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ORIGINAL ARTICLE

Urantide improves the structure and function of right ventricle as determined by echocardiography in monocrotaline-induced pulmonary hypertension rat model

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Abstract

Urotensin II (UII) has been reported to play a key role in pulmonary arterial hypertension (PAH) development. Doppler echocardiography, a noninvasive and simple tool, is recommended for diagnosing PAH. This study was designed to investigate the effect of urantide, a UII receptor antagonist, on the structure and function of the right ventricle in PAH rat models by Doppler echocardiography. A total of 60 male rats were divided into two groups: early- and late-treatment groups. Rats in the urantide and MCT (monocrotaline) subgroups were injected with 10 μg/kg urantide in the urantide group or an equal amount of normal saline in the MCT group 1 week after PAH model construction in the early-treatment group and 4 weeks after the construction in the late-treatment group. Rats in the control group received an equal volume of normal saline solution. PAH-related indexes were measured by echocardiography. PAH rat models exhibited higher right ventricular diastolic diameter and lower time to peak, ejection time, and peak flow velocity of pulmonary artery than controls ($P < 0.05$). However, compared with the MCT group, all abovementioned indexes were improved in the urantide group ($P < 0.05$). No significant differences in pulmonary artery diameter and left ventricular ejection fraction were noted among the groups. Compared with the MCT group, systolic pulmonary arterial pressure (SPAP) and mean pulmonary arterial pressure (mPAP) were significantly lower in the urantide group ($P < 0.05$). SPAP examined by echocardiography was correlated with mPAP by catheterization ($P < 0.05$). Urantide treatment improved right heart failure parameters in MCT-induced PAH rats, thus providing a potential new strategy for treating PAH.

Highlights
A monocrotaline-induced PAH rat model was established.
Urantide benefited the structure and function of the right ventricle in PAH rats.
Urantide improved the hemodynamic parameters of PAH rats.
SPAP examined by echocardiography was significantly correlated with mPAP value.

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Keywords Echocardiography · Pulmonary arterial hypertension · Urantide

Introduction

Pulmonary arterial hypertension (PAH) is characterized by endothelial and smooth muscle cell proliferation and results in progressive pulmonary vascular remodeling, mean pulmonary arterial pressure (mPAP) increase, right ventricular hypertrophy, and functional failure, leading to a very poor prognosis [1]. PAH is defined by right heart catheterization manifesting as resting mPAP ≥ 25 mmHg, pulmonary capillary wedge < 15 mmHg, and pulmonary vascular resistance (PVR) ≥ 3 Wood units. Although the mechanism of PAH has not been completely revealed, multiple pathogenic pathways, including those at the molecular and genetic levels and in the vascular smooth muscle and endothelial cells, have been proposed [2, 3]. This condition is considered to be associated with the combination of pulmonary vasoconstriction, thrombogenesis, and pneumoangiogram reconstruction [3].

Because vascular remodeling together with enhanced vasoconstriction has been associated with the development of PAH, an endothelin-1 receptor antagonist, Bosentan, has become commonly used to alleviate PAH [4, 5]. Studies have suggested that urotensin II (UII), originating from the caudal neurosecretory system of teleost fish, is a potent vasoconstrictor that is more effective than endothelin-1 [6, 7]. UII and its receptor are commonly expressed in the blood vessels, heart, liver, kidney, lung, smooth muscle, and endothelium [4, 8, 9]. UII was reported to participate in vascular tone regulation and promote the proliferation of vascular smooth muscle cells [10]. More recently, it was indicated to have potential utility for pulmonary vasoconstriction in a PAH lamb model [11]. These lines of evidence implied that UII can be a crucial factor in PAH development as well as in pulmonary vascular remodeling, suggesting that UII receptor antagonist is an effective drug for PAH.

Urantide, a UII receptor antagonist [12], can effectively alleviate MCT (monocrotaline)-induced PAH in a rat model, probably by relaxing pulmonary arteries and blocking pulmonary vascular remodeling [13]. To explore the effects of urantide on PAH and the subsequent right ventricular hypertrophy, we investigated the changes in the structure and function of the right ventricle and PAP in MCT-induced PAH rats by echocardiography.

Materials and methods

Animals and grouping

All experiments were approved by the Ethics Committee of Experimental Research in the First Affiliated Hospital of Harbin Medical University and conformed to the Guide for the Care and Use of Laboratory Animals. Sixty Wistar rats (3-month old, 180–200 g) were purchased from the Experimental Animal Center of Chinese Academy of Sciences, Beijing, China. All the rats were raised in climate-controlled conditions with a 12/12-h light/dark cycle and were fed standard food ad libitum.

The rats were randomly assigned to one of the two groups: the early-treatment group ($n = 30$) and the late-treatment group ($n = 30$). Then, rats in each group were divided into three subgroups: control group, MCT group, and urantide group ($n = 10$ per subgroup). A PAH animal model was induced by the subcutaneous injection of 50 mg/kg MCT (Sigma Chemical Co, St Louis, MO, USA) in the MCT and urantide groups. Urantide (Peptides International, Louisville, KY, USA) at 10 μg/kg/day was found to be the optimum concentration on mPAP in the PAH model based on our preliminary study. One week (early-treatment group) after PAH model construction, the surviving rats (some rats died of pulmonary edema or pulmonary hemorrhage within 1 week of MCT injection) were injected intraperitoneally with either urantide (10 μg/kg/day, urantide subgroup, $n = 8$) or an equal amount of normal saline (NS) (MCT subgroup, $n = 8$) for 3 weeks. The normal control rats ($n = 10$) received an equal volume of 0.9% saline for 3 weeks. In the late-treatment experiment group, 20 rats were also injected subcutaneously with 50 mg/kg MCT, and 4 weeks later, the surviving ones were randomly injected with either urantide at 10 μg/kg/day (urantide subgroup, $n = 7$) or an equal amount of 0.9% saline (MCT subgroup, $n = 7$) intraperitoneally for 2 weeks. Rats ($n = 10$) in the control group received an equal volume of NS for 2 weeks.

Echocardiography

Ultrasonic cardiogram was monitored at week 4 in the early-treatment group and at week 6 in the late-treatment group. After rats had been anesthetized with Nembutal (45 mg/kg IP), echocardiography was performed using a Philips 7500 ultra-phonic machine with a 12-MHz transducer. Systolic pulmonary arterial pressure (SPAP), pulmonary artery diameter (PA diam), right ventricular diastolic diameter (RVEDD), ejection time (ET), time to peak (TTP), peak flow velocity of pulmonary artery (PFVP), and left ventricular ejection fraction (LVEF) were determined. Meanwhile, blood flow signals in pulmonary artery and spectral changes of blood flow in the systole period were observed with a spectral-color Doppler ultrasound (7500, detecting head mid-frequency 12 MHz; Philips, Germany). All the results were monitored by an echocardiographyer.

Hemodynamic measurement

Rat anesthesia was performed by the intraperitoneal injection of 1.5 mL/kg pentobarbital sodium. mPAP was measured in accordance with a previously described method [13]. Briefly, a polyethylene catheter was connected to a pressure transducer (Henan Hunan Medical Science and Technology Co, China) and inserted into the right jugular vein, and then passed through the right ventricle into the pulmonary artery. The intravascular location of the catheter tip was identified by pressure tracing.

Statistical analysis

All data are presented as mean ± SD (standard deviation). Comparisons among groups were performed using two-way ANOVA followed by the LSD t-test. Pearson's correlation analysis was conducted to examine the correlation between SPAP examined by echocardiography and mPAP by catheterization. The significance level was set at $\alpha = 0.05$. Data analysis was conducted using the software SPSS 13.0.

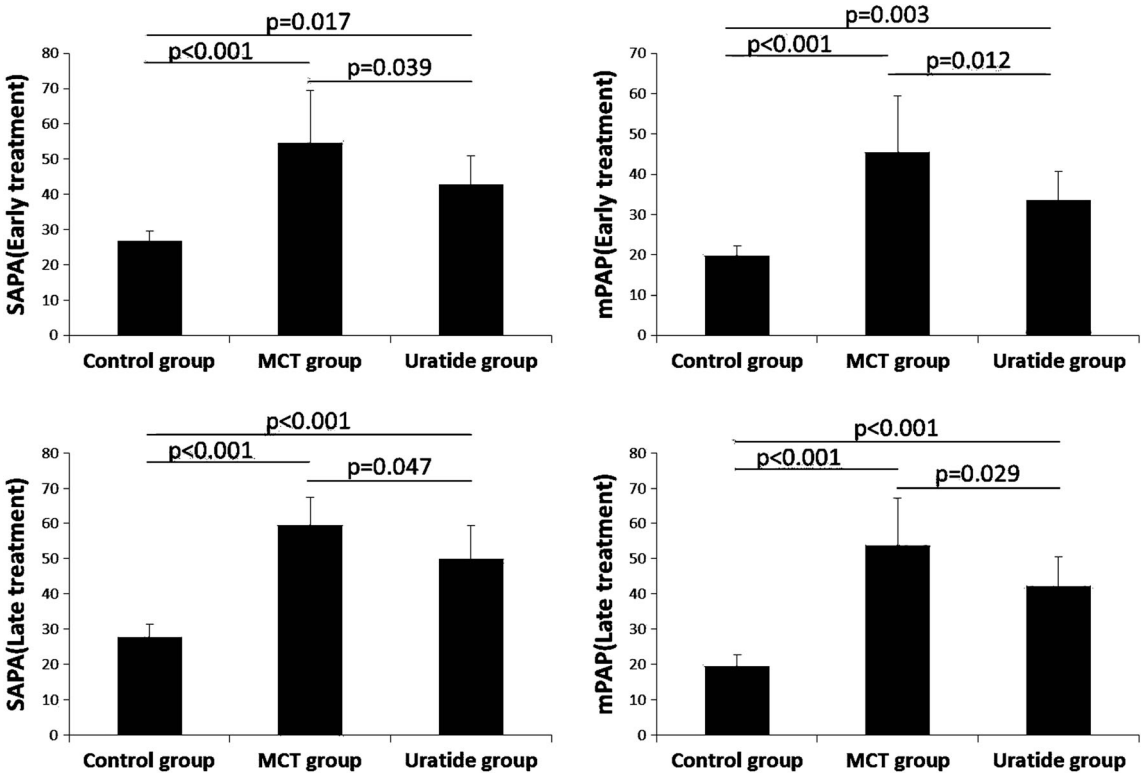

Fig. 1 Urantide on SPAP and mPAP in different groups. All data are presented as mean ± SD (standard deviation). SPAP in the early (**a**)- and late (**b**)-treatment groups. mPAP in the early (**c**)- and late (**d**)-treatment groups. *$P <$ 0.05 compared with that in the control group. **$P < 0.05$ compared with the MCT group. SPAP systolic pulmonary arterial pressure, PA diam pulmonary artery diameter

Results

Echocardiography assessment

Both in the early- and late-treatment experiment groups, rats in MCT group exhibited higher SPAP (early: $P < 0.001$; late: $P < 0.001$) and RVEDD (early: $P < 0.001$, late: $P < 0.001$) and lower TTP (early: $P < 0.001$, late: $P < 0.001$), ET (early: $P < 0.001$, late: $P < 0.001$), and PFVP (early: $P < 0.001$, late: $P < 0.001$), compared with controls. After urantide treatment for both 4 and 6 weeks, all the values were significantly improved compared with control or MCT group (all $P < 0.05$)

Table. 1 Cardiac function in the early- and late-treatment experiment

Parameters	Early treatment			Late treatment		
	Control group ($n = 10$)	MCT group ($n = 8$)	Urantide group ($n = 8$)	Control group ($n = 10$)	MCT group ($n = 7$)	Urantide group ($n = 7$)
PA diam (mm)	3.13 ± 0.17	3.15 ± 0.30	3.14 ± 0.11	3.13 ± 0.19	3.16 ± 0.35	3.15 ± 0.17
RVEDD (mm)	2.34 ± 0.15	4.40 ± 0.45*	3.55 ± 0.39*§	2.56 ± 0.30	5.2 ± 0.65*	3.80 ± 0.54*§
LVEF (%)	74.0 ± 10.2	77.9 ± 10.7	75.2 ± 8.9	74.5 ± 9.0	76.1 ± 10.1	75.7 ± 15.1
ET (ms)	81.9 ± 9.5	40.4 ± 4.1*	63.5 ± 8.2*§	78.2 ± 9.4	39.5 ± 10.0*	54.4 ± 5.5*§
TTP (ms)	30.6 ± 5.1	16.1 ± 2.1*	20.2 ± 3.8*§	33.0 ± 6.0	16.4 ± 1.9*	23.6 ± 3.7*§
PFVP (cm/s)	95.44 ± 9.80	48.23 ± 8.72*	77.18 ± 11.61*§	95.46 ± 15.22	43.22 ± 10.33*	69.79 ± 12.61*§

All the data were expressed as mean ± SD (standard deviation)

PA diam pulmonary artery diameter, *RVEDD* right ventricular diastolic diameter *LVEF* left ventricular ejection fraction, *ET* ejection time, *TTP* time to peak, *PFVP* peak flow velocity of pulmonary artery

* $P < 0.05$ compared with control group

§ $P < 0.05$ compared with MCT (monocrotaline) group

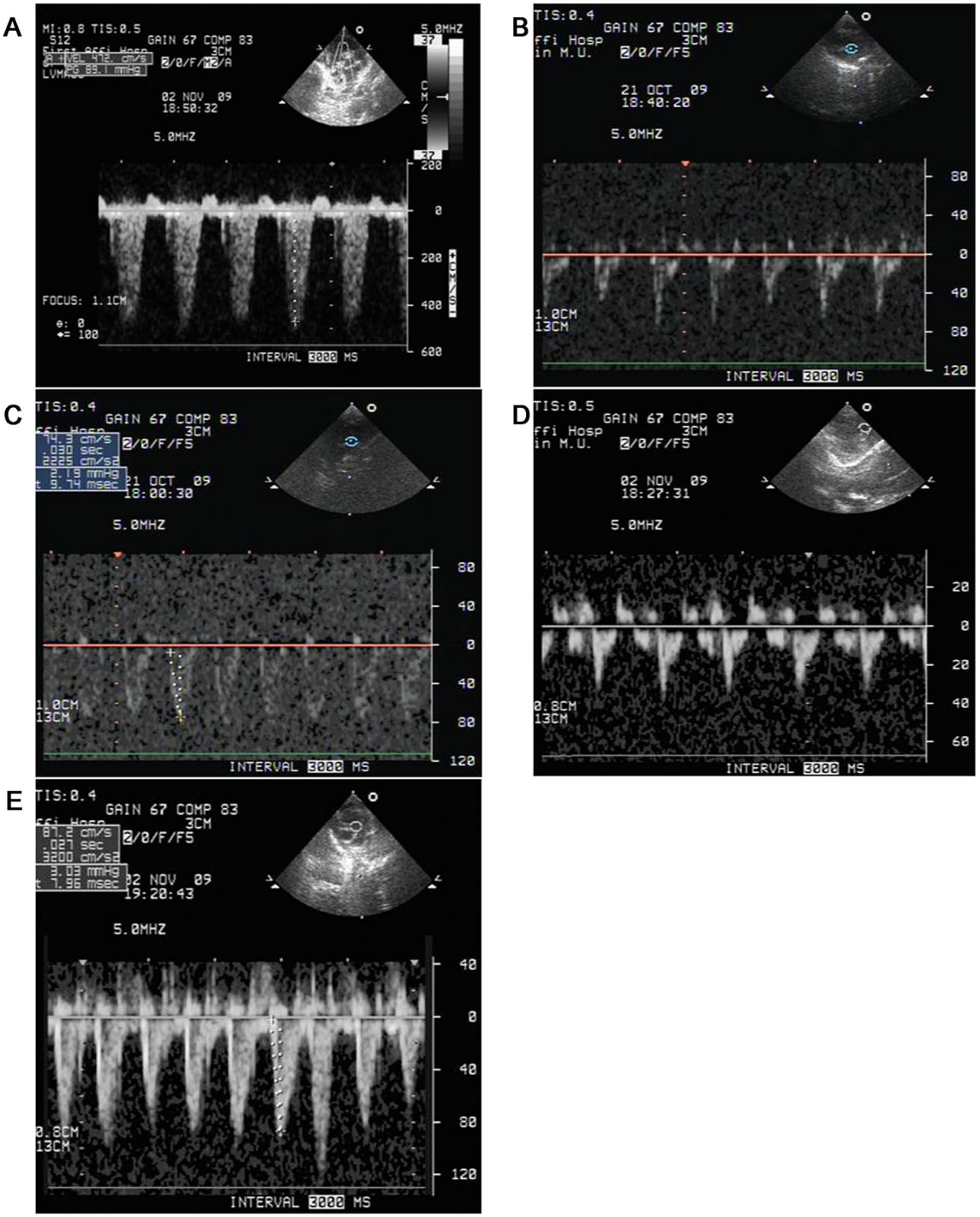

Fig. 2 Pulmonary blood flow spectra in different groups. **a** Control group. **b**, **c** The early-treatment MCT (**b**) and urantide (**c**) groups. **d**, **e** The late-treatment MCT (**d**) and urantide (**e**) groups. MCT, monocrotaline

(Fig. 1; Table 1). There were no statistically significant differences in PA diam and LVEF among the groups. Pulmonary arterial blood flow spectra are displayed in Fig. 2. In the early-treatment group, pulmonary blood flow spectra of the rats 4 weeks after MCT injection showed a typical "dirk" alteration, and limbs were steep and straight, which suggested that blood flow reached its peak speed immediately after pulmonary valve opening, and low ranges demonstrated that the speed reduced (Fig. 2B; limbs were shorter, meaning shortening of ET). Pulmonary blood flow spectra after treating rats for 3 weeks with urantide were circular and obtuse, and ET and TTP of these rats were longer than those of the control rats (Fig. 2C). In the late-treatment group, pulmonary blood flow spectra 6 weeks after MCT injection showed a typical

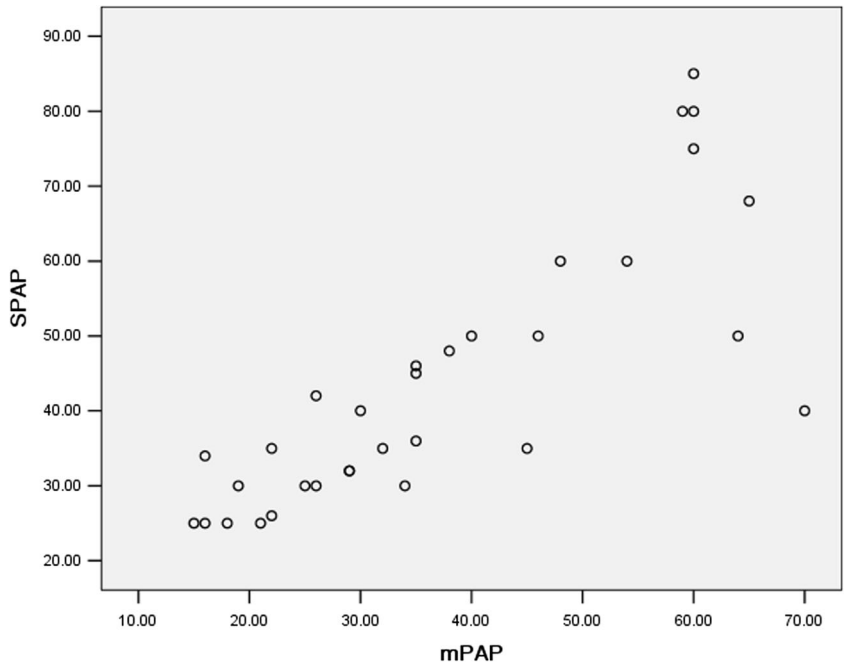

Fig. 3 Correlation between mPAP measured with a right heart catheter and SPAP measured with echocardiography. All data are presented as mean ± SD (standard deviation). SPAP systolic pulmonary arterial pressure, PA diam pulmonary artery diameter

"dirk" alteration, similar to that shown in Fig. 2B (Fig. 2D). Pulmonary blood flow spectra in the systole period in the pulmonary artery of rats from the urantide treatment group were circular and obtuse, and ET and TTP of these rats were longer than those of the rats in the MCT group (Fig. 2E).

Hemodynamic assessment

mPAP was positively correlated with the level of SPAP (Fig. 3; $r = 0.813$, $P < 0.001$).

After urantide treatment, all the rats were surviving and postmortem examination revealed that the heart and lung were better in rats of treatment group than MCT subgroup and no other organ injury was observed.

Discussion

The prognosis of progressive PAH is relatively poor because it does not only result in an increase in pulmonary arterial pressure but also evokes ventricular hypertrophy and failure and even results in death [14, 15]. Reducing pulmonary arterial pressure as early as possible will ameliorate adverse structure and functional changes of the right ventricle, thereby to avoiding mortality [16]. Over the past two decades, PAH has changed from a fatal condition to a chronic manageable disease because of the advances in early diagnosis and new therapies. Nevertheless, none of the current therapies is curative; thus, the search for new treatment strategies continues. In many PAH studies, prostacyclin, nitric oxide, and the endothelin pathways have been targeted as the main three pathways for PAH treatment [17–19]. In the past few years, although modern treatments of PAH, namely, the endothelial receptor antagonist Bosentan and phosphodiesterase type-5 inhibitors Sildenafil and Prostacyclin, have led to significant improvements in patients' symptomatic status and a lower rate of clinical deterioration [20], the development of more effective medications in terms of efficiency and safety is still expected.

The MCT-induced rat PAH model, which produces endothelial injuries and changes to the pulmonary vasculature similar to those in human PAH, is commonly used to study this condition [21]. In this study, we investigated the effects of urantide on MCT-induced PAH rats in vivo and observed that SPAP in the urantide group was clearly decreased but was still above the control levels. Moreover, PAH rats exhibited higher RVEDD and lower TTP, ET, and PFVP, which were similar to those reported in the study by Tran et al. [22].

A reliable noninvasive method to identify patients who are likely to have PAH is needed. Our present study enabled identification of the fact that pulmonary arterial pressure levels measured by echocardiography and by a right heart catheter were positively correlated. For the earlier diagnosis of PAH, receptive screening of patients with echocardiography is better than invasive catheterization [22, 23], and our work supported this conclusion. A previous study showed similar findings, and it was agreed that echocardiography should take an important place in the management of PAH [24]. Specifically, it was asserted that this technology should not be restricted merely to screen for the disease [25, 26] but should be used to predict the development of pulmonary artery and heart and its prognosis. Doppler ultrasound cannot be used to make a definitive diagnosis of PAH, but it is an atraumatic examination method that can accurately determine cardiac

hemodynamics and at the same time supply the estimated values of RVSP, cardiac function, and morphology and identify possible cardiac reasons for PAH, providing evidence for the diagnosis of PAH [27, 28]. Moreover, Shao [23] asserted that real-time three-dimensional echocardiography could conveniently accurately measure right ventricular (RV) volume and RVEF without geometric assumption and could be useful to evaluate RV dysfunction. Our results suggested that right cardiac blood-pumping function declined due to an increase in the cardiac load. We found that urantide improved the structure and function of the right ventricle.

To determine the extent of right ventricular hypertrophy, our preliminary work [13] weighed the RV free wall and the left ventricle and septum (LV + S) separately and obtained the ventricular weight ratio as determined using the following equation: RV/(LV + S). We confirmed that the RV/(LV + S) ratio increased in the control groups, but it clearly decreased in the treatment groups. Our previous data [13] also demonstrated that urantide decreased mPAP as determined by a float catheter by 27.6 and 25.8% in the early- and late-treatment groups, respectively. In this study, we focused on evaluating the right ventricle as mentioned above and reached similar conclusions.

Normal pulmonary hemodynamics has the characteristics of low resistance, low pressure, large capacity, and good compliance. When pulmonary artery pressure rises continuously, secondary changes in heart hemodynamics occur, leading to compensatory hypertrophy of myocardial cells and hyperplasia of fibroblasts in the right ventricle [29, 30]. At an advanced stage, increasing myocardial cells and capillary blood do not fit each other, leading to decrease in myocardial transmission function and active contractile force, followed by myocardial degeneration and fibrosis, increasing stiffness. The time of right ventricular pressure rising to pulmonary artery pressure increases in the early phase, whereas that of the pressure dropping to pulmonary artery pressure decreases in the late phase; thus, some corresponding shape variations occur in the blood spectrum of the pulmonary artery and the right ventricular outflow tract. Specific features of spectral parameters in this context include the shortening of TTP and ET and the reduction in peak velocity. The above indexes were clearly improved in the urantide treatment group, suggesting that urantide could not only lower PAH and protect heart function but also increase right cardiac output, decrease end-diastolic volume load, postpone right ventricular end-diastolic diameter enlargement, and improve cardiac muscle reconstruction. Therefore, urantide can prevent and improve decreases in cardiac function due to PAH.

Despite the abundance of studies performed on this issue, the pathogenesis of PAH has remained unclear. Multiple pathogenic pathways have been implicated in the development of PAH, including those at the molecular and genetic levels and in the smooth muscle and endothelial cells and adventitia. Such development was thought to involve pulmonary vasoconstriction, thrombogenesis, and pneumonic vascular system reconstruction in combination. The present results indicate that UII may be involved in the processing of MCT-induced PAH, and the effects could be blocked by the UII receptor antagonist urantide. Urantide can relieve MCT-induced PAH in rats and also postpone the increase of pulmonary arterial pressure after MCT induction. It is anticipated that further studies will be performed to confirm these results. There is also a need for strategies to overcome the challenge of translating novel experimental findings into clinical practice.

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Compliance with ethical standards All experiments were approved by the Ethics Committee of Experimental Research in the First Affiliated Hospital of Harbin Medical University and conformed to the Guide for the Care and Use of Laboratory Animals.

Disclosures None.

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