Hemodynamics and right-ventricle functional characteristics of a swine carotid artery-jugular vein shunt model of pulmonary arterial hypertension: An 18-month experimental study

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Abstract

The continuous changes in pulmonary hemodynamic properties and right ventricular (RV) function in pulmonary arterial hypertension (PAH) have not been fully characterized in large animal model of PAH induced by a carotid artery–jugular vein shunt. A minipig model of PAH was induced by a surgical anastomosis between the left common carotid artery and the left jugular vein. The model was validated by catheter examination and pathologic analyses, and the hemodynamic features and right-ventricle functional characteristics of the model were continuously observed by Doppler echocardiography. Of the 45 minipigs who received the surgery, 27 survived and were validated as models of PAH, reflected by mean pulmonary artery pressure ≥ 25 mmHg, and typical pathologic changes of pulmonary arterial remodeling and RV fibrosis. Non-invasive indices of pulmonary hemodynamics (pulmonary artery accelerating time and its ratio to RV ventricular ejection time) were temporarily increased, then reduced later, similar to changes in tricuspid annular displacement. The Tei index of the RV was elevated, indicating a progressive impairment in RV function. Surgical anastomosis between carotid artery and jugular vein in a minipig is effective to establish PAH, and non-invasive hemodynamic and right-ventricle functional indices measured by Doppler echocardiography may be used as early indicators of PAH.

Keywords: Pulmonary arterial hypertension, arterial venous shunt, animal model, minipigs, Doppler echocardiography

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Introduction

Pulmonary arterial hypertension (PAH) is one of the most common hemodynamic disorders, which can occur during the progression of congenital heart diseases (CHD) with a left to right shunt.^{1–3} Patients diagnosed with CHD and PAH are considered to have a poor prognosis since comorbidities of PAH often lead to a worsening of the pulmonary circulatory disorder. This interaction can cause right ventricular (RV) dysfunction and cardiac failure.^{4,5} Moreover, comorbidities of PAH often increase surgical risks for the treatment of CHD, and the success rate of the surgical procedure and long-term survival of these patients are much lower than CHD patients without PAH.^{6,7} Therefore, early PAH detection and treatment, particularly in pediatric patients with CHD, is of significance for the improvement of clinical outcomes.

Animal models of PAH induced by an arterial to venous (A–V) shunt are an effective way to mimic the hemodynamic characteristics of pulmonary circulation observed in patients with CHD complicated with PAH.^{8,9} However,

early studies to establish an A–V shunt *via* the carotid artery–jugular vein-induced PAH model were primarily conducted in rats¹⁰ and rabbits.^{11,12} The cardiovascular systems in pigs have anatomical properties similar to humans compared with the previously described animal models.¹³ Moreover, large animal models of PAH, like those established in minipigs, are more convenient for hemodynamic measurements, including cardiac catheter and echocardiography measurements. Therefore, the carotid artery–jugular vein shunt-induced PAH model in large animals, such as in minipigs, could be an effective tool for the study of pathophysiological features and early prevention strategies in CHD patients complicated with PAH.

Although the gold standard for PAH diagnosis and hemodynamic assessment is right heart catheter (RHC) examination, it has been accepted that non-invasive detective methods, such as Doppler echocardiographic measurements, can also be reflective of hemodynamic characteristics in patients with PAH.^{14–16} Previous studies have established echocardiographic measurements in patients and animal models with PAH which often focused on the consistency

Figure 1 Surgical establishment and confirmation of the anastomosis between the left common carotid artery and the left jugular vein. (a) An end-to-side anastomosis was performed between the left common carotid artery and the left jugular vein; (b, c) assessment of patency and the blood flow frequency spectrum of the shunt between the left common carotid artery and left jugular vein using Doppler echocardiography. A: left common carotid artery; V: left jugular vein

between echocardiographic indices of pulmonary hemodynamics and pulmonary arterial pressure (PAP) measured by RHC.^{15,17,18} In this study, we report the successful establishment of a minipig PAH model induced by surgical anastomosis between the left common carotid artery and the left jugular vein. Moreover, we applied sequential Doppler echocardiographic analyses and indices that reflect the pulmonary hemodynamic properties and RV functions in this model until 18 months postsurgery.

Materials and methods

Animal treatments and groups

Fifty-one Guangxi Bama minipigs (aged one month) were purchased from the Laboratory Animal Center of Guangxi Province. Body weights of the minipigs were 6.46 ± 0.05 kg. All animals were housed individually, in clean stainless steel cages in an animal room set at 24°C – 28°C and 55–70% relative humidity under a natural day/night light cycle, and provided with regular food and free access to water. All animal care and experiments were performed in accordance with the Guide for the Care and Use of Animals from the Laboratory Animals Center of Guangxi Medical University. All minipigs were randomly divided into two groups, the shunted ($n=45$) and sham group ($n=6$). Animals from both groups were maintained under the same conditions before and after surgery, and monitoring duration of the current study was 18 months postsurgery.

Surgical protocols for the establishment of PAH minipig models

Anesthesia of the minipigs was performed with intravenous injection of pentobarbital (40 mg/kg). Once the anesthesia took effect, a 5-cm incision was made on the left side of the neck. For minipigs in the shunted group, the left jugular

vein was exposed and then injected with 1 mg/kg heparin. The left common carotid artery and left jugular vein were dissected and the left common carotid artery was clamped at the proximal site, ligated at the distal site, and then amputated 0.5–1 cm before the bifurcation. Under an operative microscope, a 6–8 mm hole was made in the left jugular vein and an end-to-side anastomosis was performed between the left common carotid artery and the left jugular vein using a Prolene 7-0 suture (Figure 1(a)). Degree of anastomosis patency was tested by releasing the vascular clamps to visualize pulsatile blood flow at the proximal site of the left jugular vein. Penicillin was administered postoperatively to prevent secondary infections. The minipigs in the sham group underwent all of the same surgical procedures except for the end-to-side anastomosis of the left common carotid artery and the left jugular vein.

RHC examinations

Before and every two months until 18 months after the surgical procedure, a 7F Swan-Ganz catheter (Baxter Healthcare Corp., Irvine, USA) was placed in the pulmonary artery *via* the right jugular vein or right femoral vein to measure PAP (systolic, diastolic and mean), pulmonary vascular resistance (PVR), and cardiac output (CO). Cardiac index was calculated as CO divided by body surface area. Measurement of the parameters was performed in triplicate for each minipig, and data were acquired by a multilead physiologic recorder (Space Labs Medical, USA). A mean pulmonary arterial pressure (mPAP) of ≥ 25 mmHg indicated successful induction of PAH.

Echocardiographic assessment of the anastomosis patency

Patency and blood flow shunting were detected after surgical establishment of the shunt between the left common

Figure 2 Representative echocardiographic images to assess pulmonary hemodynamic characteristics and right ventricle function. (a) Echocardiographic assessment of transpulmonary forward blood flow characteristics; (b) echocardiographic assessment of Tei index of right ventricular myocardial performance; (c) echocardiographic assessment of the tricuspid annular displacement during the systolic phase. AT: acceleration time; DT: deceleration time; ET: ejection time. (A color version of this figure is available in the online journal.)

carotid artery and left jugular vein using a IE33 Color Doppler Echocardiography (Philips Instrument, Netherlands) and an L11-3 high-frequency detector (frequency: 4.0–8.0 MHz). The rate of blood flow shunting was calculated as $S \cdot VTI \cdot HR / 60$ (where S, the surface area of the anastomosis; VTI, velocity–time integration; HR, heart rate) as shown in Figure 1(b) and (c).

Echocardiographic assessment of transpulmonary forward blood flow characteristics

Characteristics of the transpulmonary forward blood flow were detected *via* the pulsed wave Doppler Echocardiography at the site of the pulmonary valve every two months following surgery for 18 months. The following parameters were recorded: pulmonary artery acceleration time (PAAT, defined as the time from beginning to maximum transpulmonary forward flow), pulmonary artery deceleration time (PADT, defined as the time from the maximum to the ending of the transpulmonary forward flow), and right ventricular ejection time (RVET, defined as the time from beginning to the ending of the transpulmonary forward flow). The ratio between PAAT and RVET (PAAT/RVET) was also calculated (Figure 2(a)).

Assessment of Tei index of the right ventricle

The Tei index of the RV was measured *via* tissue Doppler imaging (TDI) at the apical cardiac four-chamber view by detecting the frequency spectrum of tricuspid annular movement. The Tei index was calculated using the previously described¹⁹ formula $[(\text{isovolumetric contraction time} + \text{isovolumetric relaxation time}) / \text{ejection}$

time) *via* TDI analyses of the tricuspid annular movement (Figure 2(b)).

Tricuspid annular displacement (TAD) during the systolic phase of the right ventricle

Two-dimensional speckle tracking echocardiography-based TAD measurements were performed during the systolic phase of the right ventricle *via* the Qlab 8.1 system at the cardiac four-chamber view. Three points were used for initialization of the end-diastolic frame. These points were placed at the insertion of the anterior leaflet and septal leaflet into the tricuspid annulus and the RV apex (Figure 2(c)). The software automatically tracked two tricuspid annular points and calculated their absolute displacement toward the RV apex throughout the cardiac cycle. Maximal end-systolic displacement of the mid-annular point was presented as the distance of annular motion.

Lung tissue and the RV myocardium pathologic analyses

The lungs and heart were harvested and dissected. Then, the lower lobes of the left and right lungs, as well as the RV free wall were isolated separately for further analyses. Tissue blocks of lung were sliced orthogonally to the pulmonary artery, and then tissue blocks of lung and RV free wall were fixed with 4% formaldehyde phosphate buffer for 48 h, followed by alcohol dehydration and paraffin embedding. Microtomed tissue sections (5 mm thickness) were stained with hematoxylin, eosin (HE) and additional Masson staining for the RV myocardium before routine optic microscopic examination, as described

previously.¹⁰ The Heath-Edwards methods were applied to evaluate pathologic changes in lung tissues, and morphologic classification of small or middle pulmonary arteries of $\geq$ class II indicated pathologic changes of PAH.^{15,20} The fixed tissue slides were observed with optic microscope and the small- to medium-sized pulmonary arteries with similar diameters (10 selected arteries for each minipig, with a diameter between 30 and 100 μ m) were evaluated and compared. The percentile wall thickness (WT%) was defined as the ratio between medial WT and the external diameter of the artery. Moreover, the percentile of the collagen area (AREA %) stained with blue, defined as the percentile of the collagen to the area of the overall visual fields were also calculated with the pathologic analytic equipment (LEICA DMR+Q550, Germany).

Statistical analyses

The Statistical Package for Social Sciences, version 16.0, for Windows (SPSS, Chicago, IL), was used for all statistical analyses. Continuous variables are presented as mean $\pm$ standard error (SEM), and the comparisons of means between two independent variables were evaluated using a Student's *t*-test. Correlations between PAAT, PAAT/RVET, Tei index, and mPAP were analyzed using partial regression analyses, and correlations between TAD and mPAP were analyzed *via* quadratic regression analyses. $p < 0.05$ was considered statistically significant.

Results

Surgical establishment of the PAH minipig model

Of the 45 minipigs randomized to the shunted group, 13 died during the perioperative period due to anesthetic complications, postprocedural infections, or other comorbidities. In the remaining 32 surviving minipigs, results of Doppler echocardiography showed early occlusion or severe stenosis of the anastomosis in five minipigs, and the remaining 27 minipigs had patent and effective A-V anastomosis throughout the following-up duration, with a mean shunt rate of 10.67 ± 0.16 cm³/s. Twelve of the pigs underwent surgical repair of the AV shunt six months (six pigs) or 12 months (another six pigs) after AV shunt, aiming to evaluate the changes of the hemodynamics and right cardiac function after surgical treatment. Minipigs in the shunted group grew slower than the sham group. At the end of the monitoring (18 months postsurgery), results of the RHC and the pulmonary pathological analyses showed that these 27 minipigs fulfilled the criteria of successful modeling (27/45, 60%), with the body weight of 19.84 ± 0.13 kg. The six minipigs in the sham group all survived after the 18-month follow-up, with the body weight of 24.12 ± 0.44 kg.

Sequential changes of PAP, PVR in PAH minipigs as measured by RHC examinations

Sequential changes of PAP and PVR in minipigs of the two groups are shown in Table 1. The PAP (systolic, diastolic, and mean) of minipigs in the shunted group increased progressively following surgery ($p < 0.05$), with mPAP

reaching 42.18 ± 0.67 mmHg at 18 months postsurgery. Similar changes were detected in PVR whose progressive increment reaching 3.93 ± 0.08 WU (Wood unit) at end point. While for minipigs in the sham group, no significant difference in PAP and PVR was detected during follow-up ($p > 0.05$).

Sequential changes of hemodynamic parameters of the pulmonary artery

Sequential changes in hemodynamic parameters, including PAAT, RVET, PADT, and PAAT/RVET, are shown in Table 2. Generally, for the minipigs in the shunted group, the parameters of PAAT, RVET, PADT, and PAAT/RVET all showed trends of initially increasing and then decreasing. Specifically, these parameters were elevated two months postsurgery, and reached a peak at 6–8 months postsurgery, followed by a decrease. However, for the minipigs in the sham group, no significant changes in these parameters were detected. Moreover, PAAT, RVET, and PADT in the shunted group were significantly higher than those in the sham group at six months postsurgery ($p < 0.05$); however, these values were significantly lower at the 12 and 18 months postsurgery ($p < 0.05$). PAAT/RVET was significantly lower in the minipigs of the shunted group compared to those of the sham group from 10 months postsurgery ($p < 0.05$).

Sequential changes in functional parameters of the right ventricle

The Tei index of minipigs in the shunted group showed an increasing trend during follow-up, while no significant changes in Tei index were detected in the sham group (Table 3). We also found that the Tei index from the shunted group was generally higher than was found in the sham group (Table 3). In addition, TAD of minipigs from the shunted group showed a trend of initially increasing and then decreasing, with peak TAD detected at 12 months postsurgery. No significant changes in TAD were detected for animals in the sham group. Similarly, we found that the TAD of minipigs in the shunted group was generally higher compared to the sham group (Table 3).

Correlations of pulmonary hemodynamic and RV function

By performing regression analyses, we found that mPAP in the minipigs was negatively correlated with pulmonary hemodynamic parameters including PAAT ($r = -0.645$, $p < 0.05$; Figure 3(a)) and PAAT/RVET ($r = -0.603$, $p < 0.05$; Figure 3(b)). Moreover, a linear positive correlation was identified between Tei and mPAP ($r = 0.728$, $p < 0.05$; Figure 3(c)). A quadratic correlation was observed for TAD and mPAP ($r = 0.815$, $p < 0.05$; Figure 3(d)).

Pathologic changes of pulmonary arteries and the RV myocardium

As shown in Figure 4(a), minimal occurrence of hyperplasia or fibrosis was detected in the pulmonary arteries of pigs in the sham group. However, hyperplasia and thickening of

Table 1 Sequential changes of pulmonary arterial pressure and pulmonary vascular resistance of the minipigs

Groups	months	Number of minipigs	PASP (mmHg)	PADP (mmHg)	mPAP (mmHg)	PVR (WU)	CO (L/min)	CI (L/min m ²)	Wt (kg)
Shunted	0	27	14.32 ± 0.10	4.63 ± 0.07	10.13 ± 0.08	0.94 ± 0.01	1.10 ± 0.01	3.18 ± 0.03	6.46 ± 0.05
	2	27	19.81 ± 0.13	5.02 ± 0.08	14.25 ± 0.08	1.07 ± 0.01	1.56 ± 0.02 ^{ad}	4.33 ± 0.04 ^{ad}	6.97 ± 0.06
	4	27	30.65 ± 0.17 ^{ad}	11.28 ± 0.12 ^{ad}	23.71 ± 0.13 ^{ad}	1.41 ± 0.02	1.81 ± 0.01 ^{ad}	4.21 ± 0.06 ^{ad}	9.01 ± 0.10 ^a
	6	27	35.72 ± 0.20 ^{ad}	11.73 ± 0.16 ^{ad}	28.14 ± 0.18 ^{ad}	1.73 ± 0.02 ^{ad}	2.11 ± 0.01 ^{ad}	4.30 ± 0.03 ^{ad}	10.95 ± 0.11 ^a
	8	21	44.08 ± 0.36 ^{abd}	14.17 ± 0.15 ^{ad}	31.36 ± 0.23 ^{ad}	1.97 ± 0.01 ^{ad}	2.42 ± 0.03 ^{ad}	4.41 ± 0.07 ^{ad}	13.04 ± 0.09 ^a
	10	21	49.66 ± 0.48 ^{abd}	17.50 ± 0.26 ^{abd}	35.42 ± 0.35 ^{abd}	2.56 ± 0.02 ^{abd}	2.73 ± 0.03 ^{abd}	4.20 ± 0.05 ^{ad}	15.07 ± 0.12 ^{ab}
	12	21	52.35 ± 0.46 ^{abd}	17.55 ± 0.34 ^{abd}	36.31 ± 0.44 ^{abd}	2.89 ± 0.00 ^{abd}	2.91 ± 0.02 ^{abd}	4.36 ± 0.06 ^{ad}	16.89 ± 0.10 ^{ab}
	14	15	52.92 ± 0.55 ^{abd}	18.36 ± 0.48 ^{abd}	37.29 ± 0.54 ^{abd}	3.17 ± 0.01 ^{abd}	3.18 ± 0.04 ^{abd}	4.22 ± 0.04 ^{ad}	18.24 ± 0.13 ^{ab}
	16	15	56.43 ± 0.85 ^{abd}	20.89 ± 0.49 ^{abd}	40.05 ± 0.62 ^{abd}	3.59 ± 0.02 ^{abd}	3.43 ± 0.01 ^{abd}	4.48 ± 0.08 ^{ad}	19.01 ± 0.21 ^{abd}
Sham	18	15	59.26 ± 1.17 ^{abcd}	21.92 ± 0.63 ^{abcd}	42.18 ± 0.67 ^{abcd}	3.93 ± 0.08 ^{abcd}	3.50 ± 0.02 ^{abcd}	4.49 ± 0.06 ^{ad}	19.84 ± 0.13 ^{abcd}
	0	6	14.12 ± 0.57	4.60 ± 0.26	10.34 ± 0.34	0.92 ± 0.03	1.12 ± 0.05	3.20 ± 0.06	6.65 ± 0.30
	2	6	14.73 ± 0.48	4.69 ± 0.28	10.51 ± 0.45	0.93 ± 0.08	1.18 ± 0.05	3.19 ± 0.07	7.21 ± 0.30
	4	6	14.55 ± 0.40	4.71 ± 0.28	10.16 ± 0.38	0.95 ± 0.06	1.39 ± 0.08	3.16 ± 0.11	9.14 ± 0.42
	6	6	16.34 ± 0.42	5.16 ± 0.31	11.47 ± 0.65	0.97 ± 0.07	1.63 ± 0.10	3.13 ± 0.12	11.89 ± 0.36
	8	6	16.68 ± 0.63	5.52 ± 0.38	11.62 ± 0.47	0.96 ± 0.10	1.78 ± 0.06	3.16 ± 0.07	13.34 ± 0.48
	10	6	15.97 ± 0.52	5.34 ± 0.33	11.07 ± 0.38	0.94 ± 0.12	2.09 ± 0.14	3.19 ± 0.16	16.11 ± 0.50
	12	6	16.42 ± 0.57	5.56 ± 0.41	11.75 ± 0.45	0.95 ± 0.08	2.37 ± 0.11	3.07 ± 0.09	18.58 ± 0.46
	14	6	16.75 ± 0.76	6.13 ± 0.54	12.19 ± 0.42	0.97 ± 0.07	2.54 ± 0.06	3.15 ± 0.06	20.94 ± 0.43
	16	6	16.72 ± 0.53	5.65 ± 0.47	11.84 ± 0.58	0.98 ± 0.07	2.71 ± 0.07	3.23 ± 0.07	22.24 ± 0.35
	18	6	16.89 ± 0.42	5.77 ± 0.46	11.76 ± 0.52	0.97 ± 0.03	2.92 ± 0.06	3.30 ± 0.15	24.12 ± 0.44

Data are presented as mean ± SEM. a, $P < 0.05$ as compared with month 0; b, $P < 0.05$ as compared with month 6; c, $P < 0.05$ as compared with month 12; d, $P < 0.05$ as compared with the minipigs from the sham group at the same time point.

CI: cardiac index; CO: cardiac output; mPAP: mean pulmonary arterial pressure; PADP: pulmonary artery diastolic pressure; PASP: pulmonary artery systolic pressure; PVR: pulmonary vascular resistance; Wt: body weight.

Table 2 Sequential changes of pulmonary hemodynamic parameters of the minipigs

Groups	months	Number of minipigs	PAAT (ms)	RVET (ms)	PADT (ms)	PAAT/RVET
Shunted	0	27	86.33 ± 0.94	274.52 ± 0.70	190.29 ± 1.43	0.30 ± 0.00
	2	27	95.50 ± 0.96 ^a	304.50 ± 1.70 ^{ad}	226.00 ± 0.84 ^{ad}	0.32 ± 0.00
	4	27	111.33 ± 1.78 ^{ad}	299.50 ± 0.09 ^{ad}	186.33 ± 1.07 ^a	0.37 ± 0.02 ^a
	6	21	113.67 ± 2.14 ^{ad}	370.50 ± 0.55 ^{ad}	246.33 ± 1.70 ^{ad}	0.31 ± 0.00
	8	21	97.17 ± 1.27 ^{abd}	355.33 ± 0.48 ^{ad}	259.67 ± 0.26 ^{ad}	0.29 ± 0.00
	10	21	96.33 ± 0.82 ^{abd}	338.00 ± 1.97 ^{abd}	242.17 ± 1.80 ^{ad}	0.28 ± 0.00 ^{abd}
	12	21	89.00 ± 0.39 ^{bd}	326.00 ± 1.66 ^{abd}	233.00 ± 0.65 ^{abd}	0.26 ± 0.00 ^{abd}
	14	15	80.23 ± 0.60 ^{bcd}	309.75 ± 1.17 ^{abd}	221.32 ± 0.89 ^{abd}	0.25 ± 0.01 ^{abd}
	16	15	76.61 ± 0.85 ^{abcd}	304.43 ± 1.24 ^{abcd}	214.32 ± 0.95 ^{abcd}	0.24 ± 0.01 ^{abcd}
Sham	18	15	71.83 ± 0.79 ^{abcd}	300.43 ± 1.58 ^{abcd}	208.17 ± 0.66 ^{abcd}	0.23 ± 0.00 ^{abcd}
	0	6	90.00 ± 1.91	285.33 ± 2.40	196.52 ± 2.96	0.31 ± 0.00
	2	6	96.10 ± 2.69	297.21 ± 3.03	216.40 ± 2.67	0.30 ± 0.01
	4	6	98.42 ± 3.22	300.21 ± 0.51	210.44 ± 1.01	0.31 ± 0.00
	6	6	92.43 ± 3.56	296.21 ± 1.49	208.20 ± 1.66	0.30 ± 0.01
	8	6	98.52 ± 3.49	298.28 ± 2.88	204.00 ± 0.89	0.30 ± 0.00
	10	6	100.25 ± 2.67	301.22 ± 0.23	211.24 ± 2.40	0.31 ± 0.00
	12	6	98.78 ± 1.05	291.09 ± 0.10	199.86 ± 1.32	0.29 ± 0.01
	14	6	92.57 ± 1.46	295.81 ± 2.05	203.00 ± 0.90	0.30 ± 0.00
	16	6	94.57 ± 1.22	303.24 ± 1.51	205.06 ± 0.80	0.29 ± 0.01
	18	6	96.04 ± 2.54	298.44 ± 1.31	199.10 ± 2.01	0.29 ± 0.02

Data are presented as mean ± SEM. a, $P < 0.05$ as compared with month 0; b, $P < 0.05$ as compared with month 6; c, $P < 0.05$ as compared with month 12; d, $P < 0.05$ as compared with the minipigs from the sham group at the same time point.

PAAT: pulmonary artery acceleration time; PADT: pulmonary artery deceleration time; RVET: right ventricular ejection time.

Table 3 Sequential changes of functional parameters of the right ventricle

Groups	Months	Number of minipigs	RV Tei index	TAD (mm)
Shunted	0	27	0.37 ± 0.00	5.43 ± 0.08
	2	27	0.39 ± 0.01	6.86 ± 0.24 ^d
	4	27	0.43 ± 0.01 ^{ad}	8.84 ± 0.08 ^{ad}
	6	21	0.43 ± 0.01 ^{ad}	9.66 ± 0.19 ^{ad}
	8	21	0.44 ± 0.01 ^{ad}	10.80 ± 0.38 ^{ad}
	10	21	0.46 ± 0.01 ^{abd}	12.03 ± 0.31 ^{abd}
	12	21	0.56 ± 0.00 ^{abd}	12.46 ± 0.23 ^{abd}
	14	15	0.59 ± 0.00 ^{abd}	12.15 ± 0.01 ^{abcd}
	16	15	0.62 ± 0.01 ^{abd}	11.89 ± 0.33 ^{abcd}
Sham	18	15	0.67 ± 0.01 ^{abcd}	9.50 ± 0.17 ^{acd}
	0	6	0.36 ± 0.00	5.51 ± 0.33
	2	6	0.35 ± 0.01	5.87 ± 0.41
	4	6	0.37 ± 0.00	5.81 ± 0.49
	6	6	0.37 ± 0.01	6.00 ± 0.45
	8	6	0.36 ± 0.01	5.92 ± 0.37
	10	6	0.38 ± 0.00	6.05 ± 0.61
	12	6	0.37 ± 0.00	5.89 ± 0.44
	14	6	0.38 ± 0.01	6.10 ± 0.29
	16	6	0.39 ± 0.02	6.03 ± 0.22
	18	6	0.37 ± 0.01	6.12 ± 0.04

Data are presented as mean ± SEM. a, $P < 0.05$ as compared with month 0; b, $P < 0.05$ as compared with month 6; c, $P < 0.05$ as compared with month 12; d, $P < 0.05$ as compared with the minipigs from the sham group at the same time point. TAD: tricuspid annular displacement.

the arterial walls, particularly of the medial layer, was detected in the pulmonary arteries from the pigs in the shunted group, with peri-arterial fibrotic hyperplasia (Figure 4(b)). Severe stenosis or even occlusion of the lumen could be observed in pulmonary arteries of minipigs in the shunted group (Figure 4(c)). Pathologic classification in lung tissue range class II–III in 23 minipigs and class III–IV in four according to the Heath–Edwards methods. Regarding RV myocardial tissues from the minipigs of the shunted group, the nucleus of the cardiomyocytes was enlarged and the myocardial fibers were loosely distributed (Figure 5(a)), while those from the sham group showed closely distributed cardiomyocyte nuclei and regularly arranged myocardial fibers (Figure 5(b)). Using Masson staining, we found that collagen distribution in the right ventricle was more robust in the minipigs from the shunted group (Figure 5(c)) compared with those from the sham group (Figure 5(d)). The WT% and AREA% in the lung and right cardiac tissues of minipigs in shunted group were more remarkable as compared with the sham group, indicating that the extent of pulmonary artery remodeling and fibrosis of the RV myocardium was more severe (Table 4).

Discussion

In this study, we successfully established an A–V shunt-induced PAH model in minipigs by surgically forming an anastomosis between the left common carotid artery and the left jugular vein. The animal model was validated using an elevated mPAP ≥ 25 mmHg as measured by RHC

Figure 3 Correlations of pulmonary hemodynamics and right ventricle function. (a) Negative correlation between PAAT and mPAP; (b) negative correlation between PAAT/RVET and mPAP; (c) positive correlation between Tei index and mPAP; (d) quadratic correlation between TAD and mPAP. mPAP: mean pulmonary arterial pressure; PAAT: pulmonary artery acceleration time; RVET: right ventricular ejection time; TAD: tricuspid annular displacement

and typical pathological changes in the lungs 18 months following surgery. Moreover, by performing a series of sequential non-invasive measurements of pulmonary hemodynamics and RV function *via* Doppler echocardiography, our study showed dynamic changes of the above indices, including PAAT, PADT, RVET, PAAT/RVET, Tei index, as well as TAD. Of note, these indices were correlated with mPAP and could be used as a sensitive indicator of pulmonary hemodynamics and RV function during PAH pathogenesis.

A-V shunt-induced PAH animal models have been considered effective tools to investigate hemodynamic changes and preventative strategies targeting PAH induced by progression of CHD. A variety of surgically induced shunts between the pulmonary and systemic circulations have been reported in previous studies of PAH, including the induction of shunts between the aorta and pulmonary artery,²¹ abdominal aorta and vena cava,²² left subclavian artery and pulmonary artery,²³ and left common carotid artery and pulmonary artery.¹² Although these methods better mimic the blood flow shunts as well as overload-induced PAH in CHD patients, they are difficult to perform because elegant surgical techniques are needed and severe surgical trauma is common.⁹

Another strategy to induce PAH is with the induction of an A-V shunt. Recently, success of A-V shunt-induced PAH has been reported in small animals, including rats¹⁰ and rabbits.¹¹ Although these models are easy to establish, models in these small animals are often intolerant of repeated invasive hemodynamic measurements, and more importantly, the anatomic characteristics of these small animals are not similar to humans, which limits the translatable value. Minipigs have similar anatomic features of the human cardiovascular system.¹³ Moreover, the minipigs grew slower as compared with the models in small animals, which makes repetitive measurements of the related hemodynamic parameters more feasible and allows a longer follow-up duration. We developed this novel method of A-V-induced PAH in these animals by surgical formation of an anastomosis between the left common carotid artery and the left jugular vein. The extents of the shunt among the included minipigs were standardized by surgical induction of the similar sized anastomotic stoma and by the close monitoring with high-frequency ultrasonic images. RHC examinations detected continuously increasing PAP and PVR following surgery, and the model was validated using RHC and pathologic assessments 18 months after

Figure 4 Representative pathologic changes in pulmonary arteries from minipigs in the sham and shunted groups. (a) Pulmonary arteries from the sham group showed minimal fibrotic hyperplasia or thickening of the arteries; (b) hyperplasia and thickening of the arterial walls, particularly of the medial layer, could be detected in the pulmonary arteries from the pigs in the shunted group, with peri-arterial fibrotic hyperplasia (Heath and Edward Class II); (c) severe thickening of the media with presentation of artery stenosis of 50%, with remarkable peri-arterial fibrotic hyperplasia from the pigs of the shunted group (Heath and Edward Class III-IV). H&E staining, magnification of 400 $\times$. (A color version of this figure is available in the online journal.)

surgery. The success rate of the model was about 60%, which was acceptable for the experiment.

The frequency spectrum of pulmonary blood flow at the level of the pulmonary arterial valve was relatively easy to observe using Doppler echocardiography, and results of previous studies have indicated that changes in the shape of the blood flow frequency spectrum may be an early sign of PAH development.^{24,25} Results of our study indicated that following induction of an A-V shunt in the minipigs, the indices of forward pulmonary blood flow (PAAT, PADT,

RVET, and PAAT/RVET) showed a temporary increase until six months following surgery, which may indicate that with artificial induction of volume overload in pulmonary circulation, compensatory regulation of the right ventricle may increase CO. However, this compensatory regulation is temporary. With continuous volume overload in the pulmonary circulation, constriction of the pulmonary artery occurs and eventually leads to structural remodeling.^{26–28} This process is irreversible, as reflected by the eventual reductions of PAAT and PAAT/RVET in our

Figure 5 Representative pathology of the right ventricle from minipigs in the sham and shunted groups. (a) H&E staining of the myocardium from the shunted group showed enlarged cardiomyocyte nuclei and loosely distributed myocardial fibers; (b) H&E staining of the myocardium from minipigs of the sham group showed that cardiomyocyte nuclei were closely distributed and the myocardial fibers were arranged; (c) Masson staining of the myocardium from minipigs of the shunted group showed that collagen distribution was obvious and was mostly detected around the vessels; (d) Masson staining of the myocardium from minipigs of the sham group showed few collagen in the fields. Magnification for H&E. staining 400 $\times$; for Masson staining 100 $\times$. (A color version of this figure is available in the online journal.)

Table 4 Quantitative analyses of pulmonary arterial remodeling and right ventricular myocardial fibrosis

Groups	Pulmonary arterial remodeling			Right ventricular myocardial fibrosis	
	WT (μm)	ED (μm)	WT%	Area(μm^2)	Area%
Shunted (n = 27)	9.73 $\pm$ 0.19*	48.56 $\pm$ 0.63	0.193 $\pm$ 0.03 $^{\Delta}$	5106.70 $\pm$ 35.01 $^{\Delta}$	15.50 $\pm$ 0.07 $^{\Delta}$
Sham (n = 6)	5.75 $\pm$ 0.22	46.64 $\pm$ 0.57	0.123 $\pm$ 0.01	642.34 $\pm$ 17.36	2.70 $\pm$ 0.09

Data are presented as mean $\pm$ SEM.

area: area of the blue-stained collagen; area%: percentile of the blue-stained collagen to the overall visual fields; ED: external diameter; WT: wall thickness; WT%: percentile of WT to ED.

As compared with the minipigs from the sham group, * $P < 0.05$, $^{\Delta}$ $P < 0.01$.

minipig model. Our regression analyses suggested that both PAAT and PAAT/RVET are negatively correlated with mPAP measurements from RHC, indicating that these two indices may be used as dynamic markers of PAP during PAH pathogenesis.

Tei index is an accepted indicator of overall myocardial function, independent of cardiac hypertrophy, pre- and after load, as well as regurgitation.¹⁹ Tei index is sensitive and correlated with the indices of ventricular function as

measured by invasive strategies, which includes cardiac catheter examination.^{29–31} Previous studies have indicated that right ventricle contractility on the longitudinal axis is the most important contributor to overall RV output, longitudinal motion was primarily reflected by atrioventricular annulus movement, and TAD could be used as a marker for contractile movement of the RV myocardium.³² TAD is also correlated with ejection fraction in the right ventricle.³³ In our study, TAD showed initial increases until 12 months

following surgery, indicating an early compensatory over-contraction of the right ventricle. Continuous overload and over-contraction of the right ventricle may lead to myocardial fibrosis and remodeling of the heart, eventually causing decreased pump function in the right ventricle, as reflected by subsequent reductions of TAD after 12 months. The Tei index, however, continuously increased after A-V shunt formation, indicating that overall right ventricle function was continuously impaired during PAH pathogenesis. Interestingly, we found that the Tei index was linearly positively correlated with mPAP as measured by RHC, reflecting impaired RV function caused by progressive increments in PAP. For TAD and mPAP, a quadratic correlation was also observed, suggesting a compensatory increase in right ventricle contraction during the early phases of A-V shunt formation and subsequent decreases in right ventricle output.

Our study has several limitations. Firstly, surgical techniques and perioperative care of the minipigs during surgical induction of the A-V shunt should be explored and more experience should be obtained to improve modeling methods. Influence of many factors, such as the size and position of the anastomosis and length of the induction period may have an important impact on the modeling success rate. Secondly, our carotid artery-jugular vein shunt model may not perfectly mimic the actual hemodynamic characteristics of PAH in CHD patients, in which PAH is often induced by shunting between the pulmonary and systematic circulations. Therefore, translatability of our results should be cautionary.

Conclusion

In conclusion, surgical induction of anastomosis between the common carotid artery and jugular vein in minipigs is effective and feasible for the establishment of PAH. Non-invasive indices as measured by Doppler echocardiography (PAAT, PAAT/RVET, Tei index, and TAD) may be used as early indicators for the detection of pulmonary hemodynamic disorders and RV dysfunction in the pathogenesis of PAH.

Authors' contributions: JW conceived of the study, participated in its design and the sequence alignment, and drafted the manuscript. YH carried out the molecular genetic studies and performed the statistical analysis and helped to draft the manuscript. XL carried out the immunoassays and participated in the sequence alignment. YH participated in the sequence alignment. ZL participated in its design and coordination. All authors read and approved the final manuscript.

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