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MEASUREMENT OF IN-VIVO PULMONARY VASCULAR IMPEDANCE IN TWO ANIMAL MODELS OF PULMONARY HYPERTENSION

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INTRODUCTION

Pulmonary vascular input impedance has been increasingly promoted as an important diagnostic for pulmonary arterial hypertension (PAH) [1,2]. The gold-standard clinical diagnostic for the disease, pulmonary vascular resistance (PVR), quantifies only the mean resistance to flow but ignores the impact of vascular stiffness and flow pulsatility, which in PAH can represent up to 40% of the total load presented to the right ventricle. PVR has also been found to be only a moderate predictor of PAH outcomes [3]. The first of these deficiencies is not present in impedance; clinical studies have found the sum of its 1st and 2nd harmonic moduli to have good correlation (r^2=0.812) with global pulmonary vascular stiffness (PVS) [2], a hemodynamically-measured quantifier of vascular stiffness. Additionally, the 0th harmonic modulus of impedance has excellent correlation to PVR (r^2=0.974); thus, it also quantifies the resistive load. Moreover, because PVS has recently been found as a valuable determinant of mortality in PAH [4], we believe that impedance, as a combined measure of PVR and PVS, might be an excellent predictor of disease outcomes.

The human studies noted above have understandably not examined detailed associations between impedance and vascular behavior and structure, since the latter data are obtainable only through focused drug studies or ex-vivo measurements. Mechanical changes to a vascular network should be reflected in its input impedance; thus, such investigation should be useful in determining how impedance varies with changes in vascular condition, such as chronic stiffening due to vascular remodeling or acute stiffening due to smooth muscle cell response and/or pressure-induced strain-stiffening. Naturally, clinical identification of such stiffness changes on a routine basis could greatly impact diagnosis. Here, we demonstrate simple-to-implement impedance measurements in two animal models as part of a larger effort to establish said links between clinically-viable diagnostics, such as impedance, and physiological changes that occur to the vasculature as part of the progression of PAH.

METHODS

Animal Preparation: The two animal models examined here develop PAH due to chronic exposure to a hypoxic environment. The first model consisted of 10 male Sprague-Dawley rats (300-400g), half exposed to hypoxia via hypobaric chamber for 3-4 weeks (barometric pressure ≈ 410 mmHg) and half retained at standard conditions in Denver, CO (barometric pressure ≈ 630 mmHg). The second model utilized 4 male Holstein calves (70-110lb), again with half exposed to hypoxia for two weeks (barometric pressure ≈ 460 mmHg) and the other half remaining normoxic. Both models were exposed to a 12:12-h light-dark cycle, and water and appropriate food were made available ad libitum. Animal care and use committees at both the University of Colorado Health Science Center (rat) and Colorado State University (calf) approved all protocols and procedures.

Animal Data Collection and Analysis: The measurements obtained from each animal are identical; the main differences between collection methods are equipment size and type. For all measurements, rats are anesthetized with ketamine hydrochloride (40 mg/kg) and xylazine (10 mg/kg) intraperitoneally, while cows remain conscious. Right jugular access is then obtained in each animal, and a fluid filled catheter, consisting of PV1 tubing for the rat or a commercial Swan-Ganz catheter for the calf, is inserted into the main pulmonary artery (MPA) for pressure measurements. During collection of MPA pressure, blood velocity at the midline of the MPA is obtained with pulse-wave Doppler echocardiography using an FPA probe on a commercial ultrasound scanner (Vivid 5, GE Medical Systems Inc). The imaging depth dictates the probe frequency:

10MHz for the rat and 3.5MHz for the calf. Cardiac output (CO) is then obtained. In the rat, this is accomplished by taking 2D B-mode images of the aortic bulb, which yield aortic diameter values (and thus cross-sectional area), along with pulse-wave Doppler images of the midline velocity in the ascending aortic arch, which yield midline blood velocity there. Assuming spatially constant velocity across the aorta, flow – and thus, CO – is obtained as the time-integral of the velocity multiplied by the cross-sectional area [5]. For the calf, standard thermodilution methods are employed to obtain CO.

Offline, the midline velocity time history is obtained from the ultrasound image data with a simple noise-decimation method, and transient flow in the pulmonary vasculature is computed by equating CO measurements to those predicted by the velocity history multiplied by an area correlation factor [1]. A discrete Fourier transform is then performed on pressure and flow time-histories for each cardiac cycle, and impedance modulus is obtained from the ratios of corresponding harmonics of pressure and flow, i.e. $|Z(\omega)| = |P(\omega)|/|Q(\omega)|$, where $|Z|$,$|P|$, and $|Q|$ are the moduli of impedance, pressure, and flow at harmonic ω. Data are gathered from multiple cardiac cycles (n>10), enabling a comprehensive error analysis [1].

RESULTS

Figure 1A displays representative impedance curves from a normoxic rat and a hypoxic rat. We see that the absolute value of the 0^{th} harmonic modulus is far larger in rats than that seen in humans due to their much lower values of cardiac output; however, the trends seen in both figures are qualitatively very similar to impedance curves obtained in previous human and animal studies [1,2,6]. The zero harmonic modulus of impedance (Z_0) increases in subjects with hypertension from 206±82.2WU to 838±325WU (n=9, p<0.0001). Also of note is the increase in the first two higher harmonic moduli (i.e. $Z_1+Z_2 = Z_{sum}$) in the hypertensive animals (from 23.7±14.3WU to 110±74.4WU; n=9, p<0.0001), indicative of an increase vascular stiffness. We note that no significant difference in heart rates existed between the two groups.

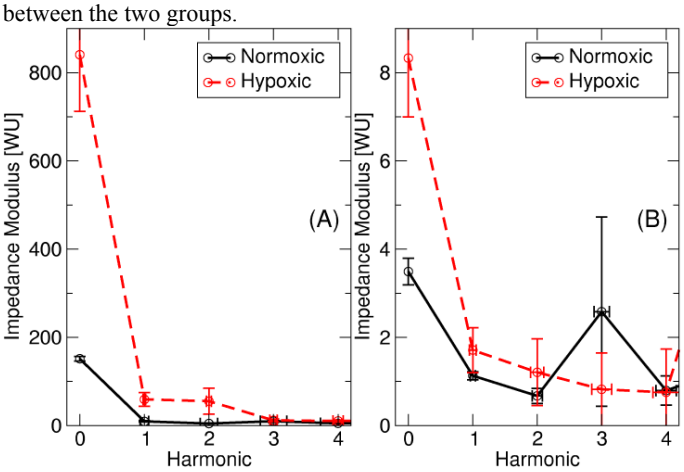

Figure 1: In-vivo impedance moduli from (A) Sprague-Dawley rat model and (B) Neonatal Holstein calf model.

In Figure 1b appear impedance results from a normoxic calf and an hypoxic calf; here, the effect of hypertension is somewhat less pronounced on the 0^{th} harmonic, although there is still a significant difference between the two small groups. The hypertensives have Z_0 of 7.27±1.31WU and a Z_{sum} of 2.83±0.65, while the normotensives have a Z_0 of 3.62±1.49WU and a Z_{sum} of 1.95±0.52. The heart rates of the calves were also significantly different (118bpm, hyper; 140bpm, normo).

DISCUSSION

This preliminary work demonstrates our ability to obtain impedance curves in vivo from two animal models of PAH using a well-established clinical method [1,2]. The ability to determine impedance in vivo, with minimally invasive catheterization, provides easier measurement capability than invasive vascular cuffs to gather flow data [6] or isolated lung (ex-vivo) configurations [7]. This method allows for serial tracking of impedance in the animal, avoids opening the thoracic cavity, which requires ventilation and could change measured values, and naturally uses the heart as the flow input device, which provides a broadband input signal unlike the single frequency flow input provided by some mechanical pumps.

Each animal model has specific advantages: rats are easier to handle and are inexpensive; calf arteries undergo remodeling that more closely resembles those of humans with PAH. These models allow several avenues of research to be explored. Studies of impedance alone include: 1) in the calf model, collection of right ventricle pressure curves for determination of the isovolumetric relaxation time (τ), a clinical measure of total afterload, to compare to Z; 2) in the rat model, collection of reactivity to individually-administered drugs to determine efficacy of each in terms of total, resistive, and compliant afterload reduction; 3) use of the beforementioned reactivity studies to categorize each drug's potential to mitigate chronic stiffening due to vascular remodeling; 4) in the rat model, collection of reactivity due to drugs administered via inhalation or injection to determine efficacy of delivery method. Lastly, fresh arterial tissue from each model can be tested *in vitro* to examine changes in biomechanical properties due to hypertension. The difference in the extent to which each animal remodels is itself useful in allowing us to study the impacts of different levels of vascular remodeling on stiffness. This work forms part of a multi-modal evaluation into pathophysiology, biomechanics and clinical evaluation of pulmonary arterial hypertension.

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