Pulmonary artery denervation improves hemodynamics and cardiac function in pulmonary hypertension secondary to heart failure

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Abstract
This study aimed to determine the benefits and correlated mechanisms of pulmonary artery denervation (PADN) for heart failure (HF) pulmonary hypertension (PH). PH secondary to HF is associated with poor clinical outcomes because there is no proper therapy for it. PADN showed improved outcomes for patients with HF-PH. However, the underlying mechanisms remain unknown. Supracoronary aortic banding (SAB) was used to create HF-PH models. Sprague-Dawley rats were randomly assigned to control, SAB, sham, SAB with PADN, and SAB without PADN groups. Surgical (longitudinally damaging vessel nerves) and chemical (10% phenol applied to the surface of nerves) PADN was performed for animals in the SAB with PADN group. Morphological, echocardiographic, hemodynamic, and protein expression changes were measured four weeks thereafter. Adrenergic receptor (AR) expressions of pulmonary arteries from four HF-PH patients and four patients without PH were measured. Ten HF-PH patients who underwent PADN were followed-up for six months. SAB-induced HF-PH was achieved by 50% of animals. Surgical and chemical PADN was associated with significant improvements in pulmonary artery muscularization, hemodynamics, and right ventricular functions. In pulmonary arterial specimens from HF-PH patients, $\beta_2$-AR and $\alpha_{1A/B}$-AR, as well as eNOS, were downregulated and $\alpha_{1D}$-AR was upregulated compared to those from patients without PH. PADN led to a mean increase of 84 m during the 6-min walk distance for HF-PH patients at six-month follow-up. HF-PH was characterized by downregulated $\beta_2$-AR, $\alpha_{1A}$-AR, and $\alpha_{1B}$-AR and by upregulated $\alpha_{1D}$-AR. PADN is associated with significant improvements in hemodynamics and pulmonary artery remodeling.

Keywords
heart failure, supracoronary aortic banding, pulmonary hypertension, adrenergic receptor

Heart failure (HF), a multifactorial and systemic disease, is estimated to affect approximately 1–2% of the adult population.1 Over the past few decades, despite dramatic reductions in morbidity and mortality and the development of numerous medical and device-based therapies,2–4 patients with pulmonary hypertension (PH) still have poor clinical outcomes due to HF.1,3,4 Based on the fifth world symposium on PH,5 long-term elevated left ventricular filling pressure triggers pulmonary arterial vasoconstriction with resultant pulmonary arteriolar remodeling, thereby shifting post-capillary PH to mixed post-capillary and pre-capillary PH, a stage defined as having a diastolic pulmonary pressure gradient (DPG) $\geq$ 7 mmHg independent of left ventricular ejection fraction (LVEF). Endothelin receptor antagonists

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and phosphodiesterase 5 inhibitors, two classes of target
drugs used for patients with World Health Organization
(WHO) class 1 pulmonary arterial hypertension (PAH),
have failed to demonstrate evidence of regression during
left ventricular (LV) remodeling or improvements in LV
function.\(^8\)

HF is associated with sustained and excessive levels of
sympathetic nerve activity.\(^8\) More importantly, increased
cardiac noradrenaline spillover was greater and occurred
before that of other organs,\(^9\) indicating that the heart is
exposed to higher levels of noradrenaline release for
longer periods than other organs. Similarly, sympathetic
overactivation should have a crucial role in the initiation
and development of pulmonary arterial constriction.\(^10\)
Although \(\beta\)-blockers have become the first line of treatment
for HF,\(^8\)\(^9\) previous clinical studies\(^11\)\(^15\) have not reached an
agreement regarding the benefits of \(\beta\)-blockers for HF-PH.
Recently, European Society of Cardiology/European
Respiratory Society PH guidelines suggested \(\beta\)-blockers as
an alternative medical therapy to treat PH-HF.\(^16\) This
implied complicated interactions between different AR in
the pathophysiology of HF-PH. Percutaneous pulmonary
artery denervation (PADN) has been used for PAH since
2013.\(^17\) A subgroup analysis (HF-PH patients) of a consen-
sus study including 66 patients with different etiologies of
PH\(^18\) showed improvements in exercise capacity, hemody-
namics, and clinical results for HF-PH patients. However,
correlated mechanisms of PADN treatment remain to be
studied. The present study aimed to elucidate the effects
and mechanisms of PADN for animals with PH induced
by supracoronary aortic banding (SAB) and to determine
the abnormal regulation of AR in pulmonary artery (PA)
specimens from HF-PH patients. Finally, we analyzed the
benefits of PADN for patients with HF-PH.

**Methods**

**Animal groupings based on supracoronary aortic banding**

Animals used in this study were on the controlled tempera-
ture (\(25 \pm 2^\circ\mathrm{C}\)) and 12-h light/dark cycle conditions and
provided with a standard rodent chow and water. Male
Sprague-Dawley rats (Nanjing Medical University Animal
Laboratory, Nanjing, China) aged four weeks and weighing
100–150 g were randomly divided into control (\(n = 6\)), sham
(\(n = 12\), thoracotomy (but not SAB), and SAB (\(n = 60\))
groups (Suppl. Fig. S1). The study protocol was approved
by the Institutional Animal Care and Use Committee and
the study was performed in accordance with the *Guide for
the Care and Use of Laboratory Animals* (National Research
Council).

**Supracoronary aortic banding procedure**

For SAB-induced HF models (Suppl. Fig. S2), animals were
administered general anesthesia and their chests were
opened using a surgical knife. A titanium clip with a residual
open diameter of 0.71 mm (to create a diameter stenosis of
70–80% by visual estimation) was then implanted on the
ascending aorta at least 1 mm distal to the origins of the
coronary arteries. At four weeks after SAB, animals with
successful HF-PH models (\(n = 30\); 50\%), defined as the pre-

cence of HF (LVEF < 60% and LV posterior wall thickness
[\(\text{LVPWT}\) > 2.5 mm] and PH (right ventricular systolic pressure
[\(\text{RVSP}\) > 35 mmHg and PA velocity time integral
\(<30\) ms), were randomly assigned to SAB (\(n = 6\)), SAB
with PADN (\(n = 12\)), and SAB without PADN (\(n = 12\))
groups (Suppl. Fig. S1).

**PADN for animals with HF-PH**

The SAB animals in the SAB with PADN group underwent
surgical and chemical PADN (Suppl. Fig. S2). First, the
main PA (MPA) and major branches were isolated from
the surrounding tissues following thoracotomy at the left
third intercostal space. Surgical PADN was then performed
according to the method reported by Jurastch et al.\(^19\)
Finally, 10% phenol was applied to the surface of the
MPA and the bifurcation area for 5 min after removal of
the deep adventitial tissue in the MPA wall. For animals in
the SAB without PADN group, surgical PADN was not
performed following thoracotomy; instead of 10% phenol,
saline was smeared on the surface of the MPA and bifurca-
tion area for 5 min. All animals were monitored for an addi-
tional four weeks after PADN.

To confirm the blocking effects by surgical and chemical
PADN, peri-vessel tissues near the main PA trunk and
bifurcation area (each 0.5 mm in length) were subsequently
sliced at a thickness of 4 \(\mu\)m. Tyrosine hydroxylase (TH)
immunostaining (Suppl. Fig. S3) was performed to compare
the changes in nervous density.

**Echocardiographic measurements for animals**

Animals were anesthetized with 1.0–1.5% isoflurane inhal-
tion. Echocardiographic measurements were then performed
(Vevo 770; VisualSonics Co. Ltd.) before and four weeks
after PADN. LVPWT, LV end-diastolic diameter, LVEF,
right ventricular (RV) end-diastolic diameter, and RV ante-
rior wall thickness were measured. The PA velocity time
integral was quantified as an indicator of longitudinal RV
systolic function and PH. Each parameter was averaged
over three cardiac cycles.

**Right heart catheterization for animals**

Right heart catheterization (RHC) was performed at four
weeks after PADN. A right heart catheter with an external
diameter of 0.9 mm was inserted through the jugular vein,
advanced to the MPA, and connected to a PowerLab system
(AD Instruments Co., Australia). Right atrial pressure
(RAP) and RVSP were then measured and recorded.
Hemodynamic data were presented as the mean of 10 measurements.

**Collection of lung and heart tissues**

After hemodynamic measurements, all animals were euthanized. The lung and heart tissues were perfused with ice-cold saline to remove the blood. The lungs, right ventricle, and left ventricle with septum were then rapidly excised, dried, and weighed. The RV hypertrophy index was defined as the ratio of the RV to the left ventricle with septum.

**Pathological assessment of pulmonary artery muscularization**

The lung issues were fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&F) (Jiangsu; KeyGen Biotech, Nanjing, China). Double-staining for von Willebrand factor and α-smooth muscle actin (α-SMA) was performed to evaluate pulmonary vessel muscularization, which was classified as none (0–25%), partial (25–75%), or full (75–100%). Two slices from each rat and 20 pulmonary vessels (diameter < 150 µm) in each slice were assessed. The medial wall thickness of the pulmonary vessels (diameter < 150 µm) was acquired according to Rabinovitch’s method. The ratio of the vascular wall thickness to its outer diameter was determined by an Olympus BHS microscope (San Jose, CA, USA) using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD, USA).

**Biochemical measurements**

Lung tissues were ground, lysed, and centrifuged at 3000 rpm, and the supernatant was collected. cAMP and cGMP concentrations were subsequently measured using an enzyme-linked immunosorbent assay kit (Sigma, St. Louis, MO, USA), as instructed by the manufacturer. The enzyme-linked immunosorbent assay was also used to assay plasma noradrenaline levels. Nitrate and nitrite levels were assayed using a nitric oxide synthase activity assay kit (KeyGen Biotech). The supernatant was collected and treated with nitrate reductase to assay nitrite levels, and the absorbance was measured at 530 nm by a spectrophotometer (NanoDrop 2000; Thermo Scientific, Waltham, MA, USA).

**Western blotting**

The lung tissues and pulmonary arterial specimens were homogenized on ice, and the proteins were extracted using the appropriate kit. Horseradish peroxidase-conjugated secondary antibody (1:4000) was purchased from Santa Cruz (Dallas, TX, USA). Primary antibodies against GAPDH (1:2000), AKT (1:1000) and its phospholate sites at 308 (1:1000) and 473 (1:1000), PKA (1:1000), and Phospho-PKA (1:1000) were obtained from Cell Signaling Technology (Danvers, MA, USA). The primary antibodies against endothelial nitric oxide synthase (eNOS; 1:1000) and its phospholate sites at Ser1177 (1:1000), Thr495 (1:1000), and Ser633 (1:1000) were from R&D Systems (Minneapolis, MN, USA). Primary antibodies against various AR (1:500) were obtained from Santa Cruz.

**Human pulmonary arterial specimens**

Pulmonary arterial branches were obtained from eight patients at Nanjing Cardiovascular Research Center: four with HF-PH who underwent a heart and lung transplantation and four patients without PH who had localized lung cancer and underwent pneumonectomy (peritumor normal tissues). The PA segments were carefully separated from the surrounding tissues. Then, the most distal segments (tertiary intralobar segment) were obtained. All patients provided informal consent to provide their blood, tissues, and organs.

**Percutaneous PADN for HF-PH patients**

The results of 10 patients with HF-PH (defined as mean pulmonary arterial pressure [PAP] ≥ 25 mmHg at rest, pulmonary arterial wedge pressure [PAWP] > 15 mmHg, pulmonary vascular resistance [PVR] ≥ 3 Wood units, and DPG > 7 mmHg) after maximal medication for 5–7 days agreed with those of the study protocol for percutaneous PADN. PADN has been described previously. Briefly, a PADN catheter with 10 electrodes was positioned at the distal MPA. Denervation was then performed at the conjunctural area between the distal main trunk and the ostial left branch with a temperature of 45–50°C, energy ≤ 15 W, and a time of 120 s/site. N-terminal brain natriuretic peptide (NT-proBNP) level, 6-min walk distance, and RHC before and six months after PADN were assessed according to standard methods. Echocardiographic parameters of 10 patients were independently measured by Dr. Jing-Ping Sun (Hongkong Chinese University), who was blinded to the study protocol. This protocol was approved by the ethics committee of Nanjing First Hospital and informal consent was obtained from all patients. After PADN, all baseline medications were continued during the six-month follow-up.

**Statistical analysis**

Parameters were reported as counts and mean ± SD, mean ± SEM, or percentages when appropriate. Categorical variables were performed to chi-square test or Fisher’s exact test. Continuous variables were performed to Student’s t test or Wilcoxon rank-sum scores for non-normally distributed data. SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Student t-tests and one-way analysis of variance were used to assess the differences in measurement data among
the multiple groups. Differences in the continuous variables between the two therapies were analyzed using a paired t-test. All experiments were repeated at least three times. A P value < 0.05 was considered statistically significant.

Results

SAB-induced severe HF-PH

Of 60 animals, SAB was successfully induced in 30 (50%) rats (Suppl. Fig. S1). Notably, 12 (20%) animals died within four weeks after SAB. Compared to the control (normal) group, animals in the SAB group or SAB without PADN group had a significant decrease in LVEF but a significant increase in RAP, RVSP, RV and LV size, and lung mass index (Table 1). Importantly, PADN was associated with a significant decrease in the density of sympathetic nerve endings (Suppl. Fig. S2).

Surgical and chemical PADN significantly improved hemodynamics and RV function

At four weeks after PADN, both PA hemodynamics (RAP and RVSP) and RV functional indexes were significantly improved by PADN treatment (Table 1) compared to those in the SAB group or SAB without PADN group. Unfortunately, slight but non-significant improvements in LV chamber size, LV mass, and LVEF were noted after PADN treatment.

Surgical and chemical PADN significantly improved PA remodeling

Although SAB led to severe PA remodeling, as reflected by the higher percentage of partial/full muscularization and media thickness (Fig. 1), PADN resulted in a significant increase in partial muscularization (53.50 ± 3.01%) but a decrease in full muscularization (21.5%) when compared with the SAB group (30.0% and 47.8%) or the SAB without PADN group (31.1% and 42.5%) (all P < 0.001) (Fig. 1).

Surgical and chemical PADN improved pulmonary arterial relaxation

As shown in Fig. 2, SAB was accompanied by significantly increased serum NE and decreased concentrations of cAMP, nitrite, and cGMP as well as protein expressions of PKA, Akt, and eNOSSer633, eNOSSer1177 in lung tissues of animals (Fig. 3a). These changes were significantly improved by PADN treatment, thus indicating at least partial recovery of pulmonary arterial relaxation.

Table 1. Hemodynamic and echocardiographic parameters before and four weeks after PADN.

|                           | Control (n = 6) | SAB (n = 5) | SAB with PADN (n = 6) | SAB without PADN (sham, n = 7) |
|---------------------------|----------------|-------------|-----------------------|-------------------------------|
| RAP (mmHg)*               | 1.87 ± 0.11†   | 2.62 ± 0.13 | 2.08 ± 0.12‡          | 2.68 ± 0.13                   |
| RVSP (mmHg)*              | 24.90 ± 1.05†  | 43.08 ± 3.67 | 36.65 ± 2.22‡         | 45.23 ± 3.35                  |
| LVPWT (mm)                | 1.37 ± 0.04†   | 3.11 ± 0.11 | 2.99 ± 0.07           | 3.01 ± 0.19                   |
| LVEDd (mm)                | 7.19 ± 0.19†   | 9.59 ± 0.39 | 9.34 ± 0.48           | 9.27 ± 0.37                   |
| LVEDs (mm)                | 4.04 ± 0.15†   | 5.81 ± 0.23 | 5.53 ± 0.34           | 5.62 ± 0.41                   |
| LVEF (%)                  | 73.65 ± 2.29†  | 47.11 ± 0.90 | 49.41 ± 1.27         | 46.83 ± 1.79                  |
| RVAVW (mm)                | 0.45 ± 0.05†   | 0.95 ± 0.03 | 0.83 ± 0.04‡          | 0.92 ± 0.09                   |
| PA VTI (%)                | 41.63 ± 1.82†  | 25.19 ± 1.26 | 29.29 ± 2.34‡         | 25.09 ± 1.33                  |
| RV mass (mg)              | 228.27 ± 5.72† | 301.02 ± 13.33 | 262.62 ± 8.96‡       | 305.54 ± 4.81                 |
| RV index (%)              | 0.59 ± 0.02†   | 0.80 ± 0.02 | 0.69 ± 0.01§          | 0.81 ± 0.01                   |
| LV mass (mg)              | 750.22 ± 57.58§ | 1389.28 ± 95.89 | 1361.03 ± 57.22      | 1391.87 ± 52.34               |
| LV index (%)              | 1.97 ± 0.18†   | 3.69 ± 0.18 | 3.63 ± 0.25           | 3.69 ± 0.16                   |
| RVHI (%)                  | 30.57 ± 2.39†  | 21.73 ± 1.42 | 19.33 ± 1.18†         | 21.98 ± 9.64                  |
| Lung (mg)                 | 3067.21 ± 78.20† | 5519.62 ± 171.01 | 4473.18 ± 98.91§     | 5602.31 ± 132.43              |
| Lung index (%)            | 8.01 ± 0.27†   | 14.67 ± 0.20 | 11.92 ± 0.28§         | 14.85 ± 0.29                  |

Values are presented as mean ± SD. All data for animals that underwent chest opening but not SBA were similar to those in the control group and are not shown in Table 1.

*Measurement by right heart catheterization.
†P < 0.01, compared with the remaining three groups.
‡P < 0.05, compared with SAB without PADN group.
§P < 0.01, compared with SAB or SAB without PADN group.

LVEDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVPWT, systolic left ventricular posterior wall thickness; LVEDd, left ventricular end-diastolic diameter; PA VTI, pulmonary artery velocity time integral; PADN, pulmonary artery denervation; RVAVW, right ventricular anterior wall thickness.
Differences in expressions of adrenergic receptors

In SAB animals, $\beta_2$-AR and $\beta_3$-AR were downregulated, but $\alpha_1$-A-AR and $\alpha_1$-D-AR were upregulated in lung tissues (Fig. 3b and 3c). In contrast, PADN was associated with upregulation of $\beta_2$-AR and $\beta_3$-AR and downregulation of $\alpha_1$-A-AR and $\alpha_1$-D-AR (Fig. 3b and 3d). There were no changes in $\beta_1$-AR and $\alpha_1$-B/C-AR in the SAB group or the SAB with PADN group.

Abnormal regulation of receptors in the PA of patients with HF-PH

Compared to patients without PH, patients with HF-PH had significantly downregulated $\beta_2$-AR, $\alpha_1$-A-AR, $\alpha_1$-B-AR, and eNOS$^{\text{Ser-633}}$; however, they had upregulated $\alpha_1$-D-AR (Fig. 4).

Clinical results of PADN for patients with combined post-capillary and pre-capillary PH

Ten HF-PH patients (seven men; average age = 67.5 years) underwent percutaneous PADN (Suppl. Table S1). Of them, three patients had ischemic cardiomyopathy after a previous myocardial infarction and stent implantation, two had hypertrophic obstructive cardiomyopathy after percutaneous trans-septal myocardial ablation, four had hypertension, and six had diabetes. Five patients had atrial fibrillation. $\beta$-blockers were prescribed for six patients and diuretics were prescribed for all patients. In particular, sildenafil was required for eight patients. Seven patients had DPG$^<7$ mmHg (combined post-capillary and pre-capillary PH [Cpc-PH]) and the remaining three patients had a DPG$^\geq7$ mmHg.

Immediately after PADN, all but one patient showed an average mPAP reduction of $>10\%$ with RHC (data not included in this analysis). No periprocedural complications were recorded. As shown in Table 2, PADN resulted in significant reductions in PAP and PVR (Fig. 5, Suppl. Fig. S4). Notably, we also found a statistically significant reduction in LVEDP at six months after PADN, with a net increase in cardiac output of 31%, which was in line with the reduction of N-proBNP.

Echocardiographic measurements showed that the significant reduction of PAP was not accompanied by significant
improvements in either LV or RV function, although those functional parameters tended to be slightly changed (Table 2). As shown in Suppl. Fig. 4, three patients had reduced LVEF at six months after PADN (case 1, from 63% to 57%; case 2, from 62% to 54%; and case 3, from 50% to 48%). Of these three patients, only case 1 had repeated LVEDP (20 mmHg) at six months after PADN compared to baseline LVEDP (19 mmHg). During six months of follow-up, no all-cause death was observed. Two patients were readmitted to the hospital because of worsening dyspnea.

Discussion

The present study is the first to demonstrate the effects and mechanisms of improvements in cardiac function and hemodynamics due to PADN. The main findings were: first, sympathetic nerve damage by surgical and chemical PADN is associated with confirmed reductions in RVSP/RAP and improvements in RV function; second, downregulation of β2-AR and upregulation of α1-D-AR may have critical roles in the development of HF-PH; and third, percutaneous PADN is feasible and effective for improving hemodynamics, exercise capacity, and cardiac function of HF-PH patients.

The pulmonary vasculature is generally innervated by sympathetic, parasympathetic, and sensory nerve fibers. Decreased arterial PO2 increases sympathetic nerve stimulation, which further stimulates baroreceptors and noradrenergic fibers in the PA with excessive release of NE and proximal airway segments.20 This is supported by our finding that SAB animals had a large number of sympathetic nervous endings. We previously reported21 that the PA sympathetic nervous bundle usually localizes at the left lateral wall of the main PA, with the shortest distance from nerve endings to the vessel lumen being <1 mm, which is an anatomic feature indicating the feasibility of using radiofrequency to damage nerves.

Two classes of abundant receptors in PA (endothelial and smooth muscle cells) are β-ARs and α1-ARs. Although increased vascular resistance is mediated by α1-AR on sympathetic nerve stimulation, Hussain et al.22 found that contractions of the PA due to phenylephrine are mediated, in part, via the α1D-subtype adrenoceptor. Our results showed that different expressions of AR might be explained by different tissues used for analysis (lung tissues from animals...
and PA from patients). However, the consistent finding was the upregulation of α1D-AR; this result further suggests that antagonists particularly targeting this subtype of α1-AR are expected. Similarly, we also found profound downregulation of β2-AR in either animal lung tissues or human PA. β2-AR has been reported to be important for mediating vasorelaxation and cAMP synthesis in lymphocytes of patients with primary pulmonary hypertension and for attenuating lung injuries. These results are in agreement with our results indicating that downregulation of β2-AR occurs parallel to reduced cAMP and cGMP in SAB. Furthermore, Pourageaud et al. described that β2-AR-mediated (but not β1-AR-mediated, β3-AR-mediated) relaxation in the PA was NO-independent and endothelium-independent, as supported by our findings. However, even the interaction between β2-AR and α1-D-AR balancing the pulmonary vascular tone was not fully understood from the present study, it was obviously that β2-AR and α1-D-AR may be the two most important AR for mediating PA contractions, smooth muscle cell proliferation, and endothelial dysfunction. This might be why nebivolol had significant advantages over older β-blockers for PAH patients. On the other hand, eNOS mainly localize at endothelium and has not been reported to be measured in smooth muscle cells. The significantly reduced expression of PKA-Akt-eNOS signaling might largely reflect the injury of endothelial cells.

SAB is an accepted model used to study human pathophysiologically compatible models of valvular aortic stenosis-induced LV hypertrophy. With the development of diastolic HF due to SAB, both diastolic and systolic HF occurred, which was in agreement with our results indicating that SAB animals had significantly reduced LVEF. Importantly, in SAB rats, we found that the presence of PH was mainly caused by progressive PA remodeling and contraction, similar to Cpc-PH in HF patients. However, non-significant improvements in the LV function of animals may be due to the short (only four weeks) follow-up.
**Fig. 4.** Receptor expressions in human pulmonary arterial tissue specimens. (a) Western blotting analysis showed downregulation of β2-AR, α1A-AR, and α1B-AR, but upregulation of α1D-AR in human pulmonary arterial tissues. (b) Protein expression levels were normalized against those of GAPDH and quantified. (c) Pulmonary arterial tissue lysates were immunoprecipitated with an anti-eNOS antibody and blotted with an anti-phospho-Ser633-eNOS antibody. (d) Bar graph shows quantitative results for phospho-Ser633-eNOS. *p < 0.05 vs. control.

AR, adrenaline receptor; eNOS, endothelial nitric oxide synthase.

**Table 2.** Changes in exercise capacity, hemodynamics, and cardiac function of 10 patients with HF-PH at six months after PADN.

|                     | Baseline          | At 6 months       | P     |
|---------------------|-------------------|-------------------|-------|
| NT-pro BNP (pg/mL)  | 2311 (580–13,876) | 1563 (550–3437)   | 0.074 |
| WHO class, III–IV (n (%)) | 9 (56.3) | 1 (6.3) | 0.003 |
| 6-min walk distance (mm) | 367.3 ± 97.9 | 451.9 ± 64.3 | <0.001 |
| Right heart catheterization |                     |                   |       |
| Right atrial pressure (mmHg) | 13.9 ± 5.2 | 12.3 ± 4.4 | 0.158 |
| Systolic right ventricular pressure (mmHg) | 67.8 ± 18.0 | 58.8 ± 14.2 | 0.001 |
| Systolic pulmonary arterial pressure (mmHg) | 66.9 ± 18.5 | 55.9 ± 13.8 | <0.001 |
| Mean pulmonary arterial pressure (mmHg) | 42.6 ± 10.9 | 33.9 ± 8.2 | <0.001 |
| Pulmonary arterial wedge pressure (mmHg) | 22.2 ± 9.7 | 18.5 ± 5.8 | 0.140 |
| Left ventricular end-diastolic pressure (mmHg) | 23.0 ± 7.6 | 18.6 ± 5.6 | 0.027 |
| Diastolic pressure gradient (mmHg) | 8.6 ± 1.6 | 5.9 ± 1.3 | 0.303 |
| Pulmonary vascular resistance (Wood units) | 8.4 ± 5.4 | 4.5 ± 2.6 | 0.005 |
| Cardiac output (L/min) | 2.9 ± 1.1 | 3.8 ± 1.4 | 0.001 |
| Pulmonary arterial compliance (mL/mmHg) | 1.2 ± 0.5 | 1.8 ± 0.9 | 0.007 |

(continued)
Left heart disease is the most common cause of PH; it is mainly caused by left heart systolic or diastolic dysfunction.29 Recently, DPG ≥ 7 mmHg has been considered an indicator of HF-PH severity regardless of LV function. Seven patients in this study were classified as having Cpc-PH. Although targeting PAH medications were not extensively accepted for the routine treatment of HF-PH, our team tested the efficacy of PADN for PH caused by left heart disease,18 thus confirming the potential of PADN for this particular PH population. In the present study, we found significant improvements in pulmonary arterial hemodynamics according to either RHC or echocardiography; furthermore, the results supported our previous report.18 Similar to an animal study, we did not observe

| Table 2. Continued. | Baseline | At 6 months | P  |
|--------------------|----------|-------------|----|
| SvO₂ (%)           | 73.2 ± 7.1 | 75.3 ± 4.9 | 0.102 |
| Echocardiographic measurements | | | |
| Left atrial diameter at end-systole (mm) | 50.7 ± 5.2 | 50.4 ± 6.6 | 0.834 |
| Left ventricular diameter at end-diastole (mm) | 58.2 ± 13.1 | 58.1 ± 11.9 | 0.444 |
| Left ventricular diameter at end-systole (mm) | 43.3 ± 15.5 | 44.4 ± 14.1 | 0.126 |
| Left ventricular ejection fraction (%) | 47.8 ± 14.0 | 49.3 ± 15.3 | 0.188 |
| Systolic pulmonary arterial pressure (mmHg) | 69.6 ± 19.0 | 58.0 ± 19.0 | 0.033 |
| Mean pulmonary arterial pressure (mmHg) | 42.6 ± 10.9 | 33.9 ± 8.2 | <0.001 |
| Right ventricular diameter (mm) | 39.4 ± 9.9 | 39.7 ± 9.1 | 0.797 |
| Pericardial fluid (mm) | 2.3 ± 3.1 | 1.9 ± 2.3 | 0.180 |
| Left ventricular Tei | 0.42 ± 0.09 | 0.40 ± 0.08 | 0.998 |
| Right ventricular Tei | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.093 |

HF, heart failure; NT-proBNP, N-terminal brain natriuretic peptide; PADN, pulmonary artery denervation; PH, pulmonary hypertension; WHO, World Health Organization.

**Fig. 5.** Changes in the 6-min walk distance (6MWD), pulmonary arterial hemodynamics, and LVEF. CO, cardiac output; m, mean; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal brain natriuretic peptide; PAC, pulmonary arterial compliance; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vessel resistance; RVP, right ventricular pressure; s, systolic; SV, stroke volume.
significant improvements in either LV or RV functions after PADN for HF-PH patients. Possible explanations could include the short (only six months) follow-up and small patient numbers ($n=10$). Furthermore, of three patients who had reduced LVEF after PADN (Suppl. Fig. 4), two had well-preserved LVEF at baseline (63% for case 1 and 62% for case 2). Even if we could find correlations between HF-PH patterns, because of the small patient population, the findings mentioned might have indicated that different mechanisms contributed to the development of PH induced by diastolic or systolic cardiac dysfunction.

**Limitations**

This study had some limitations. First, we could not measure ARs in the PA of animals because the distal vessel segments were too small to be separated. However, western blotting was performed with the PA of humans, which could conclusively analyze the abnormal regulation of adrenaline receptors. Second, we did not analyze the relationship between abnormally regulated protein expressions and the severity of PH in animals or patients. Third, endothelial cells and smooth muscle cells from human PA were not separately cultured, which caused trouble in interpreting the differences between two types of cells. As eNOS is usually expressed in endothelium, downregulated eNOS and PKA-Akt expressions strongly indicated the severe endothelial injury. Fourth, shorter follow-up and small patient numbers might have been the major reasons for not observing improvements in cardiac function. However, reduced LVEDP and PAWP at six months after PADN might underscore the potential of PADN for treating HF-PH. In the near future, we will report our first randomized study comparing PADN with sham for HF-PH patients.

**Conclusion**

The present study reported significant improvements in hemodynamics achieved by PADN. The mechanism is that upregulating $\beta_2$-AR density and downregulating $\alpha_1$-D-AR density modulate downstream signaling to improve NO bioavailability, thereby improving pulmonary vascular remodeling and contraction. Further randomized clinical
Acknowledgments
The authors thank Ms. Ling Lin, Ms. Wen Teng, and Ms. Hai-Mei Xu for their data monitoring and data collection throughout the study.

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

Funding
This study was funded by grants from the National Science Foundation of China (NSFC 91639303, NSFC 81770441), Jiangsu Natural Science Funds (2016-870), Jiangsu Six Talent Peaks (2015-WSN-069), and Nanjing Science and Technology Committee (201402045).

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