Percutaneous pulmonary artery denervation completely abolishes experimental pulmonary arterial hypertension *in vivo*

Shao-Liang Chen[1,2]*, PhD, FACC; Yao-Jun Zhang[1], MD; Lin Zhou[1], MD; Du-Jiang Xie[1], MD; Feng-Fu Zhang[1], MD; Hai-Bo Jia[1], MD; Sally S. Wong[3], PhD; Tak W. Kwan[4], MD

1. Department of Cardiology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China; 2. Department of Cardiology, Nanjing Heart Center, Nanjing, China; 3. School of Public Health, City University of New York, New York, NY, USA; 4. Division of Cardiology, Beth Israel Medical Center, New York, NY, USA

S.L. Chen and Y.J. Zhang contributed equally to this manuscript.

KEYWORDS

- denervation
- pulmonary artery
- pulmonary hypertension

Abstract

Aims: The study sought to assess the effect of percutaneous pulmonary artery denervation (PADN) on balloon-occlusion-induced acute pulmonary arterial hypertension (PAH) *in vivo*. The PADN is a minimally invasive and endovascular catheter-based interventional therapy using radiofrequency ablation to abolish the pulmonary arterial baroreceptors to pressure response.

Methods and results: To examine the efficacy of balloon-occlusion-induced PAH, twenty Mongolian dogs were randomly assigned to one of two groups: group 1 (left distal pulmonary basal trunk occlusion) and group 2 (left pulmonary interlobar artery occlusion). Afterwards, PADN treatment at the main pulmonary artery bifurcation level with left pulmonary interlobar artery occlusion in all 20 dogs was conducted. Haemodynamic parameters were measured at baseline and during balloon occlusion as well as the PADN treatment at different time points: one, two, three, five, and ten minutes. Before the PADN treatment, most haemodynamic parameters of the pulmonary artery remained unchanged in group 1 with distal pulmonary basal trunk occlusion. However, in group 2 with the occlusion of the left pulmonary interlobar artery, mean pulmonary arterial pressure, mean right ventricular pressure, and pulmonary vessel resistance gradually increased, and mean absolute difference reached peak at five minutes (Δ16.6 mmHg, Δ14.1 mmHg and Δ1,144 dye/s/cm^5, respectively; each $p<0.01$). These haemodynamic parameters at five minutes induced by left pulmonary interlobar artery occlusion were completely abolished with the PADN treatment compared to baseline (Δ0.3 mmHg, Δ0.2 mmHg, and Δ34 dye/s/cm^5, respectively).

Conclusions: Balloon occlusion of the left pulmonary interlobar artery led to a significant increase of haemodynamic parameters of the pulmonary artery. The pressure responses were completely abolished by the PADN treatment at the main bifurcation area of the left pulmonary artery.

Corresponding author: Department of Cardiology, Nanjing First Hospital, Nanjing Medical University, No. 68 Changle Road, Nanjing City, Jiangsu Province, China. E-mail: chmengx126@gmail.com

Introduction

Pulmonary arterial hypertension is defined as a group of diseases characterised by a progressive increase in pulmonary vascular resistance resulting in right heart failure and premature death[1-7]. Recent therapeutic advances have improved treatment options including prostanoids, endothelin-receptor antagonists and the phosphodiesterase type-5 inhibitors[3-6,8,9]. A meta-analysis of 23 randomised controlled trials demonstrated a reduction in mortality of the patients using the targeted therapies approved for pulmonary arterial hypertension (PAH)[10]. However, there is no cure for PAH and it remains a life-threatening disorder even though these therapies improve symptoms, haemodynamics and outcomes[11].

The pathogenesis of PAH is complicated and still largely unknown. In particular, the role of sympathetic nerve activation remains unsettled[12]. Historically, several studies have demonstrated that distention and occlusion of one branch of the pulmonary artery by the use of balloons and non-occlusive inflatable cuffed cylinders led to significant increases of pulmonary arterial pressure (PAP) and pulmonary vessel resistance (PVR)[13-18]. Of note, the increase of PAP and PVR induced by pulmo-pulmonary reflex was confirmed. To clarify the underlying mechanisms involved in this procedure, experimental study showed that pulmonary hypertension may be secondary to the distention of the main pulmonary artery by excitation of stretch receptors in or near the bifurcation area of the main pulmonary artery. In addition, the efferent limb of this reflex was predominantly mediated by the adrenergic nervous system[19]. Based on the evidence mentioned above, we hypothesised that percutaneous catheter-based pulmonary artery denervation (PADN) around the bifurcation area of the main pulmonary artery by radiofrequency ablation might abolish the baroreceptors or sympathetic nervous fibres of the pulmonary artery. Thus, it can be predicted that balloon-occlusion-induced pulmonary arterial pressure increase will be blunted.

Methods

STUDY DESIGN

The study design is shown in **Figure 1**. Briefly, twenty Mongolian dogs were randomly assigned to one of two groups: group 1 (left distal pulmonary basal trunk occlusion), and group 2 (left pulmonary interlobar artery occlusion). The occlusion model was conducted by the use of a balloon to stop the distal blood flow completely. Haemodynamic parameters were measured before and after balloon occlusion using a Swan-Ganz pulmonary artery catheter (Baxter Healthcare Corp., Irvine, CA, USA). The effect of PADN treatment at the main pulmonary artery bifurcation level was investigated by repeating the above-mentioned procedure followed by left pulmonary interlobar artery occlusion. The study was approved by the Nanjing First Hospital IRB committee (#NJMEDHOS 76895211) and followed the National Research Council for animal research guidelines.

UNILATERAL PULMONARY ARTERY OCCLUSION AND HAEMODYNAMIC MEASUREMENTS

Sequential unilateral left pulmonary artery occlusion was performed. Briefly, a 6 Fr guiding catheter was engaged in the left pulmonary
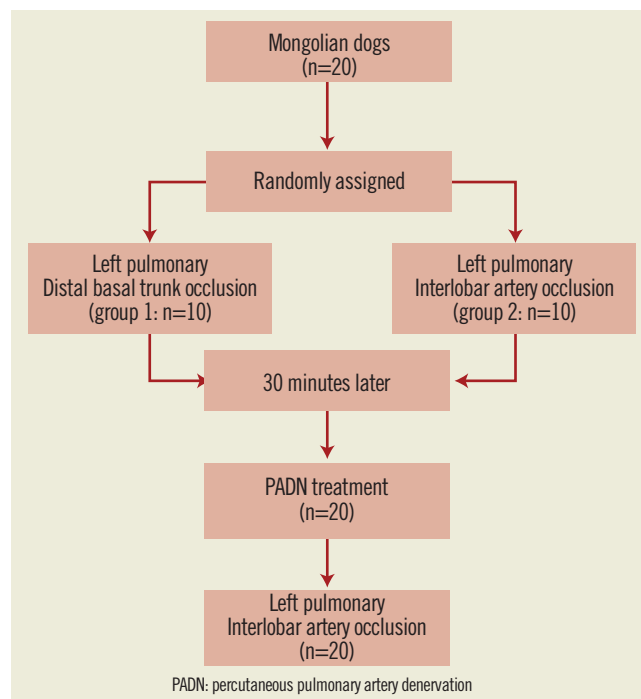

Figure 1. *Study design. PADN: percutaneous pulmonary artery denervation.*

artery and a pulmonary arteriography was performed **(Figure 2A)**. A balloon with a diameter of 2.5 mm (8 mm in length) was positioned in the distal pulmonary basal trunk and was inflated at 16 atm for ten minutes in group 1 **(Figure 2B)**. In group 2, a balloon with a diameter of 5 mm (8 mm in length) was advanced to the pulmonary interlobar artery, and was inflated at 16 atm for ten minutes **(Figure 2C)**. Pulmonary arteriography was performed to confirm the total occlusion of blood flow distal to the balloon. Haemodynamic measurements were performed before, one, two, three, five, and ten minutes after balloon occlusion.

A 7 Fr flow-directed Swan-Ganz catheter (Baxter Healthcare Corp.) was inserted percutaneously via the femoral vein for the measurement of resting right atrial pressure (RAP), right ventricular pressure (RVP), systolic/diastolic and mean PAP, pulmonary artery occlusion pressure (PAOP), thermodilution cardiac output (CO), and mixed venous oxygen saturation. PVR=(mean PAP-PAOP)/CO and transpulmonary gradient (TPG)=mean PAP-PAOP were then calculated[20-22].

DEDICATED APPARATUS FOR PULMONARY ARTERY HYPERTENSION

A dedicated 7.5 Fr triple-function (mapping, temperature-sensing, and ablation) catheter (PADN™; MicroPort Co., Shanghai, China) along an 8 Fr long sheath was advanced beyond the pulmonary valvular level. This catheter has a tapered (to 5 Fr) circular tip with 10 electrodes (each with a width of 0.75 mm and separated by 2 mm, **Figure 3A**) pre-mounted. The circular tip consists of a self-adjustable inner ring (with diameters of 15 mm, 17 mm, 20 mm, and 22 mm) as well as an outer ring (with diameters of 25 mm, 28 mm, 30 mm and
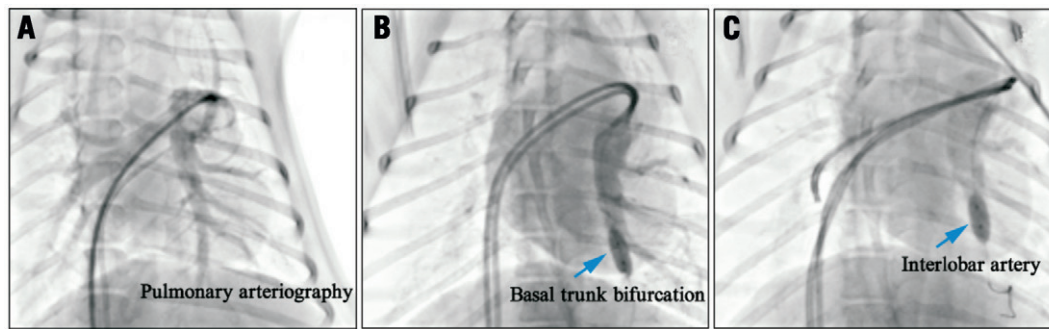
Figure 2. *Pulmonary arteriography of the animal model. A) Pulmonary arteriography showed the basal trunk and the interlobar artery. B) A balloon at the left distal pulmonary basal trunk bifurcation level. C) A balloon at the left pulmonary interlobar artery occluded distal blood flow. It is more proximally located than the basal trunk.*

33 mm, respectively). The position and direction of rings are adjusted by manipulating a rotating handle **(Figure 3B)**. There are 10 manual knobs on the surface of the radiofrequency generator, each connected to a specific electrode on the rings **(Figure 3C)**.

PULMONARY ARTERY DENERVATION PROCEDURE

Based on the anatomy of pulmonary arteriography[23], one of five different ablation levels was potentially selected as a candidate for PADN treatment **(Figure 4)**: Level 1 (<1 mm distal to orifice of left branch), Level 2 (<1 mm distal to ostial right branch), Level 3 (<2 mm proximal to bifurcation level), Level 4 (2 mm to 5 mm distal to ostial right branch), and Level 5 (2 mm to 5 mm distal to ostial left branch). Preliminary experiments in our lab showed that Level 3 was the most effective area for PADN treatment (data not shown). In addition, PADN treatment at level 4 and 5 in sites 2 mm to 5 mm distal to the ostial right or left pulmonary artery failed to abolish the pressure responses to balloon inflation at the left pulmonary interlobar artery.

In the present study, after haemodynamic parameters returned to baseline levels, all dogs in both groups (n=20) received the PADN treatment at Level 3[19]. To ensure that the electrodes are tightly contacting the arterial inner surface, three criteria are used as follows: (1) stronger resistance felt when rotating the handle, (2) inability to advance distally or ease of withdrawing proximally, and (3) angiography confirmation. After the first round of ablation, the catheter tip is withdrawn and rotated and re-advanced to ensure the whole circular axis of the vessel is ablated. The ablation parameters at each point were programmed as: temperature 50°C, energy ≤10 W, impedance <140 Ω, time 20 sec.

STATISTICAL ANALYSIS

All data are expressed as the mean ± SEM of results obtained from the number of experiments. Normal distribution was checked by the Kolmogorov-Smirnov test. Differences between data sets were assessed by paired t-tests or the Wilcoxon rank-sum test. All analyses were undertaken using SPSS 20.0 (IBM Corp., Armonk,
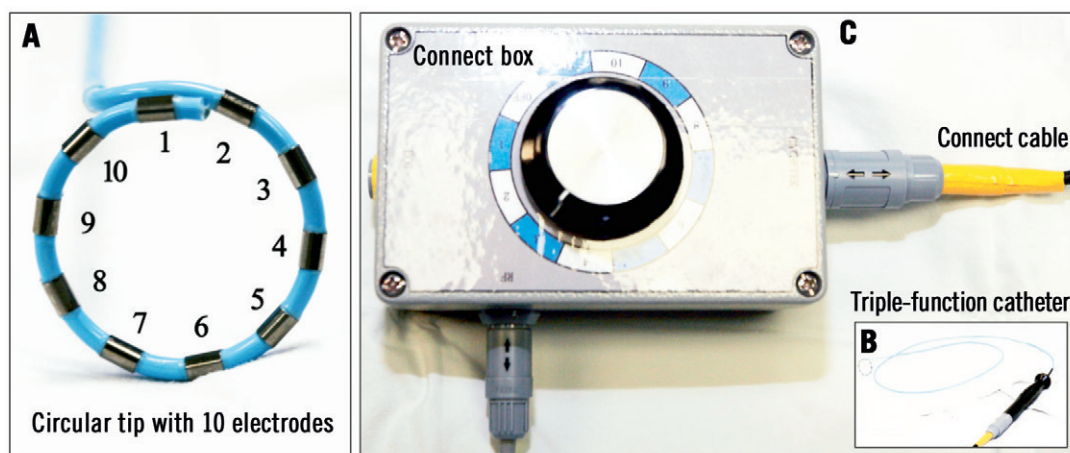

Figure 3. *A dedicated radiofrequency percutaneous catheter-based pulmonary artery denervation system. A) A dedicated 7.5 Fr triple-function catheter had a tapered (to 5 Fr) circular tip with 10 electrodes (each has 0.75 mm electrode-width and is separated by 2 mm) pre-mounted. B) The circular tip consists of an inner ring and an outer ring, on which electrodes are connected to the generator. C) Sequential ablations were performed by selecting the knob on the generator.*
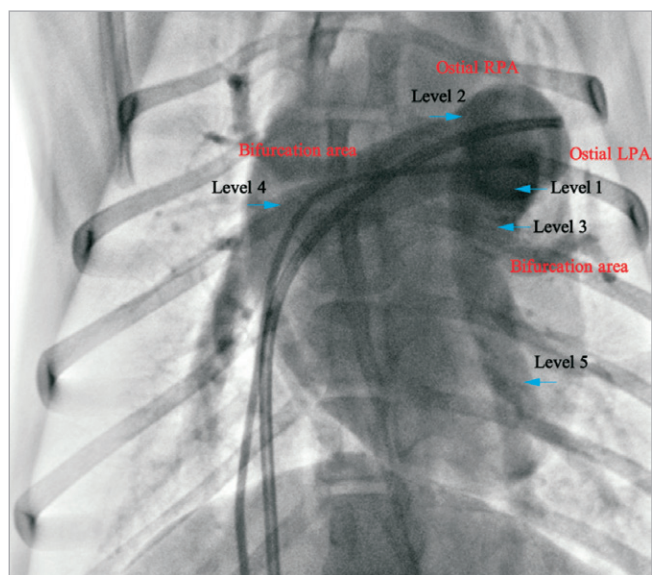
Figure 4. *Different radiofrequency ablation levels of the animal model. Five ablation levels were performed at Level 1 (<1 mm distal to orifice of left branch), Level 2 (<1 mm distal to ostial right branch), Level 3 (<2 mm proximal to bifurcation level), Level 4 (2-5 mm distal to ostial right branch) or Level 5 (2-5 mm distal to ostial left branch).*

Figure 5. *Changes of haemodynamics before and during balloon occlusion of the left distal pulmonary basal trunk *indicated p<0.05, compared with baseline (before occlusion); †indicated p<0.01, compared with baseline. DPAP: diastolic pulmonary arterial pressure; DRVP: diastolic right ventricular pressure; MPAP: mean pulmonary arterial pressure; MRVP: mean right ventricular pressure; SPAP: systolic pulmonary arterial pressure; SRVP: systolic right ventricular pressure*

NY, USA). A probability value of <0.05 was considered statistically significant (two-sided).

Results

PULMONARY ARTERIAL PRESSURE RESPONSE TO LEFT DISTAL PULMONARY BASAL TRUNK OCCLUSION BY BALLOON INFLATION BEFORE PADN (GROUP 1)

Baseline systolic PAP, mean PAP, and diastolic PAP were 25.7±1.6 mmHg, 14.1±0.7 mmHg, and 8.5±1.2 mmHg, respectively. After balloon occlusion of the left distal pulmonary basal trunk by inflation with a diameter of 2.5 mm, only at three minutes during occlusion, the pulmonary arterial parameters increased statistically compared to the baseline, but the absolute differences
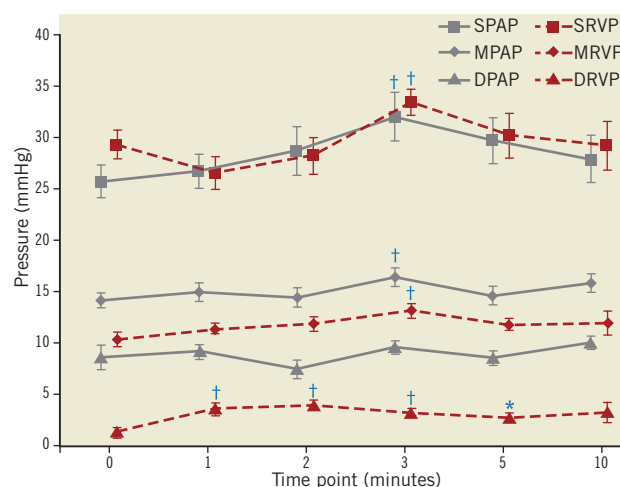
were numerical (4.3 mmHg for SPAP, 1.8 mmHg for MPAP and 1.2 mmHg for SRVP, **Figure 5**).

During continuous occlusion of the left distal pulmonary basal trunk, pulmonary artery occlusion pressure (PAOP), cardiac output (CO), pulmonary vascular resistance (PVR), and heart rate remained stable without significant changes before and during balloon inflation **(Table 1)**.

PULMONARY ARTERIAL PRESSURE RESPONSE TO LEFT PULMONARY INTERLOBAR ARTERY OCCLUSION BY BALLOON INFLATION BEFORE PADN (GROUP 2)

Baseline systolic PAP, mean PAP, and diastolic PAP were 23.4±0.9 mmHg, 13.9±0.5 mmHg, and 9.6±0.6 mmHg, respectively. During occlusion of the left pulmonary interlobar artery by balloon inflation of a diameter of 5 mm, mean PAP gradually increased and reached peak value (30.5±1.1 mmHg) at five minutes, with absolute

Table 1. Change of pulmonary vascular resistance, transpulmonary pressure gradient and heart rate before and during balloon inflation of the left distal pulmonary basal trunk before pulmonary artery denervation.

		MPAP (mmHg)	CO (L/min)	PAOP (mmHg)	PVR (dye/s/cm^5)	TPG (mmHg)	HR (bpm)
Before occlusion		14.1±0.7	2.13±0.09	3.8±1.5	586±196	9.4±3.2	167±16
During occlusion	1 min	14.9±0.9	2.10±0.15	5.6±1.4*	548±182	8.8±3.0	173±18
	2 min	14.4±1.0	2.16±0.13	6.3±2.2*	489±247	8.2±3.3	173±18
	3 min	16.4±0.9*	2.13±0.12	6.1±2.1*	647±191	10.3±2.9	175±15*
	5 min	14.6±0.9	2.09±0.12	6.1±3.4*	513±320	8.5±5.0	175±17
	10 min	15.8±0.9	2.07±0.13	5.2±2.3*	675±221	10.6±3.6	172±13

*indicated p<0.05, compared with baseline (before occlusion); CO: cardiac output; HR: heart rate; PAOP: pulmonary artery occlusion pressure; PVR: pulmonary vascular resistance; TPG: transpulmonary pressure gradient

difference of 16.6 mmHg. Between five and ten minutes, increased pulmonary arterial pressure persisted **(Figure 6)**.

Notably, because there were no significant increases of CO and PAOP, we detected that a significant increment of mean PAP resulted in the higher PVR up to three minutes (1,607±237 dye/s/cm^5), five minutes (1,753±369 dye/s/cm^5) and ten minutes (1,822±411 dye/s/cm^5), when compared to baseline PVR (609±160 dye/s/cm^5, all p<0.01, **Table 2**).

PULMONARY ARTERIAL PRESSURE RESPONSE TO LEFT PULMONARY INTERLOBAR ARTERY OCCLUSION BY BALLOON INFLATION AFTER PADN

There were no adverse outcomes and relative complications (death, perforation, acute thrombus formation) pre and post the PADN procedure. The average procedural time was 18.6 minutes.
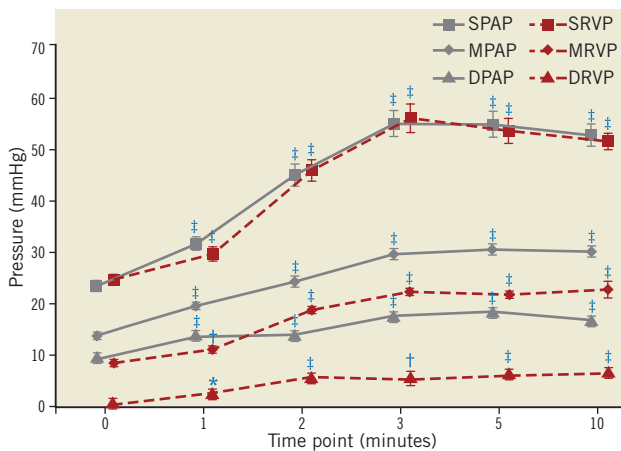
Immediately after the PADN procedure, systolic PAP, mean PAP, and diastolic PAP were 29.8±1.7 mmHg, 16.9±1.5 mmHg, and 12.8±1.7 mmHg, respectively. The peak systolic PAP, mean PAP, and diastolic PAP were observed at three minutes after balloon occlusion. At the five and ten minute time points, there was no significant difference in all of the haemodynamic parameters observed **(Figure 7)**. In addition, there was no significant change of PVR between baseline and five minutes (569±330 dye/s/cm^5 vs. 603±258 dye/s/cm^5, p=0.601). Similarly, CO, PAOP and heart rate did not change significantly **(Table 3)**.

Figure 6. *Changes of haemodynamics before and during balloon occlusion of the left pulmonary interlobar artery. *indicated p<0.05, compared with baseline (before occlusion); †indicated p<0.01, compared with baseline; ‡indicated p<0.001, compared with baseline. DPAP: diastolic pulmonary arterial pressure; DRVP: diastolic right ventricular pressure; MPAP: mean pulmonary arterial pressure; MRVP: mean right ventricular pressure; SPAP: systolic pulmonary arterial pressure; SRVP: systolic right ventricular pressure*
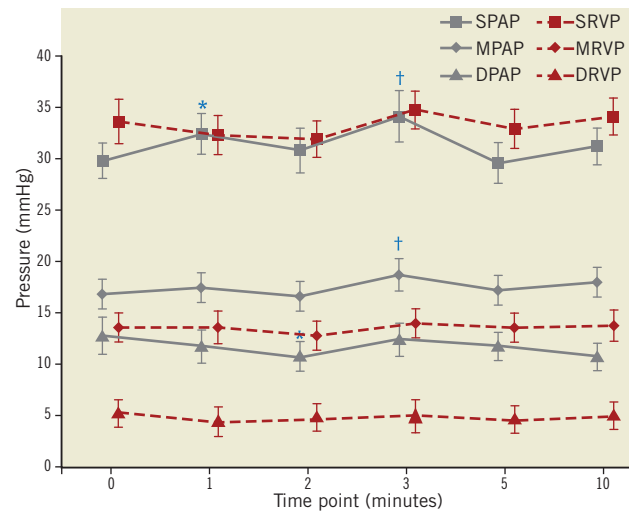

Figure 7. *Changes of haemodynamics before and during balloon occlusion of the left pulmonary interlobar artery after pulmonary artery denervation in the main pulmonary artery bifurcation area. *indicated p<0.05, compared with baseline (before occlusion); †indicated p<0.01, compared with baseline. DPAP: diastolic pulmonary arterial pressure; DRVP: diastolic right ventricular pressure; MPAP: mean pulmonary arterial pressure; MRVP: mean right ventricular pressure; SPAP: systolic pulmonary arterial pressure; SRVP: systolic right ventricular pressure*

Table 2. Change of pulmonary vascular resistance, transpulmonary pressure gradient and heart rate before and during balloon inflation of the left pulmonary interlobar artery before pulmonary artery denervation.

		MPAP (mmHg)	CO (L/min)	PAOP (mmHg)	PVR (dye/s/cm^5)	TPG (mmHg)	HR (bpm)
Before occlusion		13.9±0.5	2.25±0.15	3.5±1.3	609±160	10.9±2.0	176±15
During occlusion	1 min	19.6±0.7*	1.97±0.14*	3.6±1.4	970±160*	16.0±2.1*	151±10*
	2 min	24.2±1.1*	1.83±0.18*	3.7±1.6	1,347±353*	20.5±4.4*	166±10
	3 min	29.6±1.2*	1.98±0.31*	4.6±1.3	1,607±237*	25.2±4.2*	167±13
	5 min	30.5±1.1*	1.82±0.32*	5.1±0.6*	1,753±369*	25.4±3.4*	168±13
	10 min	30.1±1.2*	1.84±0.31*	4.9±2.4	1,822±411*	25.7±4.1*	168±10

*indicated p<0.05, compared with baseline (before occlusion); CO: cardiac output; HR: heart rate; PAOP: pulmonary artery occlusion pressure; PVR: pulmonary vascular resistance; TPG: transpulmonary pressure gradient

Table 3. Change of pulmonary vascular resistance, transpulmonary pressure gradient and heart rate before and during balloon inflation of the left pulmonary interlobar artery after pulmonary artery denervation.

		MPAP (mmHg)	CO (L/min)	PAOP (mmHg)	PVR (dye/s/cm^5)	TPG (mmHg)	HR (bpm)
Before occlusion		16.9±1.5	2.04±0.05	7.5±6.4	569±330	8.9±5.1	157±35
During occlusion	1 min	17.5±1.4	2.02±0.09	8.1±6.6	653±293	10.2±4.6	160±31
	2 min	16.6±1.5	2.04±0.14	7.9±6.9	591±325	9.3±5.1	158±33
	3 min	18.7±1.6*	2.02±0.08	8.3±6.4	720±336	11.1±4.9*	162±35
	5 min	17.2±1.4	2.06±0.08	8.0±6.9	603±258	9.5±4.0	161±34
	10 min	18.0±1.4	2.04±0.05	7.9±6.9	782±568	12.2±8.7	164±31

*indicated p<0.05, compared with baseline (before occlusion); CO: cardiac output; HR: heart rate; PAOP: pulmonary artery occlusion pressure; PVR: pulmonary vascular resistance; TPG: transpulmonary pressure gradient

Discussion

We hypothesised that experimental pulmonary hypertension can be caused by a reflex mechanism from balloon occlusion of the pulmonary artery. From this experimental study, we reported for the first time that: (1) occlusion of the left pulmonary interlobar artery rather than the left pulmonary basal trunk by balloon inflation induced significant increases of PAP and RVP; (2) PADN at the main bifurcation area of the pulmonary artery using a dedicated ablation catheter might completely abolish the pulmonary artery pressure response to the inflated balloon occlusion.

ACUTE PULMONARY ARTERIAL HYPERTENSION MODEL

According to previous experimental animal models, unilateral pulmonary arterial distention was mainly produced by stepwise inflation of a balloon or by non-occlusive inflatable cuffed cylinders[13-19].

However, controversy existed about the mechanisms resulting in a significant increase of PAP or PVR. Several studies have shown that the baroreceptors in the pulmonary artery branches contributed to the mediation of PAP but without evidence of stimulation of vagal nerves when blood flow was totally or sub-totally occluded[13,14]. However, Ueda et al did not find direct evidence of the sympathetic nerves in this pulmo-pulmonary reflex[15]. In another study, Murao et al postulated that this pressure response was solely a mechanical effect involving vasoconstriction[16]. Regardless of the mechanism, the use of the non-occlusive (triple-lumen) balloon distention (stepwise inflation) of the main pulmonary artery at the bifurcation area induced the most significant increases in PAP and PVR[18,19]. The present study is consistent with previous findings that pulmonary arterial pressure increased significantly using balloon occlusion of the pulmonary artery. This finding represented the fact that acute pulmonary arterial hypertension can be reproduced experimentally and thus it is important to validate the efficacy of the PADN treatment in our study.

BARORECEPTORS AND PULMONARY ARTERIAL HYPERTENSION

Baroreceptors are sensors located in the blood vessels of several mammals: they detect blood pressure and send messages to the central nervous system to increase resistance when necessary[24]. Baroreceptors can be classified as arterial (high-pressure) or venous (low-pressure)[25]. Accumulative evidence implied that the baroreceptors might lie in the main pulmonary artery, probably in or near the bifurcation rather than in the branches themselves. This postulation was examined by Juratsch and colleagues demonstrating that surgical dissection of the areas of the pulmonary artery under the bifurcation and adjacent to the aorta were particularly necessary to abolish the pressure response by balloon inflations[19]. In addition, the sympathetic nerves potentially providing both the afferent and efferent pathways for this reflex were investigated[14,18,19]. Efferent sympathetic nerve endings participating in the rise of pressure have been examined by both animal and clinical studies[18,19,26-30]. On the other hand, afferent sympathetic activity originating from the pulmonary artery has been found in the 3rd and 4th rami of the spinal cord[31-33].

PADN TREATMENT

The sites of the sympathetic nerve endings can also be postulated from our study. A previous study reported that the low-pressure baroreceptors are buried in the pulmonary arterial wall close to the intima[19]. However, the high-pressure baroreceptor sensory endings mostly lie in the tunica adventitia of the artery. Thus, it is crucial to remove the adventitia and some portions of the medial layers in order to abolish these baroreceptors. In the present study, PADN treatment completely abolished the pressure response to balloon inflations. Such pressure response, however, was not abolished when PADN was performed in the sites distal (2 mm to 5 mm) to the bifurcation level of the pulmonary artery. This may suggest that the sympathetic nerve endings were localised in or near the area of the bifurcation level of the pulmonary artery.

With the development of sympathetic denervation for the treatment of refractory systemic hypertension by renal ablation denervation technology[34-36], we recognised the potential of pulmonary artery denervation for the treatment of pulmonary hypertension. From our experience, there are several limitations of the conventional catheter when used for PADN including: the difficulty of contacting the vessel's inner surfaces tightly, the need for multiple rotations to complete circular ablation, and the high risk of perforation. Thus, we designed a special ablation catheter with a circular tip for this study, where only slight movements were required to keep tight contact with the vessel. As a result, we anticipate that pulmonary artery denervation therapy could be performed safely and successfully.

Clinical implications

Although medical therapy for PAH was evaluated extensively, the chronic treatment of PAH remains unsatisfactory. Symptomatic improvement is not always possible. Once the diagnosis of pulmonary hypertension is established, the prognosis remains poor. As a result, this novel PADN treatment in the present study may provide new hope for therapeutics in patients with pulmonary arterial hypertension.

Limitations

The study has several limitations. First, the balloon occlusion was not inflated in a stepwise manner. It would have induced vasoconstriction of the pulmonary artery[13-17], leading to a rise in both PAP and systolic right ventricular pressure. However, this present study was to examine the effect of PADN on PAP, and did not focus on the mechanisms in the changes of haemodynamics during balloon inflation. Second, the balloon occlusion was repeated after PADN therapy in group 1 and group 2. The tolerance effect of the pulmonary haemodynamics parameters from repeated balloon occlusions was not assessed. Third, there was an absence of histological evidence from the PADN treatment in our study. The lack of histological proof of sympathetic nerve damage weakened our hypothesis of damaged nerve endings by our ablation techniques. However, the safety of the PADN treatment was approved, as no experimental dogs died after the PADN treatment. Finally, the current study only reported that PADN treatment abolished the acute experimental pulmonary hypertension. The effect of PADN treatment in chronic pulmonary hypertension or irreversible condition remains unsettled. Further experimental studies will address the details of precise mechanisms and different settings with respect to the PADN treatment. As a result of the safety and efficacy of PADN treatment in the present study, a FIM study is expected to be organised in the near future.

Conclusions

We are the first to report that pulmonary artery denervation in the main pulmonary artery bifurcation area with our newly designed system might completely abolish the experimental pulmonary hypertension response to balloon inflations.

Acknowledgements

We appreciated the efforts of Ms. Tian Xu and Ms. Rong Wang for their data collection.

Conflict of interest statement

The authors have no conflict of interest to declare.

References

1. Galiè N, Hinderliter AL, Torbicki A, Fourme T, Simonneau G, Pulido T, Espinola-Zavaleta N, Rocchi G, Manes A, Frantz R, Kurzyna M, Nagueh SF, Barst R, Channick R, Dujardin K, Kronenberg A, Leconte I, Rainisio M, Rubin L. Effects of the oral endothelin-receptor antagonist bosentan on echocardiographic and doppler measures in patients with pulmonary arterial hypertension. *J Am Coll Cardiol.* 2003;41:1380-6.

2. Galiè N, Seeger W, Naeije R, Simonneau G, Rubin LJ. Comparative analysis of clinical trials and evidence-based treatment algorithm in pulmonary arterial hypertension. *J Am Coll Cardiol.* 2004;43:81S-88S.

3. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med.* 2004;351:1425-36.

4. Galiè N, Humbert M, Vachiery JL, Vizza CD, Kneussl M, Manes A, Sitbon O, Torbicki A, Delcroix M, Naeije R, Hoeper M, Chaouat A, Morand S, Besse B, Simonneau G. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol.* 2002;39:1496-502.

5. Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med.* 2004;351:1655-65.

6. Galiè N, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. *Circulation.* 2008;117:3010-9.

7. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Cottin V, Degano B, Jais X, Montani D, Souza R, Simonneau G. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation.* 2010;122:156-63.

8. Barst RJ, Ivy DD, Gaitan G, Szatmari A, Rudzinski A, Garcia AE, Sastry BK, Pulido T, Layton GR, Serdarevic-Pehar M, Wessel DL. A randomized, double-blind, placebo-controlled, dose-ranging study of oral sildenafil citrate in treatment-naive children with pulmonary arterial hypertension. *Circulation.* 2012;125:324-34.

9. Oudiz RJ, Brundage BH, Galiè N, Ghofrani HA, Simonneau G, Botros FT, Chan M, Beardsworth A, Barst RJ. Tadalafil for the treatment of pulmonary arterial hypertension: a double-blind 52-week uncontrolled extension study. *J Am Coll Cardiol.* 2012;60:768-74.

10. Galiè N, Manes A, Negro L, Palazzini M, Bacchi-Reggiani ML, Branzi A. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. *Eur Heart J.* 2009;30:394-403.

11. Rich S. The value of approved therapies for pulmonary arterial hypertension. *Am Heart J.* 2007;153:889-90.

12. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, Christman BW, Weir EK, Eickelberg O, Voelkel NF, Rabinovitch M. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2004;43:13S-24S.

13. Osorio J, Russek M. Reflex changes on the pulmonary and systemic pressures elicited by stimulation of baroreceptors in the pulmonary artery. *Circ Res.* 1962;10:664-7.

14. Aramendia P, Taguini CM, Fourcade A, Taguini AC. Reflex vasomotor activity during unilateral occlusion of the pulmonary artery. *Am Heart J.* 1963;66:53-60.

15. Ueda H, Murao M, Momose T, Hatano S, Koike S, Shiraishi T, Kuroiwa A, Sugimoto T, Uzawa T, Shimomura K, Saito Y, Yamada T.

Effects of unilateral pulmonary artery or vein distension with a balloon on the pulmonary and femoral arterial pressures in anesthetized dogs. *Jpn Heart J.* 1965;6:428-42.

16. Murao M, Onodera S, Homma T, Kataoka N, Takabori T. Effects of occlusion and further distension of unilateral pulmonary artery with an inflatable balloon upon systemic and pulmonary hemodynamics. *Jpn Heart J.* 1969;10:59-69.

17. Hyman AL. Pulmonary vasoconstriction due to nonocclusive distention of large pulmonary arteries in the dog. *Circ Res.* 1968;23:401-13.

18. Laks MM, Juratsch CE, Garner D, Beazell J, Criley JM. Acute pulmonary artery hypertension produced by distention of the main pulmonary artery in the conscious dog. *Chest.* 1975;68:807-13.

19. Juratsch CE, Jengo JA, Castagna J, Laks MM. Experimental pulmonary hypertension produced by surgical and chemical denervation of the pulmonary vasculature. *Chest.* 1980;77:525-30.

20. Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J.* 2009;30:2493-537.

21. Task Force for Diagnosis and Treatment of Pulmonary Hypertension of European Society of Cardiology (ESC); European Respiratory Society (ERS); International Society of Heart and Lung Transplantation (ISHLT), Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G. Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J.* 2009;34:1219-63.

22. Tonelli AR, Mubarak KK, Li N, Carrie R, Alnuaimat H. Effect of balloon inflation volume on pulmonary artery occlusion pressure in patients with and without pulmonary hypertension. *Chest.* 2011;139:115-21.

23. Wielopolski PA. Pulmonary arteriography. *Magn Reson Imaging Clin N Am.* 1993;1:295-313.

24. Stanfield, C. Principles of human physiology, 3rd edition. San Francisco, CA: Pearson Benjamin Cummings; 2008:424.

25. Cardiovascular Physiology, 9th edition. Maryland Heights, MO: Mosby Elsevier; 2007:172.

26. Coleridge JC, Kidd C. Electrophysiological evidence of baroreceptors in the pulmonary artery of the dog. *J Physiol.* 1960;150:319-31.

27. Velez-Roa S, Ciarka A, Najem B, Vachiery JL, Naeije R, van de Borne P. Increased sympathetic nerve activity in pulmonary artery hypertension. *Circulation.* 2004;110:1308-12.

28. Wensel R, Jilek C, Dorr M, Francis DP, Stadler H, Lange T, Blumberg F, Opitz C, Pfeifer M, Ewert R. Impaired cardiac autonomic control relates to disease severity in pulmonary hypertension. *Eur Respir J.* 2009;34:895-901.

29. Richards AM, Ikram H, Crozier IG, Nicholls MG, Jans S. Ambulatory pulmonary arterial pressure in primary pulmonary hypertension: variability, relation to systemic arterial pressure, and plasma catecholamines. *Br Heart J.* 1990;63:103-8.

30. Nootens M, Kaufmann E, Rector T, Toher C, Judd D, Francis GS, Rich S. Neurohormonal activation in patients with right ventricular failure from pulmonary hypertension: relation to hemodynamic variables and endothelin levels. *J Am Coll Cardiol.* 1995;26:1581-5.

31. Ueda H, Uchida Y, Kamisaka K. Distribution and responses of the cardiac sympathetic receptors to mechanically induced circulatory changes. *Jpn Heart J.* 1969;10:70-81.

32. Kostreva DR, Zuperku EJ, Hess GL, Coon RL, Kampine JP. Pulmonary afferent activity recorded from sympathetic nerves. *J Appl Physiol.* 1975;39:37-40.

33. Nishi K, Sakanashi M, Takenaka F. Afferent fibres from pulmonary arterial baroreceptors in the left cardiac sympathetic nerve of the cat. *J Physiol.* 1974;240:53-66.

34. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Bohm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet.* 2010;376:1903-9.

35. Mabin T, Sapoval M, Cabane V, Stemmett J, Iyer M. First experience with endovascular ultrasound renal denervation for the treatment of resistant hypertension. *EuroIntervention.* 2012;8:57-61.

36. Ormiston JA, Watson T, van Pelt N, Stewart R, Haworth P, Stewart JT, Webster MW. First-in-human use of the OneShot™ renal denervation system from Covidien. *EuroIntervention.* 2013;8:1090-4.