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Research paper

Stem cell therapy restores viscoelastic properties of myocardium in rat model of hypertension

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ABSTRACT

Extensive remodeling of the myocardium is seen in a variety of cardiovascular diseases, including systemic hypertension. Stem cell therapy has been proposed to improve the clinical outcomes of hypertension, and we hypothesized that changes in mechanical properties of the myocardium would accompany the progression of disease and the results of treatment conditions. Using spontaneously hypertensive rats (SHR) as a model of hypertension, we treated 13-week-old hypertensive rats with a single injection of adipose-derived stem cells (ADSC) isolated from a normotensive control. We indented the isolated ventricles of control, untreated sham-injected SHR, and ADSC-treated SHR hearts with a custom cantilever-based system and fit the resulting data to a standard linear solid model. SHR animals had higher blood pressure (198.4±25.9 mmHg) and lower ejection fraction (69.9±4.2%) than age-matched control animals (109.0±1.6 mmHg, 88.2±1.3%), and increased viscoelastic properties accompanied these clinical changes (right ventricle effective stiffness, SHR: 21.97±5.10 kPa, Control: 13.14±3.48 kPa). ADSC-treated animals saw improvement in clinical parameters compared to the untreated SHR group, which was also accompanied by a significant restoration of viscoelastic properties of the myocardium (ACSD-treated SHR: 9.77±6.96 kPa).

1. Introduction

High blood pressure or hypertension (HTN) affects approximately 80 million American adults over age 20, and in 30–40% of those individuals, HTN remains untreated or uncontrolled and their risk of stroke and heart attack remains high (Mozaffarian et al., 2015). In patients with hypertensive heart disease and in animal models of HTN, hypertrophic thickening of the myocardium and fibrosis are seen along with high blood pressure, though the relationship between the progression of HTN and stiffening of myocardium is poorly understood. Since stem cell therapies have been found to reduce post-myocardial

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infarction fibrosis generally (Madonna et al., 2009; Ammar et al., 2015) and stiffness specifically, (Berry et al., 2006) we hypothesized that stem cells may improve function in hypertensive heart disease through a remodeling mechanism as well.

Spontaneously hypertensive rats (SHR) are a murine model of HTN that develop high blood pressure and express hallmark signs of cardiovascular disease. These pathological findings correlate well with mechanical properties of the myocardial tissue itself; hearts from spontaneously hypertensive rats (SHR) with developed HTN have twice the elastic modulus of pre-hypertensive SHR hearts (Conrad et al., 1995). Using methods similar to those proposed for human stem cell therapies, we isolated adipose-derived stem cells (ADSC) from normotensive rats and administered to SHR by injection to the jugular vein to investigate effects on myocardial properties and overall cardiovascular improvement. Since prior work with simple small molecules shows a short-lived treatment effect, we chose a cell-based therapy to encourage a long-term effect.

Common techniques to characterize material properties of the myocardium include macroscale compression and tension of centimeter-scale tissue samples, nano-scale indentation by atomic force microscopy or nano-indenter instruments, and "incremental elastic modulus" calculations based on intracavitary pressure–volume data. However, the wide variety in indentation methods and material models obfuscate comparisons among the data (Fig. 1). Reported data for the elastic modulus of myocardium using these various methods and models spans three orders of magnitude, overlapping with commonly accepted values for brain tissue (~1 kPa) through collagenous bone (>100 kPa). Furthermore, the typical emphasis on left ventricle properties may ignore important changes occurring in the right ventricle and pulmonary system.

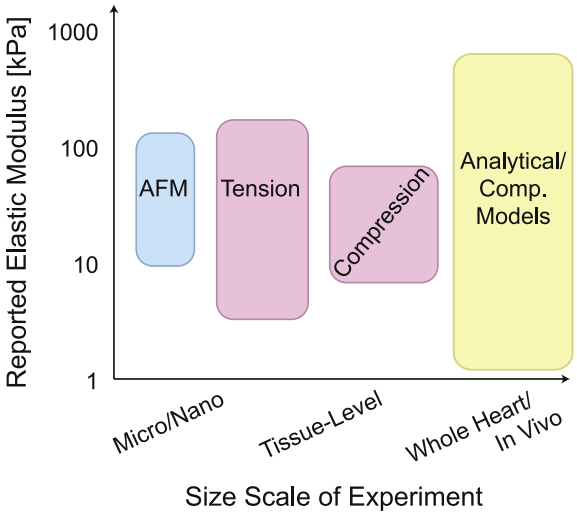

Fig. 1 – Characterization methods for mechanical properties of myocardium vary widely, as do resulting values for elastic modulus. Results from atomic force microscopy (AFM) indentation, (Berry et al., 2006; Ogneva et al., 2010; McCain and Parker, 2011) tensile testing, (Conrad et al., 1995; Ott et al., 2008; Levental et al., 2007; Heller et al., 2000) compression testing, (Bhana et al., 2010; Kissling et al., 1977) and analytical and computational models based on imaging data (Niederhoffer et al., 2000; Fuzhang Zhao et al., 2003; Higuchi et al., 1997; Janz et al., 1976; Lacolley et al., 2001', 2002; Youcef et al., 2014) span nearly four orders of magnitude for purportedly similar myocardial tissue.

To characterize the material properties of our myocardial samples, we developed a custom indentation system capable of characterizing transverse slices of intact left and right ventricle in multiple locations (Krick et al., 2011). We then fit the indentation data to the common standard linear solid viscoelastic material model, which allows us to calculate an instantaneous elastic modulus for comparison to existing data in the literature, as well as a steady-state elastic modulus and viscosity. The steady-state elastic modulus is independent of the indentation strain-rate, suggesting that comparisons among these moduli data from different researchers would be more reliable. Furthermore, using this viscoelastic model, we can quantify changes in viscosity that may inform studies of impaired relaxation and diastolic dysfunction also observed in hypertensive patients (Palmiero et al., 2015; Szelényi et al., 2015).

Here, we have used our custom indentation system to explore the effects of stem cell therapy in restoring normal viscoelastic properties to hypertensive rat myocardium. By examining the left and right ventricles separately, we have also identified unexpected remodeling in the right heart. The right ventricle appears to be very responsive to ADSC treatment. Our indentation system combined with a standard linear solid viscoelastic model demonstrates a useful way to investigate changes in tissue viscoelastic properties for disease and treatment models.

2. Material and methods

2.1. *Force–displacement quantification with custom indenter*

We modified a tool designed to measure friction forces (Krick et al., 2011) to create a custom Multi-Scale Indenter (MSI, Fig. 2). The cantilever-based probe is displaced vertically using a software-controlled piezoelectric stage. The probe is brought into contact with the tissue sample following a time-dependent displacement profile set by the user in custom LabVIEW code. The reacting normal force of the tissue bends the titanium cantilever, and the relative displacement of the cantilever tip is measured by capacitive probe. The stiffness of the cantilever, calibrated by contact with glass, is utilized by the LabVIEW code to determine the normal load throughout the indentation cycle.

2.2. *Stem cell therapy for SHR model*

To investigate the therapeutic potential of adipose-derived stem cells (ADSC) in a rat model of hypertension, ADSC from normal rats were injected into SHR. ADSCs are reasonable to obtain and isolate, rich in mesenchymal cells, and been used in scar models previously (Reviewed in Madonna et al. (2009)). First, ADSC were isolated from inguinal adipose tissue of a normotensive Wistar-Kyoto (WKY) rat and characterized by flow cytometry using common markers of stemness (CD44+, CD90+, CD34− and CD45−). Their adipocyte differentiation