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Dehydroepiandrosterone attenuates pulmonary artery and right ventricular remodeling in a rat model of pulmonary hypertension due to left heart failure

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ABSTRACT

Aim: Pulmonary hypertension due to left heart failure (PH-LHF) is the most common cause of pulmonary hypertension. However, therapies for PH-LHF are lacking. Therefore, we investigated the effects and potential mechanism of dehydroepiandrosterone (DHEA) treatment in an experimental model of PH-LHF.

Main method: PH-LHF was induced in rats via ascending aortic banding. The rats then received daily DHEA from Day 1 to Day 63 for the prevention protocol or from Day 49 to Day 63 for the reversal protocol. Other ascending aortic banding rats were left untreated to allow development of PH and right ventricular (RV) failure. Sham ascending aortic banding rats served as controls.

Key finding: Significant increases in mean pulmonary arterial pressure (mPAP) and right ventricular end-diastolic diameter (RVEDD) were observed in the PH-LHF group. Therapy with DHEA prevented LHF-induced PH and RV failure by preserving mPAP and preventing RV hypertrophy and pulmonary artery remodeling. In preexisting severe PH, DHEA attenuated most lung and RV abnormalities. The beneficial effects of DHEA in PH-LHF seem to result from depression of the STAT3 signaling pathway in the lung.

Significant: DHEA not only prevents the development of PH-LHF and RV failure but also rescues severe preexisting PH-LHF.

1. Introduction

Pulmonary hypertension due to left heart failure (PH-LHF) is considered the most common cause of pulmonary hypertension, and heart failure with concomitant pulmonary hypertension is associated with a poor prognosis [1,2]. However, therapies for this disease are limited. Limited clinical data indicate that sildenafil improves the functional capacity and clinical status of patients with PH-LHF [3–6], but these previous studies do not provide sufficient evidence to support the use of sildenafil for the clinical management of patients [1]. Therefore, more effective therapeutic treatments are needed.

Signal transducer and activator of transcription 3 (STAT3) is activated in response to cytokines, resulting in increased expression of nuclear factor of activated T-cell 2 (NFATc2) and increased activation of the proviral integration site for moloney murine leukemia virus (Pim1). When activated, Pim1 triggers NFATc2 activation, promoting $[Ca2]_i$-dependent pulmonary artery smooth muscle cells (PASMC) proliferation and inhibiting mitochondria-dependent apoptosis. The STAT3/Pim-1/NFAT axis has been demonstrated to be involved in the progression of pulmonary hypertension in different animal models (monocrotaline- or SU5416/hypoxia/normoxia-exposed rats) [7–11]. However, few studies have been performed on pulmonary hypertension caused by left heart failure. We have provided evidence that pulmonary vascular remodeling in pulmonary hypertension induced by aortic banding was improved by decreasing the expression of RhoA/Rho kinase (ROCK) [12], which was associated with activation of the STAT3 signaling pathway [9,10]. Furthermore, dominant-negative STAT3 mutants or inhibition of STAT3 tyrosine or serine phosphorylation

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completely abolished RhoA oncogenic potential [13]. We had consulted literatures when designing the study and found that it has more supportive researches in lung tissues of PH [14,15], and serine 727 have more evidence in glioma [16,17] and left heart tissues [18,19], so we choose PY750 as phosphorylation site. These findings suggest that the STAT3/pim-1/NFAT axis may also serve as a major signaling hub in a model of PH-LHF. Therefore, this axis may be a potential therapeutic target for treatment of PH-LHF.

Dehydroepiandrosterone (DHEA) is a steroid hormone derived from cholesterol and synthesized by the adrenal glands [20]. DHEA and its sulfate ester DHEAS are the most abundant steroid hormones in circulation [21] and may benefit the cardiovascular system. Farrukh et al. found that DHEA reverses acute hypoxic pulmonary vasoconstriction and prevents the development of the secondary increase in pulmonary arterial pressure during sustained alveolar hypoxia by increasing K_{Ca}-channel activity [22], after which several studies and clinical trials investigated the impact of DHEA on pulmonary hypertension [7,23–25]. A previous study demonstrated that DHEA improved MCT-induced pulmonary artery hypertension by decreasing mean pulmonary artery hypertension and right ventricle hypertrophy and that these effects were associated with inhibition of the STAT3 signaling pathway [7], Rawat et al. found that DHEA improved left ventricular diastolic function by suppressing pyridine nucleotide signaling [26]. Furthermore, Ventetuolo et al. found that higher levels of estradiol and lower levels of DHEAS were associated with pulmonary artery hypertension in men [27]. Therefore, DHEA supplementation therapy may be an effective therapeutic option [28]. However, no study has investigated the effect of DHEA on pulmonary vascular and ventricular remodeling in a PH-LHF model. We hypothesized that DHEA can improve pulmonary hemodynamics, pulmonary vascular remodeling and cardiac function in an experimental model of PH-LHF induced by supra-coronary aortic banding and that these effects are related to suppression of the STAT3 signaling pathway.

2. Materials and methods

2.1. Study design

The Animal Ethics and Research Committee of Sun Yat-sen University approved all animal protocols (22014016). PH-LHF was induced in male Sprague-Dawley rats (body weight of 250–300 g). Rats were randomly assigned to aortic-banded groups. The operation was performed as described previously [12]. Briefly, the animals were anesthetized with pentobarbital (36 mg/kg, intraperitoneal injection), a blunt-syringe needle with an inner diameter of 1.2 mm was placed along the axis of the ascending aorta, and the nylon suture was tied approximately 1 cm distal to the aortic valve around the aorta. The needle was then removed, leaving a stenosis that could be directly observed. Rats of the sham group underwent the same operation with the exception of aortic banding [29]. The rats were randomly divided into four groups: a sham group, a PH-LHF group, a reversal group, and a prevention group. The rats in both the PH-LHF group and the sham group were administered sterile water (15 ml/kg/day) for 63 days. The rats in the prevention group were administered DHEA by gavage (15 mg/kg/day) for 63 days after banding. The rats in the reversal group were administered DHEA from Day 50 to Day 63. The dosages of DHEA were previously reported as safe and effective in rats [30]. On Day 64, hemodynamics, histomorphology and biochemistry of all the rats were assessed, and the rats were then sacrificed.

2.2. Biochemistry

Plasma steroid hormone levels of DHEA, DHEAS, estradiol and testosterone were measured using ELISA kits (Shanghai yuan ye Bio-Technology, Shang Hai, China).

2.3. Echocardiographic and hemodynamic measurements

Transthoracic echocardiography and right heart catheterization were performed as previously described [7,31]. After the animals were anesthetized with pentobarbital (36 mg/kg, intraperitoneal injection), a pulmonary artery catheter filled with 1 ml heparin solution (1000 IU/ml) was inserted into the right external jugular vein, the superior vena cava, the right atrium, the right ventricle and the pulmonary artery in turn. The catheter was connected to a pressure transducer, and pressure data were recorded with a polygraph system BL-420E (Taimeng, Chengdu, China). Right catheterizations were performed to measure the right atrium pressure (RAP), the right ventricular systolic pressure (RVSP) and the mean pulmonary arterial pressure (mPAP).

After the animals were anesthetized with aether, echocardiography was performed using a 17.5-MHz linear array transducer (Vevo 2100 Imaging System, visual sonics, Toronto, Canada). Right ventricular end-diastolic diameter (RVEDd), left ventricular end-diastolic dimension (LVEDd), pulmonary artery acceleration time (PAAT), interventricular septal thickness in diastole (IVSd), left ventricular posterior wall thickness in diastole (LVPWd), left ventricular ejection fraction (LVEF) and the ratio of peak E velocity to peak A velocity (E/A) were measured.

2.4. Morphology and histology

Rats were euthanized with 15% pentobarbital sodium via intraperitoneal injection. The weights of the right ventricular (RV) and left ventricular + septum (LV + IVS) were determined relative to body weight. The wet and dry ratio of the middle lobe of the right lung (W / D ratio) was assessed to evaluate pulmonary edema. Lung slides were stained with hematoxylin-eosin. Ten areas of pulmonary arterioles from each rat were randomly selected for analysis. The medial wall thickness was measured under a microscope at 400 × magnification using the following equation: percent wall thickness (WT%) = (medial thickness × 2 / external diameter) × 100%. RV tissues were subjected to Masson's trichrome staining using Masson's Trichrome Stain Kit (Polysciences, Inc., Warrington, PA), collagen volume fraction (CVF) were analyzed by ImageJ analysis software.

2.5. Western blot

Lung tissues were lysed on ice with RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) supplemented with protease inhibitors (Goodbio Technology, Wuhan, China) and phosphatase inhibitors (Goodbio Technology, Wuhan, China). After the protein concentrations were measured using the bicinchoninic acid (BCA) method, equal amounts of protein were electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% BSA Tris-buffered saline (pH 7.6, containing 0.1% Tween and 5% bovine serum albumin) for 1 h at room temperature and then probed with anti-Pim1 antibodies (Affinity, Cincinnati, USA, diluted 1:1000) and secondary antibodies for 1 h at room temperature. The protein of interest on the membrane was detected using an enhanced chemiluminescence (ECL) detection system (Goodbio Technology, Wuhan, China).

To examine the expression of STAT3, PY750-STAT3, NFATc, Pim-1 and survivin, the same protocols were followed with the following exceptions: an 8% sodium dodecyl sulfate-polyacrylamide gel was used, and the membrane was probed with anti-STAT3 (Abcam, diluted 1:500), anti-pSTAT3 (Abcam, Cambridge, UK, diluted 1:500), anti-NFATc2 (Affinity, diluted 1:500), and anti-survivin (Affinity, diluted 1:1000).

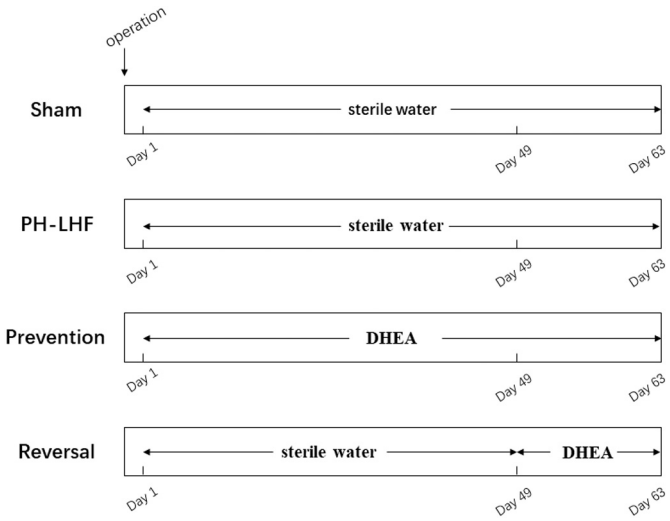

Fig. 1. Flow diagram of the experiment.

2.6. Statistical analysis

The data are presented as the means ± SD. Western blot was analyzed via densitometry. All data from the four groups were analyzed using ANOVA followed by Bonferroni correction. The values of several results (DHEAS, testosterone, RAP, WL/DL, mean wall thickness) were compared using Mann-Whitney *U* tests and the Kruskal-Wallis test for 2 or more independent groups. $P < 0.05$ was considered statistically significant. All statistical tests were performed using SPSS v13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. The effect of DHEA to hemodynamics and lung vascular remodeling

Flow diagram was shown in Fig. 1.

As presented in Table 1, the levels of DHEA, DHEAS, estradiol and testosterone were significantly decreased in the PH-LHF group compared with those in the sham group, and DHEA treatment attenuated these changes in both the prevention group and the reversal group.

Considerable lung vascular remodeling and medial wall thickening in pulmonary arteries were evident in the PH-LHF rats (Fig. 2A and B). As a result, compared with the sham-operated group, the mean PAP was approximately 10 mm Hg higher in the PH-LHF rats (13.29 ± 2.06 mm Hg vs 26.5 ± 1.2 mm Hg), indicating that the pulmonary hypertension model induced by aortic banding mimicked the human disease (Fig. 2C). Vascular permeability as an adaptive response partially compensated for the chronic elevation in lung hydrostatic pressure as shown by an increased wet-to-dry lung weight ratio in lungs from the PH-LHF rats compared with that in the sham-operated rats (Fig. 2D).

Treatment with DHEA prevented and reversed the development of lung vascular remodeling, mean pulmonary pressure and strengthened lung vascular barrier function in the prevention and the reversal group, with the improvement being most pronounced in the prevention group (Fig. 2).

3.2. The effect of DHEA to ventricular remodeling and function

9 weeks after the operation, no significant differences in the body weight were observed among the 4 groups (Table 2). Within 9 weeks, the rats with aortic banding showed significant increases in RV and LV weights (Fig. 3A and B). Aortic banding resulted in marked RV dilation as evidenced by an increased end-diastolic area (6.13 ± 0.18 mm vs 3.55 ± 0.32 mm), which was associated with signs of PH as indicated by reduced PAAT (25.69 ± 3.22 ms vs 31.11 ± 4.63 ms), and significant RV fibrosis, which was indicated by Masson's trichrome staining and increased collagen volume fraction (Fig. 5). Aortic banding also increased LVPW, IVS and LVEF. The LV end-diastolic area did not differ between the groups, and the ratio of peak E velocity to peak A velocity (E/A) decreased after banding, which are consistent with the characteristics of diastolic heart failure with a preserved ejection fraction (Fig. 4). DHEA markedly prevented RV hypertrophy and fibrosis as shown by the RV/BW ratio (0.046 vs 0.058) and Masson's trichrome staining (Fig. 5). The notion that long-term DHEA treatment may have protective effects on RV function was substantiated in echocardiographic measurements. As shown in Fig. 4, DHEA prevented the echocardiographic signs of RV dilation (3.84 ± 0.18 mm vs 6.13 ± 0.18 mm) and significantly increased the PAAT (33.17 ± 7.63 ms vs 25.69 ± 3.22 ms) but failed to attenuate LVPW, IVS, LVEF and E/A changes. After 2 weeks of DHEA reversal treatment, the rats with PH-LHF showed significantly improved RV function and a trend toward reduced RV hypertrophy as indicated by the RV/BW ratio (0.052 vs 0.058), but DHEA did not reverse LV hypertrophy and function compared with the corresponding parameters in the model group.

3.3. The effect of DHEA to STAT3/NFAT signaling pathway in lung

The STAT3/NFAT signaling pathway in rats with aortic banding was activated as indicated by the significantly increased expression levels of PY750-STAT3, Pim-1, NFATc2 and Survivin compared with those in the sham group. Treatment with DHEA prevented and reversed the expression of STAT3/NFAT signaling pathway in the prevention and the reversal group, with the improvement being most pronounced in the prevention group. There were not significant differences of STAT3 levels among the groups (Fig. 6).

4. Discussion

In the present study, we first showed that DHEA was beneficial in rats induced with PH-LHF by supra-coronary aortic banding. Specifically, in the prevention group, treatment with DHEA (15 mg/kg/day) for 9 weeks significantly attenuated pulmonary hypertension, pulmonary vascular remodeling and right ventricular remodeling. Smaller improvements in pulmonary vascular remodeling and right

Table 1
Hormone levels.

	Sham	PH-LHF	Prevention	Reversal
DHEA (ng/ml)	249.54 ± 28.47	106.97 ± 17.49*	196.17 ± 16.94#	141.76 ± 13.57#
DHEAS (ng/ml)	13.45 ± 1.27	6.51 ± 0.63*	11.10 ± 1.10#	7.89 ± 0.63#
Estradiol (pmol/L)	47.12 ± 4.93	25.24 ± 3.45*	35.88 ± 2.45#	29.28 ± 1.60#
Testosterone (ng/ml)	19.64 ± 2.32	9.56 ± 1.40*	16.29 ± 1.09#	10.84 ± 1.26#

Results are expressed as the means ± SE.
RAP, right atrium pressure; RVSP, right ventricular systolic pressure; mPAP, mean pulmonary arterial pressure.
* $P < 0.05$ versus sham.
# $P < 0.05$ versus PH-LHF.

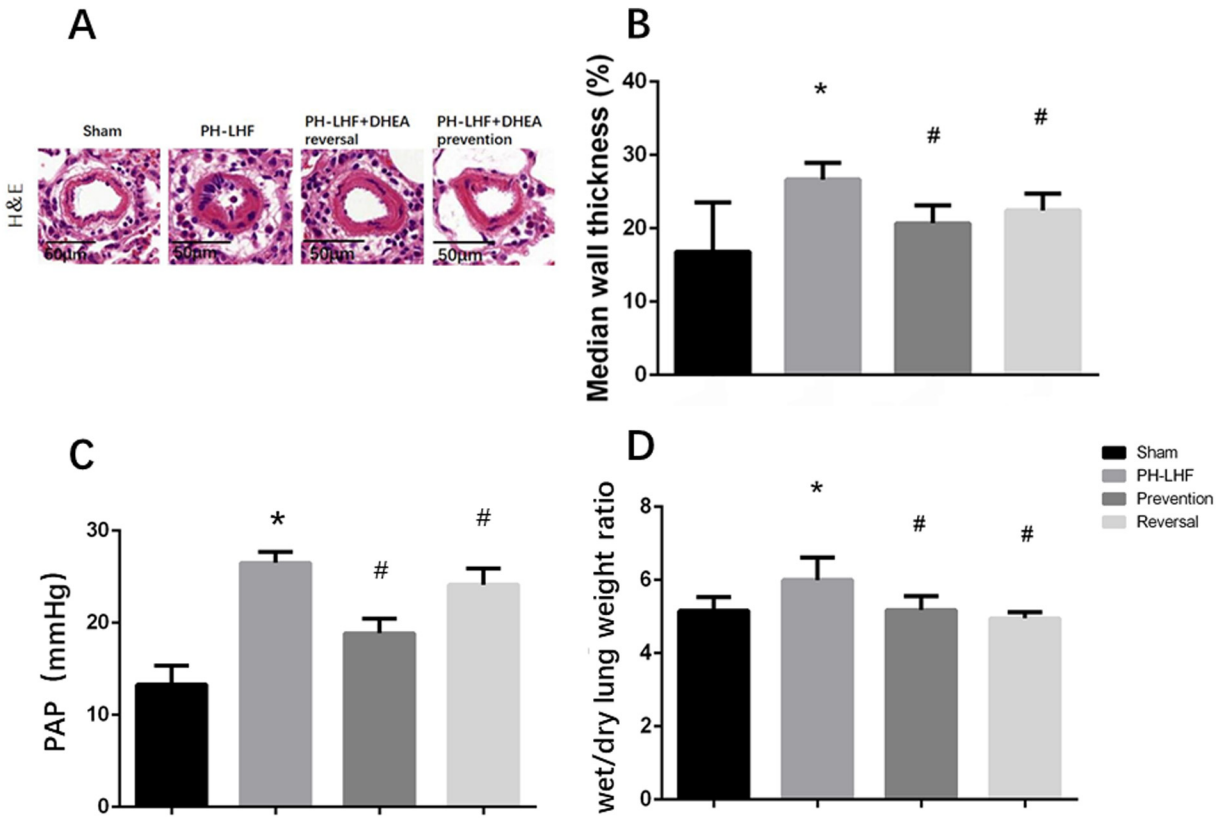

Fig. 2. DHEA attenuated pulmonary hemodynamics, improved lung vascular remodeling and barrier function. The group data show: A, representative images of hematoxylin and eosin–stained lung sections from each group of rats; B, the mean vascular wall thickness of pulmonary arterioles in lungs from sham-operated, banded (PH-LHF), DHEA-prevented banded (prevention) and DHEA-reversed banded rats (reversal); C, pulmonary arterial pressure in each group; D, the wet-to-dry lung weight ratio in each group. Data show means ± SE of pooled data from two independent trials (n = 8 rats per experiment, resulting in a total of 16 rats per group); $^{*}P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus the PH-LHF group.

Table 2
Body weight change in study.

	BW before (g)	BW after (g)
Sham	265.29 ± 25.20	507.00 ± 63.36
PH-LHF	268.25 ± 24.80	439.50 ± 82.88
Prevention	272.86 ± 38.34	447.85 ± 37.58
Reversal	235.43 ± 5.68	445.00 ± 23.61

Results are expressed as the means ± SE.
BW before: body weight before ascending aortic banding.
BW after: body weight at Day 63.

ventricular enlargement were observed in the reversal group. Mechanistically, the beneficial effects of DHEA on pulmonary vascular and right ventricle remodeling were associated with inhibition of the STAT3 signaling pathway [14].

In the present study, we used an ascending aortic banding model that represents patients with heart failure with preserved ejection fraction, a highly prevalent category of heart failure that is characterized by abnormalities in diastolic rather than systolic ventricular function [31]. A previous study reported that the mPAP of rats significantly increased as early as 4 to 6 weeks after aortic banding [32], and another study proved that LVEF would decrease after banding [33]. However, we demonstrated that a 9-week period was required for the development of pulmonary hypertension and evident pulmonary vascular remodeling [12], and after aortic banding, rats LVEF would increase mildly, which are consistent with the results described in a previous study [31]. Therefore, we selected 9 weeks as the time point.

Recent epidemiologic studies have demonstrated that despite female susceptibility to PH-LHF, female patients exhibit better survival

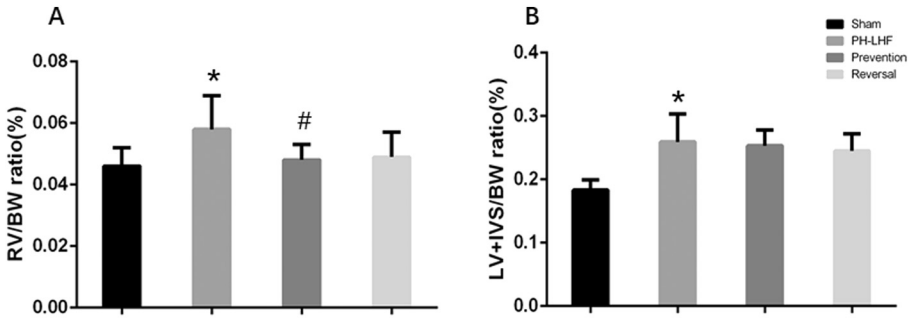

Fig. 3. DHEA attenuated right ventricular hypertrophy but fail to the left ventricular. A: Compared with the sham group, the rats subjected to aortic banding exhibited significant increases in right ventricular hypertrophy. DHEA significantly prevented RV hypertrophy, and a trend toward a decrease in RV hypertrophy was observed in the reversal group. B: Compared with the sham group, the rats subjected to aortic banding exhibited significant increases in left ventricular hypertrophy, which was not improved by DHEA. Data show means ± SE of pooled data from two independent trials (n = 8 rats per experiment, resulting in a total of 16 rats per group); $^{*}P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus the PH-LHF group.

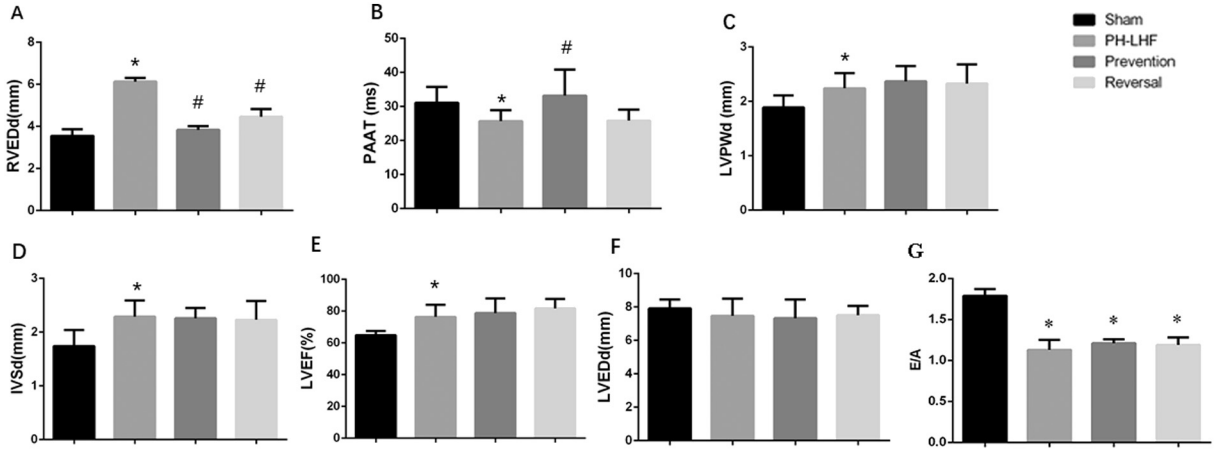

Fig. 4. DHEA improved RV dilation and systolic function but fail to the left ventricular. Note the marked dilation of the RV in banded rats, which was attenuated by DHEA. Bar graphs show the right ventricular end-diastolic diameter (RVEDd, A), pulmonary artery acceleration time (PAAT, B), left ventricular end-diastolic dimension (LVEDd, C), interventricular septal thickness in diastole (IVSd, D), left ventricular ejection fraction (LVEF, E), left ventricular posterior wall thickness in diastole (LVPWd, F) and the ratio of peak E velocity to peak A velocity (E/A, G). Data show means ± SE of pooled data from two independent trials (n = 8 rats per experiment, resulting in a total of 16 rats per group); $^{*}P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus the PH-LHF group.

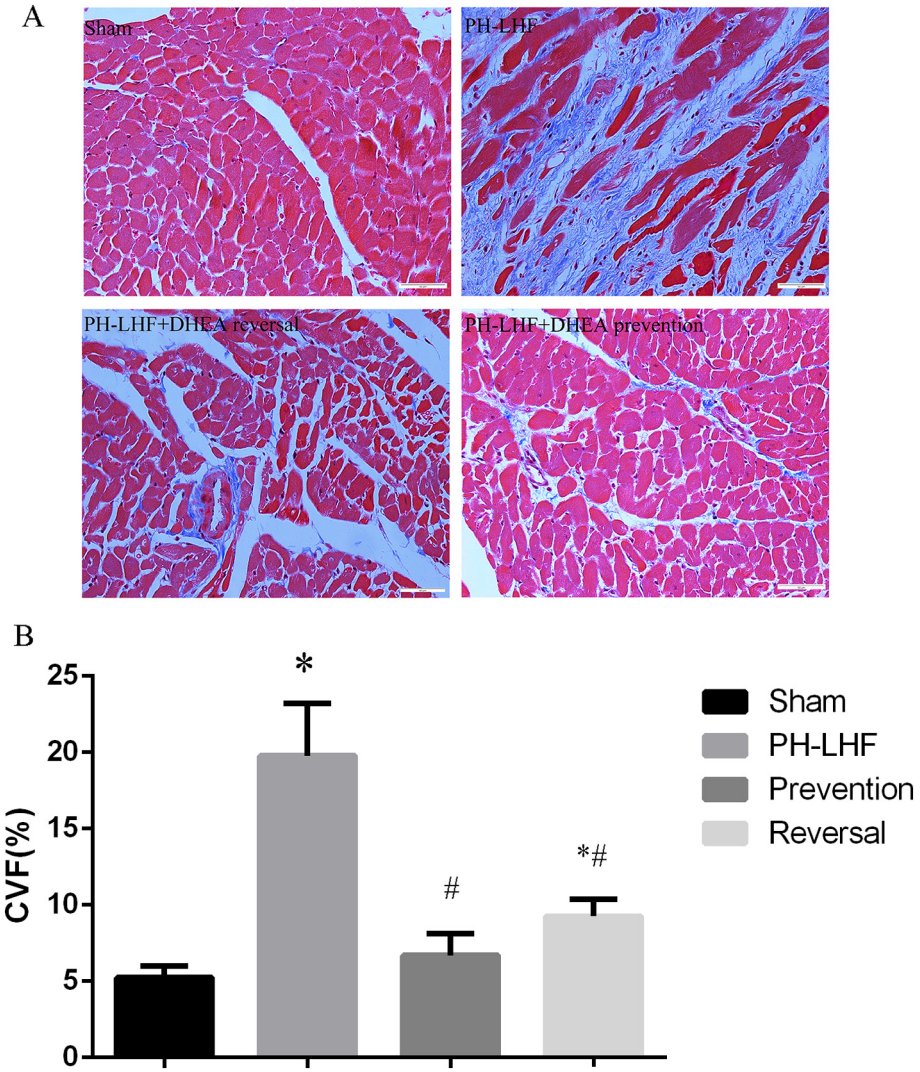

Fig. 5. DHEA prevents and reverses fibrosis in RV from rats with PH-LHF. A: Representative images of Masson's trichrome staining from each group, Bar equals 50 μm. B: collagen volume fraction from each group; the values are means ± SE, n = 5 rats per group; $^{*}P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus the PH-LHF group.

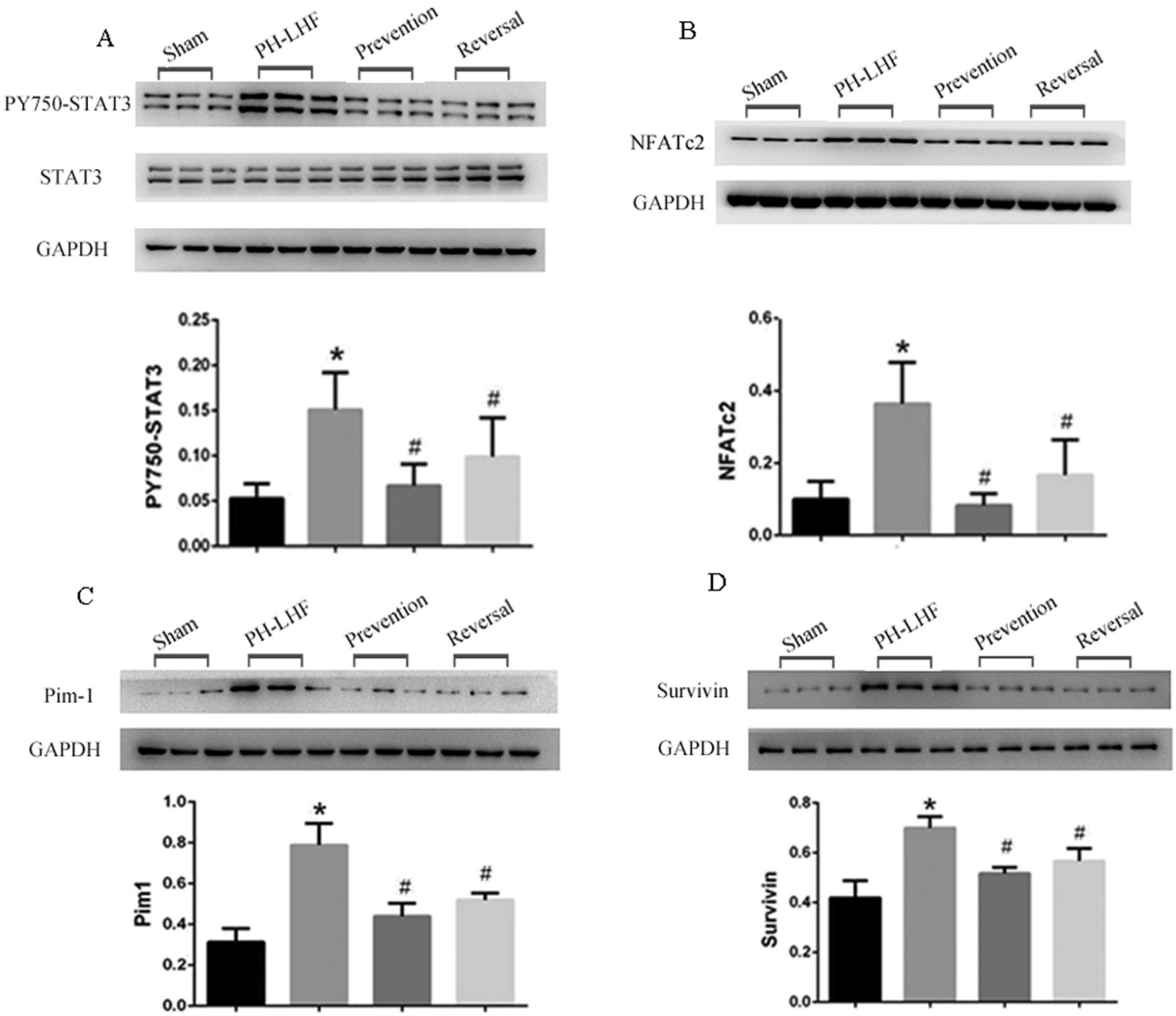

Fig. 6. DHEA suppressed STAT3/NFAT signal pathway in lung. A, PY750-STAT3 and STAT3; B, NFATc2; C, Pim-1; D, Survivin. The top shows representative immunoblots, and the bottom shows the densitometric assessment. The values are means ± SE, n = 5 rats per group; *$P < 0.05$ versus the sham group, $^{\#}P < 0.05$ versus the PH-LHF group.

than men [34,35]. This finding suggests that sex hormone levels may be an important factor in the progression of PH-LHF [27]. In the present study, we found that the levels of estradiol, testosterone and DHEAS were significantly decreased in the rats with PH-LHF, and DHEA reversed this decrease. We also demonstrated that exogenous DHEA supplementation significantly improved pulmonary hemodynamics, pulmonary remodeling and ventricular remodeling in a PH-LHF model, which is consistent with the well-documented beneficial effect of DHEA on pulmonary hypertension in different animal models [7–11]. Although the potential beneficial effects of estrogen on pulmonary hypertension remain controversial [27,36–38], some studies have shown that estradiol may protect RV structure and function and attenuate pulmonary vascular remodeling [36,37]. As DHEA is a precursor in the biosynthesis of estrogen, we speculated that the increases in estradiol may be partly associated with the positive effect of DHEA on PH-LHF. Only small increases in estradiol and DHEAS levels were observed in the reversal group, which may have contributed to a smaller improvement in pulmonary vascular remodeling and ventricular remodeling. Our findings are clinically relevant given that the safety of long-term DHEA or DHEAS ingestion by humans has been confirmed [39].

Pulmonary vascular remodeling, which is characterized by increased downstream left heart pressure that progresses to a reactive stage accompanied by increased pulmonary vascular resistance, is one of the most important characteristics of PH-LHF [2]. In the current study, pulmonary vascular remodeling was improved by DHEA in PH-LHF. However, there is concern that the improvement in pulmonary vascular remodeling may deteriorate lung edema because remodeling is an adaptive response that partially compensates for lung edema. In contrast, we demonstrated that DHEA attenuated pulmonary vascular remodeling and lung edema without improving LV hypertrophy and systolic function, as assessed by echocardiography. Some possible reasons are as follows: (1) As increased asymmetrical RV hypertrophy and septal wall thickening will compress the LV cavity and cause diastolic heart failure [26], and an increased RVEDd / LVEDd ratio is associated with the severity and prognosis of pulmonary hypertension [40], the RVEDd / LVEDd ratio was significantly decreased by DHEA in the present study. Therefore, the decreased RVEDd / LVEDd ratio and reduced RV hypertrophy indirectly improve left diastolic function, resulting in reduced lung edema. (2) As the physiological concentrations of DHEA stimulated nitrogen monoxide (NO) release and increased cyclic GMP (cGMP) levels from intact aortic endothelial cells [41], and cGMP signaling negatively regulates microvascular barrier failure [31], this protective effect of DHEA on hydrostatic edema formation may be associated with cGMP signaling, which negatively regulates microvascular barrier failure.

Mechanistically, we found that treatment with DHEA attenuated pulmonary vascular remodeling, which may have been mediated through inhibition of the STAT3 signaling pathway. This finding is consistent with a previous study that attributed mechanisms including inhibition of the STAT3/NFAT axis [14]. Although we have not elucidated the exact molecular mechanism by which DHEA decreases STAT3 activation, the findings described above suggest that the STAT3 signaling pathway serves as a major signaling hub and is therefore a potential therapeutic target for the treatment of PH-LHF.

There are several limitations to the present study. First, the level of STAT3 signal pathway should be tested in LV because STAT3 signaling is known to play a role in left heart disease, and if DHEA is postulated to affect this pathway, it would be important to see more of the LV phenotype. The reason why we did not perform this assay is that there are many evidences support that STAT3 signaling play an important role in pressure overload-induced left ventricle hypertrophy, suppressing this pathway could improve LV remodeling and left heart failure [42,43] but there were also some researches proved the protection of STAT3 signal pathway in left ventricle [44,45], thus, we thought there was controversy of the STAT3 signal pathway function in left heart failure. There were also many studies proving another important signal pathway in left heart failure [46,47]. As a result, we tend to think DHEA treatment could suppress STAT3 signal pathway in LV very little, or STAT3 signal pathway may not play a key role in LV remodeling. Second, a previous study demonstrated that DHEA treatment exerts beneficial effects on RV (more-so than on the pulmonary vasculature) following exposure to SU5416/hypoxia, suggesting an RV-specific effect [9]. Our results seem to be consistent with those of this study as we showed that the effect of DHEA is primarily exerted on pulmonary circulation and right ventricular remodeling, but we should more definitively address whether this beneficial effect is independent of the decrease in pressure overload in a future study. Further studies are needed to determine whether combination therapy with DHEA plus an angiotensin-converting enzyme inhibitor attenuates pulmonary hypertension, pulmonary vascular remodeling and biventricular remodeling in a PH-LHF model to greater degrees than monotherapy alone.

5. Conclusion

DHEA decreased mean pulmonary arterial pressure and improved distal pulmonary artery remodeling and right ventricular remodeling without affecting left ventricular structure and systolic function in a PH-LHF model. We found that the STAT3 signaling pathway can be therapeutically targeted by DHEA. Therefore, DHEA may be an attractive future therapeutic agent for treating PH-LHF.

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Conflict of interest

The authors of this manuscript have declared that no competing interests exist.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2018.12.056.

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