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Dimethylarginine dimethylaminohydrolase 1 deficiency aggravates monocrotaline-induced pulmonary oxidative stress, pulmonary arterial hypertension and right heart failure in rats

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ABSTRACT

Patients with pulmonary arterial hypertension (PAH) and right ventricular (RV) failure have a poor clinical outcome, but the mechanisms of PAH and RV failure development are not totally clear. PAH is associated with reduced NO bioavailability and increased endogenous NOS inhibitor asymmetric dimethylarginine (ADMA). Dimethylarginine dimethylaminohydrolase-1 (DDAH1) plays a critical role in ADMA degradation. Here we generated a novel DDAH1 deficiency rat strain using the CRISPR-Cas9 technique, and studied the effect of DDAH1 dysfunction on monocrotaline-induced PAH, lung vascular remodeling and RV hypertrophy. DDAH1 knockout resulted in abolished DDAH1 expression in various tissues, and significant increases of plasma and lung ADMA content. DDAH1 knockout has no detectable effect on cardiac and lung structure, and LV function under control conditions in rats. However, DDAH1 knockout significantly aggravated monocrotaline-induced lung and RV oxidative stress, lung vascular remodeling and fibrosis, pulmonary hypertension and RV hypertrophy in rats. DDAH1 KO resulted in significantly greater increases of plasma and lung ADMA content under control conditions. In the wild type rats monocrotaline resulted in significant increases of plasma and lung ADMA contents and reduction of lung eNOS protein content and these changes were more marked in DDAH1 KO rats. Together, our results demonstrated that DDAH1 plays an important role in attenuating monocrotaline-induced lung oxidative stress, pulmonary hypertension and RV hypertrophy in rats.

1. Introduction

Pulmonary arterial hypertension (PAH) is a disease characterized by progressively increased pulmonary arterial pressure due to aberrant pulmonary artery remodeling and vasoconstriction, which ultimately results in right ventricular (RV) hypertrophy and consequent uncompensated RV failure [1]. RV hypertrophy and failure in response to the increased pulmonary pressure overload are crucial determinants of prognosis in patients with PAH [2,3]. Although the detailed mechanisms of PAH and RV failure development are not totally clear, studies have consistently demonstrated that methods to enhance nitric oxide (NO)/cGMP signaling are effective in treating PAH [1].

Asymmetric dimethylarginine (ADMA) is an endogenous NO synthases (NOS) inhibitor that competes with L-arginine and, as a result, attenuates NOS activity and NO production [4]. In mammals, there are at least three main NOS isozymes, neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) [5]. Increased ADMA is associated with various cardiovascular diseases such as pulmonary hypertension, atherosclerosis, hypertension, and chronic renal diseases etc. [6,7]. Dimethylarginine dimethylaminohydrolase-1 (DDAH1) degrades ADMA [8,9], and in fact appears to be the critical enzyme for ADMA degradation [10]. Thus, global DDAH1 KO not only results in increases of tissue and plasma ADMA, but also results in abolished ADMA degradation capacity in mice [10]. Chronic hypoxia-induced PAH is associated with reduced lung DDAH1 protein expression, increased ADMA concentration and decreased NO production in rats [11]. However, it is unclear whether DDAH1 dysfunction or chronic ADAM accumulation is sufficient to cause spontaneous PAH and RV hypertrophy under control conditions or exacerbate PAH development and RV hypertrophy after stressed conditions such as after MCT injection, a commonly used method to generate PAH in rats.

Here, by using a novel DDAH1 knockout ($DDAH1^{-/-}$) rat strain generated in our laboratory, we studied the effect of DDAH1 dysfunction on monocrotaline-induced PAH, lung vascular remodeling and RV hypertrophy in rats. Our findings indicate that DDAH1 plays an important role in attenuating monocrotaline-induced PAH development and RV hypertrophy.

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2. Methods

Detailed methods are available in the online-only Data Supplement.

2.1. Animals and experimental design

CRISPR-Cas9 technique was used to generate DDAH1$^{-/-}$ rats on Sprague-Dawley background using the strategy as illustrated in Fig. 1. Male DDAH1 global-knockout rats and wild-type control rats with bodyweight ~120 g were intraperitoneally injected with a single dose of MCT, 40 mg/kg (Sigma-Aldrich, St Louis, MO, USA) or an equivalent volume of saline as a control as previously described [12]. All animals were housed in a room with a 12-hour light/dark cycle (6 AM to 6 PM light), constant temperature (24 ± 1 °C) and humidity (45–50%). Twenty-four days after MCT injection, RV pressure was determined, then the rats were sacrificed and the major organs were collected for biochemical and histological analysis. All procedures with animals were approved by the Animal Care and Use Committee of Tenth Peoples' Hospital.

2.2. Statistical analysis

All of the values are expressed as mean ± SEM or median (±SEM). Data of 2 groups were compared with unpaired *t*-test. Two-way ANOVA was used to test for differences between KO and wild-type animals under control conditions and after MCT treatment. If analysis of variance demonstrated a significant effect, post hoc pairwise comparisons were made using the Fisher's least significant difference test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. DDAH1 knockout in rats results in abolished DDAH1 expression in all tissues tested

As presented in Fig. 1, the experimental approach resulted in deletion of exon1 of DDAH1 in DDAH1$^{-/-}$ rats (Fig. S1A–B). DDAH1$^{-/-}$ totally abolished DDAH1 protein expression in heart, lung, liver, kidney, spleen, skeletal muscle and brain tissues (Fig. S1C). DDAH2 protein expression was unchanged in DDAH1$^{-/-}$ rats. The DDAH1$^{-/-}$ rats showed no detectable gross defect in development and growth, a finding that is consistent with the prior observations in global DDAH1$^{-/-}$ mouse strain [10].

3.2. DDAH1$^{-/-}$ has no detectable effect on PAH and RV hypertrophy in rats under control conditions

We found that DDAH1$^{-/-}$ had no detectable effect on PAH development and RV hypertrophy under control conditions as evidenced by similar systolic RV pressure, RV end-diastolic pressure, and RV contractility between wild type and DDAH1$^{-/-}$ rats (Fig. 1A–D, Table S1). In addition, we found that DDAH1$^{-/-}$ had no effect on bodyweight gain in rats under control conditions (Fig. 1E). DDAH1$^{-/-}$ also had no effect on RV weight, the ratio of RV weight to bodyweight or tibial length, and the ratio of RV weight to LV weight plus septum weight under control conditions (Fig. 1F–H, Table S2).

3.3. DDAH1$^{-/-}$ exacerbated MCT-induced PAH, RV hypertrophy and RV dysfunction

While MCT resulted in significant increases of RV systolic pressure in both wild type and DDAH1$^{-/-}$ rats, DDAH1$^{-/-}$ significantly exacerbated MCT-induced increases of RV systolic pressure, RV diastolic pressure, RV dp/dtmax, and RV dp/dtmin as compared to WT mice (Fig. 1A–D). DDAH1$^{-/-}$ had no detectable effect on the increase of bodyweight under control conditions, or after MCT injection (Fig. 1E). Consistent with the greater increase of RV systolic pressure in DDAH1$^{-/-}$ rats after MCT, DDAH1$^{-/-}$ rats also exhibited significantly greater increase of the ratio of RV weight to LV + septum weight, greater RV weight, and greater ratio of RV weight to body weight or tibial length (Fig. 1F–H, and Table S2). While DDAH1$^{-/-}$ had no detectable effect on MCT-induced increase of LV weight, as anticipated (Supplemental Fig. 2A, B, Table S2), DDAH1$^{-/-}$ did significantly exacerbate MCT-induced increase of the lung weight, and its ratio to bodyweight or tibial length (Supplemental Fig. 2C, D, Table S2). In addition, histological analysis showed that MCT-induced increases in RV cardiomyocyte size in both wild type and DDAH1$^{-/-}$ rats, and the increases of cardiomyocyte size were greater in DDAH1$^{-/-}$ rats as compared with wild type rats (Fig. 2A–C). Furthermore, DDAH1$^{-/-}$ significantly exacerbated MCT-induced RV fibrosis (Fig. 2D, E). DDAH1$^{-/-}$ also significantly exacerbated MCT-induced RV ANP protein content (Fig. 2F, G). These results

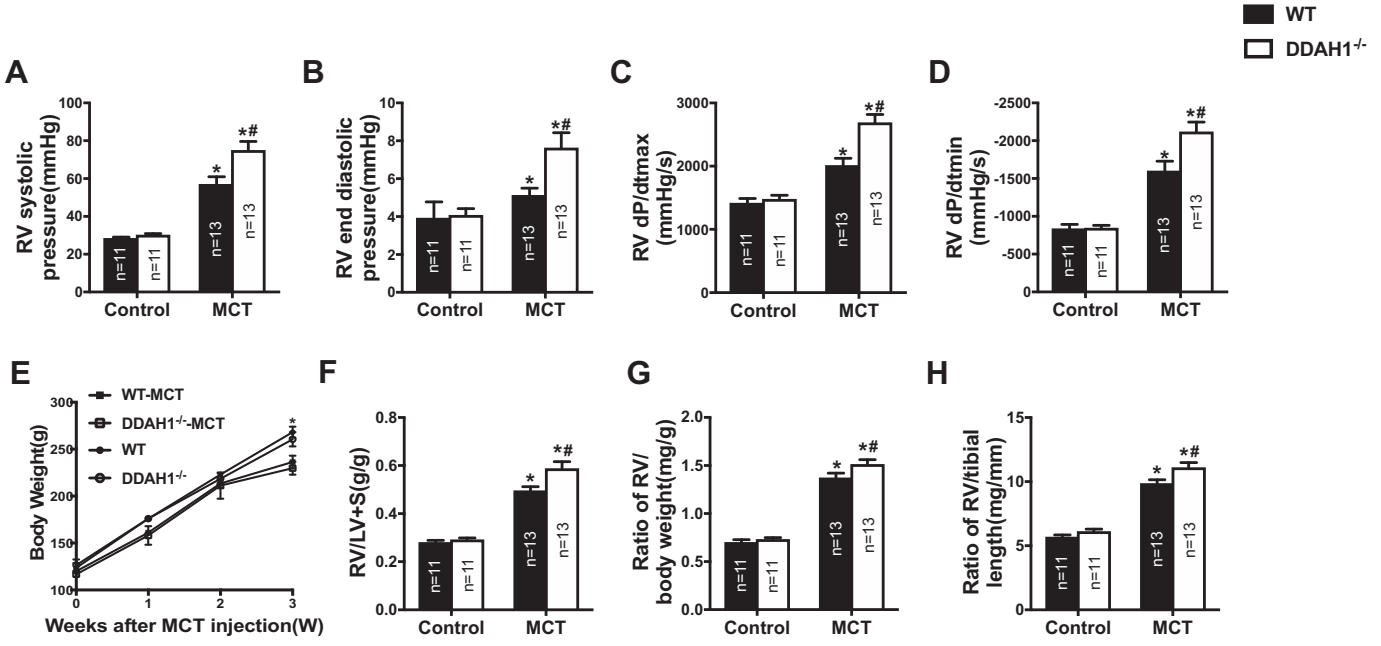

Fig. 1. DDAH1$^{-/-}$ exacerbated MCT-induced RV hemodynamic dysfunction in rats. A, RV systolic pressure (mmHg). B, RV end diastolic pressure (mmHg). C, RV dP/dtmax (mmHg/s). D, RV dP/dtmim (mmHg/s) in rats. E, Changing of body weight of rats in WT-control, DDAH1$^{-/-}$ control, WT-PAH and DDAH1$^{-/-}$-PAH groups. F, Ratio of RV weight to LV + septum weight (g/g). G, Ratio of RV weight to body weight. H, Ratio of RV weight to tibial length. Data are presented as mean ± SEM. *$p < 0.05$ vs. control group; #$p < 0.05$ vs wild-type (WT) rats.

indicated that DDAH1$^{-/-}$ aggravated MCT-induced PAH and RV hypertrophy in rats.

3.4. DDAH1$^{-/-}$ aggravated MCT-induced pulmonary vascular remodeling and lung fibrosis

We further determined lung partially muscularized (PM) and fully muscularized (FM) small arteries in wild type and DDAH1$^{-/-}$ rats under control conditions and after MCT by immunostaining of smooth muscle α-actin (Fig. 3A, B). The partially muscularized and fully muscularized small arteries in lung tissues were not different between wild type mice and DDAH1$^{-/-}$ rats under control conditions (Fig. 3A, B). MCT caused an increase of the number of lung fully muscularized small arteries in both wild type and DDAH1$^{-/-}$ rats (Fig. 3B), but the increase of lung muscularized small arteries was significantly greater in the DDAH1$^{-/-}$ rats as compared with corresponding wild type rats ($p < 0.05$) (Fig. 3B). Furthermore, HE staining also revealed severe lung vessel muscularization and lung vessel occlusion in DDAH1$^{-/-}$ rats (Fig. 3C). DDAH1$^{-/-}$ significantly exacerbated MCT-induced increase of lung medial wall thickness (Fig. 3D). Moreover, Masson's Trichrome Stain showed that lung fibrosis was not different between DDAH1$^{-/-}$ rats and wild type rats under control conditions, but MCT-induced lung fibrosis was significantly greater in the DDAH1$^{-/-}$ rats as compared with wild type rats ($p < 0.05$) (Fig. 3E, F). Overall, these data indicate that DDAH1$^{-/-}$ exacerbated MCT-induced lung vascular remodeling and fibrosis.

3.5. DDAH1$^{-/-}$ increased lung ADMA and exacerbated MCT-induced decrease of lung NO production

To further understand the impact of DDAH1$^{-/-}$ on lung NO metabolism, we determine lung DDAH1, eNOS, ADMA and NO production. As anticipated, Western blot showed that DDAH1 was totally abolished in lung tissues of DDAH1$^{-/-}$ rats (Fig. 4A, B). Interestingly, lung eNOS expression was significantly decreased in DDAH1$^{-/-}$ rats under control conditions. MCT caused significant decreases of lung eNOS protein expression in both wild type and DDAH1$^{-/-}$ rats (Fig. 4A, C), but the decrease of lung eNOS protein content was significantly greater in DDAH1$^{-/-}$ rats (Fig. 4A, C). Furthermore, DDAH1$^{-/-}$ caused a significant increase of plasma and lung ADMA under control conditions, and further exacerbated MCT-induced increase of lung ADMA content in rats (Fig. 4G, H). Moreover, MCT caused a significant decrease of lung NO production in both wild type and DDAH1$^{-/-}$ rats, and the decrease of lung NO production was significantly greater in the DDAH1$^{-/-}$ rats as compared with corresponding wild type rats ($p < 0.05$) (Fig. 4J). These results demonstrated that DDAH1$^{-/-}$ caused increase of lung ADMA content and a decrease of lung NO production in rats after MCT.

3.6. DDAH1$^{-/-}$ increased RV ADMA and exacerbated MCT-induced decrease of RV NO in rats

We further determined RV DDAH1 and eNOS protein contents, ADMA content and NO production. Western blot showed DDAH1 protein expression was undetectable in RV tissues in DDAH1$^{-/-}$ rats

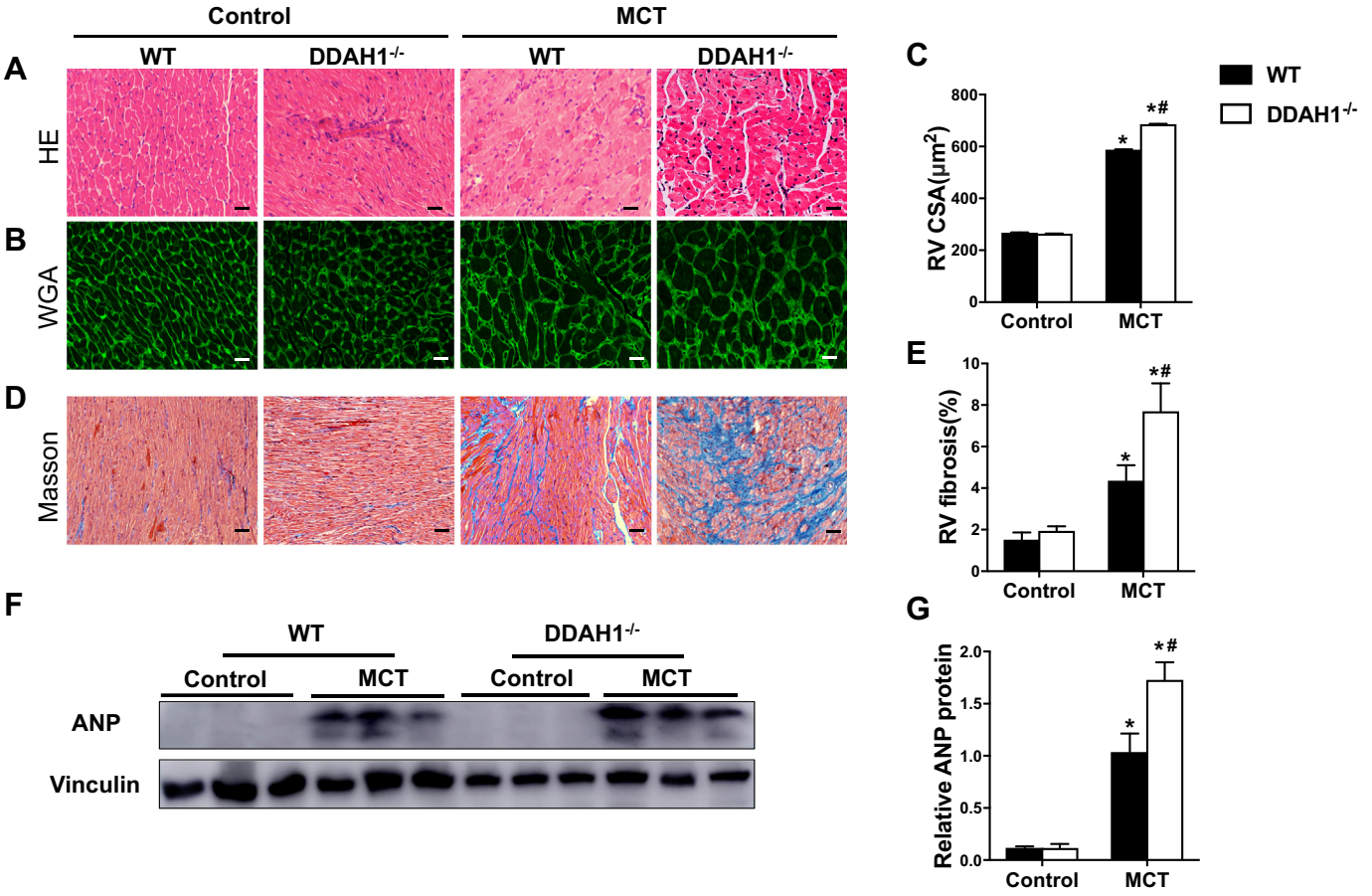

Fig. 2. DDAH1$^{-/-}$ exacerbated MCT-induced RV failure and fibrosis in rats. A, B, Representative images of HE and WGA staining for detection of myocyte size of RV. D, Masson trichrome staining for quantification of fibrotic areas in RV. C, E, Quantitative data of cross-section-area (CSA) and fibrosis in RV. n = 5 per group. F, G, Western blot and quantitative data for ANP in RV. n = 3 per group. Scale bar = 50 μm. Data are presented as mean ± SEM. *$p < 0.05$ vs. control group; #$p < 0.05$ vs wild-type (WT) rats.

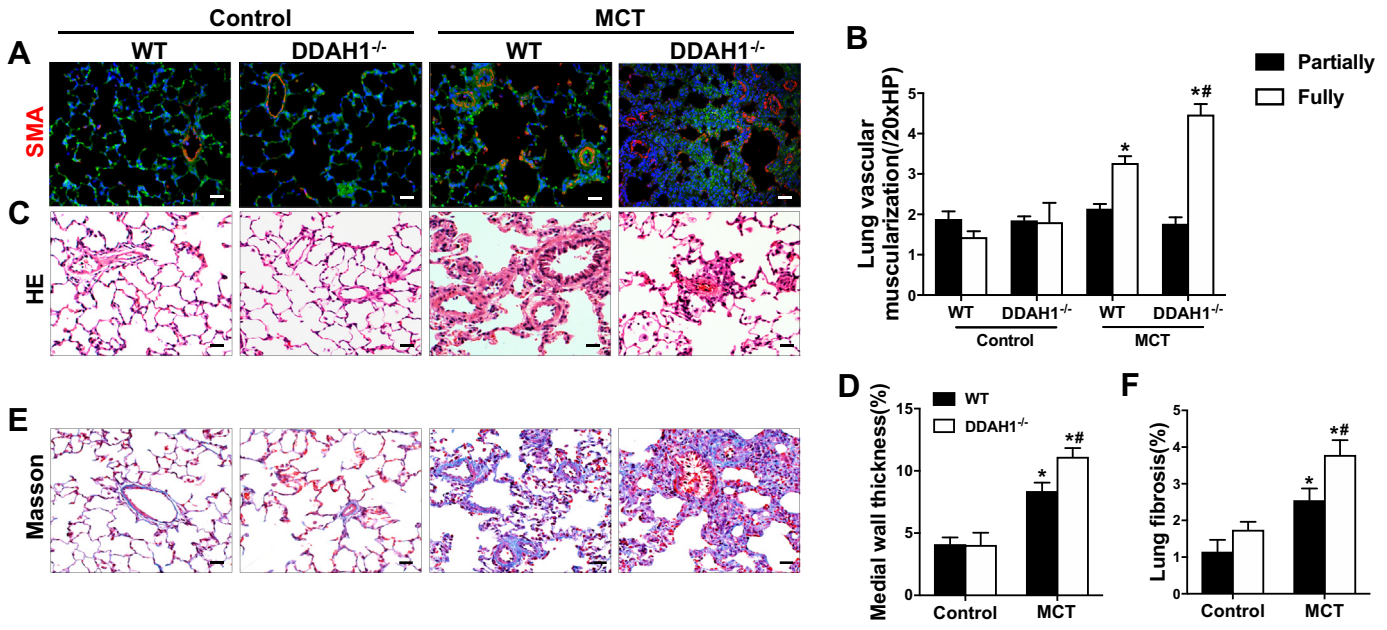

Fig. 3. DDAH1$^{-/-}$ exacerbated MCT-induced pulmonary vascular remodeling in rats. A, Representative images of lung smooth muscle α-actin staining to evaluate the extent of pulmonary vascular muscularization. C, Representative images of lung HE staining for pulmonary vascular medial wall thickness assessment. E, Representative images of lung trichrome staining for pulmonary fibrosis evaluation. B, Non-muscularized, partially muscularized, and completely muscularized pulmonary arterioles were calculated from 5 mice in each group, at least 5 images of each mouse were calculated. D, Quantitative data of pulmonary arteries (51–150 μm) medial wall thickness, at least 5 arteries (from 5 areas) and 5 samples of each group were calculated. F, Quantitative data of lung fibrosis. n = 5 per group. Scale bar = 50 μm. Data are presented as mean ± SEM. *$p < 0.05$ vs. control group; #$p < 0.05$ vs. wild-type (WT) rats.

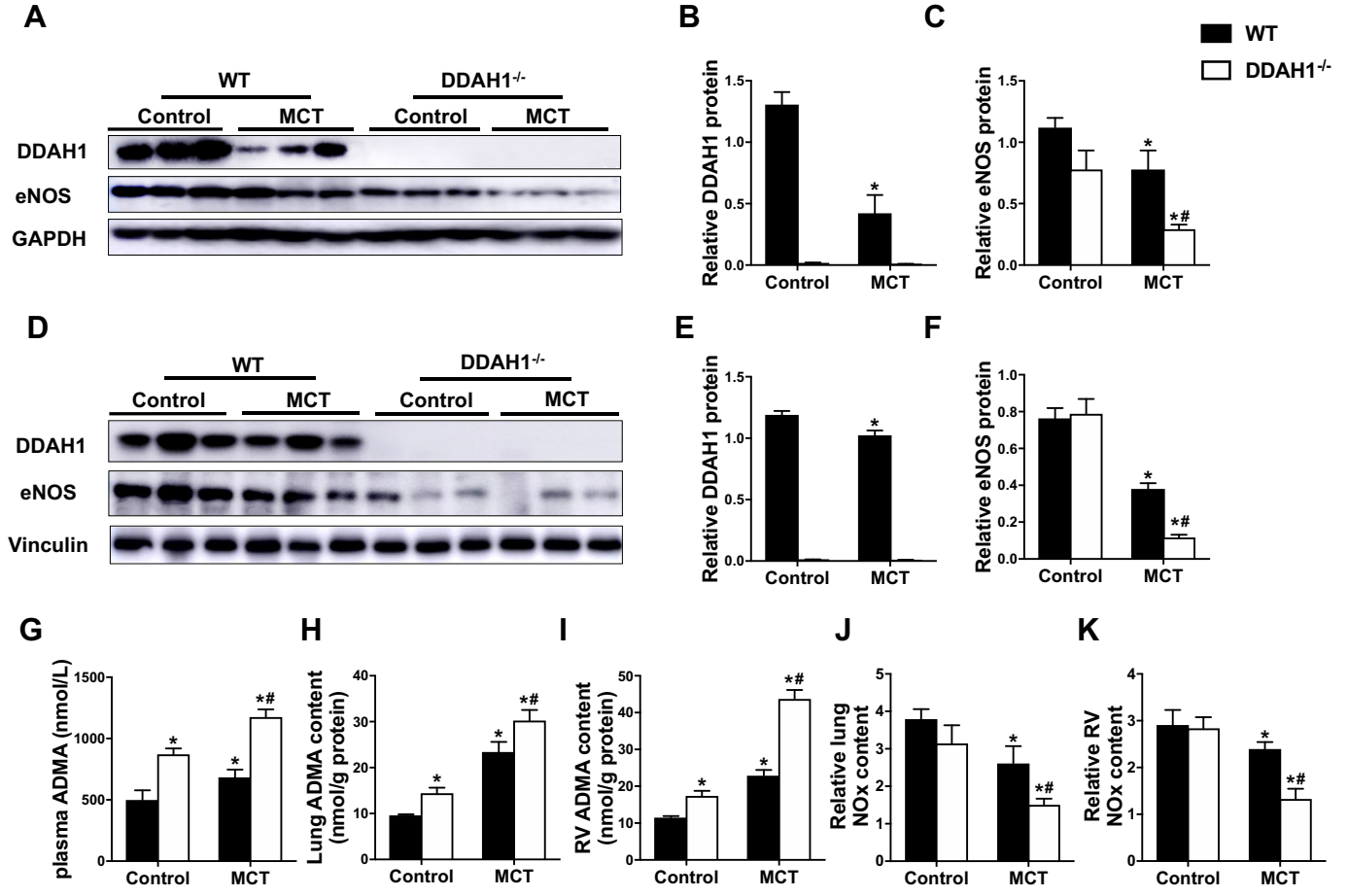

Fig. 4. DDAH1$^{-/-}$ exacerbated ADMA accumulation and eNOS reduction in lung and RV. A, Expression of DDAH1 and eNOS in lung were analyzed by western blot. B, C, Quantitative data of DDAH1 and eNOS in lung. D, Expression of DDAH1 and eNOS in RV were analyzed by western blot. E, F, Quantitative data of DDAH1 and eNOS in RV. n = 3 per group. G, H, I, Plasma, Lung and RV ADMA content. J and K, Relative NOx content in lung and RV. n = 5 per group. Data are presented as mean ± SEM. *$p < 0.05$ vs. control group; #$p < 0.05$ vs wild-type (WT) rats.

under both control conditions and after MCT (Fig. 4D, E). In addition, MCT caused a small but significant decrease of RV DDAH1 protein expression in wild type mice (Fig. 4D, E). While DDAH1$^{-/-}$ did not affect RV eNOS protein expression under control conditions (Fig. 4D, F), interestingly, MCT caused significantly decreases of RV eNOS protein expression in both wild type and DDAH1$^{-/-}$ rats (Fig. 4D, F), but the decrease of RV eNOS protein expression was significantly greater in the DDAH1$^{-/-}$ rats as compared with wild type rats ($p < 0.05$) (Fig. 4F). DDAH1$^{-/-}$ resulted in significant increases of RV ADMA content under control conditions (Fig. 4I), and DDAH1$^{-/-}$ further exacerbated MCT-induced increases of RV ADMA content in both wild type and DDAH1$^{-/-}$ rats (Fig. 4I). In addition, MCT caused significant decreases of RV NO production in both wild type and DDAH1$^{-/-}$ rats, and the decrease of NO production was significantly greater in the DDAH1$^{-/-}$ rats as compared with corresponding wild type rats (Fig. 4K). Those results demonstrated that DDAH1$^{-/-}$ caused an increase of RV ADMA, and a decrease of RV NO production in rats after MCT.

3.7. DDAH1$^{-/-}$ exacerbates MCT-induced pulmonary reactive oxygen species production

Since previous studies demonstrated that ADMA could increase NOS-derived reactive oxygen species (ROS) production and oxidative stress [13–15], and ROS contribute to PAH development [16], we postulated that DDAH1$^{-/-}$ might also exacerbate PAH development through increased lung oxidative stress. Consequently, DHE staining was performed to evaluate tissue ROS production. As expected, MCT stimulation caused a significant increase of lung ROS in wild type rats, and DDAH1$^{-/-}$ significantly exacerbated MCT-induced increase of lung ROS production (Fig. S3A, B). Moreover, DDAH1$^{-/-}$ caused a significant increase of lung 3-Nitrotyrosine (3-NT, a marker of oxidative stress) in rats under control conditions. MCT caused significant increases of lung 3-NT production in both wild type and DDAH1$^{-/-}$ rats, and the increase of lung 3-NT was ~6-fold greater in the DDAH1$^{-/-}$ rats as compared with wild type rats (Fig. S3C, D). Furthermore, DDAH1$^{-/-}$ significantly exacerbated MCT-induced increase of lung 4-hydroxynonenal (4-HNE) (Fig. S3C, E), another commonly used marker for oxidative stress.

3.8. DDAH1$^{-/-}$ exacerbates MCT-induced RV oxidative stress in rats

Since ROS also contribute to cardiac hypertrophy, and ADMA might also affect RV ROS production, we further determined RV oxidative stress in these experimental groups (Fig. S4). DHE staining demonstrated that MCT stimulation caused a significant increase of RV ROS in wild type rats, and DDAH1$^{-/-}$ significantly exacerbated MCT-induced increase of RV ROS production (Fig. S4A, B). Moreover, MCT caused significant increases of RV 3-NT and 4-HNE, and DDAH1$^{-/-}$ significantly aggravated MCT-induced increases of RV 3-NT and 4-HNE (Fig. S4C–E).

4. Discussion

The present study has several important findings. First, we demonstrated for the first time that DDAH1$^{-/-}$ rats had an increase of systemic and tissue ADMA contents, relative to wild type rats, but had no detectable effect on the growth and development. Second, we found that DDAH1$^{-/-}$ had no effect on LV or RV hypertrophy and dysfunction, and PAH development under control conditions. Third, we demonstrated that DDAH1$^{-/-}$ significantly exacerbated MCT-induced PAH, lung vascular remodeling, and RV hypertrophy. In addition, we showed that DDAH1$^{-/-}$ caused significant increase of lung and RV oxidative stress in rats.

ADMA accumulation is associated with various cardiovascular diseases, such as heart failure, pulmonary hypertension, systolic hypertension, and chronic renal diseases etc., and ADMA is recognized as an independent risk factor for cardiovascular diseases [17]. Moreover, a previous study demonstrated that hypoxia-induced PAH is associated with reduced lung DDAH1 protein expression, increased ADMA concentration, and decreased NO production in rats [11]. Thus, one of the important questions is whether chronic ADMA accumulation caused by DDAH1 dysfunction is sufficient to cause cardiovascular diseases such as PAH, systolic hypertension and heart failure. The findings that DDAH1$^{-/-}$ has no detectable effect on RV systolic blood pressure, and cardiac hypertrophy and function in rats are consistent with our previous findings in DDAH1$^{-/-}$ mice [10]. The findings from this DDAH1$^{-/-}$ rat strain, our global DDAH1$^{-/-}$ mouse strain [10], and another DDAH1$^{-/-}$ mouse strain from Dr. Leiper's group [18] consistently demonstrated that DDAH1 is not required for embryonic development and growth. Importantly, findings from these DDAH1$^{-/-}$ strains demonstrated that chronic ADMA accumulation alone is insufficient to cause abnormal cardiac structure and function and many cardiovascular diseases under control conditions. The findings that DDAH1$^{-/-}$ significantly exacerbated MCT-induced increase of RV systolic pressure, lung vascular remodeling and RV hypertrophy indicate that DDAH1 plays an important role in attenuating PAH development. In other words, these data indicate DDAH1 dysfunction or chronic ADMA accumulation alone could not cause spontaneous PAH and RN hypertrophy, but they could exacerbate PAH development under pathological conditions such as after MCT injection.

One important finding of this study is that DDAH1$^{-/-}$ caused an increase of blood and tissue ADMA contents in rats. While several DDAH1$^{-/-}$ mouse strains clearly demonstrated that DDAH1 is important for systemic and tissue ADMA metabolism in mice [10], the significant increase of blood and tissue ADMA content in this DDAH1$^{-/-}$ rat strain further confirm the important role of DDAH1 in regulating ADMA metabolism in another species beyond mice [19]. Thus, by using either a global DDAH1$^{-/-}$ mouse strain or an endothelial-specific DDAH1$^{-/-}$ mouse strain, the previous studies from our group demonstrated that DDAH1 exerts an important role in systemic ADMA metabolism and NO production [10,20]. Using a different DDAH1$^{-/-}$ mouse strain, another group also showed that DDAH1 exerts an important role in regulating systemic ADMA metabolism and NO production [21]. However, a study from a further group showed that vascular endothelial DDAH1 has a limited role in regulating systemic ADMA [18]. The discrepancy regarding vascular endothelial DDAH1 in systemic ADMA degradation may due to the different design of these two DDAH1$^{flox/flox}$ strains, as both groups used the same Tie2-Cre mice. Specifically, the endothelial DDAH1$^{-/-}$ mice from our group show diminished DDAH1 expression in several tissues [19], while the other endothelial DDAH1$^{-/-}$ mice showed a minimal effect on tissue DDAH1 expression [18]. Nevertheless, the data from these strains consistently show that DDAH1 distributed in vascular endothelium enhances angiogenesis and vascular endothelial injury repair [18,20]. Furthermore, over-expression of DDAH1 results in decreases of plasma and tissue ADMA levels in mice. Over-expression of DDAH1 also attenuated insulin resistance, and enhanced angiogenesis in mice [22]. These data all support the important role of DDAH1 in tissue and systemic ADMA metabolism.

While the mechanism by which DDAH1$^{-/-}$ exacerbates MCT-induced lung vascular remodeling and PAH is not totally clear, since reduced NO signaling and increased oxidative stress both contribute to PAH development [23–25], DDAH1 probably regulates PAH development through decreasing NO signaling and increasing oxidative stress [17]. Decrease of NO by eNOS knockout exacerbates hypoxia-induced PAH development, while eNOS overexpression attenuates hypoxia-induced PAH in mice [23,26]. These findings are conceptually consistent with our finding that DDAH1 KO does not cause spontaneous PAH under control conditions but exacerbated PAH development after MCT. The finding of reduced lung eNOS protein expression in DDAH1 KO rats after MCT indicates that the decreased NO bioavailability in these experimental animals might be a collective outcome of increased ADMA and reduced eNOS protein content. Furthermore, NO inhalation and increase in NO/cGMP signaling by PDE5 inhibitors are both effective in

attenuating PAH development [23–25]. As stated, PAH development is associated with increased lung oxidative stress [23,25]. Enhanced oxidative stress can exacerbate lung vascular remodeling and PAH development, while reduced oxidative stress can attenuate lung vascular remodeling and PAH development [12,23,25]. Since DDAH1$^{-/-}$ attenuates NO production by increasing the endogenous NOS inhibitor ADMA [8–10,21], and ADMA can also cause ROS production in various tissues [13–15], the exacerbated lung vascular remodeling and PAH in DDAH1$^{-/-}$ rats after MCT are likely the collective effect of the increased lung oxidative stress and the reduced lung NO production as observed in our study.

Since NO signaling can directly regulate cardiac hypertrophy and failure [5], the exacerbated MCT-induced RV hypertrophy seen in DDAH1$^{-/-}$ rats may not be simply an outcome of the exacerbated lung vascular remodeling and PAH in these rats. Thus, several previous studies have demonstrated that overexpression of eNOS in cardiomyocytes protects against cardiac remodeling and dysfunction under stress conditions such as myocardial infarction and pressure overload [27,28], and eNOS gene deficiency was shown to exacerbate TAC-induced LV hypertrophy and dysfunction in mice [27]. Most interestingly, over-expressing eNOS rescued global eNOS knockout mice from the exacerbated TAC-induced ventricular remodeling and dysfunction, which they otherwise demonstrated [29], indicating an important role of NO derived from cardiomyocytes in attenuating TAC-induced LV remodeling and function. In addition, progressive LV hypertrophy, fibrosis, and dysfunction that develops in surviving tissue after myocardial infarction, are exacerbated in eNOS-knockout mice as compared to wild type mice [28]. These reports consistently support the concept that reduced NO signaling either by eNOS KO or chronic ADMA accumulation after DDAH1 KO could not cause spontaneous pathological diseases such as LV failure or PAH. It is important to note that increased oxidative stress can exacerbate cardiac hypertrophy and dysfunction, and that decreased oxidative stress can attenuate cardiac hypertrophy and heart failure development [30–32]. Importantly, we recently demonstrated that cardiac-specific DDAH1 gene-deletion significantly exacerbated systolic overload-induced cardiac hypertrophy and heart failure, which is associated with increased cardiac oxidative stress [14]. Selective gene-deletion of DDAH1 also exacerbates myocardial infarction-induced ventricular remodeling and dysfunction [32]. Since DDAH1$^{-/-}$ attenuates RV NO production and increases RV oxidative stress, and NO and oxidative stress are known to directly regulate cardiac hypertrophy, this might explain the exacerbated RV hypertrophy and dysfunction observed in DDAH1$^{-/-}$ rats after MCT.

Some limitations should be noticed in present study: The study was performed only in vivo, further study should be performed to target the specific cells that are responsible for the progress of PAH-induced RV failure.

5. Conclusion

In summary, we have demonstrated that DDAH1 deficiency could increase ADMA levels in plasma and tissue and impair the capacity of the RV in response to PAH, indicating that modulation of DDAH1 activity may be a promising therapeutic approach for treatment of PAH-induced RV failure.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.07.078.

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