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Detrimental role of lysyl oxidase in cardiac remodeling

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ABSTRACT

A key feature of heart failure is adverse extracellular matrix (ECM) remodeling, which is associated with increases in the collagen cross-linking enzyme, lysyl oxidase (LOX). In this study, we assess the progression of cardiovascular remodeling from the compensatory to decompensatory phase, with a focus on the change in LOX expression and activity as it relates to alterations in ECM composition and changes in cardiac function. Adult male Sprague-Dawley rats were studied after 4, 14, or 21 weeks of aortocaval fistula-induced volume overload (VO). Progressive increases in the left and right ventricular mass indicated biventricular hypertrophy. Echocardiography revealed significant increases in the posterior wall thickness and internal diameter of the left ventricle as early as 3 weeks, which persisted until the 21 week endpoint. There were also significant decreases in eccentric index and fractional shortening in VO animals. Hemodynamic measurements showed progressive decreases in contractility, indicative of systolic dysfunction. There were progressive VO-induced increases in LOX expression and activity, collagen, and collagen cross-linking during the course of these experiments. We observed a negative correlation between LOX activity and cardiac function. Additional rats were treated with an inhibitor of LOX activity starting at 2 weeks post-surgery and continued to 14 weeks. LOX inhibition prevented the cardiac dysfunction and collagen accumulation caused by VO. Overall these data suggest a detrimental role for the chronic increase of cardiac LOX expression and activity in the transition from compensated remodeling to decompensated failure.

1. Introduction

Significant progress has been made in understanding cardiovascular disease and in improving mortality rates; however, the incidence of heart failure continues to increase [1]. The compensatory response to chronic volume overload, pressure overload, and hypertension is primarily driven by hypertrophic and fibrotic responses, which are associated with reorganization of the cardiac ECM [2–4]. The major structural component of the cardiac ECM is collagen, which is produced by fibroblasts [5–8]. Lysyl oxidase (LOX), also produced by fibroblasts, is a collagen cross-linking enzyme and is significantly elevated in failing human hearts [9,10]. LOX plays a critical role in collagen maturation and deposition by oxidizing peptidyl lysines to peptidyl semi-aldehydes, which then condense with neighboring amino or peptidyl groups creating intra- and inter-molecular covalent cross-links [11–13]. The degree of collagen crosslinking among collagen fibrils determines the strength of these fibers and their ability to resist degradation [14–16]. Previous studies from our laboratory have demonstrated the beneficial effects of reducing LOX activity in rodents with established disease [3]. However, little is known about the temporal changes in cardiac LOX during progressive remodeling and if this enzyme plays a role in the development of heart failure. Here, we used the rat aortocaval fistula (ACF) model of chronic volume overload (VO) as a model of progressive cardiac remodeling to evaluate changes in LOX expression and activity at key stages in ventricular remodeling. Further, we used an inhibitor of LOX activity to assess the role of LOX in VO-induced cardiac dysfunction and fibrosis.

Disease progression in the VO model has been characterized into three stages: acute stress, which occurs from 12 h to 7 days; compensatory remodeling, which occurs from 3 to 10 weeks; and decompensated failure, which occurs 14 weeks and beyond [5,17]. The acute phase, which has been studied by various groups, is characterized by an overall increase in compliance, mild left ventricular (LV) dilation, and rapid ECM turnover [18–20]. However, the mechanisms underlying ECM remodeling during the compensated and decompensated phases have not been fully elucidated. The compensated phase is characterized by progressive increases in LV end diastolic pressure (EDP) and end diastolic diameter (EDD) [21]. The decompensated phase is characterized by both diastolic and systolic dysfunction, and ultimately, congestive heart failure [8,22]. All three stages of the VO model are marked with progressive increases in ventricular volume, leading to a corresponding

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increase in wall stress [5]. Few studies have examined the role of LOX in cardiovascular disease, and little is known about its role in the transition from compensated function to heart failure or if increased LOX activity promotes the progression to failure [23,24]. Thus, the purpose of this study was to: 1) assess cardiac remodeling and associated changes in LOX from early compensation to decompensated using a volume overload model of heart failure; and 2) evaluate the potential cardioprotective effects of inhibiting LOX during progressive ventricular remodeling.

2. Methods

Studies were performed using eight week old male Sprague Dawley (Hsd:SD) rats that were housed under standard conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed to the principles of the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee. Isoflurane was used for anesthesia (4% induction / 3% maintenance, balance oxygen). Post-operative analgesia was provided by buprenorphine HCl (1 mg/kg, subcutaneous) administered to the rats immediately post-surgery. At the experimental endpoints, animals were anesthetized with isoflurane and killed by removal of the heart.

2.1. Rat model of chronic volume overload

Infrarenal aortocaval fistula was created in rats as previously described [4]. Briefly, an 18-gauge needle was used to create a shunt between the abdominal aorta and vena cava. The needle was inserted into the aorta and advanced into the vena cava; the needle was then withdrawn and the aortic puncture sealed with surgical glue. Mixing of deoxygenated and oxygenated blood within the vena cava was apparent, and indicated successful shunting of aortic blood and volume overload condition.

2.2. Experimental timeline

The experimental timeline is depicted in Fig. 1. Experimental endpoints of 4, 14 and 21 weeks post-surgery were selected to assess the progressive temporal alterations induced by volume overload. Previous studies indicate that these time points were associated with early compensated, late compensated, and failing stages of remodeling [3,5,14,21]. Rats were subjected to VO or sham operation. Sham-operated controls received the same surgical procedure as the VO group, except that the shunt was not made. Prior to surgery, a baseline echocardiogram was collected on all animals. Following surgery, echocardiograms were collected periodically under anesthesia to assess the temporal alterations in cardiac structure and function. At the 4 week endpoint, a subset of rats from each group was randomly selected and their tissue collected for molecular analysis of cardiac protein expression, activity, and collagen staining. Samples from another cohort of animals were collected at 14 weeks, and the remaining animals were maintained to 21 weeks post-surgery, which allowed progression to symptomatic

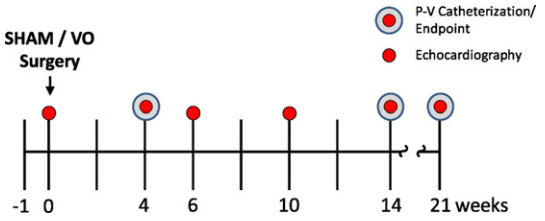

Fig. 1. Experimental timeline. Echocardiography and pressure-volume (P-V) catheterization were used to assess cardiac function at the given time points. Tissues were collected at 4, 14, and 21 weeks post-surgery.

congestive heart failure. Failing animals were defined by both outward signs of heart failure (i.e., labored respiration, bulging abdomen and lethargy and increased lung mass due to pulmonary edema (>2500 g)) [21,25,26]. In another group of VO and Sham animals, the LOX inhibitor β-aminopropionitrile (BAPN) was administered at 100 mg/kg/day via osmotic minipump (i.p.; Alzet, Cupertino, CA) to assess the impact of LOX inhibition on the progression of LV remodeling and function. The experimental timeline is shown in Fig. 8a. Four groups were studied, SHAM and VO, either with BAPN or vehicle saline. The inhibitor was administered starting at 2 weeks post-surgery and continued for the duration of the 14 week protocol. At the 14 week endpoint, LV tissue was collected for molecular analysis.

2.3. Left ventricular structural and functional assessment

Structural and functional parameters were assessed in sedated (Isoflurane 1.0%) rats by echocardiography (Visualsonics VEVO 770). M-mode and B-mode images were collected at baseline, as well as at 4, 6, 10, 14, and 21 weeks. For the experiments with the LOX inhibitor, images were collected at baseline, as well as at 2, 5, 8, 10, 12, and 14 weeks. M-mode echo was processed using the VEVO software package. Pressure-volume catheterization was performed as previously described [3]. Briefly, at the endpoints, animals were anesthetized and LV pressure and volume loops were recorded using a Millar conductance catheter introduced via the carotid artery. For the VO rats, a custom variable segment length (VSL) catheter was used. The VSL catheter was necessary for accurate assessment of LV volume and compliance in these enlarged, dilated hearts. Conductance readings were adjusted by the method of parallel conductance (15% saline bolus) and calibrated using blood-filled cuvettes of known diameter. Pressure-volume catheter data were processed using PVAN analysis package (Millar). The echocardiogram provides LV chamber and wall dimensions, heart rate, and fractional shortening under light anesthesia, while the pressure-volume catheter provides heart rate, end diastolic (ED) and end systolic (ES) pressure and volume, stroke volume, ejection fraction, dP/dt min and max, cardiac output, stroke work, preload recruitable stroke work, elastance (t), ED and ES pressure-volume relation (PVR), compliance and contractility. Cardiac output by transonic flow probe was also collected and provided confirmation of catheter chamber volume estimates.

2.4. Western blot analysis

Total proteins were extracted from LV free wall tissue using RIPA lysis and extraction buffer (Thermo Scientific). Protein concentrations were determined (Bio-Rad) and equal samples were loaded, separated by SDS-PAGE, and transferred onto nitrocellulose membranes (Amersham). Membranes were blocked with 5% nonfat milk in TBS and 0.01% Tween for 1 h at room temperature and then incubated with a primary antibody for lysyl oxidase (Novus; NB110), collagen I (Abcam; Ab34710) and collagen III (Abcam; Ab7778). GAPDH (Abcam; Ab9485) was utilized to normalize loading of the blots. Secondary HRP antibodies (Santa Cruz Biotechnology) and enhanced chemiluminescence (Pierce) were used for detection. Data were collected and analyzed using a Carestream Gel Logic 2200 Pro imaging system.

2.5. Cardiac lysyl oxidase activity

LOX activity in LV homogenates was assessed using a commercial assay, according to the manufacturer's protocol (AAT Bioquest, Inc., Sunnyvale, CA).

2.6. Analysis of the collagen matrix

Collagen content was determined by hydroxyproline assay. A portion of LV free wall (30–50 mg) was dried at 60 °C and weighed. Dried