Influence of gender on ethanol-induced ventricular myocyte contractile depression in transgenic mice with cardiac overexpression of alcohol dehydrogenase

Jinhong Duan, Lucy B. Esberg, Gang Ye, Anthony J. Borgerding, Bonnie H. Ren, Nicholas S. Aberle, Paul N. Epstein, Jun Ren

Division of Pharmaceutical Sciences, University of Wyoming College of Health Sciences, Laramie, WY 82071-3375, USA
University of North Dakota School of Medicine, Grand Forks, ND 58203, USA
Department of Pediatrics, University of Louisville School of Medicine, Louisville, KY 40202, USA
Department of Chemistry, University of St. Thomas, St. Paul, MN 55104, USA

Received 21 August 2002; received in revised form 25 November 2002; accepted 25 November 2002

Abstract

Acute ethanol exposure depresses ventricular contractility and contributes to alcoholic cardiomyopathy in both men and women chronically consuming ethanol. However, a gender-related difference in the severity of myopathy exists with female being more sensitive to ethanol-induced tissue damage. Acetaldehyde (ACA), the major oxidized product of ethanol, has been implicated to play a role in the pathogenesis and gender-related difference of alcoholic cardiomyopathy, possibly due to its direct cardiac effect and interaction with estrogen. This study was designed to compare the effects of cardiac overexpression of alcohol dehydrogenase (ADH), which converts ethanol into ACA, on the cardiac contractile response to ethanol in ventricular myocytes isolated from age-matched adult male and female transgenic (ADH) and wild-type (FVB) mice. Mechanical properties were measured with an IonOptix SoftEdge system. ACA production was assessed by gas chromatography. The ADH myocytes from both genders exhibited similar mechanical properties but a higher efficacy to produce ACA compared to FVB myocytes. Exposure to ethanol (80–640 mg/dl) for 60 min elicited concentration-dependent decrease of cell shortening in both FVB and ADH groups. The ethanol-induced depression on cell shortening was significantly augmented in female but not male ADH group. ADH transgene did not exacerbate the ethanol-induced inhibition of maximal velocity of shortening/relengthening in either gender. In addition, neither ethanol nor ADH transgene affect the duration of shortening and relengthening in male or female mice. These data suggest that females may be more sensitive to ACA-induced cardiac contractile depression than male, which may attribute to the gender-related difference of alcoholic cardiomyopathy.

Keywords: Gender; Transgene; Alcohol dehydrogenase; Acetaldehyde; Ventricular myocyte; Shortening; IonOptix SoftEdge system; Alcoholic cardiomyopathy

1. Introduction

The evolution and clinical presentations of several cardiomyopathies have shown clearly gender-related differences. Women generally possess different responsiveness such as myocardial adaptation to noxious cardiac agents or insults than men. Epidemiological evidence revealed that women may have distinct metabolic and pathophysiological characters in response to ethanol exposure, making them more sensitive to ethanol-induced...
cardiac damage (Kennedy et al., 2002). In preclinical ethanol-induced ventricular dysfunction, women were more susceptible to the toxic effects of ethanol than men (Fernandez-Sola and Nicolas-Arfeis, 2002). In overt alcoholic cardiomyopathy, women exhibited similar prevalence of cardiomyopathy as men, despite far less ethanol consumption, supporting a greater female propensity to ethanol-induced cardiac damage (Urbano-Marquez et al., 1995). Ethanol intake is a common cause of dilated cardiomyopathy, a condition characterized by dilated left ventricular diameters, reduced ejection fraction and cardiac output, depressed mechanical function, enhanced risk of stroke, hypertension and sudden death, in the absence of causes of myocardial damage other than ethanol ingestion (Fernandez-Sola et al., 1994; Urbano-Marquez et al., 1989; Patel et al., 1997; Richardson et al., 1998). Several mechanisms have been postulated toward the development of alcoholic cardiomyopathy, including direct and indirect cardiotoxicity of ethanol (Preedy et al., 1999), the major metabolic products of ethanol—acetaldehyde (ACA) (Ren et al., 1997; Liang et al., 1999; Duan et al., 2002), and accumulation of fatty acid ethyl esters (Laposata and Lange, 1986). Many of the chronic ethanol intake-induced cardiac dysfunctions such as inhibition of membrane ionic currents may be duplicated and interpreted by the acute effect of ethanol on the hearts (Thomas et al., 1994; Richardson et al., 1998; Preedy et al., 1999). Nevertheless, none of these speculations have been shown to be responsible for the gender-related difference in alcoholic cardiomyopathy.

ACA has been suggested to play a crucial role in ethanol-induced myocardial depression because it concentrates in the heart (Espinet and Argiles, 1984; Ren et al., 1997) and its ability to directly inhibit cardiac excitation–contraction (E–C) coupling and sarco(endoplasmic reticulum Ca$^{2+}$ release function (Ren et al., 1997; Brown et al., 1999; Ren and Brown, 2000). The advancement of the ACA toxicity theory in alcoholic cardiomyopathy was relatively trivial over the past years, due to lack of a suitable model or method to alter the ACA concentration in vivo for a definitive test of ACA. Recently, our group developed a transgenic mouse model to over-express alcohol dehydrogenase (ADH) specifically in the hearts. Our results indicated that elevated cardiac ACA exposure due to ADH overexpression increased the severity of alcoholic cardiomyopathy, functionally and morphologically at the whole heart (Liang et al., 1999) and myocyte (Duan et al., 2002) levels. Occurrence of elevated blood ACA levels during alcohol oxidation was established in both normally cycling women and ones taking oral contraceptives, but not in men. An association between elevated ACA levels and high estrogen phases was observed in both groups of women (Eriksson et al., 1996). Estrogen-related ACA elevation following ethanol ingestion, either acutely or chronically, may be the key factor explaining the gender differences of the adverse effects of alcohol. Thus, the aim of the present study was to investigate the influence of gender on elevated cardiac ACA exposure-induced cardiac depression in isolated ventricular myocytes from ADH transgenic mice and age-matched wild-type controls (FVB).

2. Materials and methods

2.1. Development of the ADH transgenic mice

All animal procedures have been approved by the University of North Dakota School of Medicine Institutional Animal Care and Use Committee. Briefly, ADH transgene was constructed to produce local overexpression of ADH in the heart. This gene includes the mouse $\alpha$-myosin heavy chain promoter to drive cardiac specific expression. The cDNA for murine class I ADH (Edenberg et al., 1985) was inserted behind the promoter. This cDNA was chosen because class I ADH is the most efficient in the oxidation of ethanol. FVB mice obtained from the University of North Dakota Biomedical Research Center were used to produce transgenic lines containing the ADH transgene (Taketo et al., 1991). Standard procedures were used for producing transgenic animals. A second transgene containing a cDNA for the enzyme tyrosinase was coinjected with ADH. The enzyme tyrosinase produces coat color pigmentation in albino mice (Overbeek et al., 1991) and was used to conveniently identify transgenic animals (Liang et al., 1999).

2.2. Assessment of the ACA production after acute ethanol challenge

Isolated ventricular myocytes (200 000 cells per ml) from either FVB or ADH mice were exposed to 640 mg/dl ethanol for 15 min in a sealed vial
before the reaction was terminated by 4-methylpyrazole (1 mM). Vials were then stored at −80 °C until analysis. Immediately before analysis, the samples were warmed to 25 °C. Two milliliter of the headspace gas from each vial was removed through the septum on the cap with a gas tight syringe and transferred to a 200 μl loop injection system on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector. ACA and other components were separated on a 9-m VOCOL capillary column (Supelco, Inc.) with a 1.8 μm film thickness and an inner diameter of 0.32 mm. The temperature was held isothermally at 30 °C, and the carrier gas was helium at a flow rate of 1.8 ml/min. Under these conditions, separation of ACA from ethanol and other compounds was complete in 1 min. Quantitation was achieved by calibrating the gas chromatography areas against those from headspace samples of known ACA standards, over a similar concentration range as the cell samples, in the same buffer.

Cell shortening and relengthening were assessed using a video-based edge-detection system (IonOptix, Milton, MA) as described (Duan et al., 2002). In brief, cells were placed in a chamber mounted on the stage of an inverted microscope (Olympus IX-70) and superfused at 25 °C with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 mM glucose, 10 HEPES, at pH 7.4. The cells were field stimulated to contract at a frequency of 0.5 Hz. Changes in cell length during shortening and relengthening was captured and converted to digital signal before being analyzed with pClamp software. The myocyte being studied was rapidly scanned with a camera at 120 Hz to ensure recording with good fidelity. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS) and time-to-90% relengthening (TR₉₀), maximal velocities of shortening and relengthening (± dL/dr). Steady-state contraction of myocyte was achieved before application of ethanol.

2.5. Data analysis

Six FVB mice and six ADH mice were used from each gender. Four to six ventricular myocytes from each mouse were used to conduct the ethanol concentration response. Data are reported as mean ± S.E.M. Statistical significance ($P < 0.05$) for each variable was estimated by one-way analysis of variance or t-test, where appropriate. A Dunnett’s test was used for post hoc analysis when required.

3. Results

3.1. General features of male and female FVB and ADH transgenic mice

As shown in Table 1, male FVB and ADH mice exhibit significantly higher body and heart weights compared to the age-matched female littermates. Transgenic overexpression of ADH itself did not

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Heart weight (mg)</th>
<th>Heart/body weight (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB-Male (6)</td>
<td>26.4 ± 0.4</td>
<td>194 ± 5</td>
</tr>
<tr>
<td>FVB-Female (6)</td>
<td>23.8 ± 0.3*</td>
<td>177 ± 5*</td>
</tr>
<tr>
<td>ADH-Male (6)</td>
<td>25.6 ± 0.7</td>
<td>190 ± 7</td>
</tr>
<tr>
<td>ADH-Female (6)</td>
<td>23.3 ± 0.3*</td>
<td>176 ± 4*</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.; (n), number of animals.

* $P < 0.05$ vs. male counterpart.
elicit any effect on body and heart weights in either male or female animals. Despite the difference in body weight between the age-matched male and female mice, the heart-to-body weight ratio was similar among all the groups studied. The ability of ventricular myocytes to oxidize ethanol into ACA measured by gas chromatography and flame ionization detection was significantly higher in myocytes from the ADH group compared to the FVB group (Fig. 1), validating the transgenic elevation of cardiac ADH activity (by ~40 times) (Liang et al., 1999). However, no overt difference was observed in the ability to convert ethanol into ACA between male and female myocytes.

3.2. Effects of ethanol on peak cell shortening

Our earlier study has revealed that cardiac overexpression of ADH transgene augments the ethanol-induced cardiac depression (Duan et al., 2002). To determine if there is a gender difference in the ADH transgene-induced augmented cardiac depression, mechanical properties were evaluated in myocytes exposed to different concentrations of ethanol (0–640 mg/dl) for 60 min. As shown in Fig. 2, the baseline (0 mg/dl ethanol) PS was similar in ventricular myocytes from FVB and ADH groups of either gender, suggesting that the ADH transgene itself was not innately harmful to the ventricular mechanical function. Ethanol caused an appreciable decrease in the extent of cell shortening (PS) in both FVB and ADH mice of either gender. The threshold of inhibition was between 80 and 120 mg/dl in all groups studied. Myocytes from male and female FVB mice displayed similar degree of inhibition induced by ethanol at 120, 240, and 640 mg/dl. ADH transgene did not induce any significant effect in ethanol-induced inhibition compared to that of the FVB group in male mice. However, the ADH transgene exacerbated the ethanol-induced depression of PS in female mice. The ethanol-induced depression of PS is also significantly greater in female ADH mice than the male ADH mice.

3.3. Effects of ethanol on maximal velocity and duration of shortening and relengthening

The baseline maximal velocity of shortening and relengthening (±dL/dt) was similar in ventricular myocytes from FVB and ADH groups of either gender. Similar to its effect on PS, exposure of myocytes to ethanol for 60 min elicited a concentration-dependent depression of ±dL/dt in FVB and ADH mice of either gender. The threshold of inhibition for ±dL/dt was between 80 and 240 mg/dl. Unlike its effect on PS, ethanol elicited similar pattern of response between male and female myocytes in either FVB or ADH group. Ventricular myocytes from the ADH group also
Fig. 3. Effect of 60 min ethanol exposure (0–640 mg/dl) on (a) maximal velocity of shortening (+dL/dt) and (b) maximal velocity of relengthening (−dL/dt) in ventricular myocytes isolated from age-matched male and female FVB and ADH mice. Mean ± S.E.M., *P < 0.05 vs. baseline (0 mg/dl ethanol), n = 30 myocytes per mouse group.

displayed similar response to ethanol-induced inhibition compared to those of the FVB wild-type group (Fig. 3). The ethanol-induced attenuation of ±dL/dt in either FVB or ADH myocytes was not associated with altered duration of shortening (TPS) and relengthening (TR90) in response to ethanol exposure. It is worthy mentioning that the baseline TPS but not TR90 is significantly less in female ADH group compared to all other groups, demonstrating potential hazardous effect of the ADH transgene in certain contractile parameter(s) such as the sarco(endo)plasmic reticulum Ca2+ release in female hearts (Fig. 4).

4. Discussion

The scenario of this study stems from the fact that a gender-related difference exists in ethanol-induced cardiac depression, which may be associated with a higher ACA responsiveness with estrogen. ACA is being speculated as an ultimate candidate toxin for alcoholic cardiomyopathy following ethanol ingestion. Since acute ethanol exposure-induced cardiac contractile depression is often reminiscent of the cardiac dysfunctions following chronic ethanol intake (Thomas et al., 1994; Preedy et al., 1999), we used a short-term model of ethanol exposure (60 min) in the present study. Our major finding revealed that overexpression of ADH specifically in the hearts is likely to lead to equally enhanced cardiac ACA exposure in both male and female mice. However, augmented ethanol-induced depression in cardiac contractility (PS) was only observed in female mice. Different from its effect on peak cell shortening (PS), ADH transgene did not induce any gender-
related effects on any mechanical indices tested including $\pm dL/dt$, TPS and TR$_{50}$, following an acute ethanol exposure in vitro. These results suggested that, although ACA may not be a significant factor in the ethanol-induced cardiac depression in males, elevated ACA levels certainly deteriorates the cardiac contractility in females once it exceeds certain points.

Ethanol has been shown to directly disrupt myofibrillary morphology and depress myocardial contractility (Patel et al., 1997; Richardson et al., 1998). Depressed cardiac contractile function is the hallmark of ethanol-induced cardiomyopathy (Tepper et al., 1986; Capasso et al., 1991; Thomas et al., 1994; Ren and Brown, 2000). It has been reported that impaired intracellular Ca$^{2+}$ handling, such as decreases sarcoplasmic reticulum Ca$^{2+}$ uptake and binding, may be mainly responsible for altered cardiac contraction following ethanol exposure (Guarnieri and Lakatta, 1990; Danziger et al., 1991; Thomas et al., 1994). It has been postulated that inhibition of ethanol on Ca$^{2+}$ regulatory proteins such as Ca$^{2+}$ pumps and channels may play a role in the decreased cytosolic Ca$^{2+}$ concentration. However, whether these inhibitory effects were due to the direct action of ethanol or its metabolic products such as ACA is still debatable.

ACA is known to exert negative myogenic inotropic effects independent of cholinergic or purinergic mechanisms (Brown et al., 1999). This is further supported by our recent observations that cardiac overexpression of ADH, which exposes the heart to much more ACA, deteriorates the ethanol-induced cardiac contractile depression (Liang et al., 1999; Duan et al., 2002). The potential mechanism involved in the augmented cardiac depression in the ADH heart, likely attributed to ACA, is largely unknown. It may be postulated that ACA impairs the cardiac E–C coupling through one or more of the following mechanisms. First, patients with alcoholic cardiomyopathy possess circulating antibodies to cardiac ACA-protein adducts (Harcombe et al., 1995). Protein adducts are polypeptides bound with other molecules (usually reactive biochemicals), which either renders the protein inoperative or immunogenic. The formation of protein adducts may contribute to the pathogenesis of cardiomyopathy by evoking an immunological reaction against functional proteins (e.g. those involved in cardiac E–C coupling) or by causing the added proteins to be selectively targeted for more rapid degradation. Secondly, the ethanol-induced cardiac depressive actions may be attributed to changes in oxidant–antioxidant balance (McDonough, 1999), which is essential in normal cardiac function. ACA was shown to directly enhance free radical generation by its oxidation via aldehyde oxidase and/or xanthine oxidase, leading to accumulation of superoxide anions (Guerri et al., 1994). Metabolism of ACA through xanthine oxidase plays an important role in the production of oxidative stress in the heart and may be one of the mechanisms mediating cardiac pathology in alcoholism (Oei et al., 1986). Lastly, ACA may directly attenuate intracellular Ca$^{2+}$ mobilization and inhibit membrane voltage-dependent Ca$^{2+}$ channels (Morales et al., 1997; Ren et al., 1997; Brown et al., 1999). The blood ACA level may reach very high (mM) in certain populations, such as Asians and African Americans, due to lack or mutation of aldehyde dehydrogenase (Yoshida, 1992), validating the clinical relevance of cardiac toxicity of ACA and value of our ADH transgenic mice. It may be helpful to mention that earlier studies using metabolic inhibitors of ethanol or ACA to alter blood or organ ACA level were suffered from the nonspecific and ineffective features of the inhibitors, and the toxic effect of ACA (Preedy and Richardson, 1994).

Increased susceptibility of ethanol-induced cardiac dysfunctions in females is believed to be directly associated with estrogen. Although ethanol is one of the oldest known substances of abuse, the mechanism(s) whereby this compound causes cardiac dysfunction remain only partially understood, especially with the impact of estrogen. At present, little information exists regarding the cross-action of ethanol and estrogen on myocardial contractile function. The more severe alcoholic cardiomyopathy in female may be the result of a direct impact of estrogen on the interaction between ethanol and nitric oxide (NO) system. NO is an important messenger in the regulation of cardiac function. Several lines of evidence have suggested that NO mediated the adverse effect of ethanol (Wang and Pang, 1993). Interestingly, estrogen acts in a gender-specific way on vascular endothelial cells and other components of the vessel wall, enhancing the synthesis and release of NO (Venkov et al., 1999), therefore may directly potentiate the interaction between ethanol and NO. In addition, estrogen has been show to interact...
with the ACA metabolism, and may underscore, at least in part, the enhanced ACA toxicity en route to severe alcoholic cardiomyopathy in female subjects. The information gained from this study may be important clinically as well as socially in the development of treatment regimens that could hasten the reversal of many of the negative inotropic effects of ethanol exposure characteristic of alcoholic cardiomyopathy in women. In our study, only PS but not other mechanical parameters tested exhibited gender-differences following ethanol exposure. This discrepancy may be due to the intrinsic properties in myocardial function between male and female, such as estrogen-related disparity in contractile protein expression (Capasso et al., 1983). In addition, estrogen has been reported to affect myofilament Ca$^{2+}$ sensitivity without any significant change in the maximum myocardial contractility (Wattanapermpool and Reiser, 1999), which may directly involve acute ethanol exposure-induced cardiac contractile depression. Further study is still warranted to investigate the impact of gender-related intrinsic difference on ethanol- or ACA-induced cardiac contractile response.

In conclusion, data from the present study provides convincing evidence that elevated cardiac ACA exposure exaggerated acute ethanol exposure-induced cardiac contractile depression in female but not male ventricular myocytes, supporting a role of ACA in gender-related difference of alcoholic cardiomyopathy. The fact that only PS but not any other cardiac contractile parameters tested were exaggerated by acute ethanol exposure in female ADH mice indicated potential gender-related specificity in ethanol- or ACA-induced effects in cardiac contractile proteins. Future work with short- and long-term ethanol intake and the assessment of cardiac contractile protein function using this transgenic mouse line are warranted to better understand the precise interaction of female sex hormones such as estrogen with ACA in the development of alcoholic cardiomyopathy.

Acknowledgments

This research was supported in part by the Ronald McNair Program (LBE), a New Faculty Scholar Award from University of North Dakota and NIH 1R15AA13575-01 (JR).

References


