Cystamine slows but not inverses the progression of monocrotaline induced-pulmonary arterial hypertension in rats
Cystamine slows but not inverses the progression of monocrotaline-induced pulmonary arterial hypertension in rats

Han-Ming Wang, Wan-Zhu Liu, Fu-Tian Tang, Hai-Juan Sui, Xing-Jie Zhan, Hong-Xin Wang

H.-M. Wang, F.-T. Tang, H.-J. Sui. Department of Pharmacology, College of Basic Medicine, Jinzhou Medical University, Jinzhou, 121001, China.

W.-Z. Liu. Experimental Teaching Center of Basic Medicine, Jinzhou Medical University, Jinzhou, 121001, China.

X.-J. Zhan. Grade 2012 Clinical Class 6, Jinzhou Medical University, Jinzhou, 121001, China.

H.-X. Wang. Department of Pharmacology, College of Basic Medicine; Key Laboratory of Cardiovascular and Cerebrovascular Drug Research of Liaoning Province, Jinzhou Medical University, Jinzhou, 121001, China.

Corresponding author: H.-X. Wang (email: hongxinwang@lnmu.edu.cn).
Abstract:

Tissue transglutaminase (TG2) plays an important role in pulmonary arterial hypertension (PAH). Previous researches indicate that TG2 and protein serotonylation catalyzed by TG2 are upregulated in PAH. Serotonin transporter inhibitor fluoxetine ameliorates PAH via inhibition of protein serotonylation. It is still unknown whether PAH is inhibited through direct inhibition of TG2. Therefore, the present study aimed to investigate the effects of TG2 inhibitor cystamine on monocrotaline-induced PAH in rats. Rats were treated with monocrotaline (60 mg·kg\(^{-1}\), i.p.) in combination with or without cystamine (20, 40 mg·kg\(^{-1}\)·d\(^{-1}\), o.p.). The results showed that compared with monocrotaline alone, combination of monocrotaline with cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\), o.p.) relieved right ventricle hypertrophy, inhibited pulmonary arteriolar remodeling and downregulated protein expression of TG2, phosphorylated protein kinase B (Akt) and extracellular regulated protein kinase (ERK) at day 21. However, except for TG2 expression, these changes were not significantly inhibited by cystamine at day 35. In addition, cystamine dose-dependently enhanced the survival rate of rats injected with monocrotaline at day 35. The findings suggest that cystamine slows but not reverses monocrotaline-induced PAH in rats, which was largely associated with the inhibition of TG2 protein expression and Akt and ERK activation.

Keyword:

Akt; Cystamine; ERK; Monocrotaline; Pulmonary arterial hypertension; Serotonin; Transglutaminase
**Introduction**

Pulmonary arterial hypertension (PAH) is characterized by an increase in mean pulmonary artery pressure, due to pulmonary vascular remodelling that leads to an imbalance between excessive vasoconstrictors and low levels of vasodilators (Siqueira et al. 2017). The treatment of PAH involves the use of a diverse group of drugs that target the pulmonary vascular bed to decrease pulmonary arterial pressure (Pankey et al. 2016). These agents include prostacyclin and its analogs, calcium channel blockers and endothelin receptor antagonists, phosphodiesterase type 5 inhibitors, anticoagulants, and soluble guanylate cyclase stimulator (Pankey et al. 2016). Current therapies predominantly target pulmonary vasoconstriction rather than proliferative vascular remodeling, and therefore, new strategies are urgently required to directly address the pathological remodeling that underpins the disease (Thompson and Lawrie 2017).

Extensive clinical and experimental studies have implicated serotonin (5-HT) and serotonin transporter (5-HTT) in the pathologic pulmonary vascular smooth muscle remodeling that underlies PAH (Penumatsa et al. 2014a; Thomas et al. 2013). Increased 5-HT has been demonstrated in both patient and rodent models with PAH (Thomas et al. 2013). 5-HT induces the proliferation of pulmonary artery smooth muscle cells (PASMCs) derived from animal and human-being (Han et al. 2015; Maclean and Dempsie 2010). The serotonin transporter (5-HTT) has also been shown to be involved in proliferation of human, bovine and rodent PASMCs (Maclean and Dempsie 2010). The transgenic mice overexpressing the 5-HTT gene globally, display an exaggerated pulmonary arterial remodeling in response to chronic hypoxia, while mice genetically lacking 5-HTT develop less PAH and vascular remodeling in response to chronic hypoxia (Thomas et al. 2013). Moreover, our previous study showed that 5-HTT inhibitor fluoxetine inhibited monocrotaline (MCT)-induced PAH in rats (Wang et al. 2012).

There is increasing awareness of the participation of protein serotonylation, the interaction of protein and serotonin, and tissue transglutaminase (TG2), catalyzing the
reaction, in PAH (Wei et al. 2012). For example, Guilluy et al. reported that lungs, platelets, and PASMCs from patients with PAH were characterized by marked elevation in RhoA serotonylation (Guilluy et al. 2009). The 5-HTT inhibitor fluoxetine and TG2 inhibitor monodansylcadaverin prevented 5-HT-induced RhoA serotonylation and RhoA/Rho kinase activation, as well as proliferation of PASMCs from pulmonary hypertensive patients (Guilluy et al. 2009). Our previous studies have shown elevated RhoA serotonylation in the lungs of pulmonary arterial hypertensive rats, which was inhibited by fluoxetine (Wang et al. 2012). Furthermore, the marked elevation of TG2 activity was detected in lungs of a hypoxia/Sugen mouse model of pulmonary hypertension (DiRaimondo et al. 2014). TG2 was also significantly upregulated, which was associated with right ventricular hypertrophy in rats induced by chronic hypoxia (Baandrup et al. 2011). In addition, TG2 is directly responsive to hypoxia by enhancement of both its protein expression and activity in PASMCs (Penumatsa et al. 2014b). However, it remains unclear whether the TG2 inhibitors inhibit MCT-induced PAH in vivo.

Serotonin-induced mitogenesis of PASMCs requires the coordinate signaling of the protein kinase B (Akt) and extracellular regulated protein kinase (ERK) pathways (Liu and Fanburg 2006; Liu et al. 2004). The TG2 inhibitor monodansylcadaverin was reported to exhibit inhibitory effect on Akt and ERK activation, just as fluoxetine inhibits 5-HT-induced these pathways in PASMCs (Guilluy et al. 2007; Penumatsa et al. 2014a; Song et al. 2005). Furthermore, these effects on the Akt pathway were confirmed by studies using TG2 knock-down (Penumatsa et al. 2014a). The precipitated material was immunoblotted for both Akt and TG2, and it was found that 5-HT treatment results in increased binding of Akt with TG2 (Penumatsa et al. 2014a). And this association was reduced upon pre-treatment of cells with monodansylcadaverin (Penumatsa et al. 2014a). Therefore, in order to ascertain the effect of TG2 inhibitor against PAH, cystamine, which is extensively used in vivo study compared with others (Nurminskaya and Belkin 2012), inhibites both inward and outward remodelling (Eftekhari et al. 2007) and moderates right ventricular hypertrophy induced by hypoxia (Li et al. 2009), was applied in the present study to
focus on its effect against MCT-induced PAH and relationship between TG2 and Akt and ERK pathways in rats.

**Method**

**Animal and group**

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Jinzhou Medical University. All the animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011, published by The National Academies Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). Ninety-eight male Sprague-Dawley rats (156 to 182 g body weight) were used. All rats were housed under controlled temperature (20–26°C) and humidity (55%–70%) in a 12 h light/dark cycle, with access to water and food ad libitum. In experiment I, 40 rats were randomly divided into control (CTL) group, MCT group, MCT+CYS20 and MCT+CYS40 groups, containing 10 animals in each group. The preparation for rat model of severe PAH with a high mortality was as described previously (Abe et al. 2004). Briefly, except for the rats in the CTL group, all rats in other groups were intraperitoneally administered with MCT (60 mg·kg\(^{-1}\)) once followed by intragastric administration of vehicle in MCT group, of cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)) in MCT+CYS20 group or cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)) in MCT+CYS40 group for 3 weeks. In experiment II, fifty-eight rats were also randomly divided into four groups: CTL group (n=10), MCT group (n=24, MCT 60 mg·kg\(^{-1}\)), MCT+CYS20 group (n=12, MCT 60 mg·kg\(^{-1}\) and cystamine 20 mg·kg\(^{-1}\)·d\(^{-1}\)), MCT+CYS40 group (n=12, MCT 60 mg·kg\(^{-1}\) and cystamine 40 mg·kg\(^{-1}\)·d\(^{-1}\)). But all the live rats were dealt at the 35\(^{th}\) day. The route for administration of each drug is same as that in experiment I.

**Survival analysis**

Survival rates of rats were examined in experiment II. The day of MCT injection was defined as day 0. The survival analysis covered the entire experimental period to the 35\(^{th}\) day.

**Assessment of PAH**

At the 21\(^{st}\) day, the rats in experiment I were anesthetized with sodium pentobarbital
(40 mg·kg$^{-1}$, i.p.). Pulmonary arterial pressure and systemic arterial pressure were measured as described previously (Wang et al. 2012). After the assessment of PAH, the pulmonary vessels of all rats from each group were perfused with normal saline. Then some rats were euthanized with an overdose of sodium pentobarbital and their hearts were dissected and weighted. The weight ratio of the right ventricle (RV) to the left ventricle plus septum (LV+S) was used to evaluate the extent of right ventricular hypertrophy.

At the 35$^{th}$ day, after perfusion with normal saline, some rats in experiment II were euthanized and their hearts were also dissected, weighted and evaluated using the same method.

**Morphometric analysis of pulmonary arterioles**

After perfusion with normal saline, the pulmonary vessels of 3 rats from each group in Experiment I and II were continuously perfused with 4% paraformaldehyde. Then, the rats were euthanized and the lower lobes of the right lungs were placed into 4% paraformaldehyde for fixing. The lung tissue sections were prepared for morphometric analysis by using hematoxylin and eosin staining. Arterioles (3 rats each group, 8 pulmonary arterioles each rat) of about 50 µm diameter were evaluated for measurement of medial wall thickness and wall area of pulmonary arterioles at a magnification of 400 ×. For each arteriole, the medial wall thickness and wall area were expressed as following formula:

$$\text{Medial wall thickness(%) } = \frac{\text{external diameter} - \text{internal diameter}}{\text{external diameter}} \times 100\%$$

$$\text{Wall area(%) } = \frac{\text{total vessel area} - \text{lumen area}}{\text{total vessel area}} \times 100\%$$

**Western Blot analysis**

After the rats were euthanized at day 21 or day 35 in each group. These lower lobes of right lungs were immediately frozen with liquid nitrogen. The proteins were extracted and separated by SDS polyacrylamide gel electrophoresis followed by being transferred to PVDF membranes (Bio-Rad). After incubation in blocking buffer (5% nonfat dry milk) at room temperature for 2 h, the PVDF membranes were incubated
with anti-TG2 antibody (1:800; Cell Signaling Technology), anti-5-HTT antibody (1:400; Biosynthesis Biotechnology), anti-Akt antibody (1:800; Cell Signaling Technology), anti-phospho-Akt antibody (1:800; Cell Signaling Technology), rabbit polyclonal anti-ERK antibody (1:800; Cell Signaling Technology), mouse monoclonal anti-phospho-ERK antibody (1:800; Cell Signaling Technology), or rabbit polyclonal anti-β-actin antibody (1:2000; Santa Cruz Biotechnology) overnight at 4°C. Proteins were detected using HRP-conjugated secondary antibody (1:2000; Abcam). The signal was visualized with SuperSignal West Pico chemiluminescent substrate (Thermo Fisher). After the images were acquired, the bands were quantified using Quantity One. The results are presented as relative protein expression normalized to signal intensity of β-actin protein.

Statistical Analysis

Data are expressed as mean ± S.D. Survival curves were analyzed by Kaplan Meier method followed by a log-rank test. Differences in all other parameters were evaluated by ANOVA followed by Tukey-Kramer’s multiple comparison test with SPSS 13.0. A value of \( P < 0.05 \) was considered to be statistically significant.

Results

Effects of cystamine on survival rate of rats treated with MCT

As shown in Fig. 1, survival rate of the MCT group at day 35 was only 33.3% (\( n=24 \)). The cystamine treatment (20 and 40 mg·kg\(^{-1}·d^{-1}\)) dose-dependently improved the survival rate which were 75% in the MCT+CYS20 group (\( n=12 \)) and 91.7% in the MCT+CYS40 group (\( n=12 \)).

Effect of cystamine on pulmonary arterial pressure

The rats exposed to MCT for 3 weeks, except for two rats which have died before detection, showed a strong increase in mean pulmonary arterial pressure. Concurrent cystamine administration led to a dose-dependent reduction in mean pulmonary arterial pressure compared with that in MCT group, which was not statistically significant (Tab. 1). Although the cystamine dosing schedule showed mild reduction of mean systemic arterial pressure compared with MCT group, the difference was not
statistically significant (Tab. 1). Similarly, the difference in heart rate and body weight among groups was not statistically different (Tab. 1).

**Effect of cystamine on the weight ratio of RV to LV+S**

MCT induced increase in the weight ratio of RV to LV+S of rats at day 21 and cystamine (20 and 40 mg·kg\(^{-1}\)·d\(^{-1}\)) inhibited the alteration in a dose dependent manner (Fig. 2A). At day 35, the weight ratio of RV to LV+S of rats in MCT group was still significantly higher than that in CTL group. But, cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)) did not significantly inhibit the increased ratio induced by MCT, which is different from day 21 (Fig. 2B).

**Effect of cystamine on pulmonary arteriolar remodeling**

Pulmonary arteriolar remodeling was determined by hematoxylin and eosin staining (Fig. 3A-3H). At day 21, the percent medial wall thickness and the percent wall area of pulmonary arterioles were significantly increased in the MCT group compared with that in CTL group, which were significantly reduced in MCT+CYS20 and MCT+CYS40 groups (Fig.3I, 3K). At day 35, the percent medial wall thickness and the percent wall area of pulmonary arterioles were also significantly increased in the MCT group, but cystamine showed no effect on these parameters compared with that in the MCT group (Fig.3J, 3L).

**Effects of cystamine on transglutaminase and its downstream signaling pathway**

Compared with that of rats in CTL group, MCT markedly increased the protein expression of TG2, phosphorylated Akt and phosphorylated ERK in lung tissues at day 21 (Fig. 4A, 4C, 4E). But these MCT-induced changes were inhibited by TG2 inhibitor cystamine in a dose dependent manner (Fig. 4A, 4C, 4E). At day 35, the protein expression of TG2, phosphorylated Akt and phosphorylated ERK in the MCT group exhibited the similar pattern as in the MCT group at day 21 (Fig. 4B, 4D, 4F). Despite the lack of change in Akt phosphorylation and ERK phosphorylation observed, treatment with cystamine was associated with an markedly decrease in TG2 protein expression in the lung of rats in MCT+CYS20 and MCT+CYS40 groups (Fig. 4B, 4D, 4F).

**Effect of cystamine on serotonin transporter**
5-HTT protein expression was significantly upregulated in the lungs of rats from the MCT group compared with CTL group at day 21 and 35, respectively (Fig. 5). Cystamine (20, 40 mg·kg\(^{-1}\)·d\(^{-1}\)) had no significant effect on 5-HTT expression at day 21 or day 35 (Fig. 5).

**Discussion**

We demonstrate in this study that TG2 inhibitor cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)) decreased pulmonary arterial pressure of rats injected with MCT, which was not statistically significant, but markedly relieved the right ventricle hypertrophy and alleviated the pulmonary arteriolar remodeling at day 21. At day 35, cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)) had no effects on the above mentioned parameters, but observably enhanced survival rate of rats injected with MCT. The results suggest that although cystamine does not reverse MCT-induced PAH in rats, it delays the process of PAH.

An increasing number of studies have implicated TG2 and TG2 catalytic protein serotonylation in PAH. For example, the previous findings demonstrate that enhanced serotonylation of fibronectin occurs in sera of patients with PAH (Wei et al. 2012). Elevated transglutaminase 2 activity is associated with hypoxia-induced experimental pulmonary hypertension in mice (DiRaimondo et al. 2014). Based on the facts that TG2 enhanced inward remodeling of small arteries at the cell membrane of smooth muscle cells (van den Akker et al. 2011), increased the more contractile response of the aortic rings from TG2\(^{-/-}\) mice than those from wild type mice (Steppan et al. 2017), contributed to pulmonary vascular remodeling associated with PASMCs proliferation (Penumatsa et al. 2014b), and was identified as a potential target for intervention in the limitation of allergic inflammation (Soveg et al. 2015), we consider that the inhibition of TG2 might benefit for PAH. Cystamine, the TG2 expression and activity inhibitor (Kawabe et al. 2015), was used in the study, because it induced vasodilatation in rat mesenteric small arteries by inhibition of receptor-coupled TG2 (Engholm et al. 2016) and displayed decreased numbers of inflammatory cells in the lungs (Soveg et al. 2015). The results showed that although cystamine downregulates TG2 protein overexpression at either day 21 or day 35, it alleviated MCT-induced

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PAH in rats at day 21 rather than at day 35. The results suggest that inhibition of TG2 by cystamine is effective for delay of PAH, but not enough to reverse MCT-induced PAH in rats.

Since Akt and ERK, the downstream signaling component of TG2, were involved in PAH (Penumatsa et al. 2014a), we detected the Akt and ERK activity in lungs of rats. The results show that cystamine reduces Akt and ERK activation accompanied by the inhibition of PAH in rats injected by MCT at day 21. Our previous studies and others reported that different kinds of drugs, such as fluoxetine, epigallocatechin-3-gallate, nitrite, etc, inhibit PAH in rats accompanied by the decrease in Akt or ERK activation in vivo (Hu et al. 2017; Wang et al. 2012; Zhu et al. 2017). Moreover, in vitro, TG2 inhibitor monodansylcadaverin inhibited Akt activation down to background levels, and also inhibited ERK activation (Penumatsa et al. 2014a). In addition, monodansylcadaverin inhibited the mitogenesis of PASMCs (Penumatsa et al. 2014a).

In contrast, the present study demonstrated that cystamine with different doses had no effect on Akt and ERK activation and pulmonary arteriolar remodeling in MCT-induced pulmonary arterial hypertensive rats at day 35. That suggests that whether pulmonary arterioles are remodeled are consistent with whether Akt and ERK activate. Taken together, these results provide strong evidence for the important role of Akt and ERK in the PAH.

5-HT and 5-HTT has long since been found to be an important regulator of PAH. 5-HT is a vasoconstrictor and a vascular smooth muscle cell mitogen and the role of 5-HTT in the mitogenic effect of 5-HT on vascular smooth muscle cells has been ascribed to 5-HT internalization (Guilluy et al. 2007). The processes are linked to protein serotonylation and Akt and ERK activation, and was abolished by inhibition of 5-HTT (Guilluy et al. 2007; Song et al. 2005). Notably, 5-HT induced protein serotonylation, Akt and ERK activation and proliferation of PASMCs were also suppressed by inhibition of TG2 (Penumatsa et al. 2014a). Thus, 5-HTT and TG2 are both upstream signal component of Akt and ERK. In vivo, previous studies at our laboratory and others have indicated that established PAH was reversed to the almost normal level at day 21 and day 35 with the 5-HTT inhibitor fluoxetine treatment.
(Wang et al. 2012; Zhu et al. 2009). Meanwhile, our previous results connected inhibition of PAH with decrease of 5-HTT protein expression, protein serotonylation, and ERK and Akt activation (Wang et al. 2012). By contrast, in the present study, cystamine, which did not inhibit protein expression of 5-HTT, downregulated protein expression of TG2, the enzyme that catalyzes protein serotonylation, but did not alleviate MCT-induced PAH when over activation of Akt and ERK were not reduced at day 35. Thus, we think that inhibition of 5-HTT is better for inhibition of PAH than that of TG2. One possible interpretation is that 5-HTT activate downstream cell growth signal transduction pathways, such as Akt and ERK, via dependent or independent of TG2. In addition, serotonylation is not only TG2 mediated post-translational protein modification, other monoaminylation occurs simultaneously (Walther et al. 2011). They maybe affect Akt and ERK activity. Anyway, there may also be a few other potential reasons for those, so the correlated mechanisms worth continuing to study.

In conclusion, cystamine slows but not reverses MCT-induced PAH in rats via inhibition of TG2, which might be associated with suppression of Akt and ERK activation.

Acknowledgements

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References


Wei, L., Warburton, R.R., Preston, I.R., Roberts, K.E., Comhair, S.A., Erzurum, S.C., Hill, N.S., and


**Figure Legend**

1. **Fig. 1.** Effects of cystamine on survival of (A) rats with pulmonary arterial hypertension induced by monocrotaline. Survival rates were monitored during the treatment period from day 0 to 35. At day 0, monocrotaline and vehicle was injected intraperitoneally once and the different doses of cystamine begin to be administrated by gavage once a day for 5 weeks. \( n = 10 \sim 24; ^{**}, P < 0.01 \) compared with CTL; \(^{*}, P < 0.05 \) compared with MCT; \(^{##}, P < 0.01 \) compared with MCT. CTL: control group; MCT: monocrotaline (60 mg·kg\(^{-1}\)); MCT+CYS20: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)); MCT+CYS40: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)).

2. **Fig. 2.** Comparison of the weight ratio of RV to LV+S of rats in different groups at (A) day 21 and (B) day 35. Data are expressed as the mean ± SD. \( n = 5 \sim 8 \); \(^{**}, P < 0.01 \) compared with CTL; \(^{##}, P < 0.01 \) compared with MCT. CTL: control group; MCT: monocrotaline (60 mg·kg\(^{-1}\)); MCT+CYS20: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)); MCT+CYS40: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)).

3. **Fig. 3.** Pulmonary arteriolar remodeling in rats were illustrated by representative photomicrographs of pulmonary arterioles stained with hematoxylin and eosin from (A, E) the CTL group, (B, F) MCT group, (C, G) MCT+CYS20 group, (D, H) MCT+CYS40 group; and pulmonary arteriolar remodeling were measured as (I, J) the percentage of the medial wall thickness and (K, L) the percentage of the wall area of pulmonary arterioles at (A-D, I, K) day 21 and (E-H, J, L) day 35, respectively. Data are expressed as the mean ± SD. \( n = 24 \) measurements from 3 rats each group. Bar = 20 µm; \(^{**}, P < 0.01 \) compared with CTL; \(^{##}, P < 0.01 \) compared with MCT. CTL: control group; MCT: monocrotaline (60 mg·kg\(^{-1}\)); MCT+CYS20: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)); MCT+CYS40: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)).

4. **Fig. 4.** Comparison of (A, B) TG2 protein expression and (C, D) Akt and (E, F) ERK phosphorylation in lungs of rats from different groups at (A, C, E) day 21 and (B, D, F) day 35. Data are expressed as the mean ± SD. \( n = 3 \sim 4 \); \(^{**}, P < 0.01 \) compared with CTL; \(^{*}, P < 0.05 \) compared with MCT; \(^{##}, P < 0.01 \) compared with MCT. CTL: control group; MCT: monocrotaline (60 mg·kg\(^{-1}\)); MCT+CYS20: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)); MCT+CYS40: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)).

5. **Fig. 5.** Comparison of 5-HTT protein expression in lungs of rats from different groups at (A) day 21 and (B) day 35. Data are expressed as the mean ± SD. \( n = 3 \sim 4 \); \(^{**}, P < 0.01 \) compared with CTL. CTL: control group; MCT: monocrotaline (60 mg·kg\(^{-1}\)); MCT+CYS20: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (20 mg·kg\(^{-1}\)·d\(^{-1}\)); MCT+CYS40: monocrotaline (60 mg·kg\(^{-1}\)) + cystamine (40 mg·kg\(^{-1}\)·d\(^{-1}\)).
Tab. 1. Comparison of hemodynamic measurement of rats in the different groups at day 21

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MCT</th>
<th>MCT+CYS20</th>
<th>MCT+CYS40</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>330±18</td>
<td>238±40**</td>
<td>264±41</td>
<td>261±44</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>354±31</td>
<td>334±44</td>
<td>333±32</td>
<td>336±39</td>
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<tr>
<td>mSAP (mm Hg)</td>
<td>141±10</td>
<td>132±19</td>
<td>124±16</td>
<td>124±13</td>
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<tr>
<td>mPAP (mm Hg)</td>
<td>17.0±1.8</td>
<td>35.3±8.4**</td>
<td>33.2±8.6</td>
<td>26.7±7.2 (P=0.06)</td>
</tr>
</tbody>
</table>

Note: BW, body weight; HR, heart rate; bpm, beats per minute; mSAP, mean systemic arterial pressure; mPAP, mean pulmonary arterial pressure. At day 0, monocrotaline and vehicle was injected intraperitoneally once and the different doses of cystamine begin to be administrated by gavage once a day for 3 weeks. All data are expressed as the mean ± SD. n = 8 ~ 10. **, P < 0.01 compared with CTL. CTL: control group; MCT: monocrotaline (60 mg·kg⁻¹); MCT+CYS20: monocrotaline (60 mg·kg⁻¹) + cystamine (20 mg·kg⁻¹·d⁻¹); MCT+CYS40: monocrotaline (60 mg·kg⁻¹) + cystamine (40 mg·kg⁻¹·d⁻¹).
Fig. 1. Effects of cystamine on survival of (A) rats with pulmonary arterial hypertension induced by monocrotaline. Survival rates were monitored during the treatment period from day 0 to 35. At day 0, monocrotaline and vehicle was injected intraperitoneally once and the different doses of cystamine begin to be administrated by gavage once a day for 5 weeks. n = 10 ~ 24; **, P < 0.01 compared with CTL; #, P < 0.05 compared with MCT; ##, P < 0.01 compared with MCT. CTL: control group; MCT: monocrotaline (60 mg•kg-1); MCT+CYS20: monocrotaline (60 mg•kg-1) + cystamine (20 mg•kg-1•d-1); MCT+CYS40: monocrotaline (60 mg•kg-1) + cystamine (40 mg•kg-1•d-1).
Fig. 2. Comparison of the weight ratio of RV to LV+S of rats in different groups at (A) day 21 and (B) day 35. Data are expressed as the mean ± SD. n = 5~8. **, P < 0.01 compared with CTL; #, P < 0.05 compared with MCT; ##, P<0.01 compared with MCT. CTL: control group; MCT: monocrotaline (60 mg•kg⁻¹); MCT+CYS20: monocrotaline (60 mg•kg⁻¹) + cystamine (20 mg•kg⁻¹•d⁻¹); MCT+CYS40: monocrotaline (60 mg•kg⁻¹) + cystamine (40 mg•kg⁻¹•d⁻¹).
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Fig. 5. Comparison of 5-HTT protein expression in lungs of rats from different groups at (A) day 21 and (B) day 35. Data are expressed as the mean ± SD. n = 3 ~ 4. **, P < 0.01 compared with CTL. CTL: control group; MCT: monocrotaline (60 mg•kg-1); MCT+CYS20: monocrotaline (60 mg•kg-1) + cystamine (20 mg•kg-1•d-1); MCT+CYS40: monocrotaline (60 mg•kg-1) + cystamine (40 mg•kg-1•d-1).