Original article

Beneficial Effects of Renal Denervation on Pulmonary Vascular Remodeling in Experimental Pulmonary Artery Hypertension

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A B S T R A C T

Introduction and objectives: Activation of both the sympathetic nervous system and the renin-angiotensin-aldosterone system is closely associated with pulmonary arterial hypertension. We hypothesized that renal denervation decreases renin-angiotensin-aldosterone activity and inhibits the progression of pulmonary arterial hypertension.

Methods: Twenty-two beagles were randomized into 3 groups. The dogs’ pulmonary dynamics were measured before and 8 weeks after injection of 0.1 mL/kg dimethylformamide (control dogs) or 2 mg/kg dehydromonocrotaline (pulmonary arterial hypertension and pulmonary arterial hypertension + renal denervation dogs). Eight weeks after injection, neurohormone levels and pulmonary tissue morphology were measured.

Results: Levels of plasma angiotensin II and endothelin-1 were significantly increased after 8 weeks in the pulmonary arterial hypertension dogs and were higher in the lung tissues of these dogs than in those of the control and renal denervation dogs [mean [standard deviation] angiotensin II: 65 [9.8] vs 38 [6.7], 46 [8.1]; endothelin-1: 96 [10.3] vs 54 [6.2], 67 [9.4]; P < .01]. Dehydromonocrotaline increased the mean pulmonary arterial pressure [16 [3.4] mmHg vs 33 [7.3] mmHg; P < .01], and renal denervation prevented this increase. Pulmonary smooth muscle cell proliferation was higher in the pulmonary arterial hypertension dogs than in the control and pulmonary arterial hypertension + renal denervation dogs.

Conclusions: Renal denervation attenuates pulmonary vascular remodeling and decreases pulmonary arterial pressure in experimental pulmonary arterial hypertension. The effect of renal denervation may contribute to decreased neurohormone levels.

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Efectos beneficiosos de la simpatectomía renal sobre el remodelado vascular pulmonar en la hipertensión arterial primaria experimental

R E S U M E N

Introducción y objetivos: La activación tanto del sistema nervioso simpático como del sistema renina-angiotensina-aldosterona está estrechamente relacionada con la hipertensión arterial pulmonar. Nuestra hipótesis era que la simpatectomía renal reduce la actividad del sistema renina-angiotensina-aldosterona e inhibe la progresión de la hipertensión arterial pulmonar.

Métodos: Se asignó aleatoriamente a un total de 22 perros beagle a tres grupos de estudio. Se efectuaron determinaciones de la dinámica pulmonar de esos animales antes y 8 semanas después de la inyección de 0.1 mL/kg de dimetilformamida (perros de control) o de 2 mg/kg de deshidromonocrotalina (perros con simpatectomía arterial pulmonar y perros con simpatectomía arterial pulmonar + simpatectomía renal). Ocho semanas después de la inyección, se determinaron las concentraciones de neurohormonas y se evaluó la morfología del tejido pulmonar.

Resultados: Se observó un aumento significativo de la concentración de angiotensina II y endotelina-1 en plasma después de 8 semanas en los perros con simpatectomía arterial pulmonar, y los valores obtenidos en los tejidos pulmonares de estos animales eran superiores a los de los perros del grupo de control y el grupo de simpatectomía renal [medias: angiotensina II, 65 ± 9.8 frente a 38 ± 6.7 y 46 ± 8.1; endotelina-1, 96 ± 10.3 frente a 54 ± 6.2 y 67 ± 9.4; p < .01]. La deshidromonocrotalina aumentó la presión arterial pulmonar media (16 ± 3.4 frente a 33 ± 7.3 mmHg; p < 0.01), y la simpatectomía renal evitó que se
**INTRODUCCIÓN**

La hipertensión pulmonar (PAH) es una enfermedad fatal caracterizada por la presión arterial pulmonar elevada en el sistema vascular pulmonar y en el lado derecho del corazón. Aunque la patogenia de la PAH no está completamente comprendida, los estudios previos han implicado un aumento de la actividad simpática del sistema nervioso y el aumento de renin–angiotensina–aldosterona. \(^1\) Ha sido sugerido que la activación simpática del sistema nervioso contribuye a la hiperventilación alveolar observada en pacientes con PAH. \(^2,3\) El aumento de los niveles plasmáticos de renin y angiotensina II (Ang II) han sido estrechamente asociados con la PAH y su progresión.\(^6\) Numerosos tratamientos han demostrado ser útiles para disminuir la PAH, pero no han demostrado efectos a largo plazo.\(^7,8\)

Los estudios previos han demostrado que un reducción significativa en la liberación de noradrenalina intravascular y la presión arterial puede ser alcanzada después de la inyección analgésica.\(^9,10\) Nuestro estudio previo demostró que la concentración de Ang II en plasma fue disminuida después de la RSD.\(^11,12\) Es desconocido si la RSD tiene un impacto en los otros neurohormonas y la progresión de la PAH. Por lo tanto, en el presente estudio, nos examinamos la influencia de RSD sobre PAH, la remodelación vascular pulmonar y los neurohormonas en estudios experimentales de PAH.

**MÉTODOS**

**Preparación del Modelo Animal**

Veintidue beagles de cualquier sexo, con un peso medio (desviación estándar) de 13.5 (2.4) kg, fueron usados en el presente estudio. Este estudio se conforma con el actual Guía para el cuidado y uso de animales de laboratorio, publicado por el Instituto Nacional de Salud (no. 85-23, revisado en 1996). El protocolo del estudio fue aprobado por el Comité de Ética de Wuhan University. El manejo de los animales fue realizado de acuerdo con el Protocolo de Wuhan para la Investigación Animal.

Se administró una inyección intramuscular de 25 mg/kg de ketamina sulfato previa a la administración de pentobarbital sódico para anestesia. Todo el grupo de animales fue intubado y ventilados con aire suplementado con oxígeno a través de un respirador (MAO01746, Harvard Apparatus; Holliston, Massachusetts, Estados Unidos). Se realizó una monitorización electrocardiográfica continuada, utilizando un catéter de Swan-Ganz en cuanto a la presión arterial pulmonar y la presión arterial pulmonar media.\(^13\) El catéter fue conectado a la transductor Vigilance買い物 ( Strait Digital, San Juan, Puerto Rico) y con la ayuda de un equipo de sistema Vigilance買い物. La presión arterial pulmonar y la presión arterial pulmonar media fueron medidos a intervalos de 5 minutos y las mediciones eran recogidas y almacenadas en un sistema Vigilance買い物.

**Echocardiografía**

La ecocardiografía bidimensional y Doppler de la cavidad torácica se realizaron en todos los animales (IE33, SS-1, PHILIPS; The Netherlands) a base y después de 8 semanas. Se realizó una ecocardiografía 2-dimensional de corto y largo parasternal y 4-, 2-, y 3-cámara apical. Obtenido el dimensiones longitudinales, transversales y apicales.
right atrial dimension, left ventricular diastolic dimension, and right ventricular diastolic dimension were measured using Simpson’s biplane formula. All volumes were measured in triplicate, and the averages were reported. The right ventricular end-systolic longitudinal strain was analyzed by 2-dimensional speckle tracking. An independent echocardiography expert reviewed the images and the parameters.

Biochemical Studies

Four milliliters of venous blood was collected in EDTA (ethylenediaminetetraacetic acid) Vacutainer tubes and centrifuged at 2310 g for 10 minutes at 4 °C (Beckman Coulter, Avanti J-E) at baseline and again after 8 weeks. The serum was separated, kept in microtubes, and stored at −80 °C until assay. Levels of creatinine, Ang II, prostaglandin E₂, and endothelin-1 (ET-1) were examined by ELISA. The inferior lobe of the left lung was isolated after thoracotomy. Levels of Ang II, prostaglandin E₂ and ET-1 were measured in the lung as previously described. Tissues were obtained from the right ventricular base in all animals after 8 weeks. Levels of aldosterone and B-type natriuretic peptide were measured by ELISA.

Histological Studies

The excised left lungs were processed for light microscopic observation according to standard procedures. Tissue samples were chopped into small pieces, embedded in paraffin, cut into 3–μm slices and stained with hematoxylin and eosin. The sections were observed, analyzed, and photographed using a Nikon eclipseCi light microscope. The pulmonary arteries were identified as vessels with 2 clearly defined elastic laminae, with a layer of smooth muscle cells between the 2 laminae. The wall thickness of the arteries was measured for 15 muscular arteries at 400 magnification.

Expression of Ang II type 1 (AT₁) receptor and Ang II type 2 (AT₂) receptors in the pulmonary artery was measured using Western blot. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween for 1 h and incubated overnight at 4 °C with the primary antibodies (monoclonal rabbit anti-AT₁, and anti-AT₂ antibodies [Santa Cruz Biotechnology Inc.; Dallas, Texas, United States]; used at 1:500; rabbit antiactin antibody, antibody [Santa Cruz Biotechnology Inc.; used at 1:1000). They were then washed in tris buffered saline tween 3 times, incubated with second antibody for 1 h at 37 °C, and revealed by Immun-Star horseradish peroxidase substrate. The relative expression of protein was determined with image analyzer software (AlphaEase FC, United States).

Sections of ventricle were dissected from the heart and rapidly stored at −80 °C. Right ventricular sections were taken from the right ventricular outflow. Masson’s trichrome staining was used to identify increased concentration of interstitial fibrosis. Connective tissue was differentiated on the basis of its color and expressed as a percentage of the reference tissue area (NIKON Ti-s, Japan). Blood vessels and perivascular interstitial cells were excluded from the connective tissue quantification. Interstitial collagen volume fraction in the ventricular was determined by quantitative morphometry with an image analyzer (IPP 6.0, Media Cybernetics; Georgia, United States).

Statistical Analysis

Values are shown as means (standard deviation). The echocardiography results of the PAH and PAH + RSD groups were compared for the poststudy period using the paired t test, and 2-sample independent Student’s t tests were used to compare the means of the 2 groups. Analysis of variance in the form of Neuman–Keuls tests were used to compare the means of the continuous variables among multiple groups, and any significant difference was further analyzed using the Tukey–Kramer test. All statistical tests were 2-sided, and a probability value < .05 was required for statistical significance.

RESULTS

Characteristics of Animal Models

There were no significant differences in arterial blood pressure, heart rate or body weight at baseline among the 3 experimental groups. In the PAH + RSD group, systolic blood pressure was decreased after 8 weeks, but this decrease was not statistically significant. The PAH dogs began to display rapid breathing and decreased appetite beginning at 10 days after injection. Lung color was paler in the PAH and PAH + RSD dogs than in the control dogs. The characteristics at baseline and after 8 weeks are shown in Table 1.

Echocardiography and Hemodynamic Parameters

In contrast, right atrial dimension significantly increased after 8 weeks in the PAH dogs (P = .02). No significant differences in the right atrial dimension were observed prior to or after 8 weeks in the control and PAH + RSD groups. Significant increases in the right ventricular diastolic dimension were also noted in the PAH dogs. The right ventricular diastolic dimension was increased in PAH + RSD group after 8 weeks, but this increase was not statistically significant (P = .09). After 8 weeks, the longitudinal strain of right ventricular lateral wall was reduced in PAH group and PAH + RSD group. However, the longitudinal strain was higher in the PAH + RSD group than in the PAH group (Table 2).

The hemodynamic data at baseline and after 8 weeks among the 3 groups are shown in Table 3. There was no significant difference in the baseline pulmonary hemodynamic indices among the 3 groups. Compared with baseline, pulmonary artery pressure and right ventricular pressure were higher after 8 weeks in the PAH group. There was no significant difference in the hemodynamic parameters at baseline and after 8 weeks in the control group. The pulmonary artery pressure and right ventricular pressure increased after 8 weeks in the PAH + RSD group, but these hemodynamic parameters were lower in the PAH + RSD dogs than in PAH dogs.

ELISA Studies

The PAH group showed a statistically significant increase in the plasma and lung tissue levels of Ang II at the endpoint of the protocol (P < .01) compared with baseline (Table 4). There were no significant differences in the plasma levels of Ang II between the baseline and the study endpoint in the control and PAH + RSD groups. There were no significant differences in the plasma creatinine and lung tissue levels of prostaglandin E₂ between baseline and the study endpoint in the 3 groups.

There was no significant difference at the baseline levels of serum ET-1 concentration among the 3 groups. After 8 weeks, serum ET-1 levels increased (P < .01) in the PAH dogs, but no statistically significant difference was observed in the control dogs. In the PAH + RSD group, the serum ET-1 concentration was decreased after 8 weeks, but this decrease was not statistically
significant ($P = .19$). The ET-1 concentration in the lung tissue was significantly higher in the PAH group than in the control group ($P < .01$). Furthermore, the ET-1 concentration in the lung tissue was higher in the PAH + RSD group than in the control group ($P = .01$).

We further investigated levels of aldosterone and B-type natriuretic peptide in the right ventricle. The results showed that the levels of aldosterone and B-type natriuretic peptide in the right ventricular base tissue samples were higher in both groups with PAH induction than in control dogs. Interestingly, the levels of aldosterone and B-type natriuretic peptide in the right ventricle in the PAH group were higher than those in the PAH + RSD groups. This confirms that the PAH + RSD group had less severe right ventricle remodeling than the PAH group (Table 4).

### Morphological and Western-blot Studies

Hematoxylin and eosin staining staining demonstrated significant intimal thickening and luminal stenosis in the PAH group compared with the control and PAH + RSD groups. Figure 1 shows the representative changes in the vascular structure for the 3 groups. The neointimal occlusion was evaluated using the vascular occlusion score. An average vascular occlusion score of the 30 selected vessels was calculated for each dog as an index of vascular occlusion. The results showed that neointimal lesions occurred in 64% of the chosen small pulmonary arteries in the PAH group. The mean vascular occlusion score was 1.17 in these arteries. In contrast, the dogs in the PAH + RSD group developed neointimal lesions in 27% of the selected pulmonary arteries, and the mean vascular occlusion score was 0.24. The difference between the PAH group and the PAH + RSD group was significant ($1.17$ [0.13] vs 0.24 [0.10]; $P < .01$). The small pulmonary arteries in the control group showed that the mean vascular occlusion score was 0.

Figure 2 illustrates an example of Masson’s trichrome staining in the tissue sections. In contrast, the ventricular sections from PAH + RSD animals had significantly less fibrosis than PAH dogs, while PAH + RSD animals had higher fibrosis than control dogs. For instance, images from PAH hearts revealed a large amount of fibrosis (19.4 [3.8%]), whereas control dogs showed minimal fibrous tissue (4.1 [0.9%]) and PAH + RSD dogs showed moderate fibrous tissue (11.2 [2.6%]).

Figure 3 compares the Western blots of the pulmonary artery in the 3 groups. All immunoblot band intensity measurements were normalized to the β-actin band intensity of the loaded sample. Band densities were determined and allowed for the relative quantification of AT$_1$ and AT$_2$ densities. As shown in Figures 3A and B, the expression of AT$_1$ in the pulmonary artery was higher in the PAH group than in the control and PAH + RSD groups ($1.02$ [0.11] vs $0.39$ [0.04] and $0.38$ [0.05]; $P < .01$). There were no significant differences in AT$_2$ densities in the 3 groups ($0.44$ [0.04] vs $0.41$ [0.05] and $0.42$ [0.04]; $P = .38$) (Figures 3C and D).

### Table 2

| Changes of Echocardiographic Parameters at Baseline and After 8 Weeks in the 3 Groups |
|----------------------------------------|----------------|----------------|----------------|----------------|
| Control                                |               |                |                |                |
| Baseline                               | 17.1 (1.3)    | 17.5 (1.2)     | 11.3 (0.6)     | 27.4 (2.1)     |
| After 8 weeks                          | 16.9 (1.2)    | 17.6 (1.1)     | 11.7 (0.7)     | 27.6 (2.3)     |
| PAH                                    |               |                |                |                |
| Baseline                               | 16.6 (1.5)    | 16.9 (1.3)     | 11.8 (0.5)     | 28.1 (2.5)     |
| After 8 weeks                          | 18.9 (1.1)$^a$| 17.1 (1.3)     | 13.5 (1.1)$^a$ | 27.3 (2.3)     |
| PAH + RSD                              |               |                |                |                |
| Baseline                               | 16.8 (1.2)    | 17.3 (1.5)     | 11.1 (0.7)     | 27.9 (2.4)     |
| After 8 weeks                          | 17.2 (1.4)    | 17.5 (1.4)     | 12.1 (0.7)     | 27.7 (1.9)     |

LAD, left atrial dimension; LVDD, left ventricular diastolic dimension; PAH, pulmonary arterial hypertension; RAD, right atrial dimension; RSD, renal sympathetic denervation; RV, right ventricular; RVDD, right ventricular diastolic dimension.

Data are expressed as mean (standard deviation).

$^a$ $P < .01$ for pulmonary arterial hypertension compared with baseline.

$^b$ $P < .05$ for pulmonary arterial hypertension vs renal sympathectomy compared with baseline.

$^c$ $P < .05$ for pulmonary arterial hypertension vs renal sympathectomy compared with pulmonary arterial hypertension.
Table 3
Changes of Hemodynamic Parameters at Baseline and After 8 Weeks in the 3 Groups

<table>
<thead>
<tr>
<th></th>
<th>RVSP, mmHg</th>
<th>RVMP, mmHg</th>
<th>PASP, mmHg</th>
<th>PAMP, mmHg</th>
<th>PAWP, mmHg</th>
<th>PVR, dyn s/cm²</th>
<th>CO (L/min)</th>
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</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
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</tr>
<tr>
<td>Baseline</td>
<td>23 (6.1)</td>
<td>11 (4.2)</td>
<td>24 (7.6)</td>
<td>15 (3.5)</td>
<td>8.3 (1.5)</td>
<td>119 (65)</td>
<td>4.5 (0.3)</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>23 (5.7)</td>
<td>11 (4.1)</td>
<td>25 (7.1)</td>
<td>14 (3.7)</td>
<td>8.1 (1.6)</td>
<td>105 (69)</td>
<td>4.5 (0.3)</td>
</tr>
<tr>
<td><strong>PAH</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>22 (5.8)</td>
<td>12 (4.4)</td>
<td>26 (7.2)</td>
<td>16 (3.4)</td>
<td>8.1 (1.6)</td>
<td>137 (71)</td>
<td>4.6 (0.4)</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>43 (8.7)²</td>
<td>25 (6.3)²</td>
<td>45 (11.2)²</td>
<td>33 (7.3)²</td>
<td>8.7 (1.8)</td>
<td>550 (94)²</td>
<td>3.5 (0.2)²</td>
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<td><strong>PAH + RSD</strong></td>
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<tr>
<td>Baseline</td>
<td>21 (6.2)</td>
<td>11 (3.8)</td>
<td>23 (7.2)</td>
<td>14 (3.6)</td>
<td>8.4 (1.7)</td>
<td>97 (75)</td>
<td>4.6 (0.4)</td>
</tr>
<tr>
<td>After 8 weeks</td>
<td>31 (6.8)²</td>
<td>18 (4.5)²</td>
<td>33 (7.9)²</td>
<td>25 (3.9)²</td>
<td>8.0 (1.8)</td>
<td>358 (88)²</td>
<td>3.8 (0.4)²</td>
</tr>
</tbody>
</table>

CO, cardiac output; PAH, pulmonary arterial hypertension; PAMP, pulmonary arterial mean pressure; PASP, pulmonary arterial systolic pressure; PAWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RSD, renal sympathetic denervation; RVMP, right ventricular mean pressure; RVSP, right ventricular systolic pressure. Data are expressed as mean (standard deviation).

² P < .01 for pulmonary arterial hypertension compared with baseline.
³ P < .05 for pulmonary arterial hypertension + renal sympathectomy compared with pulmonary arterial hypertension.
⁴ P < .05 for pulmonary arterial hypertension + renal sympathectomy compared with baseline.

**DISCUSSION**

The present study explored the impact of RSD on pulmonary vascular remodeling in experimental PAH. We provide evidence: a) that RSD attenuated the PAH induced by DHMC treatment in the beagles, as evidenced by the absence of a significant increase in pulmonary artery pressure, vascular wall thickness, and increased cardiac output, and b) that decreased levels of Ang II and ET-1 after RSD were associated with pulmonary vascular remodeling, which was induced by the DHMC treatment. The present findings suggest that catheter-based RSD is a potentially effective alternative for PAH treatment.

Pulmonary arterial hypertension is characterized by elevations in pulmonary artery pressure and pulmonary vascular resistance. Multiple factors, including vasoconstriction, pulmonary vessel remodeling, and thrombosis, are involved in the pathogenesis of PAH. 1718 Sympathetic nerve activity is also an important causal factor in the development of PAH. 219 In the animal model of acute PAH, the increased pulmonary artery pressure were completely abolished by pulmonary artery denervation. 2021 In patients with PAH, it has been suggested that systemic renin–angiotensin–aldosterone system activity is upregulated, based on findings of upregulated SNS activity. Previous studies have shown that Ang II can cause the growth/proliferation and hypertrophy of pulmonary artery muscle cells, thereby inducing pulmonary arterial resistance. 2223

In our previous studies, we conducted tests to measure Ang II levels before and after RSD. We found that the decreased release of Ang II demonstrated the effectiveness of the device in achieving an efferent RSD. 1224 To investigate whether RSD has an impact on pulmonary vascular remodeling, the experimental PAH model was investigated in the present study. Monocrotaline is a pyrrolizidine alkaloid plant toxin that, when activated, causes structural remodeling of pulmonary blood vessels and an increase in pulmonary arterial pressure. 2526 Our results are in accordance with previous investigations showing that the pulmonary artery pressure and right ventricular pressure in PAH dogs were significantly higher and that the cardiac output was lower than in the control dogs. Interestingly, in the present study, we found that the pulmonary artery pressure and right ventricular pressure

Table 4
Changes of Neurohormone Concentrations in Blood, Lung and Ventricular Tissues at Baseline and After 8 Weeks in the 3 Groups

<table>
<thead>
<tr>
<th></th>
<th>Creatinine Plasma, μmol/L</th>
<th>Aldosterone Ventricule, pg/mg</th>
<th>BNP Ventricule, pg/mg</th>
<th>Angiotensin II, pg/mL</th>
<th>Prostaglandin, pg/mL</th>
<th>Endothelin-1, pg/mL</th>
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<td><strong>Control</strong></td>
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<tr>
<td>Baseline</td>
<td>76 (9.2)</td>
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<td>After 8 weeks</td>
<td>79 (9.4)</td>
<td>67 (14)</td>
<td>0.5 (0.1)</td>
<td>131 (35)</td>
<td>38 (6.7)</td>
<td>561 (131)</td>
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<td>Baseline</td>
<td>79 (10.6)</td>
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<tr>
<td>After 8 weeks</td>
<td>82 (11.4)</td>
<td>172 (25)³</td>
<td>1.9 (0.4)³</td>
<td>228 (41)³</td>
<td>65 (9.8)³</td>
<td>683 (158)</td>
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<td><strong>PAH + RSD</strong></td>
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<tr>
<td>Baseline</td>
<td>74 (10.7)</td>
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<tr>
<td>After 8 weeks</td>
<td>77 (11.6)</td>
<td>105 (21)²</td>
<td>1.0 (0.2)²</td>
<td>136 (43)</td>
<td>46 (8.1)</td>
<td>486 (138)</td>
</tr>
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</table>

BNP, B-type natriuretic peptide; PAH, pulmonary arterial hypertension; RSD, renal sympathetic denervation; SD, standard deviation. Data are expressed as mean (standard deviation).

² P < .01 for pulmonary arterial hypertension compared with the control and pulmonary arterial hypertension + renal sympathectomy groups.
³ P < .01 for pulmonary arterial hypertension compared with the baseline.
⁴ P < .01 for pulmonary arterial hypertension + renal sympathectomy compared with the control group.
⁵ P = .01 for pulmonary arterial hypertension + renal sympathectomy compared with the control group.
in the PAH + RSD dogs were significantly lower than those in the PAH dogs. Furthermore, the pulmonary arterial wall thickening was decreased after RSD. A previous study has shown that plasma levels of circulating ET-1 are elevated in animals treated with monocrotaline.27 Our data shows that RSD decreases plasma Ang II and ET-1 levels in lung tissue. The circulating plasma levels of ET-1 are elevated in patients with PAH, and increased circulating levels of ET-1 correlate with increased pulmonary vascular resistance and increased mortality in patients with PAH.23,28 Early studies by Nakamura and Johns29 reported that a mild stimulation of the renal nerve increased angiotensinogen levels in the rat kidney. In another study, Girchev et al.30 demonstrated that RSD decreases the papillary ET-1 concentration in Wistar-Kyoto rats. These results showed that renal nerves participate in the regulation of ET-1 production within the kidney.30 A recent study demonstrated that RSD prevented aortic regurgitation-induced increases in kidney norepinephrine and Ang II levels.31 Angiotensin II infusion led to an enhanced vascular ET that converts enzyme activity and renal ET-1 content in the rat. These results suggest that Ang II is a strong stimulus of ET-1 synthesis.32

Interestingly, in the present study, we found that AT₁-receptor expression was upregulated in experimental PAH, but this change was suppressed after RSD. The effect of Ang II on PAH is mediated via AT₁ receptors. De Man et al.32 have demonstrated that enhanced AT₁-receptor signaling was associated with increased pulmonary artery smooth muscle cell proliferation in PAH patients compared with controls. These data suggest the concept that, after RSD, the activity of the systemic renin–angiotensin–aldosterone system was inhibited, leading to local renin–angiotensin–aldosterone system-activity and AT₁-receptor expression downregulation. Taken together, our results suggested that RSD treatment, prior to induction of PAH, prevents increases in right ventricular systolic pressure, and attenuates thickening of pulmonary vessels. The influence of RSD on PAH is mediated mainly by the activity of the renin–angiotensin–aldosterone and ET systems.

**Potential Significance**

Pulmonary arterial hypertension is a rapidly progressive disease that ultimately leads to right-heart failure and death.

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**Figure 1.** Representative changes in the vascular structure for the 3 groups. AH, pulmonary arterial hypertension; RSD, renal sympathetic denervation. Sections were stained with hematoxylin and eosin (×400).

**Figure 2.** Representative of histological changes in the 3 groups. Red areas represent myocytes and blue areas represent collagen (×400). A: examples of the right ventricular outflow in the 3 groups; B: summary of changes in the right ventricular outflow. In contrast, the right ventricular sections in pulmonary arterial hypertension dogs had higher fibrosis than in control and pulmonary arterial hypertension + renal sympathetic denervation dogs, and the fibrosis in pulmonary arterial hypertension + renal sympathetic denervation dogs was higher than that in control dogs. PAH, pulmonary arterial hypertension; RSD, renal sympathetic denervation. A: P < .01 vs control group and pulmonary arterial hypertension + renal sympathetic denervation group. B: P < .01 vs control group.
Advances in understanding the pathobiology over the past 2 decades have led to therapies directed at reversing pulmonary vasoconstriction. Despite these advances, disease progression is common, even with the use of combination regimens targeting multiple mechanistic pathways. We showed that the pulmonary artery pressure was attenuated by RSD in experimental PAH. Renal sympathetic denervation may alter pulmonary vascular remodeling by inhibiting the renin–angiotensin–aldosterone system and

**Figure 3.** Western blot analysis of angiotensin II type 1-receptor and angiotensin II type 2-receptor in the protein samples extracts from the pulmonary artery. A: examples of the Western blot bands recognized by the angiotensin II type 1-receptor antibodies. B: mean band densities expressed as the ratios of the 3 groups. The pulmonary arterial hypertension group over the control group and the pulmonary arterial hypertension + renal sympathetic denervation group. C: examples of the Western blot bands recognized by angiotensin II type 2-receptor antibodies. D: mean band densities expressed as the ratios of the 3 groups. There was no significant difference in angiotensin II type 2 densities in the 3 groups. AT1, angiotensin II type 1; AT2, angiotensin II type 2; PAH, pulmonary arterial hypertension; RSD, renal sympathetic denervation. *P < .01.
ET activity. Our data is preliminary but renal artery ablation may be an effective alternative for the treatment of PAH patients. This finding might raise concerns about the conventional pathogenesis of PAH, and might suggest the effectiveness of RSD for PAH, a similar new understanding on the effect of pulmonary artery denervation for treatment of PAH patients.33

Limitations

The present study has several limitations. First, the time course of the hemodynamic and structural derangements induced by DHMC was not studied. Thus, it is unknown whether hemodynamic and structural remodeling begins shortly after DHMC injection and whether these alterations are irreversible. Second, we performed renal denervation at the same time as pharmacological induction of PAH. Thus, the effects of RSD when the PAH is well established, as in the clinical scenario, cannot be ascertained. However, our results suggested that RSD were efficacious at initial stages of PAH. Future studies should investigate the effects of RSD on established PAH. Third, in control and PAH dogs, the radiofrequency ablation catheter was not positioned in the renal arteries. The control and PAH groups were not real sham groups. However, our results revealed RSD not only attenuated hemodynamic changes, but suppressed pulmonary vascular remodeling. Our findings set the stage for further comprehensive mechanistic study. Fourth, we did not investigate the anatomic distribution of periarterial sympathetic nerves around renal arteries by dual immunofluorescence staining using antibodies targeted for antityrosine hydroxylase and anticalcinin gene–related peptide in control and RSD dogs. Whether all the plexus were destroyed around the renal artery is unknown. Finally, Aguero et al34 reported that right ventricular remodeling occurred at the structural, histological, and molecular level in a pulmonary vein banding model of chronic postcapillary pulmonary hypertension. In our study, we found that the right ventricle had higher levels of aldosterone, B-type natriuretic peptide, and interstitial fibrosis in PAH dogs. However, we did not investigate the changes of right ventricular mass and molecular level in experimental PAH by DHMC. The structural and molecular changes in the right ventricle in experimental PAH by DHMC should be further investigated.

CONCLUSIONS

Renal sympathetic denervation suppressed pulmonary vascular remodeling and the decrease of pulmonary arterial pressure in experimental PAH. The effect of RSD on PAH may contribute to the decreased levels of neurohormones.

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CONFLICTS OF INTEREST

None declared.

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