Journal of Molecular and Cellular Cardiology 59 (2013) 30–40

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Chronic ethanol consumption increases cardiomyocyte fatty acid uptake and decreases ventricular contractile function in C57BL/6J mice

Chunguang Hu [a,1], Fengxia Ge [a,1], Eiichi Hyodo [b], Kotaro Arai [b], Shinichi Iwata [b], Harrison Lobdell IV [a], José L. Walewski [a], Shengli Zhou [a], Robin D. Clugston [c], Hongfeng Jiang [c], Cynthia P. Zizola [b], Kalyani G. Bharadwaj [c], William S. Blaner [c], Shunichi Homma [b], P. Christian Schulze [b], Ira J. Goldberg [c], Paul D. Berk [a,c,*]

[a] The Department of Medicine, Division of Digestive & Liver Disease, Columbia University College of Physicians & Surgeons, New York, NY 10032, USA
[b] The Department of Medicine, Division of Cardiology, Columbia University College of Physicians & Surgeons, New York, NY 10032, USA
[c] The Department of Medicine, Division of Preventive Medicine, Columbia University College of Physicians & Surgeons, New York, NY 10032, USA

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 1 February 2013
Accepted 2 February 2013
Available online 16 February 2013

Keywords:
Ethanol consumption
Fatty acid transport
PGC-1α
Cardiac function
ATP
Gene expression

ABSTRACT

Alcohol, a major cause of human cardiomyopathy, decreases cardiac contractility in both animals and man. However, key features of alcohol-related human heart disease are not consistently reproduced in animal models. Accordingly, we studied cardiac histology, contractile function, cardiomyocyte long chain fatty acid (LCFA) uptake, and gene expression in male C57BL/6J mice consuming 0, 10, 14, or 18% ethanol in drinking water for 3 months. At sacrifice, all EtOH groups had mildly decreased body and increased heart weights, dose-dependent increases in cardiac triglycerides and a marked increase in cardiac fatty acid ethyl esters. [^{3}H]-oleic acid uptake kinetics demonstrated increased facilitated cardiomyocyte LCFA uptake, associated with increased expression of genes encoding the LCFA transporters CD36 and Slc27a1 (FATP1) in EtOH-fed animals. Although SCD-1 expression was increased, lipidomic analysis did not indicate significantly increased de novo LCFA synthesis. By echocardiography, ejection fraction (EF) and the related fractional shortening (FS) of left ventricular diameter during systole were reduced and negatively correlated with cardiac triglycerides. Expression of myocardial PGC-1α and multiple downstream target genes in the oxidative phosphorylation pathway, including several in the electron transport and ATP synthase complexes of the inner mitochondrial membrane, were down-regulated. Cardiac ATP was correspondingly reduced. The data suggest that decreased expression of PGC-1α and its target genes result in decreased cardiac ATP levels, which may explain the decrease in myocardial contractile function caused by chronic EtOH intake. This model recapitulates important features of human alcoholic cardiomyopathy and illustrates a potentially important pathophysiologic link between cardiac lipid metabolism and function.

1. Introduction

Excessive chronic alcohol ingestion causes both histopathologic and functional changes in multiple organs, especially in the liver, heart, and pancreas, and can result in serious or even fatal illness. Chronic, excessive alcohol consumption has been reported to be the most frequent identifiable cause of heart muscle disease [1], and has been shown to decrease the contractility of heart muscle in both animals and humans [2]. Its classical clinical cardiac consequence is a dilated cardiomyopathy, which can lead to congestive heart failure and even to death. However, key features of human heart disease caused by chronic alcohol intake, including congestive heart failure, are not reproducibly recapitulated in animal models [2]. Chronic alcohol ingestion, for example, has not heretofore been reported to produce a cardiomyopathy in wild type mice.

Many cellular and molecular mechanisms of injury have been proposed as the basis for alcoholic cardiomyopathy, including apoptosis [3], mitochondrial dysfunction [4], acetaldehyde protein adduct formation [5], oxidant stress [6–8], and imbalance between protein catabolism and protein synthesis [8–10]. However, intervening steps between exposure of cardiac muscle to ethanol (EtOH) and initiation of the cascade of responses leading to decreased cardiac contractility and output remain poorly understood.

Long chain fatty acids (LCFA), a key cardiac energy source, are taken up by cardiomyocytes via facilitated transport mechanisms [11–15] and either utilized rapidly for β-oxidation in mitochondria or re-esterified and stored as cytosolic triglycerides (TG). In well-fed animals this usually does not involve formation of obvious lipid droplets detectable by light microscopy, however such droplets may be seen in otherwise healthy animals after a prolonged fast [16,17]. While TG accumulation

* Corresponding author at: Divisions of Digestive & Liver Diseases and Preventive Medicine, Columbia University Medical Center, William Black Building 1006A, 650W 168 St, New York, NY 10032, USA. Tel.: +1 212 305 4491; fax: +1 646 426 0060.
E-mail address: pb2158@columbia.edu (P.D. Berk).
[1] The contributions of Dr. Hu and Dr. Ge to this study were equivalent.

0022-2828/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.yjmcc.2013.02.005

in cardiomyocytes in alcoholic cardiomyopathy has been described in both patients and some animal models [18], this report is the first description and functional characterization of an alcohol-induced cardiomyopathy in wild-type, genetically unmodified mice. As such, it provides a valuable model for additional, future investigations.

Our laboratory has long had an interest in LCFA transport mechanisms [19,20] and their roles in the pathogenesis of disease states such as obesity [21–23] and its co-morbidities, such as hepatic steatosis [24] and obesity-associated cardiomyopathy [25]. Increased facilitated LCFA uptake was found to be a key process responsible for *hepatic* steatosis both in mouse models of obesity and in the presence of chronic EtOH consumption [24]. LCFA uptake is also important in the development of obesity-associated cardiomyopathy in mice [25]. In the present studies we applied kinetic methods developed to quantify cellular LCFA uptake in these earlier studies to cardiomyocytes from chronically EtOH-fed mice, used gene expression methods to examine the effects of EtOH on other putative mechanisms which could contribute to TG accumulation, and examined quantitative relationships among EtOH intake, cardiomyocyte LCFA uptake, TG accumulation, and cardiac function. The results suggest that increased facilitated LCFA uptake is a major contributing factor to the increased myocardial lipid accumulation that occurs with chronic EtOH consumption, and suggest possible ways by which this could lead to decreased cardiac function.

2. Materials and methods

2.1. Mice and diet

Six-week-old male C57BL/6J mice, purchased from Jackson Laboratories (Bar Harbor, ME), were maintained in a temperature-controlled facility with a 12-h light: dark cycle, with free access to water and to a standard chow diet (LabDiet 5001, PMI, St. Louis, MO). After a two week acclimatization period, the control and EtOH groups were created by random separation of mice from the same lot. EtOH at 10% (v/v) was provided in the drinking water as the sole water source for the first 4 weeks for all mice designated for EtOH feeding. In a randomly selected subset of these animals 10% EtOH was replaced with 14% EtOH after 4 weeks (E14 group), and in a subset of these, 14% EtOH was replaced with 18% EtOH after a further 4 weeks (E18). Age-matched controls were given the same distilled water as that used for mixing the EtOH solutions. Mice were sacrificed after a total of 12 ± 1 weeks of treatment. Thus, out of the 12-week EtOH feeding period, the E10 group received 10% EtOH for the full 12 weeks, those designated as E14 received that dose for 8 weeks, and those designated as E18 EtOH received that dose for 4 weeks. This protocol was necessitated by the fact that C57BL/6J mice will initially drink EtOH consistently only at concentrations of $\leq$10%. After 4 weeks on a 10% regimen they will readily consume 14% EtOH, and after 4 weeks at that concentration, they can be switched successfully to 18% EtOH. In short, a conditioning period was needed to successfully achieve each increment in EtOH consumption. Given these gustatory preferences of the mice, there was no way to design a study in which duration of EtOH intake was equivalent at each of the 3 EtOH doses.

Metabolic cages were not available for this study. Animals were housed individually in standard plastic cages. Weights were recorded weekly, as was consumption of water or water/EtOH and food, until the final week before sacrifice when they were measured daily. The daily measurements of both food and water or water:EtOH consumption were highly reproducible both in individual animals and within treatment groups, as indicated by the standard errors recorded in Table 2. During that final week the blood alcohol concentration (BAC) was measured in plasma separated from tail vein blood samples obtained between midnight and 1 AM, using a NAD-alcohol dehydrogenase reagent (Sigma-Aldrich, St Louis, MO), as previously described [26]. Mice were euthanized at 20 ± 1 weeks of age after an overnight (12 h) fast. All applicable institutional and governmental regulations concerning ethical use of animals were followed during this research. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University Medical Center.

2.2. Tissue harvesting and determination of cardiac tissue triglyceride and cholesterol

At sacrifice hearts were removed and weighed. Portions of each were placed in neutral buffered formalin for hematoxylin and eosin (H&E) staining; or embedded in OCT compound (Tissue-Tek, Sakura Finetek USA, Inc., Torrance, CA), frozen on dry ice and stored at −80 °C for future oil red O (ORO) staining. The remainder was fixed in glutaraldehyde for electron microscopy (see below), preserved in RNAlater (Qiagen, Carlsbad, CA) at −80 °C for subsequent qRT-PCR studies, or weighed and homogenized in PBS for biochemical studies. The total tissue protein content was determined from the homogenate with the BCA™ protein analysis kit (Thermo Scientific, Rockford, IL), and triglyceride and cholesterol contents were determined, after Folch extraction, with Wako kits (Cholesterol E and L-Type TG H) according to the manufacturer's instructions.

2.3. Blood and serum analysis

Whole blood glucose was measured with a glucose meter (One-Touch, LifeScan, Inc., Milpitas, CA) in tail vein samples obtained before induction of anesthesia. The concentrations of serum free fatty acids, triglycerides (TG), total cholesterol, ALT and AST were measured on blood samples obtained from the inferior vena cava during anesthesia, using commercial kits as previously reported [24,25].

2.4. Lipid analyses

In addition to the kit assays used for serum free fatty acids and TG, noted above, high-sensitivity liquid chromatography/tandem mass spectrometry (LC/MS/MS) and liquid chromatography/mass spectrometry (LC/MS) were used to study the cardiac content of selected lipids of interest in the hearts of both control and 18% EtOH-fed mice. The methods for tissue handling, sample preparation and LC/MS/MS or LC/MS employed in this study were identical to those reported previously in an analogous study of EtOH-induced fatty liver [26]. By using specific internal standards, this method allows the quantification of 62 defined lipid species. The results presented below focus on specific intermediates of LCFA metabolism. A report on the effects of EtOH on the total myocardial lipidome will be published separately.

2.5. Cardiac ATP assay

For the analysis of ATP concentrations, hearts were removed immediately after sacrifice and quickly frozen in liquid nitrogen. Cardiac tissue samples (20 mg) were subsequently homogenized in 10 volumes of 0.25% trichloroacetic acid/2 mM EDTA using 1.0 mm Zirconia/Silica beads (BioSpec Products). Homogenates were spun at 4500 rpm for 10 min at 4 °C. The supernatant was diluted 1:10 with 250 mM Tricine buffer, pH 7.8, 50 mM MgSO4, 1 mM EDTA and 1 mM sodium azide. ATP concentrations in the diluted supernatants were determined using a firefly luciferase bioluminescence based ATP assay kit (Invitrogen) [27], following the manufacturer's instructions. Corresponding protein concentrations were determined with the BCA™ protein assay kit.

2.6. Histologic estimation of tissue neutral lipids

Histological images of oil red O (ORO)-stained cardiac sections were observed at 250× with a Nikon Eclipse 80i microscope and captured with a Nikon Digital DXM 1200C camera using standard exposure settings for all photographs. Semi-quantitative estimates of neutral lipids