Development of a servo pump system for in vivo loading of pathological pulmonary artery impedance on the right ventricle of normal rats

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Am J Physiol Heart Circ Physiol 310: H973–H983, 2016. First published February 12, 2016; doi:10.1152/ajpheart.00813.2015.—Pulmonary artery (PA) impedance provides detailed information on right ventricular (RV) afterload in pulmonary hypertension (PH). This study aimed to examine PA impedance in a rat model of monocrotaline-induced PH (MCT-PH) and to develop an experimental system for in vivo loading of pathological PA impedance on the RV of normal rats. PA impedance was quantified in normal (n = 10) and MCT-PH rats (n = 10) using a three-element Windkessel (3-WK) model. Compared with normal rats, MCT-PH rats had higher characteristic impedance (Zc) and peripheral pulmonary resistance (Rp) (Zc: 0.121 ± 0.039 vs. 0.053 ± 0.017 mmHg-min·ml−1, R:P < 0.001; Rp: 0.581 ± 0.334 vs. 0.252 ± 0.105 mmHg-min·ml−1, P = 0.013) and lower pulmonary artery compliance (Cp) (0.242 ± 0.131 vs. 0.700 ± 0.186 ml/mmHg, P < 0.001). In another group of 10 normal rats, a computer-controlled servo pump was connected to the left PA for loading PA impedance with parameters in pathological ranges designed by the 3-WK model. Activation of the servo pump decreased the error of measured vs. target PA impedance (modulus; from 0.047 ± 0.020 without pump activation to 0.019 ± 0.007 with pump activation, P < 0.001; phase: 0.085 ± 0.028 to 0.043 ± 0.012 radians, P < 0.001). In conclusion, MCT-PH increases Zc and Rp and decreases Cp. Our servo pump system, which is capable of imposing arbitrary PA impedance with pathological parameters, may offer a unique opportunity to delineate the pathological significance of PA impedance in PH.

NEW & NOTEWORTHY

Abnormality of pulmonary artery (PA) impedance was quantified in rats with monocrotaline-induced pulmonary hypertension. A servo pump system was developed for in vivo loading of arbitrary PA impedance with pathological parameters on the right ventricle of normal rats.

PULMONARY HYPERTENSION (PH) causes right ventricular (RV) maladaptation to an increase in afterload. The increased RV afterload eventually leads to RV failure and premature death. Therefore, understanding the pathological significance of RV afterload is important for risk stratification and improving treatment strategy for PH. Although pulmonary vascular resistance (PVR) is commonly used as an index of increased RV afterload, it reflects only the static or direct current (DC) properties of the pulmonary circulation in a state of steady flow. Because the in vivo pulmonary circulation is a pulsatile flow, evaluation of RV afterload should include the dynamic properties of the vascular bed. The dynamic properties of the pulmonary vascular bed can be described by pulmonary artery (PA) impedance (32, 33). PA impedance provides information on the pulsatile pressure-flow relationship that is determined by the physical characteristics of the pulmonary vascular bed (20). Several clinical studies have reported the significance of PA pressure waveforms (21) and PA impedance (17) in differentiating the etiologies of PH. According to previous studies, PA impedance may be approximated by a three-element Windkessel (3-WK) model (13, 32, 33); the three elements are characteristic impedance of the proximal PA (Zc), pulmonary artery compliance (Cp), and peripheral pulmonary resistance (Rp) (Fig. A1). PVR, which is given from the difference between mean PA pressure and mean PA wedge pressure [or mean left atrial (LA) pressure] divided by mean PA flow, is different from Rp of the 3-WK model. Total pulmonary resistance (TPR), which is calculated from mean PA pressure divided by mean PA flow, is also different from Rp.

Although the above-mentioned studies have promoted the understanding of PA impedance as RV afterload, the pathological significance of PA impedance in PH remains to be fully established. To the best of our knowledge, abnormality of PA impedance has not been thoroughly quantified even in the most commonly used rat model of monocrotaline-induced PH (MCT-PH). Therefore, the first purpose of this study was to compare PA impedance between normal rats and rats with MCT-PH. One limitation of the animal PH model is that the parameters of PA impedance (Zc, Cp, and Rp) cannot be altered independently, hampering clear-cut interpretation of the significance of each element in the pathology of PH. In this regard, Piene et al. (24, 25) and Elzinga et al. (5) examined the effects of changes in PA impedance on PA flow and pressure waveforms and RV pump function using an isolated feline heart preparation. In their model, however, the heart is devoid of neurohumoral regulation. To understand the pathological importance of PA impedance in PH, in vivo studies may also be required, where the heart is under neurohumoral regulation. As an example of such an attempt, the native PA is replaced with an artificial chamber to examine the effects of PA impedance on RV function (12, 14, 26). This method allows alteration of Cp but has limited ability to arbitrarily control Zc.

Although Miyashita et al. (19) developed an experimental system that imposes arbitrary systemic aortic impedance on the left ventricle (LV) of normal rats in vivo, the system is not directly applicable to the pulmonary circulation because the
pulmonary circulation is distinctly different from the systemic circulation. Therefore, the second purpose of this study was to newly develop a servo pump system that is able to impose arbitrary PA impedance on the RV of normal rats in vivo. The performance of the servo pump system was examined by selectively changing the parameters of PA impedance ranging from normal to pathological values obtained by measurements in normal and MCT-PH rats.

METHODS

Animal care was conducted in accordance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” approved by the Physiological Society of Japan. The experimental protocols were reviewed and approved by the Animal Subjects Committee at the National Cerebral and Cardiovascular Center.

Experimental protocols. The study consisted of the following two protocols and was performed on 30 male Sprague-Dawley rats.

Protocol 1 was performed to compare PA impedance between normal (n = 10) and MCT-PH rats (n = 10). In the MCT-PH model, PH was induced by a single subcutaneous injection of MCT (crotaline; Sigma-Aldrich, St. Louis, MO) at a dose of 80 mg/kg at 8 wk of age. After 29.0 ± 3.8 days (mean ± SD) from the MCT injection, hemodynamics were examined, and PA impedance was measured. The data obtained from the MCT-PH rats were compared with those obtained from age-matched normal rats.

Protocol 2 was conducted to develop a servo pump system to impose arbitrary PA impedance in vivo in normal rats (n = 10). The performance of the system was tested by independently manipulating the parameters of the 3-WK model. The target PA impedance was designed based on the PA impedance estimated in normal or MCT-PH rats in protocol 1.

Surgical preparation. The animal was anesthetized with an intraperitoneal injection of 2 ml/kg of a-chloralose (40 mg/ml) and urethane (250 mg/ml). Artificial ventilation via a tracheotomy was performed at a rate of 80 breaths/min with a tidal volume of 2.0–2.5 ml. Inspired room air was mixed with oxygen to prevent hypoxic pulmonary vasoconstriction (4). Partial pressure of oxygen in arterial ml. Inspired room air was mixed with oxygen to prevent hypoxic pulmonary vasoconstriction (4). Partial pressure of oxygen in arterial ml. Inspired room air was mixed with oxygen to prevent hypoxic pulmonary vasoconstriction (4).

The right femoral vein was cannulated with a catheter (ET-126; Labworks) attached to a custom-made piston pump (Fig. 1). The shaker side of the piston pump was fabricated using the barrel of a 2.5-ml syringe (SS-02SZ; Terumo, Tokyo, Japan) and was connected to the left PA by 10.220.33.5 on October 15, 2017 http://ajpheart.physiology.org/ Downloaded from

Fig. 1. Schematic illustration of in vivo pulmonary artery (PA) impedance loading system. The servo pump system, which consists of a direct current (DC) amplifier, a shaker, and a custom-made piston pump, is connected to the left PA. The right ventricle ejects blood into the main PA, and measured PA flow and pressure are fed back to the algorithm for command calculation. Activating the servo pump system alters the PA pressure waveform via withdrawal and restoration of blood in the main PA.
calculated from the product of target $Z(f)$ and $Q(t)$. The frequency spectra of pressure, which should be imposed by the servo pump ($Pc(f)$), was obtained by subtracting $Pm(f)$ from $Pt(f)$. The frequency spectra of the servo pump command [$Cmd(f)$] was then calculated by multiplying the reciprocal of a mathematical model of the servo pump transfer function [$H(f)$] and $Pc(f)$. $H(f)$ was predetermined using a binary white noise input (see Appendix C). $Cmd(f)$ was converted back to the time domain $[Cmd(t)]$ via inverse FFT. To avoid amplification of high-frequency noise, $Cmd(t)$ was high cut filtered above 50 Hz. In addition, $Cmd(t)$ was accumulated with a small updating factor ($K$) ranging from 0.05 to 0.1, to prevent instability induced by overcompensation of PA pressure. The command signal was iteratively updated every cycle (8.192 s per cycle) to decrease the difference between $Pt(f)$ and $Pm(f)$.

**Evaluation of accuracy of PA impedance loading.** To evaluate the accuracy of PA impedance loading, root mean square of errors for modulus expressed in common logarithm ($RMSE_{logM}$) and that for phase ($RMSE_{ph}$) for target vs. measured impedance values were calculated as follows:

$$RMSE_{logM} = \sqrt{\frac{1}{N} \sum_{i} \left\{ \log_{10} \left[ \frac{\text{Gain}_{\text{Target}}(i)}{\text{Gain}_{\text{Measured}}(i)} \right] \right\}^2}$$

$$RMSE_{ph} = \sqrt{\frac{1}{N} \sum_{i} \left\{ \text{Phase}_{\text{Target}}(i) - \text{Phase}_{\text{Measured}}(i) \right\}^2}$$

where $N$ represents the number of data points, which was set at 200 corresponding to 24.4 Hz, and $i$ represents the index of frequency in the discrete Fourier transform. The error was weighted by reciprocal of $i$, taking into account the increased number of data points at higher frequencies on the Bode plot. Note that measured PA impedance, which was estimated via an ensemble averaging operation (Appendix A), was not used during the calculation of the servo pump command in PA impedance loading.

The performance of PA impedance loading was also analyzed in the time domain by root mean square of errors ($RMSE_{t}$) for target ($PAP_{\text{Target}}$) vs. measured PA pressure ($PAP_{\text{Measured}}$) waveforms using Eq. 3 as shown below:

$$RMSE_{t} = \sqrt{\frac{1}{M} \sum_{i} \left\{ PAP_{\text{Target}}(i) - PAP_{\text{Measured}}(i) \right\}^2}$$

where $M$ is the number of data points, which was set at 1,000 corresponding to 1,000 ms, and $i$ represents the index of sampling point in a given segment. We also calculated the coefficient of determination ($R^2$) between target and measured PA pressure waveforms. Note that the target PA pressure in the time domain is not actually used during PA impedance control (Fig. 2), but it was calculated offline for evaluation purposes. Note also that the target PA pressure waveform at baseline was not identical to that at steady state of PA impedance loading. This is because changing PA impedance affects the PA flow waveform, which modifies the target PA pressure waveform in the iterative cycles of PA impedance loading.

**Statistics.** All data are expressed as means ± SD. In protocol 1, hemodynamic parameters were compared between normal and MCT-PH rats using Wilcoxon rank sum test for independent two groups. In protocol 2, $RMSE$ values were compared between baseline and steady state during PA impedance loading by Wilcoxon signed-rank test for paired comparison. $P$ values <0.05 were considered statistically significant.

**RESULTS**

**Protocol 1.** Group-averaged body weight and baseline hemodynamics are shown in Table 1. There was no significant difference in baseline HR between normal and MCT-PH rats. Body weight was lower in MCT-PH compared with normal rats. Compared with the normal group, the MCT-PH group had higher mean PA pressure and TPR and lower mean PA flow. Mean systemic arterial pressure was lower in MCT-PH than in normal rats.

<table>
<thead>
<tr>
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<th>Normal ($n = 10$)</th>
<th>MCT-PH ($n = 10$)</th>
<th>$P$ Value</th>
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<tr>
<td>Body weight, g</td>
<td>437 ± 60</td>
<td>378 ± 31</td>
<td>0.021</td>
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<tr>
<td>HR, beats/min</td>
<td>421 ± 37</td>
<td>383 ± 49</td>
<td>0.162</td>
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<tr>
<td>PAF, ml/min</td>
<td>47.4 ± 9.3</td>
<td>26.9 ± 13.6</td>
<td>0.003</td>
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<tr>
<td>SAP, mmHg</td>
<td>17.2 ± 3.0</td>
<td>35.1 ± 8.1</td>
<td>&lt;0.001</td>
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<tr>
<td>SAP, nmHg</td>
<td>106.2 ± 24.6</td>
<td>76.3 ± 18.7</td>
<td>0.007</td>
</tr>
<tr>
<td>TPR, mmHg/min·ml⁻¹</td>
<td>0.37 ± 0.09</td>
<td>1.66 ± 0.80</td>
<td>&lt;0.001</td>
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</table>

Values are expressed as means ± SD. MCT-PH, monocrotaline-induced pulmonary hypertension; HR, heart rate; PAF, mean pulmonary arterial flow; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure; TPR, total pulmonary resistance calculated from PAP/PAF. $P$ values were calculated using Wilcoxon rank sum test for 2 independent groups.
Typical time series of PA pressure and flow under irregular pacing obtained from normal and MCT-PH rats are shown in Fig. 3, A and B. In the MCT-PH rat, mean and pulse PA pressure were higher, and peak PA flow was lower than those in the normal rat.

Figure 4, A and B, summarizes the power spectra of PA flow and pressure and PA impedance and coherence function averaged from the normal (left) and MCT-PH (right) groups. In the normal group, the power spectra of PA flow and pressure were spread over a wide frequency range attributable to irregular pacing. The PA flow power, averaged from 0.1 to 15.0 Hz, was 7.0 ± 4.0 (ml/min)². The PA pressure power, averaged from 0.1 to 15.0 Hz, was 0.05 ± 0.02 mmHg². The PA impedance modulus remained relatively constant at ~0.2 mmHg-min·ml⁻¹ until around 1 Hz and then decreased with increasing frequency up to ~10 Hz. Above this frequency, the impedance modulus was relatively constant at ~0.06 mmHg-min·ml⁻¹. The PA impedance phase was negative at frequencies below ~17 Hz but became slightly positive thereafter, which means that flow oscillations lagged behind pressure oscillations above 17 Hz. The coherence function showed that PA pressure correlated almost linearly with PA flow up to ~15 Hz, except for troughs at the artificial ventilation frequency (~1.3 Hz) and its harmonics. Coherence decreased and showed large variance above 15 Hz.

In the MCT-PH group (Fig. 4A, right), the PA flow power, averaged from 0.1 to 15.0 Hz, was 3.0 ± 3.0 (ml/min)² and was significantly smaller compared with the normal group (P = 0.014 by Wilcoxon rank sum test). The PA pressure power, averaged from 0.1 to 15.0 Hz, was 0.13 ± 0.06 mmHg² and was significantly higher compared with the normal group (P < 0.001). The average impedance modulus was relatively constant at ~0.6 mmHg-min·ml⁻¹ in the frequency range up to ~1 Hz, decreased with increasing frequency up to ~15 Hz, and then became relatively constant at ~0.1 mmHg-min·ml⁻¹ (Fig. 4B, right). Compared with the normal group, the MCT-PH group had significantly higher Zc and Rp and significantly lower Cp based on the 3-WK model fitted to the measured PA impedance (Table 2). The coherence functions in the MCT-PH and normal groups showed similar characteristics.

Protocol 2. The servo pump system was activated to load PA impedance with pathological parameters, which mimicked the PA impedance measured in the MCT-PH rats in protocol 1. Figure 5 demonstrates the time-dependent changes of PA impedance modulus and phase, coherence function between PA flow and pressure, command signal, PA flow, and PA pressure in a representative normal rat. The target impedance was designed based on measurements in MCT-PH rats. Before the activation of the servo pump system, a large difference was observed between measured (black solid line) and target (red smooth curve) impedance (Fig. 5A, top two rows). After activating the servo pump system, the difference between the measured (black line) and target PA (red line) pressure decreased gradually, along with increased amplitude of the servo pump command (Fig. 5B, bottom three rows). The PA impedance loading typically reached a steady state within 20 iterations. After convergence, the differences between measured and target PA impedance were markedly attenuated (Fig. 5C, top two rows).

By selectively altering the parameters of target PA impedance, i.e., Zc, Cp, and Rp, the developed system was able to impose specific PA impedance at pathological range. Figure 6 illustrates typical traces obtained in one rat, showing impedance modulus and phase, coherence function between PA flow and pressure, command signal, and PA flow and pressure waveforms at baseline (Fig. 6A) and after convergence of PA impedance loading with parameters in normal values (Fig. 6B) or with parameters in pathological values (Fig. 6C–E). The black solid lines and red smooth

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**Fig. 3.** Representative time series of PA pressure (PAP) and PA flow (PAF) during irregular pacing in a normal rat (A) and a monocrotaline-induced pulmonary hypertension (PH) rat (B) obtained in protocol 1. Data from 1 segment (8.192 s) are shown.
curves in the top two rows represent measured PA impedance and target PA impedance at convergence, respectively. As reference, baseline PA impedance was duplicated as black dotted lines in Fig. 6, B–E. In the bottom row, because the respiratory change was not synchronized with the segment length of PA impedance loading, the measured PA pressure (black lines) showed some deviation from the target PA pressure (red lines) in a few beats. Nevertheless, measured PA impedance matched the target PA impedance reasonably well. Note that PA impedance at baseline before activating the servo pump system (Fig. 6A) was different from that after loading of normal PA impedance (Fig. 6B) because of the ligation of the left PA and the connection to the servo pump.

A total of 16 trials (12 trials to impose pathological PA impedance, 4 trials to impose normal PA impedance) were performed in 10 rats. The target $Z_C$ ranged from 0.07 to 0.15 mmHg-min·ml$^{-1}$, $C_P$ from 0.1 to 0.9 ml/mmHg, and $R_P$ from 0.2 to 1.0 mmHg-min·ml$^{-1}$. These parameters were designed based on the values observed in normal and MCT-PH rats in protocol 1. $RMSE_{log|Z|}$ decreased significantly from 0.047 ± 0.020 at baseline to 0.019 ± 0.007 at convergence of PA impedance loading ($P < 0.001$, Fig. 7A). $RMSE_{c}$ was reduced from 0.085 ± 0.028 to 0.043 ± 0.012 radians ($P < 0.001$, Fig. 7B). $RMSE_{r}$ at convergence was 1.6 ± 0.6 mmHg, which corresponded to 7.8 ± 2.4% of the target systolic PA pressure. The $R^2$ between measured and target PA pressure waveforms at convergence was 0.85 ± 0.07, indicating reasonable accuracy of PA impedance control.

Table 2. Parameters of three-element Windkessel model obtained in protocol 1

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<tr>
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<th>Normal $(n = 10)$</th>
<th>MCT-PH $(n = 10)$</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td>$Z_C$, mmHg·min·ml$^{-1}$</td>
<td>0.053 ± 0.017</td>
<td>0.121 ± 0.039</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$C_P$, ml/mmHg</td>
<td>0.700 ± 0.186</td>
<td>0.242 ± 0.131</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$R_P$, mmHg·min·ml$^{-1}$</td>
<td>0.252 ± 0.105</td>
<td>0.581 ± 0.334</td>
<td>0.013</td>
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</table>

Values are expressed as means ± SD. $Z_C$, characteristic impedance; $C_P$, pulmonary artery compliance; $R_P$, peripheral resistance in the 3-element Windkessel model fitted to the measured pulmonary artery impedance. $P$ values were calculated using Wilcoxon rank sum test for 2 independent groups.
DISCUSSION

To the best of our knowledge, the present study is the first to quantify PA impedance in MCT-PH rats. Furthermore, we developed a new computer-controlled servo pump system that can impose arbitrary PA impedance in vivo in normal rats. We demonstrated that this system was able to impose pathological PA impedance by independently altering the parameters in the 3-WK model (i.e., $Z_C$, $C_P$, and $R_P$) based on the values obtained in MCT-PH rats.

PA impedance in MCT-PH model. In the present study, the peripheral pulmonary resistance of the pulmonary vascular bed, $R_P$, is discriminated from DC resistance because the DC component of PA impedance theoretically equals to $Z_C//R_P$ in the 3-WK model (Eq. A3, Fig. A1B). In systemic circulation, $R_P$ is usually treated as approximately equal to DC resistance (32) because $Z_C$ has a negligible impact on DC resistance (20). However, because the ratio of $Z_C$ to $R_P$ in the pulmonary circulation is not as small as that in the systemic circulation (20), $R_P$ in pulmonary circulation is not approximately equal to DC resistance (13).

In protocol 1, both TPR and PA impedance modulus were elevated in the MCT-PH rats compared with the normal rats. The results are consistent with a previous study using lambs in which an exposure to MCT increased TPR and $Z_C$ and decreased cardiac output (7). $C_P$ was lowered and $R_P$ was elevated in the MCT-PH rats, which may be attributed to damage of the peripheral pulmonary arteries caused by MCT (27a). Increased $Z_C$ in MCT-PH rats is consistent with clinical reports showing elevated $Z_C$ in patients with PH (9, 17) despite enlargement of the proximal PA in advanced PH (34). Stiffening of the proximal PA may be the reason for increased $Z_C$ in MCT-PH rats because $Z_C$ varies with the elastic modulus of the artery and changes inversely with its cross-sectional area (20). Our findings in protocol 1 demonstrate that MCT-PH results in simultaneous changes in the three parameters of PA impedance. To examine the pathological significance of $Z_C$, $C_P$, and $R_P$ in PH separately, however, independent alteration of each element becomes necessary.

Development of PA impedance control system. Only a few attempts on the development of experimental systems for quantitative loading of PA impedance have been previously reported. Piene et al. (25) altered PA impedance by acutely activating a piston pump connected to the PA in isolated feline hearts. In isolated hearts, coronary perfusion pressure is kept constant, which is different from in vivo circulation. The absence of neurohumoral regulation may be another limitation to the isolated heart preparation. Sato et al. (26) and Kuo et al. (14) controlled RV afterload in vivo by replacing the native PA with an artificial chamber in sheep. These physical manipulations on the pulmonary circulation allow modification of $C_P$ but are not versatile in controlling $Z_C$.

As mentioned above, Miyashita et al. (19) developed a control system to impose desired systemic arterial impedance in vivo in rats. The present study employed a similar strategy to develop a PA impedance control system, but it was necessary to build an entirely new system. First, the pulmonary circulation is characterized by low pressure and low impedance (20) and large recruitment and distension of small vessels in

![Fig. 5. A representative time course for controlling PA impedance and associated changes in PAF and PAP waveforms in a normal rat. Black solid lines denote measured data, and red solid lines denote target data. Target impedance is the pathological PA impedance designed based on measurement in monocrotaline-induced PH rats. A: PA impedance at baseline without activating the servo pump system. B: PA impedance measured at the early phase of PA impedance loading. C: PA impedance measured at the convergence phase. Measured PA impedance modulus and phase (black solid lines) gradually approach the target PA impedance (red smooth curves) as the amplitude of the servo pump command signal increases.](http://ajpheart.physiology.org/doi/fig/10.1152/ajpheart.00813.2015)
response to hydraulic loading (31). Therefore, compared with the systemic circulation, loading of a larger volume is required to change the PA pressure waveform for controlling PA impedance. To meet the demand, the piston pump was fabricated using an LDPE barrel with an effective stroke volume of 10 ml. Second, the new system uses target PA impedance based on a mathematical arterial model, rather than modifying the native impedance (19). Therefore, in principle it is applicable to other arterial models such as the four-element Windkessel model (28). Nevertheless, the 3-WK model was adopted in the present study because preliminary analyses indicated that inertance had only a negligible impact on the accuracy for the prediction of PA pressure waveform from the measured PA flow waveform in the present experimental setting (data not shown). The system may be able to impose pathological RV afterload for different types of PH such as pulmonary arterial hypertension (PAH), chronic thromboembolism PH, and PH associated with congenital heart disease.

PA impedance calculation under regular pacing. Under sinus rhythm, PA impedance can be accurately estimated only at the native pulse frequency and its harmonics because of insufficient input power at other frequencies (see Appendix B). To provide sufficient input power over a wide frequency range, irregular pacing was used in protocol 1. We anticipated that PA impedance could be estimated only at the regular pacing frequency and its harmonics in protocol 2 because irregular pacing was not used. Contrary to our expectation, however, PA impedance was estimated reasonably well at frequencies outside the regular pacing frequency and its harmonics. As shown in Fig. 6A, connection of the servo pump system to the left PA increased the coherence between PA flow and pressure under regular pacing even without activating the servo pump system. These rather unforeseen results may be explained as follows.

The pulmonary circulation exhibits a significant nonlinear relationship between PA flow and pressure (22), which might have lowered coherence at frequencies outside the native pulse frequency and its harmonics (see Appendix B). Because the servo pump system has a simple structure made of nonbiological material, its dynamic characteristics may be much more...
linear than the native vascular bed. Attaching a linear structure with a large volume (~10 ml, ~50 times the stroke volume of the rat) to the left PA may increase the overall linearity between PA flow and pressure measured at the main PA, allowing estimation of PA impedance with small input power at frequencies outside the regular pacing frequency and its harmonics in protocol 2. Although this speculation needs to be confirmed, the reasonable estimation of PA impedance under regular pacing is a merit of the developed system, which allows checking of the accuracy of PA impedance control over a wide frequency range.

Possible clinical implications. Although there have been several reports investigating the PA impedance in animals and humans (8, 16, 27, 30), the pathological significance of PA impedance in PH is not well defined. PA impedance has potential prognostic value in patients with PH (10, 18). Therefore, identifying changes in the parameters of PA impedance, i.e., $Z_C$, $C_P$, and $R_P$, may further contribute to our understanding of RV dysfunction in PH. With the use of the developed PA impedance loading system, we may be able to identify the most important mechanical stimulus responsible for RV dysfunction in PH, by imposing PA impedance with pathological parameters and observing the resulting changes in RV function in animal experiments.

The present experimental system is only feasible for acute studies. Nevertheless, we may be able to obtain information on the pathology of the PA within the time window (~60 min) of an acute study. For instance, early gene expression has been observed in pressure-overloaded adult rat hearts (23), and it is likely possible to identify PA impedance parameters that trigger this gene expression and contribute to RV hypertrophy in PH. Detailed investigations of the effects of PA impedance on RV function could lead to new strategies for the treatment of PH based on abnormality in mechanical characteristics of the pulmonary vascular bed.

Limitations. First, anesthesia might have suppressed neurohumoral regulation to a variable extent. However, in a previous study (11), we were able to observe baroreflex-mediated responses in sympathetic nerve activity, arterial pressure, and HR in Sprague-Dawley rats using the same anesthesia of α-chloralose and urethane. Therefore, neurohumoral regulation may be observed in the present experimental setting although the results need to be carefully interpreted when extrapolating them to the physiological neurohumoral regulation in conscious animals.

Second, in the present study, an MCT-PH model was used because it has been extensively used to identify the effect of an increased RV afterload in PH. Although the MCT-PH model has advantages in technical simplicity, reproducibility of PH, and low cost, it is not an ideal model of human PAH (6). Further investigation in other models of PH, including the SU5416/hypoxia model (1), is required to fully understand pathological RV afterload in human PAH.

Third, this system was not able to control the static properties due to the nature of the push-pull piston pump. Whereas PA pressure waveform and the parameters of PA impedance ($Z_C$, $C_P$, and $R_P$) changed markedly by PA impedance loading, mean PA pressure and TPR did not alter significantly. Hence the effects of changes in DC resistance of the pulmonary circulation may not be evaluated properly by this system alone. Regarding this limitation, increasing the segment length of FFT would extend the lower frequency bound of PA impedance control. In addition, obstruction of the right PA or continuous volume loading may be combined with the system to increase the DC resistance measured at the main PA.

Fourth, positive pressure ventilation and thoracotomy were required in this experiment. Although quiet spontaneous respiration has no impact on PA impedance in humans (20), mechanical ventilation with a higher tidal volume has been reported to increase PVR and $Z_C$ in closed-chest swine (3). In the present study, mechanical ventilation and thoracotomy were responsible for the troughs observed in the coherence function between PA flow and pressure at the ventilation frequency and its harmonics. Synchronizing the ventilation frequency with the segment length of PA impedance control may improve the accuracy of PA impedance control because the PA pressure waveform fluctuates at the ventilation frequency. Further experiments are required to quantify the effects of ventilatory parameters on PA impedance in the present experimental setting.

Finally, to simplify surgical preparation and reduce surgical damage, the downstream pressure of the pulmonary circulation, i.e., LA pressure, was not measured or taken into account in the calculation of PA impedance. This is also because a preliminary investigation indicated little impact of LA pressure on the estimation of overall PA impedance modulus and phase (see Appendix B). Nevertheless, using the information of LA pressure may increase the accuracy of PA impedance estimation at the frequency of ventilation and its harmonics, improving the performance of PA impedance loading.

Conclusions. We have developed a computer-controlled servo pump system capable of loading arbitrary PA impedance on the RV in vivo in normal rats and succeeded in imposing pathological PA impedance designed based on the PA impedance measured in MCT-PH model rats. This system will offer a unique opportunity for systematic analysis of the effect of changes in PA impedance on RV function, which cannot be easily clarified using conventional experimental systems (e.g., an experimental system that focuses on changes in DC resistance). Once the mechanical stress responsible for the development of RV failure is identified, in terms of parameters of PA impedance, new strategies using pharmacological and/or surgical intervention can be applied to alter the corresponding parameters and treat RV failure in PH.

APPENDIX A: CALCULATION OF PA IMPEDANCE AND TOTAL PULMONARY RESISTANCE

The main PA flow and pressure waveforms were digitized at 1,000 Hz using a 16-bit analog-to-digital converter and stored on a computer dedicated for data recording. Based on a preliminary investigation (see Appendix B), PA impedance was calculated as a transfer function from PA flow to PA pressure without taking into account LA pressure as follows. The input-output data pairs were divided into 12 sets of 50% overlapping segments of 217 data points each (one segment = 8.192 s), indicating that the frequency resolution was 0.122 Hz. The first segment was from 0 to 8.192 s, the second segment was from 4,096 to 12,288 s, the third segment was from 8,192 to 16,384 s, and so forth. The total data length was 53,248 s. For each segment, a linear trend was subtracted, and a Hanning window was applied. The frequency spectra of the input and output were obtained by FFT. Next, the power of PA flow [$S_{QPA}(f)$], power of PA pressure [$S_{PAP}(f)$], and cross spectra between PA pressure and PA flow [$S_{PAP}(f)$] were ensemble averaged over the 12 segments. Finally, PA impedance was estimated as the transfer function [$Z(f)$] from PA flow to PA pressure using Eq. A1 as shown below (2).

\[
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\]
We also calculated the magnitude-squared coherence function \( \text{Coh}(f) \) that represents the linear dependence between the input and output using Eq. A2 as shown below (2).

\[
\text{Coh}(f) = \frac{\left| \frac{S_{pp}(f)}{S_{qq}(f)} \right|^2}{S_{pp}(f)S_{qq}(f)} \quad (A2)
\]

PA impedance was described by fitting a 3-WK model (Fig. A1A) (13, 32) to the data using an iterative nonlinear least-squares method. In the 3-WK model, the input impedance \([Z_{in}(f)]\) is expressed by Eq. A3 as shown below (29).

\[
Z_{in}(f) = Z_c \left(1 + \frac{R_p}{2\pi f C_p R_p} \right) \quad (A3)
\]

where \( f \) and \( j \) represent the frequency and imaginary units, respectively. As can be seen in Fig. A1B, when the frequency approaches zero, PA impedance at 0 Hz, i.e., DC resistance, is given by \( Z_c + R_p \).

In protocol 1, baseline hemodynamics were determined by averaging HR, systemic arterial pressure, PA pressure, and PA flow over 1 min without pacing. TPR was obtained by the ratio of mean PA pressure to flow, without taking LA pressure into account.

**APPENDIX B: PA IMPEDANCE ESTIMATION UNDER DIFFERENT CONDITIONS**

As is apparent from Eq. A1, sufficient input power over the frequency of interest is needed for stable estimation of PA impedance because the small input power led to inaccurate calculation of PA impedance as a division with a small denominator at the corresponding frequencies. Figure B1, A and B, demonstrates a typical example of PA impedance estimation during sinus rhythm and irregular pacing in a normal rat. Without irregular pacing, the PA impedance values are estimated only at the frequencies related to heart and mechanical ventilation rates and their harmonics, judging from the coherence values. Irregular pacing significantly improves the estimation of PA impedance over a wide frequency range of physiological interest. The ventilation frequency, which appears to improve estimation of PA impedance during sinus rhythm, actually hampers accurate estimation.

**Fig. B1.** A: PA impedance calculated as the transfer function from PAF to PAP during sinus rhythm. B: PA impedance calculated as the transfer function from PAF to PAP during irregular pacing. C: PA impedance calculated as the reciprocal of the hydraulic admittance using the framework of a 2-input/1-output model consisting of transfer functions from both PAP and left atrial pressure to PAF.
of PA impedance during irregular pacing. This is because LA pressure affects PA impedance estimation, as explained in the next paragraph.

In this study, PA impedance was calculated as the transfer function from PA flow to PA pressure without taking into account LA pressure. To examine the impact of LA pressure on PA impedance estimation, PA impedance was also calculated by the framework of a two-input/one-output model (2) using simultaneously measured LA pressure. Briefly, PA impedance is calculated by the reciprocal of hydraulic admittance, which consists of the transfer functions from two-input (PA pressure and LA pressure) to one-output (PA flow). The two-input/one-output model improved the coherence function at the frequencies of ventilation and their harmonics but had no significant impact on the PA impedance modulus and phase at other frequencies (Fig. B1C). Based on this preliminary result, LA pressure was not measured to simplify surgical preparation.

APPENDIX C: CHARACTERISTICS OF SERVO-PUMP SYSTEM

The frequency response of the servo pump system was calculated as a transfer function \([H(f)]\) between a random command signal (binary white noise with switching interval of 10 ms) and the PA pressure waveform (Fig. C1). The transfer function demonstrated low-pass characteristics of the servo pump system. To describe the estimated transfer function of the servo pump system, we employed a following mathematical model:

\[
H(f) = K_s \left( \frac{1 + \frac{f}{F_{C_2}}}{1 + \frac{f}{F_{C_1}}} \exp(-2\pi fL) \right)
\]  
\[(C1)\]

where \(K_s\) represents the steady-state gain; \(F_{C_1}\) is the corner frequency defining the low-pass characteristics (Hz); \(F_{C_2}\) is the corner frequency defining the beginning of the frequency-independent gain term (Hz) \((F_{C_1} < F_{C_2})\); and \(L\) is the pure dead time (s). These parameters were determined by nonlinear least-squares fitting to the measured transfer function. The reciprocal of this model was incorporated into the algorithm for calculating the servo pump command (Fig. 2); hence the delay in the servo pump system was canceled.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.F., T.K., S.S., K.U., and M.S. conception and design of research; M.F., T.K., S.S., and M.J.T. performed experiments; M.F., T.K., S.S., and M.S. analyzed data; M.F., T.K., and M.S. interpreted results of experiments; M.F. prepared figures; M.F., T.K., and M.S. drafted manuscript; M.F., T.K., and M.S. edited and revised manuscript; M.F. and M.S. approved final version of manuscript.

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