Basic Science and Experimental Studies

Ethanol-Associated Cardiomyocyte Apoptosis and Left Ventricular Dilation Are Unrelated to Changes in Myocardial Telomere Length in Rats

ANDREW R. RAYMOND, PhD, JASON BECKER, BScHons, ANGELA J. WOODIWISS, PhD, HENDRIK L. BOOYSEN, PhD, GAVIN R. NORTON, MD, PhD, AND RICHARD L. BROOKSBANK, PhD

Johannesburg, South Africa

ABSTRACT

Aim: The aim of this work was to determine whether ethanol-associated myocardial apoptosis and cardiac dilation are related to myocardial telomere shortening in rats.

Methods and Results: Sprague-Dawley (SD) rats received either drinking water with (ethanol: n = 19) or without (control: n = 19) 5% (v/v) ethanol ad libitum, for 4 months. Left ventricular (LV) dimensions and function (echocardiography and isolated perfused heart preparations), cardiomyocyte apoptosis (terminal deoxynucleotide transferase−mediated dUTP nick-end labeling), and leukocyte and myocardial telomere length (real-time polymerase chain reaction) were determined at the end of the study. Ethanol administration resulted in a marked increase in cardiomyocyte apoptosis (ethanol 0.85 ± 0.13% vs control 0.36 ± 0.06%; $P = .0021$) and LV dilation (LV end-diastolic diameter: ethanol 8.20 ± 0.14 mm vs control 7.56 ± 0.11 mm [$P = .0014$]; volume intercept at 0 mm Hg (V_0) of the LV end-diastolic pressure-volume relationship: ethanol 0.40 ± 0.03 mL vs control 0.31 ± 0.02 mL [$P = .020$]). However, there were no changes in systolic chamber function as indexed by LV endocardial fractional shortening or the slope of the LV systolic pressure-volume relationship (end systolic elastance). The percentage of myocardial apoptosis was correlated with the degree of LV dilation (% apoptosis vs LV EDD: $r = 0.39$; n = 38; $P = .021$; vs V_0: $r = 0.44$; n = 19; $P = .046$). No differences in leukocyte or cardiac telomere length were noted between the ethanol and control groups. Furthermore, cardiac telomere length was not associated with indexes of LV dilation (LVEDD and V_0) or cardiomyocyte apoptosis.

Conclusions: Chronic ethanol-associated myocardial apoptosis and adverse remodeling occurs independently from changes in cardiac telomere length. Telomere shortening may not be a critical mechanism responsible for cardiomyocyte apoptosis and adverse cardiac remodeling. (*J Cardiac Fail 2016;22:294−302*)

Key Words: Ethanol, LV dilation, cardiomyocyte apoptosis, telomere length.

A reduced leukocyte telomere length is associated with the risk, presence, and severity of cardiovascular disease.[1−6] Possible consequences of telomere attrition are cardiomyocyte apoptosis and premature senescence[7−10] and the repression of mitochondrial biogenesis.[11,12] Therefore, telomere attrition may be a fundamental mechanism responsible for the development of adverse cardiac remodeling (dilation) and dysfunction. Indeed, patients with chronic heart failure have a reduced average leukocyte telomere length,[3,5] and telomere length is associated with the severity of heart failure.[5] A decreased average leukocyte telomere length predicts deaths and hospitalization in patients with heart failure.[13] Moreover, in the very elderly, a shorter leukocyte telomere length is related to a reduced left ventricular ejection

From the Cardiovasular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Manuscript received February 12, 2015; revised manuscript received April 7, 2015; revised manuscript accepted June 15, 2015.

Reprint requests: Richard L. Brooksbank, PhD, Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193, Johannesburg, South Africa. Tel: +27 11 717 2363; Fax: +27 11 643 2765. E-mail: richard.brooksbank@wits.ac.za

Funding: National Research Foundation and Faculty Research Committee of the University of the Witwatersrand.

See page 300 for disclosure information.

1071-9164/$ - see front matter

http://dx.doi.org/10.1016/j.cardfail.2015.06.013

fraction.[14] However, several studies suggest that leukocyte or myocardial telomere length is not associated with adverse cardiac remodeling or dysfunction.[15,16] Whether telomere length in these studies was similarly unrelated to cardiomyocyte apoptosis was not evaluated. The maintenance of telomere length has the potential to delay the activation of apoptotic pathways.[17,18] Because cardiomyocyte apoptosis is thought to be an important mechanism explaining adverse cardiac effects,[19] whether myocardial telomere shortening accounts for cardiac pathology associated with the activation of apoptotic pathways requires further study.

Chronic alcohol consumption promotes cardiomyocyte apoptosis.[20–25] Through mitochondrial dysfunction,[23] and oxidative stress,[24,26,27] cardiomyocyte apoptosis could potentially be a factor involved in alcohol-associated left ventricular (LV) dilation, hypertrophy, myocardial wall thinning, and ventricular dysfunction.[28–30] Reduced leukocyte telomere length has been demonstrated in individuals consuming excessive alcohol,[31] and as such, telomere-induced myocardial apoptosis may play a role in alcoholic cardiomyopathy. To further elucidate the importance of relationships between telomere length and cardiac disease, we therefore aimed to determine whether myocardial telomere attrition is associated with cardiomyocyte apoptosis and adverse LV remodeling or dysfunction following chronic alcohol administration to rats.

Methods

Animal Model

This study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Ethics no. 2011/49/24). Thirty-eight male Sprague-Dawley rats were studied. The experimental group (n = 19) received drinking water with 5% (v/v) ethanol and the control group (n = 19) received normal water for a 4-month period. No pair feeding was performed and no micro- or macronutrient enrichment was provided, because a previous study[32] demonstrated that 5% v/v ethanol in the drinking water has no effect on food or fluid intake but is still capable of producing ultrastructural changes in the myocardium. Indeed, in the present study, as assessed over a 7-day period, in a subset of rats from each group, food intake (ethanol group 24.6 g/d [n = 5] vs control group 23.9 g/day [n = 10]) did not differ between rats receiving or not receiving ethanol. Similarly, fluid intake assessed throughout the study did not differ between rats receiving or not receiving ethanol (ethanol group 39.1 mL/d [n = 19] vs control group 42.8 mL/d [n = 19]). Importantly, in this study, with the use of the aforementioned approach to ethanol administration, rats consumed on average (±SD) 3.2 ± 0.1 g ethanol/kg body weight per day. Accounting for the 2.235-times faster ethanol metabolism in rats compared with humans,[33] this equates to the average ethanol intake above which alcoholic cardiomyopathy has been reported to occur in humans (>90 g ethanol per day, or assuming an average body weight of 70 kg, 1.29 g ethanol/kg body weight per day).[29]

Echocardiography

Echocardiography was performed in all rats with the use of a 7.5-MHz linear array transducer and an Acuson Cypress echocardiograph as previously described.[34,35] Echocardiography was performed after 18 hours of fasting to assess the chronic as opposed to the acute cardiac depressant effects of ethanol.[36] LV chamber dimensions and posterior wall thickness at end-diastole and end-systole were measured in anesthetized rats by means of 2-dimensional targeted-M-mode echocardiography 15 minutes after an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg). All LV dimensions were determined with the use of the American Society for Echocardiography leading edge method.[37] Chamber dimensions and LV relative wall thickness at end-diastole were used to estimate the extent of LV dilation and wall thinning, and LV dimensions during systole and diastole were used to calculate LV midwall (FS_{mid}) and endocardial (FS_{end}) fractional shortening as previously described.[34,35] Relative wall thickness was calculated as [(2 × LV end-diastolic posterior wall thickness [EDPWT])/LV end-diastolic diameter (LV EDD)]. FS_{mid} was calculated from the following formula: [(LV EDD + LV EDPWT) − (LV end-systolic diameter [ESD] + LV end systolic posterior wall thickness [ESPWT])]/(LVEDD + LV EDPWT) × 100.[34,35] FS_{end} was determined from: [(LV EDD − LV ESD)/LV EDD] × 100.[34,35] Values for FS_{mid} and FS_{end} represent LV intrinsic myocardial and chamber systolic function respectively.

Isolated Perfused Heart Preparations

To assess LV dimensions and systolic chamber and intrinsic myocardial function independently from the effects of loading conditions and heart rate, 10 rats from each of the experimental and control groups had LV dimension and function measurements determined with the use of isolated perfused heart preparations as previously described.[34,35,38] Briefly, hearts were removed via thoracotomy and rinsed in ice-cold physiologic solution (118.0 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, and 10.0 mmol/L glucose). The hearts were then immediately mounted on a perfusion apparatus and retrogradely perfused through the aorta at a constant rate with the physiologic solution, which was warmed to 37°C and saturated with 95% O_2 and 5% CO_2. Platinum wire electrodes were placed on the right atrium and at the apex of the heart and were used to pace the hearts at a constant rate of 330 beats/min. Left ventricular systolic and diastolic pressures were measured with the use of a pressure transducer and recorded on a Hellige polygraph. LV volumes were manipulated with the use of a water-filled balloon-tipped cannula coupled to the pressure transducer and inserted into the LV cavity via the left atrium. A micromanipulator was used to increase the volume of the water-filled balloon and thus LV volumes until there were no further increases in LV developed pressures (difference between systolic and diastolic pressures). This technique was performed to determine cardiac function at controlled heart rates, loading conditions, and without any interference from anesthetic agents. LV diastolic remodeling was assessed from the LV diastolic pressure-volume (P-V) relationships, using the volume intercept at zero pressure (V_0) for comparative purposes, with an increased V_0 representing an increased LV chamber size in diastole.[34,35] A load-independent measure of LV systolic chamber function was determined from the slope of the linear portion of the LV systolic P-V relationship (LV end-systolic elastance [E_{es}]). A load-independent measure of intrinsic myocardial systolic function was assessed by constructing LV developed stress-strain relationships and comparing the slopes (LV end-systolic