Exposure to chronic alcohol accelerates development of wall stress and eccentric remodeling in rats with volume overload

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ABSTRACT

Chronic alcohol abuse is one of the leading causes of dilated cardiomyopathy (DCM) in the United States. Volume overload (VO) also produces DCM characterized by left ventricular (LV) dilatation and reduced systolic and diastolic function, eventually progressing to congestive heart failure. For this study, we hypothesized that chronic alcohol exposure would exacerbate cardiac dysfunction and remodeling due to VO. Aortocaval fistula surgery was used to induce VO, and compensatory cardiac remodeling was allowed to progress for either 3 days (acute) or 8 weeks (chronic). Alcohol was administered via chronic intermittent ethanol vapor (EtOH) for 2 weeks before the acute study and for the duration of the 8 week chronic study. Temporal alterations in LV function were assessed by echocardiography. At the 8 week end point, pressure–volume loop analysis was performed by LV catheterization and cardiac tissue collected. EtOH did not exacerbate LV dilatation (end-systolic and diastolic diameter) or systolic dysfunction (fractional shortening, ejection fraction) due to VO. The combined stress of EtOH and VO decreased the eccentric index (posterior wall thickness to end-diastolic diameter ratio), increased end-diastolic pressure (EDP), and elevated diastolic wall stress. VO also led to increases in posterior wall thickness, which was not observed in the VO + EtOH group, and wall thickness significantly correlated with LV BNP expression. VO alone led to increases in interstitial collagen staining (picrosirius red), which while not statistically significant, tended to be decreased by EtOH. VO increased LV collagen I protein expression, whereas in rats with VO + EtOH, LV collagen I was not elevated relative to Sham. The combination of VO and EtOH also led to increases in LV collagen III expression relative to Sham. Rats with VO + EtOH had significantly lower collagen I/III ratio than rats with VO alone. During the acute remodeling phase of VO (3 days), VO significantly increased collagen III expression, whereas this effect was not observed in rats with VO + EtOH. In conclusion, chronic EtOH accelerates the development of elevated wall stress and promotes early eccentric remodeling in rats with VO. Our data indicate that these effects may be due to disruptions in compensatory hypertrophy and extracellular matrix remodeling in response to volume overload.

1. Introduction

Chronic alcohol abuse is one of the leading causes of dilated cardiomyopathy (DCM), and has a 4-year mortality rate near 50% [1]. The form of DCM caused by alcohol abuse is known as alcoholic cardiomyopathy (ACM). ACM is characterized by two distinct phases: an asymptomatic phase characterized by diastolic dysfunction and a symptomatic phase characterized by LV hypertrophy and systolic dysfunction [2].

The aortocaval fistula model of volume overload (VO) produces a DCM with similar pathological features to ACM. Like ACM, VO is characterized by both systolic and diastolic dysfunction. VO-induced diastolic dysfunction is characterized by increased end-diastolic pressure (EDP), increased diastolic wall stress, and a decreased slope of the end-diastolic pressure volume relation (EDPVR) [3,4]. Increased diastolic wall stress in response to VO is thought to contribute to eccentric hypertrophy. VO-induced systolic dysfunction is characterized by right ventricular (RV) and LV dilatation, and reduced ejection fraction.

The cardiac extracellular matrix (ECM) plays an important role during the pathogenesis of several cardiovascular diseases [5]. Normal cardiac ECM is primarily composed of the fibrillar collagens, which consists of collagen types I and III in proportions of ~85% and ~11% of total collagen, respectively. Collagen I is responsible for tensile strength of the ECM, whereas collagen III helps confer compliance. Cardiac fibroblasts are the major cell type in the heart responsible for synthesis and degradation of the ECM [6]. The conversion of fibroblasts to collagen-secreting myofibroblasts is enhanced by transforming growth factor beta (TGF-β), and myofibroblasts are typically characterized by their expression of alpha-smooth muscle actin.

The progression of VO in the aortocaval fistula model occurs in three distinct phases: acute stress (0 to 2 weeks), compensatory remodeling

(2 to 10 weeks), and decompensated heart failure (10 weeks and beyond) [7]. Acute stress is characterized by net degradation of extracellular matrix and collagen isoform switching, resulting in a more compliant ventricle. The compensatory phase is characterized by wall thickening that normalizes wall stress imposed by the increased preload [8,9]. This compensatory hypertrophy is accompanied by increases in extracellular matrix content, such as collagen I [10]. Decompensated heart failure occurs when the stress imposed by VO exceeds the ability of the ventricle to normalize or compensate. Many comparable mechanisms have been shown to contribute to both VO and alcohol-induced cardiac dysfunction, including adverse extracellular matrix ECM and hypertrophic remodeling [7,9,11–13].

The purpose of this study was to determine if alcohol exposure during the progression of VO would exacerbate VO-induced cardiac dysfunction and remodeling. Since both VO and alcohol produce DCM phenotypes, we hypothesized that these two stressors to the heart would act synergistically to deteriorate cardiac function and remodeling. In our long term study, we found that chronic alcohol exposure prevented compensatory hypertrophy due to VO, and accelerated the development of elevated EDP and LV wall stress in rats with VO. These changes were associated with decreased LV collagen I expression, increased collagen III expression, and decreased collagen I/III ratio. In our short term study, we found that EtOH prevented VO-induced increases in collagen III expression. By disrupting compensatory hypertrophy and normal ECM remodeling during both the acute and chronic stages of VO, alcohol may prematurely induce the transition into decompensated heart failure due to VO.

2. Materials and methods

2.1. Dual-hit model of volume overload and alcohol

Two durations of VO with and without alcohol exposure were studied: 3 days and 8 weeks. Rats were divided into four groups: Sham (acute n = 8, chronic n = 8), Sham + alcohol (Sham + EtOH; acute n = 9, chronic n = 9), volume overload (VO; acute n = 7, chronic n = 6), and volume overload + alcohol (VO + EtOH; acute n = 7, chronic n = 8). For the Sham + EtOH and VO + EtOH groups, rats were exposed to alcohol via chronic intermittent ethanol vapor inhalation (14HR ON/10HR OFF) in alcohol vapor chambers (La Jolla Alcohol Research, Inc.). Vapor settings were adjusted to produce blood alcohol levels (BALs) of 150-200 mg/dl. BALs were measured from tail venous blood samples using an Analox GM7 analyzer (London, UK). This exposure pattern and dose mimics daily intoxication and withdrawal patterns observed in humans [14]. Weekly BALs averaged 155 ± 45 mg/dl for the Sham + EtOH group and 167 ± 51 mg/dl for the VO + EtOH group. For the 3 day short-term study, rats were pre-exposed to EtOH for 2 weeks before receiving VO. Rats continued to receive alcohol exposure for 3 days before sacrifice. For the long-term study, rats were exposed to EtOH for a total of 8 weeks following aortocaval fistula surgery.

2.2. Aortocaval fistula surgery

All experimental procedures were approved by LSU Health Sciences Center's Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (225–250 g) were purchased from Harlan Laboratories (Indianapolis, IN). VO was induced surgically via infrarenal aortocaval fistula surgery prior to alcohol administration. Rats were anesthetized with 3.5% isoflurane. A laparotomy was performed to expose the abdominal aorta and inferior vena cava. An 18-gauge needle was inserted into the aorta below the renal arteries and advanced into the vena cava. Successful fistula was visually confirmed by the presence of arterial blood in the vena cava. Sham animals were exposed to the same procedure with the exception that no fistula was created.

2.3. Echocardiography

LV chamber dimensions and function were monitored weekly in sedated animals (1% isoflurane) for the 8-week protocol by echocardiography (VEVO 770, VisualSonics; Toronto, CA). Rats were sober (BALs = 0 mg/dl) for all functional assessments. The LV short-axis view was used to obtain B-mode two-dimensional images and M-mode tracings of the LV posterior and anterior wall. LV end-diastolic and end-systolic diameter (LVEDD and LVESD) and posterior wall thickness (LVPW) at diastole (d) were measured. Fractional shortening (%FS) was calculated as %FS = (LVEDD − LVESD/LVEDD) ∗ 100. Eccentric index was used to assess eccentric hypertrophy and was calculated as 2 ∗ LVPWd/LVEDD.

2.4. Pressure-volume loop analysis

At the end of the 8-week protocol, LV diastolic and systolic function was assessed by pressure-volume loop analysis. All rats were sober during functional assessment (BALs = 0 mg/dl). Rats were weighed, anesthetized with 3.5% isoflurane, intubated, and ventilated. The chest was opened and a Scisense (Ontario, CA) pressure-volume catheter was advanced into the LV via the apex (product #: FTS-1912B-9018, fixed segment for SHAM; FTE-1918B-E218, multi-segment for VO). After establishing stable baseline function for approximately 5 min, pressure and volume signals were recorded using the Advantage PV System (model FY897B Scisense). Data were acquired and analyzed with iWorx 308T data acquisition system (Dover, NH) and Labscribe software. Steady state parameters that were calculated include end-systolic and diastolic pressure and volume (ESP, EDP, ESV, EDV), stroke volume (SV), heart rate (HR), cardiac output (CO), stroke work (SW), dP/dt max, and ejection fraction (EF). For load-independent parameters, including end-systolic and -diastolic pressure volume relationships (ESPVR and EDPVR) and preload-recruitable stroke work (PRSW), the vena cava was occluded for ~3 s with a cotton tip swab during recording. Following this procedure, the heart and lungs were removed and weighed. Hearts were separated into LV plus septum and RV before weighing. LV tissue was then snap frozen in liquid nitrogen for further analysis. Meridional diastolic and systolic wall stress was calculated using the following formula: $\sigma = PR/((2h)(1 + h/2R))$, in which P = ventricular pressure, R = ventricular radius, and h = wall thickness [15].

2.5. Western blot

LV tissue was homogenized in RIPA buffer (#89900; Pierce Antibody Products; ThermoFisher Scientific; Waltham, MA) with HALT protease inhibitor cocktail (ThermoFisher Scientific #78430). Protein concentration was determined by a Bradford assay. Protein (50 μg) was separated via SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% BSA and incubated with primary antibodies overnight at 4 °C against collagen I (1:1000, Product #34710; Abcam; Cambridge, UK) and collagen III (1:5000, Abcam #7778). Histone H3 (1:5000, Abcam #1791) was used as a loading control. The following day, membranes were incubated with secondary antibody (1:1000, Abcam #97051) for 2 h at room temperature. Membranes were exposed using a Western ECL Substrate kit (Bio-Rad; Hercules, CA) and visualized with an ImageQuant LAS 4000 imager (GE Healthcare Life Sciences; Little Chalfont, UK). Densitometry was performed with ImageJ software. All data were normalized to histone H3 expression.

2.6. Histology

Mid LV-sections were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm on microscope slides. Sections were cleared, rehydrated, and stained with picrosirius red, a stain specific