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Progressive white matter atrophy with altered lipid profiles is partially reversed by short-term abstinence in an experimental model of alcohol-related neurodegeneration

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ABSTRACT

Chronic ethanol exposure causes white matter (WM) atrophy and degeneration with major impairments in the structural integrity of myelin. Since myelin is composed of oligodendrocyte lipid-rich membranes, understanding the consequences and reversibility of alcohol-related oligodendrocyte dysfunction in relation to myelin structure could provide new insights into the pathogenesis of WM degeneration and potential strategies for treatment. Adult male Long Evans rats were pair-fed with isocaloric liquid diets containing 0% or 26% ethanol (caloric) for 3 or 8 weeks. During the last 2 weeks of feeding, the ethanol groups were binged with 2 g/kg of ethanol by intraperitoneal (i.p.) injection on Mondays, Wednesdays, and Fridays; controls were treated with i.p. saline. For recovery effects, at the 6-week time point, ethanol exposures were tapered over 2 days, and then discontinued, rendering the rats ethanol-free for 12 days. Anterior corpus callosum WM lipid ion profiles were analyzed using matrix-assisted laser desorption ionization-imaging mass spectrometry (MALDI-IMS) and correlated with histopathology. Ethanol exposures caused progressive atrophy and reductions in myelin staining intensity within the corpus callosum, whereas short-term recovery partially reversed those effects. MALDI-IMS demonstrated striking ethanol-associated alterations in WM lipid profiles characterized by reduced levels of phosphatidylinositols, phosphatidylserines, phosphatidylethanolamines, and sulfatides, and partial "normalization" of lipid expression with recovery. Ethanol exposure duration and recovery responses were further distinguished by heatmap hierarchical dendrogram and PCA plots. In conclusion, chronic+binge ethanol exposures caused progressive, partially reversible WM atrophy with myelin loss associated with reduced expression of WM phospholipids and sulfatides. The extent of WM lipid abnormalities suggests that ethanol broadly impairs molecular and biochemical functions regulating myelin synthesis, degradation, and maintenance in oligodendrocytes.

Introduction

Chronic heavy alcohol abuse leads to disproportionate atrophy of white matter (WM) (S. M. de la Monte, 1988; C. G. Harper, Kril, & Holloway, 1985) with cognitive impairment characterized mainly by loss of executive functions (Chanraud et al., 2007), which can progress to dementia and disability (Li, 2008; Schmidt et al., 2005). Neuroimaging and postmortem studies have shown that severities of WM atrophy and degeneration correlate with maximum daily and lifetime alcohol exposures (S. M. de la Monte & Kril, 2014; C. Harper, Dixon, Sheedy, & Garrick, 2003). Despite its diffuse nature, alcohol-related brain degeneration (ARBD) most prominently targets the corpus callosum, and prefrontal, temporal, and cerebellar WM (S. M. de la Monte & Kril, 2014; Phillips, Harper, & Kril, 1987), indicating that the distribution of injury is non-random. Studies have focused on corpus callosum atrophy in ARBD (Chanraud et al., 2007; Estruch et al., 1997; Pfefferbaum, Lim, Desmond, & Sullivan, 1996; Pfefferbaum, Rosenbloom, Adalsteinsson, & Sullivan, 2007); damage to the corpus callosum disrupts inter-hemispheric communication,

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compromising exchange of sensory, motor, and cognitive information. Diffusion Tensor Imaging revealed that corpus callosum atrophy correlates with altered WM micro-structural integrity (Pfefferbaum, Adalsteinsson, & Sullivan, 2006; Schulte, Sullivan, Müller-Oehring, Adalsteinsson, & Pfefferbaum, 2005). These observations are supported by recent *in vivo* studies showing that ARBD-associated WM atrophy is mediated by combined effects of demyelination, dysmyelination, and axonal degeneration (Papp-Peka, Tong, Kril, de la Monte, & Sutherland, 2016). However, clinical studies provide some evidence that abstinence can partially reverse WM pathology in humans (Bartsch et al., 2007; Estilaei et al., 2001; Gazdzinski, Durazzo, Mon, Yeh, & Meyerhoff, 2010; Monnig, Tonigan, Yeo, Thoma, & McCrady, 2013). Therefore, improved understanding of its pathogenesis could lead to novel therapeutic interventions that reduce the burden of ARBD-related cognitive impairment, dementia, and disability.

Despite the wealth of information about ethanol's adverse effects on WM, the underlying basis of its degeneration in ARBD is not well understood, in part because the tools needed to study WM myelin have not been widely accessible until recently. WM myelin is a major component of the oligodendrocyte membrane that functions to insulate nerve fibers and thereby support neurotransmission. Myelin is largely composed of lipids (70–85% of its dry weight), including cholesterol, sphingolipids, and phospholipids (Schmitt, Castelvetri, & Simons, 2015). Sphingolipids (sphingomyelins, cerebrosides, and sulfatides) are located in the extracellular membrane leaflet and have a major role in myelin formation and maintenance and neuronal plasticity (Honke, 2013; Schmitt et al., 2015; Takahashi & Suzuki, 2012). Sphingolipids, together with cholesterol, form membrane microdomains (lipid rafts) that regulate membrane fluidity, protein trafficking, and signal transduction (Korade & Kenworthy, 2008). Phospholipids (phosphatidylserines, phosphatidylethanolamines, and phosphatidylinositols) are localized along the inner cytosolic leaflet exposed to the cell surface, and function by regulating intracellular signaling and membrane trafficking (Di Paolo & De Camilli, 2006; Fernandis & Wenk, 2007; Van Meer, Voelker, & Feigenson, 2008). Given these varied and critical functions of myelin lipids, specialized analytical approaches are needed to gain a better understanding of how alcohol compromises WM integrity. Fortunately, recent advances in lipidomics now enable efficient *in situ* characterization of myelin lipid profiles in relation to disease, including experimental ARBD (Yalcin, Nunez, Tong, & de la Monte, 2015), without the need to generate tissue extracts (Roux et al., 2016, 2015; Wang, Jackson, Post, & Woods, 2008). We hypothesize that progressive ethanol-mediated WM atrophy and degeneration are associated with major alterations in myelin lipid composition, reflecting oligodendrocyte dysfunction, and that abstinence-induced recovery and restoration of WM integrity will at least in part normalize myelin lipid profiles. This study utilized matrix-assisted laser desorption ionization-imaging mass spectrometry (MALDI-IMS) to address this hypothesis in an experimental chronic+binge ethanol exposure model with a subgroup evaluated after short-term recovery.

Methods

Experimental model

The use of experimental animals in this research was approved by the Institutional Animal Care and Use Committee (IACUC) at the Lifespan/Rhode Island Hospital. The protocols followed guidelines established by the National Institutes of Health. Rats were housed under standardized conditions with 12-h light/dark cycles and controlled temperature (70–74 °F). The chronic+binge ethanol exposure model was used because it is an accepted approach for producing alcohol-related diseases that more closely mimic human pathology (Bertola, Mathews, Ki, Wang, & Gao, 2013; Bertola, Park, & Gao, 2013), including WM degeneration and cognitive impairment (Tong, Yu, Deochand, & de la Monte, 2015; Tong et al., 2015). Male 6-week-old Long Evans rats were pair-fed isocaloric liquid diets containing 0% or 26% ethanol by caloric content (0 or 6% v/v) for 3 or 8 weeks (n = 6/group). During the last 2 weeks of ethanol feeding, the ethanol groups were binged with 2 g/kg of ethanol by intraperitoneal (i.p.) injection on Mondays, Wednesdays, and Fridays; controls were treated with i.p. saline. To study the effects of abstinence/recovery, an additional six rats were subjected to 6 weeks of chronic+binge ethanol exposures, followed by tapering of ethanol to 0% over a 2-day period, after which the rats were rendered ethanol-free for 12 days.

On the morning of sacrifice, 30 min after binge exposure, peak blood alcohol concentrations were measured using an Analox GM7 analyzer (Analox Instruments, MA, USA). Rats were sacrificed by isoflurane inhalation and their brains were harvested immediately. A standardized 3-mm thick coronal slice that flanked the infundibulum was frozen and stored at −80 °C for later MALDI-IMS studies, and the adjacent posterior 3-mm slice was fixed in 10% neutral buffered formalin for paraffin embedding. Histological sections (8-μm thick) were stained with Luxol fast blue hematoxylin and eosin (LHE).

MALDI-IMS

Detailed methods have been described in previous reports (Yalcin & de la Monte, 2015). In brief, cryo-sections (8-μm thick) were thaw-mounted onto indium tin oxide (ITO)-coated slides (Delta Technologies, Loveland, CO), rinsed with 50-mM ammonium formate buffer (pH 6.4), and sublimed with 5-dehydroxybenzoic acid (DHB; Sigma-Aldrich Co, St. Louis, MO). Adjacent sections stained with LHE were used to determine the region of interest (ROI) for imaging data acquisition. MALDI-IMS was performed with MALDI-time-of-flight (TOF/TOF) Ultraflextreme mass spectrometer (Bruker Daltonics, Billerica, MA) in the negative ion mode by focusing a Smartbeam II Nd:YAG laser onto ~100-μm^2 areas in corpus callosum WM. After determining that the within-group variability was low, four brains per group were used for IMS data acquisitions. The spectra from three datasets were combined for data reduction and further analysis in ClinProTools. External mass calibration was performed using 1 μL of a mixture of standard peptides (Peptide Calibration Standard II, Bruker Daltonics) and matrix (α-Cyano-4-hydroxycinnamic acid [HCCA]; Bruker Daltonics) deposited onto the slide after sublimation. This mixture provides seven calibration points in the mass range between 377 and 2463 Da and allows mass accuracy for phospholipids and sphingolipids.

Data analysis

MALDI-IMS data normalization to total ion count and visualization was performed with FlexImaging v4.0 software. Imaging spectra were processed by ClinProTools v3.0 (Bruker Daltonics). Each spectrum was baseline corrected, recalibrated, and a peak picking procedure was applied for further statistical analysis. Lipid identification was achieved by comparing mass-to-charge (m/z) values of precursor and product ions obtained by tandem mass spectrometry (MS/MS) analysis with those cataloged in the LIPIDMAPS database. When MS/MS was inconclusive for lipid structure assignments, identification was made based on previously published reports. The average intensity of lipid ions per ROI was used in R-generated heatmaps and data barplots (version 3.2) to compare time course and recovery responses of ethanol relative to control samples. Barplots were generated in R using the ggplot2