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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

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Disclosures

NWG is a consultant for Glauser Life Sciences.

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.

Keywords

alcohol; heart failure; extracellular matrix; collagen; hypertrophy; ventricular remodeling

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.

MATERIALS AND METHODS

Dual-hit Model of Volume Overload and Alcohol

Two durations of VO with and without alcohol exposure were studied: 3 days and 8 wks. 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 150–200mg/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.

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 grams) 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.

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 $2 * LVPWd / LVEDD$.

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-recrutable 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].

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 hours 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.

Histology

Mid LV-sections were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μ m on microscope slides. Sections were cleared, rehydrated, and stained with picrosirius red, a stain specific for collagen. For collagen volume fraction (CVF), fluorescent images (20X, 20 per slide) were taken with a fluorescent microscope (Nikon Eclipse TE2000-U; Tokyo, JP) and processed with NIS-Elements software by an individual blinded to the study groups. Perivascular collagen was excluded from measurement. CVF was expressed as percent of total myocardial area.

Quantitative Real-Time PCR

RNA was extracted from LV samples using RNeasy Fibrous Tissue Kit (Qiagen; Limburg, Netherlands) according to the manufacturer's instructions. RNA (1 μ g) was reverse transcribed into cDNA using a reverse transcription supermix (iScript™; Bio-Rad; Hercules, CA). Quantitative PCR was performed using iTaq™ Universal SYBR® Green Supermix (Bio-Rad) with cDNA (50 ng/reaction) and primers against ANP, BNP (Sigma-Aldrich; St. Louis, MO), Myh6, Myh7, LOX, Acta2, TGFβ1, and RPS13 (Integrated DNA Technologies; Coralville, IA). Thermocycling was performed using a CFX96 Thermocycler (Bio-Rad). All data were analyzed using the C_T method and gene expression was normalized to RPS13 values.

Measurement of Collagen Cross-Linking

Collagen cross-linking was assessed by pyridinoline (PYD) content in LV acid hydrolysates using a commercial ELISA kit (Quidel #8010; San Diego, CA) according to the manufacturer's instructions. PYD concentration was normalized to tissue dry weights.

Statistics

Data were processed and analyzed using Microsoft Excel and Graphpad 5.0 (Prism; La Jolla, CA). Comparisons were made using 1-way analysis of variance (ANOVA) with Dunnett's post-hoc analysis. Main effects, as well as interactions between VO and EtOH, were determined using 2-way ANOVA. A p-value of less than 0.05 was considered significant. All values are presented as mean $\pm$ SEM.

RESULTS

Morphological Parameters and Survival

Morphological parameters including were measured to determine cardiac hypertrophy and pulmonary edema. After sacrifice, LV, RV, and lungs were weighed and normalized to tibial length. VO (8wk) led to significant increases in LV mass/tibia length, RV mass/tibia length, and lung mass/tibia length (Table 1). EtOH had no significant effects on these morphological parameters. Bodyweight was significantly lower in VO+EtOH animals versus VO alone. Two of 8 animals in the VO+EtOH group did not survive the 8-week protocol, succumbing to cardiac failure. No differences in any of the morphological parameters were observed in the acute study.

EtOH disrupted VO-induced wall thickening and increased wall stress in VO hearts

To determine whether EtOH affected VO-induced cardiac dysfunction, we measured cardiac function using non-invasive echocardiography every 2 weeks and invasively using pressure-volume catheterization before sacrifice. LV diameter, systolic function (%FS), and wall thickness were assessed by echocardiography. VO led to significant LV dilatation as indicated by increased LVEDD and LVESD starting at week 2 (Figure 1). VO also significantly decreased %FS by week 5 and continued to decline through 8 weeks. EtOH did not affect chamber diameter or systolic function in either Sham or VO treated animals. VO led to significant increases in LVPWd (2.2 ± 0.1 mm versus 1.9 ± 0.1 mm Sham; $p < 0.05$ significant interaction; Figure 1D), which was not observed in the VO + EtOH group. The stress of EtOH combined with VO significantly decreased eccentric index by 8 weeks (0.36 ± 0.03 VO+EtOH versus 0.48 ± 0.03 Sham; Figure 1E). EtOH significantly increased the heart rate in Sham animals only (Figure 1F). Pressure-volume loop analysis was performed at 8 weeks to further evaluate changes in LV function. VO significantly increased ESV, EDV, SV, SW, and CO and decreased EF (Table 2), but EtOH did not affect any of these parameters. However, VO combined with EtOH significantly increased EDP compared to Sham, Sham+EtOH, and VO (Figure 2C; 11.0 ± 3.2 mmHg versus 3.0 ± 0.7 mmHg for Sham, $p < 0.01$; 4.9 ± 0.6 mmHg for Sham+EtOH, $p < 0.05$; 4.9 ± 1.4 for VO, $p < 0.05$). To determine whether these animals were able to compensate for the increase in EDP, we calculated diastolic and systolic wall stress (σ). Although VO alone did not significantly increase diastolic σ , the combination of VO and EtOH did, suggesting that EtOH prevented the ability of these animals to normalize increases wall stress due to elevated EDV and EDP. The combination of VO and EtOH also increased systolic wall stress relative to Sham, and this effect was not observed in the VO group.

Myosin Heavy Chain Isoforms and Cardiac Hypertrophy Markers

We assessed common LV mRNA levels of markers of cardiac hypertrophic remodeling, including Myh6 and 7, to determine whether alcohol affected VO-induced sarcomeric remodeling. We also assessed mRNA levels of atrial and brain natriuretic peptides (ANP and BNP, Figure 3), markers of pathological cardiac hypertrophy. VO led to decreased Myh6 mRNA in the LV, which was not affected by EtOH (Figure 3A). No differences were observed in LV Myh7 mRNA among any of the groups. We then calculated the Myh6/7 ratio, in which a decrease is indicative of adverse cardiac remodeling and fetal gene reprogramming. VO significantly decreased the Myh6/7 ratio, and this was not affected by EtOH (Figure 3C). VO also led to significant increases in both LV ANP and BNP (Figure 3D, E) but these effects were not observed in the VO + EtOH group. We then determined correlations between ANP/BNP expression and posterior wall thickness at diastole (LVPWTd, Figure 3F, G). No correlation was observed between ANP mRNA and LVPWTd; however, there was a significant positive correlation between BNP mRNA and LVPWTd (Figure 3G).

EtOH disrupted compensatory collagen remodeling due to chronic VO

We then assessed LV ECM remodeling, which plays an important role during compensatory hypertrophy. VO led to significant increases in total interstitial collagen volume fraction

(Figure 4A, $2.6 \pm 0.1\%$ versus $1.5 \pm 0.1\%$ of total area for Sham), and collagen I protein expression (Figure 4B, $147 \pm 16\%$ Sham control; $p < 0.05$ significant interaction). EtOH did not affect total interstitial collagen levels, but VO-induced increases in collagen I were not observed in VO + EtOH (Figure 4B). Although neither EtOH nor VO alone increased collagen III expression, the combination of VO and EtOH significantly increased collagen III expression relative to Sham control ($151 \pm 11\%$ Sham control; Figure 4C). Collagen I/III was significantly lower in the VO + EtOH versus VO group (Figure 4D). VO also increased Acta2 expression, a marker of myofibroblast activation, but this effect was not observed in the VO + EtOH group (Figure 4E). VO increased TGF β 1 expression, which was not affected by EtOH (Figure 4F).

Collagen Cross-Linking and Lysyl Oxidase Expression

To determine whether collagen cross-linking was altered in addition to changes in collagen isoforms, we assessed the content of pyridinoline (PYD), a component of the collagen cross-link produced by LOX, in the left ventricle. No differences were observed in PYD content among any of the groups (Figure 5A). However, VO led to significant increases in expression of lysyl oxidase (LOX) (Figure 5B). Interestingly, EtOH completely attenuated VO-induced increases in LOX expression.

EtOH disrupted acute VO-induced increases in LV collagen III expression

As in the chronic study, collagen was assessed in the hearts of animals with short-term VO (3 days) pre-exposed to EtOH for 2 weeks. Neither interstitial collagen volume fraction nor collagen I protein expression was significantly different between groups. Acute VO led to increases in collagen III expression, which was not observed in the VO + EtOH group. No differences in collagen I/III expression were observed.

DISCUSSION

Our data show that chronic alcohol exposure prematurely induces the development of cardiac abnormalities in volume-overloaded rats, including decreased wall thickening and eccentric index, increased end-diastolic pressure and wall stress, and adverse changes in LV fetal gene reprogramming and extracellular matrix (ECM). These changes appear to be due to interruption of normal compensatory ventricular remodeling by EtOH, including altered collagen I and III expression, and decreased collagen I/III ratio. We also observed 25% mortality in the VO + EtOH group, whereas we observed no mortality in the other groups. We propose that EtOH disrupts the compensatory hypertrophy and ECM remodeling processes in response to chronic VO. These studies provide clinical perspectives for patients with heart failure or other cardiac diseases who abuse alcohol. They also provide important mechanistic insight into disparities affects the cardiac ECM. While our lab and others have shown a pro-fibrotic effect in the naïve heart, these current studies support an anti-fibrotic effect in the pathologically hypertrophied heart [16, 17]. Thus, certain patients may benefit from pro-fibrotic therapy rather than anti-fibrotic in the context of their history and diagnosis.

The progression of volume overload due to aortocaval fistula can be divided into three phases: acute stress, compensatory hypertrophy/remodeling and decompensated heart failure [18, 19]. The compensatory phase is characterized mainly by concentric hypertrophy, which involves increases in myocyte diameter that result in thickening of the ventricular wall to maintain a normal wall thickness-to-chamber diameter ratio [9], or eccentric index. We found that 8 weeks of VO led to concentric hypertrophy as assessed by increased LV posterior wall thickness at diastole, and a preserved eccentric index. During decompensated heart failure, however, eccentric hypertrophy becomes dominant and the wall thickness-to-chamber-diameter (eccentric index) ratio falls dramatically. In rats exposed to chronic EtOH, the compensatory wall thickening response to VO was not observed, and the eccentric index was decreased. Interestingly, wall thickening due to VO was not significantly increased until week 8, the final week of the study; however, rats with VO + EtOH showed no wall thickening at any time point during the study. This shows that volume overloaded rats exposed to EtOH showed no compensation whatsoever to the hemodynamic stress, which may have resulted in the increased mortality that we observed. According to the Law of Laplace, ventricular wall stress is directly related to ventricular volume (or chamber diameter) and pressure, and inversely related to ventricular wall thickness [20]. Increased wall stress is a consequence of inadequate hypertrophy and has a strong correlation with poor prognosis in patients with heart failure [13, 20]. While rats with VO alone were able to normalize wall stress after 8 weeks, we found that EtOH exposure in rats with VO resulted in elevated LV systolic and diastolic wall-stress. These increases in wall stress were primarily due to increased LV pressure and decreased wall thickness, as there were no differences in end-diastolic diameter or volume between the untreated and EtOH-treated VO groups. We also observed an increase in heart rate (HR) in the Sham + EtOH group that was not observed in other groups. Alcohol exposure increases sympathetic nerve activity and also suppresses baroreceptor reflex [21]. There was no change in HR in the VO + EtOH group relative to the Sham control group. This is most likely due to the fact that during chronic VO, the heart becomes less responsive to sympathetic stimulation, which can be attributed to decreases in beta-1 adrenergic receptor (β_1 -AR) expression and decreased responsiveness to β_1 -AR stimulation by agonists such as dobutamine [22]. Interestingly, we also found that the VO + EtOH group had a lower body weight than the VO group. Given that both the aortocaval fistula surgery and alcohol exposure were given to the rats while they were still growing, the double hit of volume overload and alcohol exposure may have prevented normal growth in these rats.

Cardiac hypertrophic remodeling involves several changes at the level of the sarcomere, specifically in the expression of myosin heavy chain (MHC) isoforms. In the fetal rodent heart, β -MHC is the predominant isoform, whereas α -MHC becomes the predominant isoform in adulthood [23]. These isoforms drastically differ in function, as α -MHC has higher ATPase activity and contraction velocity. In several rodent pathological models, a shift in the relative expression of the adult α -MHC (Myh6) to the fetal β -MHC (Myh7) has been observed [23, 24]. This shift is more common in pathological and not physiological hypertrophy, as rodent models of exercise-induced cardiac hypertrophy exhibit increased expression of α -MHC relative to β -MHC [25]. Increased β -MHC expression relative to α -MHC appears to directly cause cardiac dysfunction [26]. Consistent with previous studies,

we found that VO led to significant decreases in LV Myh6 mRNA and Myh6/Myh7 gene expression, without significantly affecting Myh7 expression. However, EtOH had no effect on Myh6 or Myh7 expression in either rats with Sham or VO surgery. This is consistent with our functional results, in which systolic function as determined by fractional shortening and ejection fraction, was not significantly different between rats with VO alone and VO rats exposed to EtOH.

The natriuretic peptide system, which consists of the atrial and brain natriuretic peptides (ANP and BNP), becomes activated during cardiac hypertrophy caused by hemodynamic stress [27]. ANP and BNP are released by the ventricles in response to stretch due to increased blood volume, and act peripherally to promote vasodilation and fluid excretion. Consistent with the literature, we found that long-term VO (8 weeks) significantly increased LV ANP and BNP expression [28]. Interestingly, rather than further increasing expression, we found that rats with VO + EtOH did not display these increases in ANP and BNP. While these results could be interpreted as beneficial, we suggest that decreased natriuretic peptide expression in volume-overloaded rats exposed to EtOH reflects their lack of compensatory hypertrophy. Indeed, in patients with essential hypertension, both ANP and BNP levels are positively correlated with posterior wall thickness (LVPW) [29]. In patients with premature ventricular contractions, BNP levels are positively correlated with LVPW [30]. Consistent with this, we found a significant positive correlation between BNP expression and LVPW. Furthermore, in patients with systolic heart failure, BNP levels are positively correlated with serum levels of carboxy-terminal propeptide of procollagen type I (PICP), a biomarker of collagen I synthesis [31]. Our results are consistent with these studies in that compensatory increases in LVPW and collagen I expression due to VO were not observed in rats with VO + EtOH, and thus prevented increases in ANP and BNP relative to Sham.

Compensatory hypertrophy due to VO involves remodeling of the ECM, including increased deposition of collagen [32]. Pathological ECM remodeling during LV dilatation involves reorganization of collagen fibrils characterized by increased collagen III and/or reductions in cross-linking, which may contribute to dilatation [33]. Our studies showed that while EtOH did not prevent accumulation of total collagen due to VO, rats with VO + EtOH showed no significant change in LV collagen I expression relative to Sham, and increased collagen III expression relative to the Sham control group, favoring a lower collagen I/III ratio compared to VO. By disrupting compensatory hypertrophy and promoting a more compliant collagen phenotype, EtOH may decrease the ability of the LV to compensate for increases in preload, thus leading to the increased end-diastolic pressure and wall stress observed in this study. Consistent with the unaltered collagen I expression in the VO + EtOH group, we found that rats with VO + EtOH did not show elevations in Acta2 gene expression, which encodes for α -SMA, a marker of myofibroblast activation. Thus, in the volume-overloaded heart, EtOH may impair myofibroblast function during compensatory remodeling. Interestingly, while VO led to increases in TGF β 1 expression, alcohol did not affect its expression.

We also assessed the role of LV collagen cross-linking, in which collagen fibers are covalently bound to each other by lysyl oxidase [34]. This cross-linking increases the tensile strength of collagen and renders it insoluble, thus making it less prone to degradation. Although studies in our lab have shown cardioprotective effects of lysyl oxidase inhibition in

the decompensated failure stage of VO, the role of collagen cross-linking during the compensatory phase remains unclear. We found no significant differences in LV collagen cross-linking between any of the experimental groups in our study. However, we found that EtOH completely attenuated VO-induced increases in LOX mRNA expression, suggesting that impaired cross-linking may also play an important role in the effects of EtOH on VO-induced ECM remodeling.

Finally, we wanted to determine whether changes in the ECM that we observed during the compensatory phase of VO could be attributed to disruption of ECM remodeling during the early, acute phase of remodeling. Acute VO (0–2 weeks post-surgery) is characterized by rapid degradation of the collagen matrix by MMPs, with collagen loss peaking at approximately 12 hours and then returning to normal by 5 days post-surgery [35, 36]. This loss of collagen increases ventricular compliance in response to elevated diastolic volumes. We did not detect any changes in interstitial collagen volume fraction, or collagen I expression, due to EtOH after 3 days of VO. Interestingly, VO-induced increases in collagen III expression were not observed in the VO + EtOH group. These results suggest that during the acute remodeling phase, EtOH may disrupt VO-mediated increases in ventricular compliance. Consistent with our chronic studies, EtOH appears to oppose normal VO-induced ECM remodeling during both the acute and compensatory remodeling phases.

In the present study, we found that 8 weeks of EtOH alone did not significantly affect LV collagen expression. These findings seemingly contradict a previous study by our laboratory in which EtOH vapor inhalation for 2 weeks led to increases in LV interstitial collagen, as well as increased expression of collagens I and III [16]. This disparity may be due to the different durations and levels of EtOH exposure, and the exact nature of LV ECM dynamics during chronic EtOH exposure remains to be determined.

In conclusion, chronic EtOH exposure accelerates elevations in LV wall stress and decreases in eccentric index due to VO, which drive progression to decompensated heart failure. These effects appear to be caused by disruption of LV wall thickening and normal ECM remodeling during the compensatory phase of VO. Future studies will determine how chronic EtOH exposure affects cardiac function and remodeling in the decompensated heart failure (>10 weeks) stage of VO.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Volume overload (VO) produced compensatory hypertrophy in rats.
- Rats with VO given chronic alcohol exhibited increased cardiac wall stress.
- Alcohol prevented the compensatory hypertrophy response to VO.
- Combined VO and alcohol produced diastolic dysfunction with ECM alterations.
- Alcohol abuse may accelerate decompensation of the stressed heart.

Figure 1. Echocardiography parameters

EtOH did not affect dilatation or systolic dysfunction due to VO. Fractional shortening (A, %FS) was significantly decreased by VO around 5 weeks and continued to decrease until the end of the study. Left ventricular end-diastolic (B, LVEDD) and systolic (C, LVESD) diameter were significantly increased in both VO and VO + EtOH groups beginning at 2 weeks post-surgery and continued to increase throughout the 8 week protocol. LV posterior wall thickness (PWD) was significantly increased in the VO group but not the VO + EtOH group (D). VO + EtOH led to significant decreases in the eccentric index at 8 weeks post-surgery (E). EtOH also caused a significant increase in heart rate at 8 weeks (F). * $p < 0.05$ (VO vs Sham), # $p < 0.05$ (VO + EtOH vs Sham), ## $p < 0.01$ (VO + EtOH vs Sham), \$\$ $p < 0.01$ (Sham + EtOH vs Sham).

Figure 2.

EtOH prematurely elevates diastolic and systolic wall stress in VO hearts (chronic 8 week study). (A) The combination of VO and EtOH significantly increased end-diastolic pressure (EDP) relative to Sham (11.0 ± 3.2 mmHg versus 3.0 ± 0.7 mmHg for Sham). (B) VO + EtOH led to significant increases in diastolic wall stress relative to both Sham and VO (significant interaction), and (C) systolic wall stress in the VO + EtOH group relative to Sham only.

* $p < 0.05$, ** $p < 0.01$, versus Sham).

Figure 3. LV markers of fetal gene reprogramming (chronic 8 week study)

VO led to significant decreases in LV Myh6 expression (A) and the Myh6/Myh7 ratio (C), which EtOH did not affect. No differences in Myh7 expression were observed between groups (B). VO increased expression of ANP (D) and BNP (E) in air exposed rats but not EtOH exposed rats. There was a significant correlation between wall thickness (LVPWTd) and BNP mRNA levels in rats with VO (G).

Figure 4.

EtOH prevents compensatory extracellular matrix remodeling due to VO (chronic 8 week study). (A) VO increased left ventricular interstitial collagen volume fraction ($2.6 \pm 0.1\%$ versus $1.5 \pm 0.1\%$ of total myocardial area for Sham). Although there was a trend to decrease, EtOH did not significantly reduce the increase in total interstitial collagen due to VO. (B) VO increased collagen I protein expression ($146 \pm 16\%$ Sham control; significant interaction), but not in rats exposed to EtOH. (C) While neither EtOH nor VO alone increased collagen III expression, the combination of VO and EtOH significantly increased collagen III expression relative to Sham control ($151 \pm 11\%$ Sham control). (D) VO increased the collagen I/III ratio relative to the Sham group (1.31 ± 0.18 versus 0.95 ± 0.06 Sham), which EtOH completely prevented. (E) Acta2 expression was significantly increased by VO but not VO + EtOH. (F). VO led to significant increases in TGFβ1 expression, which was unaltered by EtOH. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Sham. # $p < 0.05$ versus VO.

Figure 5.

LV collagen cross-linking was determined by pyridinoline (PYD) content (chronic 8 week study). (A) No significant differences in PYD content were observed among groups. (B) VO significantly increased mRNA levels for lysyl oxidase (LOX), the major collagen cross-linking enzyme, which was completely attenuated by EtOH. * $p < 0.05$ vs Sham, # $p < 0.05$ vs VO.

Figure 6. Effects of EtOH on ECM remodeling during acute VO (3 days)

(A) Collagen volume fraction was not significantly affected by EtOH. (B) Collagen I expression was not significantly different in any group. (C) VO increased collagen III expression after 3 days, which was not observed in the VO + EtOH group. (D) The collagen I/III ratio was not significantly different in any group.

Table 1

Morphological Parameters.

Group	Sham	Sham + EtOH	VO	VO + EtOH
Long-term 8 week				
LV mass/tibia (mg/mm)	20.4 ± 0.6	19.6 ± 0.5	31.4 ± 0.9 *	31.8 ± 1.5 *
RV mass/tibia (mg/mm)	5.3 ± 0.2	5.3 ± 0.2	10.8 ± 0.5 *	10.5 ± 0.6 *
Lung mass/tibia (mg/mm)	43.1 ± 1.5	44.5 ± 1.2	59.4 ± 6.6 *	60.3 ± 4.7 *
Bodyweight (g)	416 ± 7	403 ± 3	426 ± 7	386 ± 9 [#]
Short-term 3 day				
LV mass/tibia (mg/mm)	18.5 ± 0.9	17.1 ± 1.0	19.3 ± 1.1	18.9 ± 1.7
RV mass/tibia (mg/mm)	4.0 ± 0.6	3.9 ± 0.5	4.4 ± 0.5	4.5 ± 0.6
Lung mass/tibia (mg/mm)	39.8 ± 2.4	37.7 ± 2.4	40.7 ± 1.9	39.9 ± 2.9
Bodyweight (g)	294 ± 20	282 ± 12	286 ± 17	269 ± 23

Volume overload (VO) significantly increased LV, RV, and lung mass in the long-term groups (*p<0.05 versus Sham control, #p<0.05 versus VO). Ethanol (EtOH) treatment reduced body weight in the long-term VO animals, but not in Sham. No significant differences were found in the short-term study groups.

Table 2

Cardiac functional parameters.

Group	Sham	Sham + EtOH	VO	VO + EtOH
ESP (mmHg)	90.3±3.7	94.8±6.0	85.2±3.8	93.5±5.5
ESV (μl)	147.8±17.8	188.3±19.0	393.0±44.4 *	404.2±74.9 *
EDV (μl)	349.0±28.5	366.4±17.9	715.0±17.8 *	718.5±10.6 *
SV (μl)	201.2±11.9	178.1±9.2	321.9±51.7 *	314.3±82.7 *
HR (bpm)	328±8	369±8 ***	334±5	335±7
EF (%)	58.3±1.9	49.3±5.2	44.6±6.4 *	43.5±10.8 *
CO (ml/min)	69.9±4.5	62.1±4.5	104.0±17.2 *	98.9±25.3 *
dP/dt max (mmHg/s)	6362±442	7114±611	6571±702	6896±141
SW (Joules)	2.3±0.1	2.6±0.6	4.2±0.5 *	4.2±0.8 *
ESPVR (mmHg/μl)	1.11±0.14	1.11±0.16	0.41±0.06	0.61±0.14
EDPVR (mmHg/ml)	8.8±0.8	10.0±2.0	4.5±2.3	6.2±2.3
PRSW (mmHg)	87.9±5.8	88.3±4.2	63.7±7.4	85.6±5.7

Pressure-volume catheterization data are presented. ESP=end-systolic pressure, ESV=end-systolic volume, EDV=end-diastolic volume, SV=stroke volume, EF=ejection fraction, CO=cardiac output, dP/dt max=rate of pressure development during early systole, SW=stroke work, ESPVR=end-systolic pressure volume relationship, EDPVR=end-diastolic pressure volume relationship, PRSW=preload-recruitable stroke work. Volume overload (VO) significantly increased ESV, EDV, SV, CO, SW and decreased EF.

* p<0.05 versus Sham control;

*** p<0.001.