

HYPOXIA-INDUCED CHANGES IN THE MECHANICAL PROPERTIES OF THE MOUSE PULMONARY ARTERY

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ABSTRACT

Hypobaric hypoxia produces pulmonary hypertension in mice which causes pulmonary vascular remodeling. To study the biomechanics of this process, mice were exposed to hypoxia for 0- (control), 10-, and 15-days. Using a pressurized arteriograph system, mechanical properties of the main pulmonary artery were measured and compared to the biological changes in the vessel wall measured histologically. 10- and 15-day hypoxic vessels were significantly stiffer when compared to 0-day vessels. This stiffness correlated with greater elastin and collagen content in the vessel wall.

INTRODUCTION

Primary pulmonary hypertension (PPH) is a deadly disease of the pulmonary vasculature that results in substantially elevated pulmonary pressures and eventual right ventricular failure (Division of Lung Diseases and Office of Prevention and Control 1996). These elevated pressures induce vascular remodeling, which can reduce the lumen size such that pulmonary resistance increases and pulmonary pressures rise even higher. A better understanding of pressure-induced pulmonary vascular remodeling may lead to improved treatments for patients with PPH.

Pulmonary hypertension can be created in mice through exposure to hypobaric hypoxia, which increases pulmonary pressures by 50% (Ozaki et al. 2001). In response, the pulmonary arteries (PAs) must adapt both mechanically and biologically. Large elastic arteries such as the PAs experience large deformations with every heartbeat, which help store hemodynamic energy. If the mechanical properties of the PAs change, their ability to deform and store energy will change. Extracellular matrix proteins in the vessel wall, e.g. elastin and collagen, are responsible for most of this energy storage. These proteins can be degraded or synthesized in the wall as part of the biological component of vascular remodeling. The goal of this study was to measure and correlate mechanical and biological features of pulmonary vascular remodeling. We hypothesize that remodeling of the vessel wall involves changes in its mechanical properties caused

by an increase in elastin and collagen content and wall thickness (WT).

METHODS

26 six-week old wild-type C57BL/6 male mice were exposed to hypobaric hypoxia (380 mmHg, $FiO_2 = 10\%$) for 0-days (control, n=8), 10-days (n=9), and 15-days (n=9). Animals were euthanized by pentobarbital injection as approved by the Institutional Animal Care and Use Committee. The left and right main PAs were harvested and subjected to the following protocols.

The left PA was mounted in a pressurized arteriograph system, in which changes in lumen diameter and wall thickness were detected optically (Coulson et al. 2002). A baseline pressure of 5 mmHg was used to prevent lumen collapse and the vessel was stretched by 12% to approximate *in vivo* length. In the first test, five sinusoidal pressure-loading cycles from 5 to 25 mmHg at 0.014 Hz were used to precondition the left PA and measure stiffness and damping. In the second test, the left PA was subjected to five 45 second pressure steps to 10, 15, 20, 25, and 10 mmHg separated by 450 seconds of rest at 5 mmHg. This test was used to determine the isochronal response, stretch 15 seconds into the step, and creep of the vessel. The right PA was perfused with tissue freezing medium and flash frozen for histological analysis. Vessels were sectioned to 5 μ m and stained with hematoxylin to measure average WT histologically, Verhoff van Giesen (VVG) stain to visualize elastin, and Sirius red (SR) stain to visualize collagen. Images were captured and analyzed using MetaVue software.

Calculations

Stress (σ) was calculated using the simple hoop stress formula assuming thin walls; strain (e) was calculated using the Almansi formulation for large deformations as follows:

$$\sigma = \frac{Pr_i}{WT} \quad e = \frac{1}{2} \left(1 - \frac{1}{\lambda^2} \right) \quad (1,2)$$

where P is the transmural pressure, r_i is the internal radius, and λ is the circumferential stretch ratio equal to new outside diameter (OD) divided by original OD. Due to optical variations at low pressures, all WT values were calculated from the optically measured WT at 25 mmHg assuming incompressibility and no axial extension (Faury et al. 1999). Linear viscoelastic parameters elastic modulus (E) and damping ($\tan \delta$) were calculated from the stress-strain slope in the fifth loading cycle at the mid-strain point (Lakes 1999). Creep (strain vs. time) was plotted for each step and fit to the power function

$$e = At^b \quad (3)$$

where t is seconds, A is a deformation coefficient, and b is a slope coefficient.

RESULTS

Mechanical Properties

E was significantly greater in 10-day (275 ± 53 kPa) and 15-day (296 ± 50 kPa) arteries when compared to 0-day (214 ± 28 kPa), $p < 0.01$. Damping coefficient $\tan \delta$ also was significantly greater in both

hypoxic groups when compared to 0-day arteries, $p < 0.001$ (Fig. 1A, bars represent mean \pm SD for all graphs). In response to step changes in pressure, vessels from both hypoxic groups stretched significantly less at pressures of 15, 20, and 25 mmHg when compared to 0-day (Fig. 2). The creep deformation coefficient A was significantly greater in 10- and 15-day vessels than 0-day vessels for pressures 15, 20, and 25 mmHg ($p < 0.005$). Slope coefficient b showed creep was occurring, but was not significantly different between any groups. Differences between 10- and 15-day vessels were noted in most tests, but none were statistically significant.

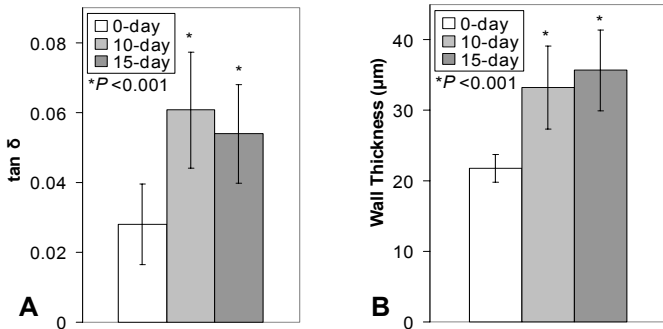


Figure 1. (A) $\tan \delta$ and (B) histologically measured WT for 0-, 10- and 15-day vessels.

Histological Measurements

Measured WT values (Fig. 1B) for 0-day vessels were significantly less than those of 10- and 15-day vessels ($p < 0.001$). Qualitative analysis of VVG staining indicated a substantial thickening of the elastic lamina (Fig. 3) and SR staining showed an increase in collagen deposition (not shown) in hypoxic vessels.

DISCUSSION

Our results indicate that hypoxic vessels are stiffer (lower stretch response, higher E, and greater creep coefficient A) and have a higher damping coefficient than normal vessels, which is caused by an increase in elastic lamina thickness, collagen, and WT. The greater stiffness in the 10- and 15-day hypoxic arteries indicates an adaptation to higher blood pressure which reduces wall deformation during systole. The higher $\tan \delta$ values in hypoxic vessels show that energy from the heart is being lost in the vessel walls during systole due to inelastic tissue mechanisms. This loss of energy in the PAs may affect the maintenance of pulmonary blood pressure during ventricular diastole and affect the pressure waveform transmitted to the pulmonary arterioles. Under higher pressures, a vessel with increased elastin and collagen content may have improved circulatory function over a non-adapted vessel.

Limitations of this study should also be noted. These include difficulties in optically measuring wall thickness at low pressures due to wall curvature and short load periods for creep measurements which may explain the lack of changes in the creep slope variable b. Future work will quantify tissue viscoelastic behavior with non-linear parameters.

In conclusion, investigating PA remodeling under pulmonary hypertension is an effective way to uncover the changing mechanical properties induced by this stress. Elastin and collagen deposition

appear to produce thicker and stiffer vessels better adapted to higher blood pressures. Understanding these mechanical and biological correlations under pulmonary vascular remodeling may give some insight into PPH progression and treatment.

ACKNOWLEDGEMENTS

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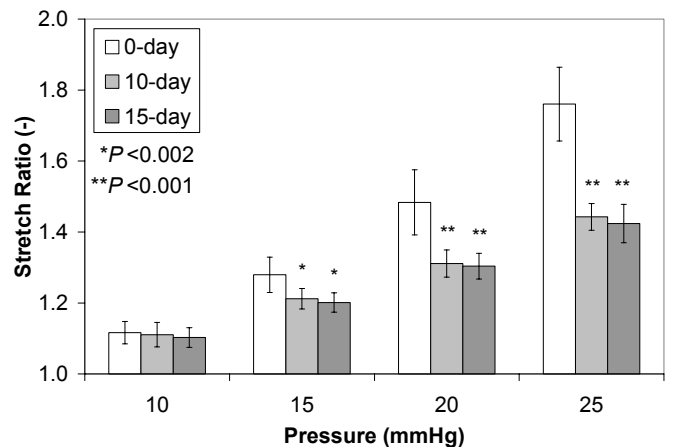


Figure 2. Isochronal stretch ratio for 0-, 10- and 15-day vessels. Significance is compared to 0-day vessels.

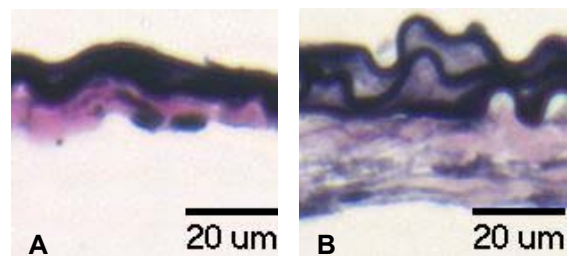


Figure 3. VVG stain showing elastin (black) in (A) a 0-day and (B) a 10-day vessel. Cell nuclei also appear dark.