Rho-kinase inhibitor decreased pulmonary artery resistance, whereas increased compliance in a rat model of pulmonary hypertension.

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Abstract: Although the dynamic mechanical properties such as arterial compliance (C) have been shown to predict increased mortality in patients with pulmonary arterial hypertension (PAH), a simple static index, i.e., pulmonary arterial resistance (R) has been exclusively used in clinical settings. We examined how a Rho-kinase inhibitor, fasudil, that is known to suppress vasoconstriction, affects pulmonary artery input impedance (Z) in Sugen/Hypoxia (SuHx) PAH in rats. We measured Z before PAH induction (Normal), and re-measured before/after fasudil injection (10 mg/kg) (PAH/Fasudil). PAH increased R while Fasudil decreased R (Normal: 16.3±2.6, PAH: 56.5±6.9, Fasudil: 39.9±5.2 mmHg/ml/sec, p<0.01). In contrast, PAH decreased C while Fasudil increased C (Normal: 3.6±0.6, PAH: 1.8±0.4, Fasudil: 2.5±0.8 ×10³ ml/mmHg, p<0.01). We conclude that the pulmonary arterial impedance may serve as a new tool in analyzing vascular mechanics to assess the severity or the drug efficacy in PAH patients.

Keywords: Pulmonary arterial hypertension, impedance, pulmonary circulation, Rho-kinase inhibitor

1. Introduction
Pulmonary arterial hypertension (PAH) is characterized by elevated mean pulmonary arterial pressure (PAP) above 25 mmHg resulting from pulmonary arterial vasoconstriction and remodeling, and leads to end-stage right heart failure. Although the decrease in pulmonary arterial compliance (C) has been shown to predict an increased mortality in PAH patients [1], we have been using, in clinical settings, the static vascular properties, resistance (R) alone, while ignoring the dynamic mechanical properties. It has been well established that vascular impedance (Z) is capable of characterizing the dynamic mechanical properties [2] and might uncover hidden changes leading to the pathogenesis of PAH. Rho-kinase which involves a diverse range of cellular functions, in particular smooth muscle contraction plays a key role in the development of PAH. A Rho-kinase inhibitor, Fasudil, suppress abnormal hyper constriction in animals and humans [3, 4]. We examined how Fasudil affects Z of pulmonary artery in Sugen/Hypoxia (SuHx) model rats which has pulmonary vascular macro/micromorphology indistinguishable from those of human PAH [5].

2. Method

Surgical Procedures
In male Sprague-Dawley rats (190 - 210 g), we induced PAH by a subcutaneous injection of Sugen5416 (20 mg/kg) and exposure to hypoxia (10% O2) for 3 weeks. We measured Z before PAH induction as a normal group (Normal, n=6) and re-measured Z before (PAH) and 20 minutes after Fasudil injection (10 mg/kg) (Fasudil, n=6) in anesthetized (urethane and α-chloralose), mechanically ventilated (respiratory rate: 120 bpm) conditions. We measured instantaneous pulmonary artery flow (PAF) and PAP under irregular cardiac pacing to broaden frequency range of PAF (Fig. 1). All data were recorded for 90.112 seconds at a sampling rate of 1000Hz.

Analysis
We segmented data into 10 sets of 50% overlapping bins of 16,384 data points each. We Fourier transformed both PAF and PAP to derive power spectra and crosspower spectra and obtained high resolution Z (from 0.07 to 100 Hz with the resolution of 0.07 Hz) by the following equation.

$$Z(f) = \frac{PAF(f) \cdot PAF(f)^*}{|PAF(f)|^2}$$

We fitted the 2-element Windkessel model to Z and estimated R and C. We estimated characteristic impedance (Rc) from the upstroke limb of instantaneous PAF-PAP relationship.

Fig. 1. Hemodynamic data under irregular cardiac pacing

3. Results
Compared to Normal, SuHx significantly increased PAP while Fasudil decreased PAP (Normal: 18.8±1.1, PAH: 50.5±6.4 and Fasudil 38.2±3.2 mmHg, p<0.01). PAF remained unaltered (Normal: 34.7±5.9, PAH: 33.0±2.5, Fasudil: 34.5±4.8 ml/min, n.s.) (Fig. 2). PAH shifted Z upward whereas Fasudil attenuated it (Fig. 3).
PAH increased R while Fasudil decreased R (Normal: 16.3±2.6, PAH: 56.5±6.9, Fasudil: 39.9±5.2 mmHg/ml/sec, p<0.01). In contrast, PAH decreased C while Fasudil increased C (Normal: 3.6±0.6, PAH: 1.8±0.4, Fasudil: 5.8±1.8 ×10³ ml/mmHg, p<0.01). Neither PAH nor Fasudil altered Re (Normal: 7.4±0.7, PAH: 6.2±1.8, Fasudil: 5.8±1.8 mmHg/ml/sec, p=n.s.) or the time constant (Normal: 126.6±15.9, PAH: 106.6±16.1, Fasudil: 108.4±26 msec, n.s.) (Fig. 4).

4. Discussion

In this study, Rho-kinase inhibitor decreased R as well as PAP and increased C, whereas the time constant, the product of R and C, hardly changed. In the systemic circulation, compliance is mainly located in the aorta, whereas resistance is in arterioles [6]. Therefore, it is conceivable R can change without affecting C. In contrast, in the pulmonary circulation, R and C are widely distributed across the arterial system [7]. Therefore R and C vessels may well be anatomically inseparable and the increases of R may reflect the distributed increases in arterial stiffness which in turn decreases C. This mechanism may explain why changes in R always couple with those in C in pulmonary arterial system in PAH.

5. Conclusion

SuHx increased PAP and R while decreased C. In contrast, Rho-kinase inhibitor decreased PAP and R while increased C. Re and the time constant remained unaltered. We conclude that the impedance analysis of pulmonary artery is a powerful tool in analyzing the pathological states and/or their alterations in response to interventions. Hence the impedance analysis is clinically applicable, its importance in the management of PAH cannot be overemphasized.

Reference