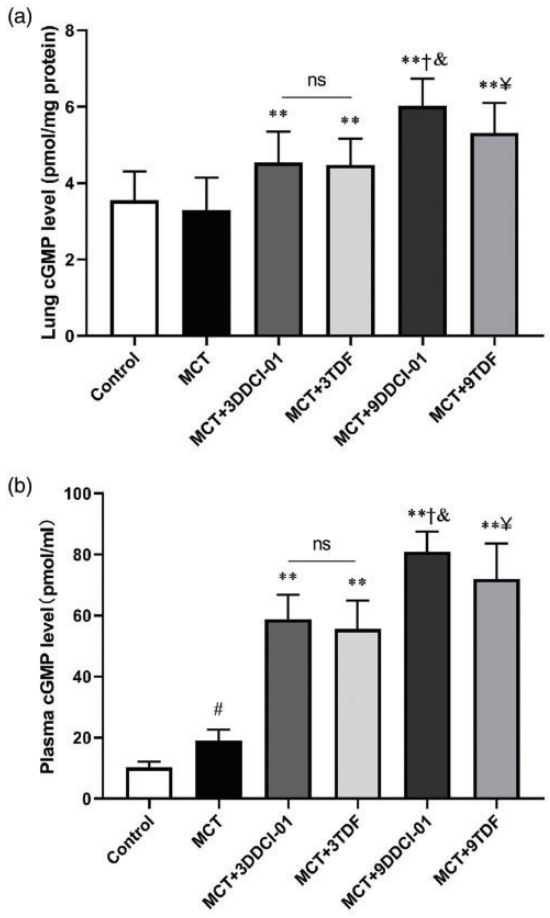

Fig. 5. (a) The pulmonary cGMP levels increased by DDCI-01 and tadalafil. (b) Both DDCI-01 and tadalafil increased the cGMP levels in plasma. $^{\#}P < 0.01$ vs. control group; $^{**}P < 0.01$ vs. MCT group; $^{\dagger}P < 0.05$ vs. MCT + 3DDCI-01 group; $^{¥}P < 0.05$ vs. MCT + 3TDF group; $^{\&}P < 0.05$ vs. MCT + 9TDF group; NS: not significant.

The activated PDE-5 concentration was much higher in pulmonary tissue than in aortic tissue in chronically hypoxia-induced PH rats.[29] Certain PDE-5 selective inhibitor, including sildenafil and TDF have been approved for treatment of patients with PAH.[30] In the present study, DDCI-01 had shown improved survival rate, increased levels of cGMP, attenuated mPAP, and improved pulmonary vascular remodeling and was not inferior to TDF. PH is the consequence of an increase in PVR due to remodeling of the small distal pulmonary arteries and arterioles, causing adaptive RVH, right heart failure.[31] In the present study, RV/(LV + S) was considered the RVH index. In addition, the effects of DDCI-01 on RV(/LV + S) were dose-dependent.

As a novel long-acting PDE-5 inhibitor, DDCI-01 enhanced the concentration of cGMP, which relaxes the pulmonary vascular, reduces PA pressure, and delays myocardial hypertrophy and remodeling. The mechanisms behind this phenomenon might be as follows (Fig. 6b): on the one hand, for pulmonary artery, PDE-5 inhibitor inhibits PDE-5 decomposition to increase its intercellular concentration, then cGMP activates PKG. Cellular hyperpolarization inhibited Ca^{2+} influx, decreasing intracellular Ca^{2+} concentration. Further, the large conductance Ca^{2+}-activated K^+ (BKca) channels open and intracellular K^+ concentration decreases, reducing pulmonary artery smooth muscle cell (PMSCs) proliferation, increasing apoptosis, pulmonary artery vasodilation, inhibiting pulmonary artery remodeling and reducing right ventricular afterload[32]; on the other hand, for RV, PDE-5 inhibitor inactivates cGMP-sensitive PDE-3, thereby elevating cardiac cyclic adenosine monophosphate (cAMP) and PKG to improve RV contractility.[33]

PAH is a progressive and fatal disease. Early detection of PAH and prompt initiation of effective therapy are considered an essential component of disease management, because patients diagnosed in the earlier course of their disease tend to derive a more pronounced benefit from therapy.[34,35] In a similar way, early intervention on experiential PH is also effective.[13,36] Exercise-induced PAH is considered to be the early stage of PAH.[37–39] In our previous study, the max mPAP was shown at exercise >30 mmHg at Day 7 was observed after MCT injection (Supplementary material); moreover, early intervention with TDF at Day 7 improved the prognosis in the MCT rats.[15] Therefore, early treatment with DDCI-01 or TDF was conducted in the rats starting at Day 7 after MCT injection.

Ultrasound imaging has advanced continuously and has become an important noninvasive accessory PAH examination.[40] In patients with idiopathic PAH, a larger RV diameter is a marker of a poor prognosis,[41] while RVTD is an important mid-cavitary diameter in clinical practice. Previous work has postulated that the dilatation of the PA itself or in relation to the diameter of the ascending aorta (PA/AO ratio) predicts PAH.[42] TAPSE is also a good parameter used to assess the RV function, which is considered as a prognostic marker.[43] Right heart failure caused by PAH can lead to progressive decrease of CO,[1] while CO improvement suggests that RV function has been ameliorated. The present study also showed that DDCI-01 can significantly improve RV function and delay RVH caused by PH.

DDCI-01 and TDF both improved the MCT-induced PH. A slight difference was observed between effects of the high dose (9 mg/kg/day) DDCI-01 and TDF. This variation might be a result of difference in pharmacokinetics and other unknown mechanisms. However, although DDCI-01 at 9 mg/kg/day attenuated mPAP and WT% a greater extent than TDF at the same dose, both had same survival rate at Day 21 after MCT injection. This result suggests that follow-up period may need to be extended to 28 days or longer to in future studies to better evaluate the survival rate.

There were some limitations to this study. First, in the present study, although DDCI-01 had the potential to improve PH, and attenuated mPAP and WT% to a greater extent than TDF, increased more cGMP than TDF at high dose, the underlying molecular mechanisms of DDCI-01 may involve in NO-cGMP pathway, which need to be

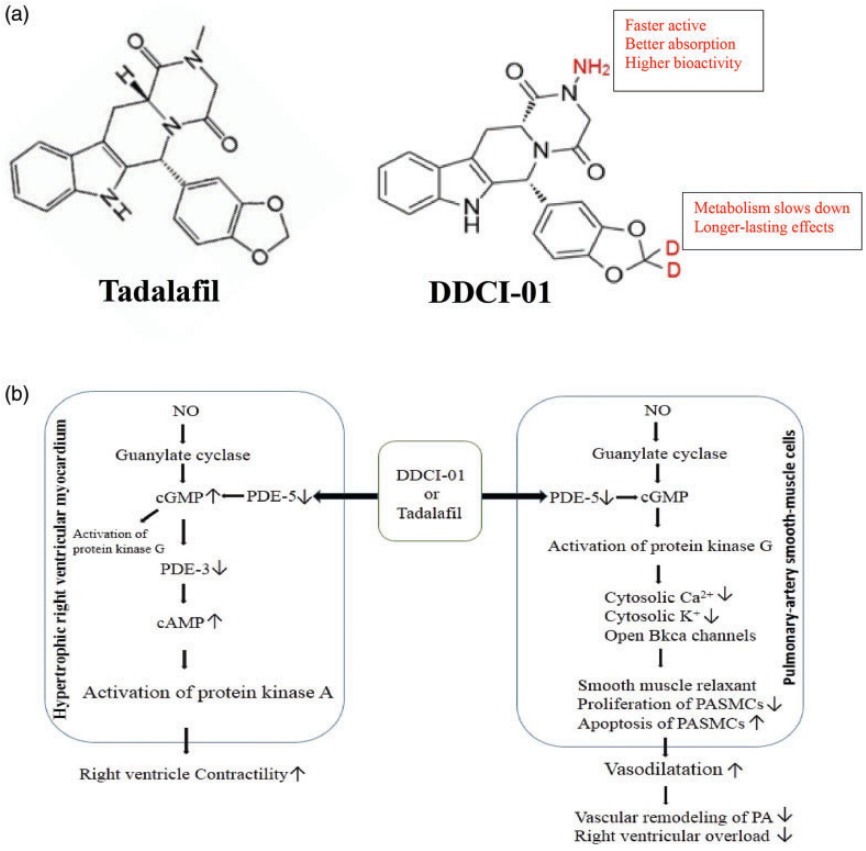

Fig. 6. (a) Compared the chemical formula of tadalafil and DDCI-01. DDCI-01: ([6R,12aR]-2-amino-6 - [2,2-dideuteriumbenzo{d {1,3}dioxol-5-yl]-2,3,12,12 a-tetrah dropyrazino[1′,2′:1,6]pyrido[3,4-b]indole- 1,4[6H,7H]-dione). (b) Proposed mechanisms of DDCI-01 on pulmonary hypertension (PH).

further explored. Second, there was only one PH model which was used in the present study, and the effects of DDCI-01 on other PH models could be further tested and verified, such as hypoxia-induced PH.

In summary, DDCI-01 treatment improves survival rate, reduces mPAP, pulmonary artery medial wall thickness, and is not inferior to TDF in a rat model of MCT-induced PH. Therefore, DDCI-01 is expected to be a novel, effective, and long-acting selective PDE-5 inhibitor in patients with PAH.

Authors' contributions

Ailing Li and Zhongkai Zhu analyzed the data. Wei Huang and Zhongzhu Chen designed the research studies. Ailing Li, Yangke He and Qian Dong performed the experiments. Ailing Li and Zhongkai Zhu analyzed the data. Dianyong Tang, Zhongzhu Chen provided technical support. Ailing Li wrote the manuscript.

Conflict of interest

The author(s) declare that there is no conflict of interest.

Ethical approval

This study was approved by the Ethics Committee of Chongqing Medical University (Chongqing, China).

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Guarantor

Not applicable.

Supplemental material

Supplemental material for this article is available online.

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