Acta Biomaterialia 31 (2016) 276–287

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Validation of an arterial constitutive model accounting for collagen content and crosslinking

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ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 20 November 2015
Accepted 29 November 2015
Available online 30 November 2015

Keywords:
Hydroxyproline
Pyridinoline
Modeling
β-Aminopropionitrile (BAPN)
Large pulmonary artery

ABSTRACT

During the progression of pulmonary hypertension (PH), proximal pulmonary arteries (PAs) increase in both thickness and stiffness. Collagen, a component of the extracellular matrix, is mainly responsible for these changes via increased collagen fiber amount (or content) and crosslinking. We sought to differentiate the effects of collagen content and cross-linking on mouse PA mechanical changes using a constitutive model with parameters derived from experiments in which collagen content and cross-linking were decoupled during hypoxic pulmonary hypertension (HPH). We employed an eight-chain orthotropic element model to characterize collagen's mechanical behavior and an isotropic neo-Hookean form to represent elastin. Our results showed a strong correlation between the material parameter related to collagen content and measured collagen content ($R^2 = 0.82$, $P < 0.0001$) and a moderate correlation between the material parameter related to collagen crosslinking and measured crosslinking ($R^2 = 0.24$, $P = 0.06$). There was no significant change in either the material parameter related to elastin or the measured elastin content from histology. The model-predicted pressure at which collagen begins to engage was ~25 mmHg, which is consistent with experimental observations. We conclude that this model may allow us to predict changes in the arterial extracellular matrix from measured mechanical behavior in PH patients, which may provide insight into prognoses and the effects of therapy.

Statement of significance

The literature has proposed several constitutive models to describe the mechanical effects of arterial collagen but none separates collagen content from crosslinking. Given that both are critical to arterial mechanics, the novel model described here does so. Furthermore, our novel model is well tested by experimental data; model parameters were reasonably correlated with measured collagen content and crosslinking and the model-predicted collagen transition stretch was consistent with that obtained experimentally. Given that arterial collagen structural changes and collagen engagement are critical to arterial stiffening in several disease states, this model, by linking mechanical and biological properties, may allow us to predict important biological changes during disease progression from measured mechanical behavior.

1. Introduction

During the progression of pulmonary hypertension (PH), proximal pulmonary arteries (PAs) become stiffer due to extrinsic and intrinsic remodeling including arterial wall thickening (extrinsic remodeling) and increased elastic modulus (intrinsic remodeling) [1–7]. Arterial stiffening can increase right ventricular (RV) afterload, which causes RV hypertrophy and eventually RV failure [8–14]. Clinical studies have found that proximal PA stiffness and its inverse, compliance, are strongly related to mortality in patients with PH [12,15–20]. Therefore, it is important to understand the extrinsic and intrinsic changes in proximal PAs that are responsible for stiffening in PH.

Many mechanical testing methods including uniaxial, planar biaxial and pressure-inflation tests have been used to characterize

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http://dx.doi.org/10.1016/j.actbio.2015.11.058
1742-7061/

the extrinsic and intrinsic, or more generally, mechanical properties of proximal PAs and their components [1,3,4,6,21–23]. The role of smooth muscle cell activity in proximal PA remodeling, without experimental or pharmacological activation, appears minimal [23,24]. Instead, changes in the extracellular matrix (e.g., elastin and collagen) can be dramatic. Elastin, which bears most of the mechanical load in the low stretch region [4,25–27], was found to increase elastic modulus significantly in a neonatal calf model of hypoxic pulmonary hypertension (HPH) and to contribute importantly to increased arterial stiffness [4]. In the adult mouse model of HPH, however, elastin was not found to change significantly; instead, collagen accumulation was found to correlate to the PA elastic modulus in the physiological strain range [3,5]. The effect of collagen on the mechanical properties of proximal PAs has thus been a focus of recent studies [3,5,23,28,29]. In several studies, collagen content has been found to correlate well with the arterial elastic modulus in the physiological strain range [3,5,28,29]. In addition, a recent study by our group showed that collagen crosslinking is better correlated to elastic modulus at high stretch levels than collagen content [23].

Increased collagen crosslinking increases the elastic modulus of collagen fibers [30–34]. At the microscopic level, collagen crosslinking can restrict the slippage between collagen fibrils and between tropocollagen molecules, which results in early stretching of tropocollagen molecules [35]. Stretching itself stiffens tropocollagen molecules, which in turn leads to a stiffer collagen fiber. Therefore, crosslinks also play an important role in the mechanical behavior of collagen fibers and thus artery. The separate effects of collagen content and crosslinking on mechanical behavior were experimentally studied recently by our group [23,29]. In these studies, we utilized a novel experimental design to decouple changes in collagen content from crosslinking during the progression of HPH in mice. In particular, transgenic mice with collagen type I resistant to collagenase degradation were used, and when exposed to chronic hypoxia, the PAs of both homozygous mutant and wildtype mice increased collagen content and crosslinking. However, when treated with the antifibrotic agent β-aminopropionitrile (BAPN) during the hypoxic exposure, crosslink formation was prevented and thus content and cross-linking were decoupled. Using this experimental approach, we found that the arterial elastic modulus correlated well with collagen content below 25 mmHg and crosslinking above 25 mmHg.

These results motivated us to use a constitutive model to differentiate the mechanical effects of changes in collagen content and crosslinking. Such a constitutive model can be used to study the effects of biology on mechanics broadly and may be used to predict the biological changes responsible for mechanical changes evident during the progression of disease. Many constitutive models, either phenomenological or structural, have been proposed to describe the mechanical properties of large elastic arteries. The phenomenological approach with polynomial, exponential or logarithmic function [36–39] captures hyperelastic behavior, but does not provide information about arterial material and structure. Structural strain-energy functions (SEFs) with terms representing elastin and collagen have been used as well [21,26,40,41], but none of these models has material parameters related to collagen crosslinking. One model that does effectively capture the effects of fiber crosslinking is an eight-chain isotropic element model that uses statistical mechanics to model the hyperelastic behavior of macromolecules [42]. This model was later revised to an orthotropic model by Bischoff and co-workers [43] and has then been applied to the large PAs of a rat model of HPH to predict crosslinking from the PA mechanical behavior [7]. However, the latter study did not distinguish between elastin and collagen content or crosslinking.

In this study, we adapted the microstructurally based eight-chain orthotropic element model used by Bischoff and co-workers [43] by adding a neo-Hookean form to represent elastin fibers and then investigated the revised model's ability to distinguish the effects of collagen content from collagen crosslinking on the elastic modulus of PAs during HPH. We first found model parameters for collagen content and crosslinking by fitting to experimental stress–stretch data. We then studied the correlations between the model parameters and the measured collagen content and crosslinking in the experimental groups with decoupled collagen content and crosslinking [23,29]. Finally, we compared the model-predicted collagen engagement or transition stretch to that obtained with two previously established methods and then used the model-predicted transition stretch to interpret the experimental observations of pressure-dependent contributions of collagen content and crosslinking to arterial mechanics [23,29].

2. Materials and methods

2.1. Materials

Data used to populate the model were derived from animals experiments that have been previously reported [23,29]. Briefly, homozygous mutant ($Col1a1^{R/R}$) and wildtype ($Col1a1^{+/+}$) mice were exposed to 10 days of chronic hypoxia to induce PH. In the mutant animals, the alpha-1 subunit of type I collagen is resistant to degradation whereas in the wildtype animals, collagen type I is degraded normally. Half of the animals exposed to chronic hypoxia were also treated with BAPN, which prevents new crosslink formation. Additional mutant and wildtype mice without any hypoxia exposure or treatment were used as controls.

2.2. Isolated vessel pressure-inflation test

Details of the pressure-inflation test are available elsewhere [23,29]. Briefly, extralobar left pulmonary arteries (LPAs) were harvested from mice post-euthanasia and mounted on glass microcannulas in an isolated vessel mechanical testing chamber with an optical viewing window directly below the artery for transillumination microscopy measurement of diameter. Calcium- and magnesium-free PBS medium was used for perfusion and superfusion to ensure the passive state of smooth muscle cells. The distance between the microcannulas was increased to stretch the vessels longitudinally to a near in vivo stretch. All tests were performed at a fixed longitudinal stretch ratio of 140%, a frequently used estimate of in vivo PA stretch and only pressure-circumferential deformation data (not axial force–length data) were obtained. A steady flow pump (LSI; Burlington, VT) with closed loop feedback control was used to pressure-inflate the arteries at transmural pressures of 5, 10, 15, 20, 25, 30, 35, 40 mmHg. LPA outer diameter and transmural pressure were simultaneously recorded.

2.3. Calculations: stretch and stress

To obtain circumferential stress–stretch curves, the simultaneously obtained pressure-outer diameter (OD) data were used. Note that due to residual strain, the artery under no-load state is under tension at the outer wall [27,44,45]. A combination of a longitudinal stretch (140% in this study) and a small intramural pressure can result in an approximate stretch-free state at the outer wall of the artery [27]. Therefore, the OD at 5 mmHg (OD_5) was taken as the reference state as in previous studies [5,23,29] such that the circumferential stretch was approximated as $\lambda = OD/OD_5$. The arterial wall volume was calculated using the optically measured inner diameter (ID) and OD at 40 mmHg and the longitudinal

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