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Wave reflection correlates with pulmonary vascular wall thickening in rats with pulmonary arterial hypertension

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ABSTRACT

Background: Wave reflection is enhanced in patients with pulmonary arterial hypertension (PAH), which may be derived from a mismatch of pulmonary artery (PA) impedance between proximal and distal sites of arteries. Whether enhanced wave reflection correlates with histological remodeling remains unknown, partly because lung biopsy is not clinically recommended for PAH patients due to substantial risks of mortality and morbidity.
Methods: Pulmonary hypertension was induced by SU5416 injection and 3-week hypoxic exposure (SuHx-PH) in rats, and hemodynamic and histological examinations were performed at 4 weeks (SuHx-PH_{4W}) and 8 weeks (SuHx-PH_{8W}) after SU5416 injection ($n = 7$ each). Two groups of age-matched normal rats were also analyzed ($n = 7$ each). Using an elastic tube with a 3-element Windkessel model, PA impedance was parameterized as pulmonary artery compliance (C_P), peripheral resistance (R_P), characteristic impedance (Z_C), and transmission time (T_D) in conducting arteries. Wave reflection was quantified as reflection gain at 0 Hz (Γ_{gain}) in the frequency domain, and as the ratio of peak backward pressure to peak forward pressure ($K_{B/F}$) in the time domain.
Results: The SuHx-PH groups demonstrated increased R_P and Z_C, and decreased C_P and T_D compared with normal groups. Γ_{gain} and $K_{B/F}$ were significantly higher in the SuHx-PH_{8W} group than in the SuHx-PH_{4W} group, and correlated strongly with a histological index of vascular wall thickening ($R^2 = 0.839$, $P < 0.001$ for Γ_{gain} and $R^2 = 0.775$, $P < 0.001$ for $K_{B/F}$).
Conclusions: Enhanced wave reflection caused by abnormal PA impedance correlates with histological remodeling, and may have a diagnostic value in clinical staging of PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a fatal disease characterized by increased right ventricular (RV) afterload due to progressive remodeling of pulmonary vasculature. Because an abnormal increase in RV afterload causes RV failure and ultimately death [1], analyses of abnormal RV afterload at different stages of vascular remodeling may help understand the pathophysiology of PAH. RV afterload may be quantified by static and dynamic properties of the pulmonary vascular bed. The static properties, which can be assessed by total pulmonary resistance (TPR) or pulmonary vascular resistance (PVR) [2], determine pulmonary artery (PA) pressure in static (steady) flow. In contrast, dynamic properties, which can be described by PA impedance, determine PA pressure in dynamic (pulsatile) flow. Previous study has shown that not only static but also dynamic properties of RV afterload are important in determining clinical outcome of patients with PAH [3]. Decreased pulmonary artery compliance (C_P), increased peripheral resistance (R_P), and increased characteristic impedance of proximal arteries (Z_C) have been reported in patients with PAH [4,5,6]. Enhanced wave reflection has also been observed in patients with PAH [7], which may be derived from a mismatch of PA impedance between proximal and distal sites of arteries. Although abnormal wave reflection may reflect the degree of obstruction in pulmonary vasculature in PAH, clinical research has not confirmed the relation between wave reflection and histological remodeling in the pulmonary vascular bed, partly because lung biopsy is not recommended for patients with PAH due to substantial risks of mortality and morbidity [8].

Abbreviations: C_P, pulmonary arterial compliance; T_D, transmission time; $K_{B/F}$, ratio of peak backward pressure to peak forward pressure, (Peak P_B)/(Peak P_F); PA, pulmonary artery; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; R_P, peripheral resistance; RV, right ventricle; SuHx-PH, SU5416/hypoxia-induced pulmonary hypertension; TPR, total pulmonary resistance; VWT, vascular wall thickening; Z_C, characteristic impedance; Γ_{cutoff}, a cutoff frequency of reflection coefficient spectrum; Γ_{gain}, a reflection gain at 0 Hz.

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Animal models of PAH have contributed to understanding the pathophysiology of human PAH [9]. Particularly, a rat model of PAH induced by injection of SU5416, an inhibitor of vascular endothelial growth factor receptor tyrosine kinase, followed by chronic exposure to hypoxia (SuHx-PH) has the advantage of mimicking human PAH histologically [10]. Neointimal obstructive thickening is reproduced in the SuHx-PH rats, but not in other models. Identifying the progression of PA impedance and wave reflection abnormalities over time in the SuHx-PH rats is expected to provide critical insights for the staging of PAH patients. While wave reflection abnormalities in SuHx-PH mice have been demonstrated recently [11], the relationship between the degree of wave reflection and vascular wall remodeling in animal models of PAH remains unquantified.

We have reported a technique to estimate PA impedance over a wide frequency range of interest in anesthetized rats [12,13]. The present study used this technique to 1) characterize PA impedance in the SuHx-PH rats at different time points (4 and 8 weeks after SU5416 injection), and 2) to analyze the relationship between parameters of wave reflection and a histological index of vascular wall thickening of the pulmonary vascular bed.

2. Methods

2.1. Animal model and study design

Experiments were performed in 28 male Sprague-Dawley rats. All animals received humane care. The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocols were reviewed and approved by the Animal Subjects Committee at the National Cerebral and Cardiovascular Center, Japan.

According to a previous report by Abe et al. [9], pulmonary hypertension was induced in 5-week-old rats by a subcutaneous injection of SU5416 (S8442, Sigma-Aldrich, USA) at a dose of 20 mg/kg followed by 3-week exposure to hypoxia (10% O_2). Thereafter, the animals were returned to normoxia. Abe et al. [10] have shown that neointimal obstructive lesion can be developed at 5 weeks after returning to normoxia. Thus, hemodynamic and histological examinations were performed at 1 week and 5 weeks after returning to normoxia; the former and the latter groups were designated SuHx-PH_{4W} and SuHx-PH_{8W}, respectively, based on the duration after SU5416 administration (Fig. 1A). Normal rats aged 9 weeks ($Normal_{4W}$) and 13 weeks ($Normal_{8W}$) were also analyzed as age-matched controls. The Normal groups received no subcutaneous vehicle injections. Seven rats were examined in each group.

2.2. Hemodynamic study

Animals were anesthetized with an intraperitoneal injection (2 ml/kg) of a mixture of α-chloralose (40 mg/ml) and urethane (250 mg/ml). The anesthetic mixture was diluted to a 1/18 concentration and administered intravenously (2–3 ml/kg) to maintain the level of anesthesia. Volume-controlled mechanical ventilation was performed with oxygen-enriched room air. Ventilation rate was 80 breaths/min and a tidal volume was 6.5 ml/kg. In a preliminary investigation, partial pressure of oxygen in arterial blood (PaO_2) was supranormal (>200 mm Hg) and that of carbon dioxide in arterial blood ($PaCO_2$) was within normal (<40 mm Hg) in both Normal and SuHx-PH rats. These ventilatory settings suppressed spontaneous breathing without the use of muscular relaxants. Through a left thoracotomy, PA flow and pressure were measured, using an ultrasound Doppler flowmeter and a catheter-tip micromanometer, respectively, under open-chest conditions. The PA flow and pressure waveforms were recorded under sinus rhythm and irregular left atrial pacing as described previously [12,13].

2.3. Histopathologic study

After hemodynamic examination, the rats were euthanized by an overdose of intravenous pentobarbital sodium. The right lung was removed and fixed in 10% formaldehyde solution for histological investigation. The samples were embedded in paraffin, and 2–3 μm sections were prepared and stained with hematoxylin-eosin and Elastica-van Gieson stain. Vascular remodeling was quantified in small arteries <50 μm in diameter [14]. After measurement of outer and lumen diameters, the following index was used to assess vessel wall thickening (VWT) in percentage.

$$\mathrm{VWT} = 100 \times \frac{outer\ diameter - lumen\ diameter}{outer\ diameter} \quad \text{Eq.(1)}$$

2.4. Calculation and parameterization of pulmonary artery impedance

PA flow and pressure waveforms were simultaneously recorded during irregular pacing to estimate PA impedance over a wide frequency range. Without irregular pacing, PA impedance can be estimated only at the frequencies related to heart and mechanical ventilation rates and their harmonics where input power (i.e., PA flow power) is sufficiently large [12]. PA impedance and the magnitude-squared coherence function were calculated at a frequency resolution of 0.122 Hz [12,13]. Although PA impedance approximated a 3-element Windkessel (3-WK) model in normal rats in our previous studies, a preliminary analysis in SuHx-PH rats indicated that including an additional element of wave reflection was necessary to better describe PA impedance abnormality. Hence, PA impedance was parameterized using a combination of a lossless elastic tube and a 3-WK model (Fig. 1B) [15]. The tube corresponds to conducting arteries from the main PA to a reflection site in the pulmonary vascular bed. The characteristic impedance of the tube, Z_C, is independent of the length of the tube. The 3-WK model corresponds to the arterial system distal to the reflection site, and is described by three parameters (C_P, R_P, and Z_C). Impedance of the combined tube and 3-WK model [$Z_{model}(f)$] can be expressed by the following equation.

$$Z_{model}(f) = Zc\left\{\frac{1+[\Delta(f)]^2\Gamma(f)}{1-[\Delta(f)]^2\Gamma(f)}\right\} \quad \text{Eq.(2)}$$

where f represents frequency. $\Delta(f)$ is a frequency-domain expression of the time delay (T_D) from the inlet of the proximal PA to the reflection site (see Appendix for details). $\Gamma(f)$ is a reflection coefficient spectrum. $\Gamma(f)$ has low-pass characteristics defined by a reflection gain at 0 Hz [$\Gamma_{gain} = \Gamma(0)$] and a cutoff frequency (Γ_{cutoff}), which can be derived from C_P, R_P, and Z_C (see Appendix for details). The parameters of the tube and 3-WK model were estimated by fitting Eq. (2) to measured PA impedance spectra in the frequency range up to 48.8 Hz.

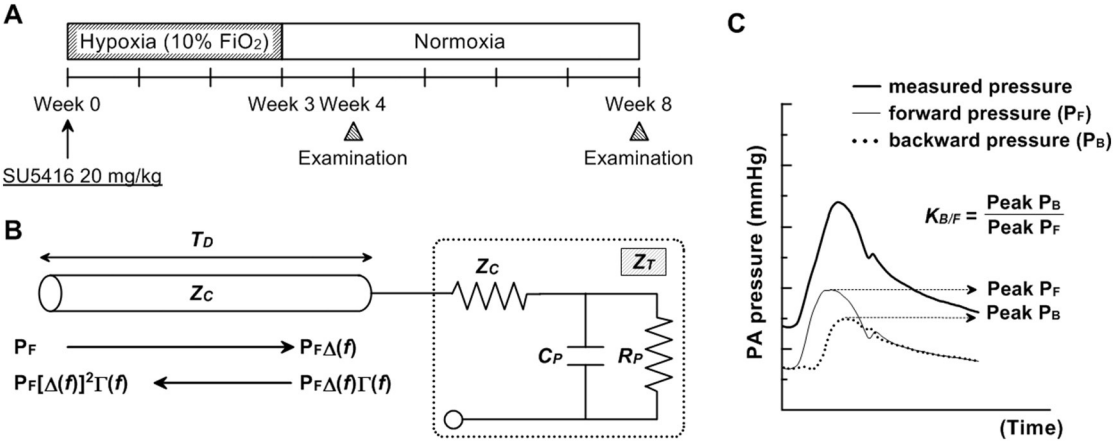

Fig. 1. A: Experimental protocol. **B**: Schematic representation of an elastic tube connected to a 3-element Windkessel model. The model can be characterized by peripheral resistance (R_P), pulmonary arterial compliance (C_P), characteristics impedance (Z_C), and transmission time from the inlet of the tube to a reflection site (T_D). Z_T: impedance beyond the reflection site; P_F: forward pressure; $\Delta(f)$: frequency-domain representation of T_D; $\Gamma(f)$: reflection coefficient spectra. **C**: Measured pulmonary arterial (PA) pressure (bold line) waveform can be separated into its forward (P_F; fine line) and backward (P_B; dot line) pressure waveforms.

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