Alcohol 67 (2018) 51–63

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Imaging mass spectrometry of frontal white matter lipid changes in human alcoholics

Suzanne M. de la Monte [a, b, c, d, *], Jared Kay [a, d], Emine B. Yalcin [a, d], Jillian J. Kril [e], Donna Sheedy [e], Greg T. Sutherland [e]

[a] Department of Pathology, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[b] Department of Neurology, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[c] Department of Neurosurgery, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[d] Department of Medicine, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI, USA
[e] Discipline of Pathology, Sydney Medical School, University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 20 February 2017
Received in revised form
28 June 2017
Accepted 8 August 2017

Keywords:
Alcohol-use disorder
White matter
Neurodegeneration
Mass spectrometry
Human
Sphingolipids
Phospholipids
Imaging

ABSTRACT

Background: Chronic alcohol use disorders (AUD) are associated with white matter (WM) degeneration with altered myelin integrity. Matrix assisted laser desorption ionization-imaging mass spectrometry (MALDI-IMS) enables high throughput analysis of myelin lipid biochemical histopathology to help characterize disease mechanisms.
Purpose: This study utilized MALDI-IMS to investigate frontal lobe WM myelin lipid abnormalities in AUD.
Methods: Standardized cores of formalin-fixed WM from Brodmann Area 4 (BA4) and BA8/9 of 20 postmortem AUD and 19 control adult human brains were embedded in carboxymethyl-cellulose, cryosectioned (8 μm), thaw-mounted onto indium tin oxide (ITO) -coated glass slides, and sublimed with 2,5-dihydroxybenzxoic acid (DHB) matrix. Lipids were imaged by MALDI-time of flight in the negative ionization mode. Data were visualized with FlexImaging software v4.0 and analyzed with ClinProTools v3.0.
Results: Principal component analysis (PCA) and data bar plots of MALDI-IMS data differentiated AUD from control WM. The dominant effect of AUD was to broadly reduce expression of sphingolipids (sulfatides and ceramides) and phospholipids. Data bar plots demonstrated overall similar responses to AUD in BA4 and BA8/9. However, differential regional effects of AUD on WM lipid profiles were manifested by non-overlapping expression or discordant responses to AUD for a subset of lipid ions.
Conclusions: Human AUD is associated with substantial inhibition of frontal lobe WM lipid expression with regional variability in these effects. MALDI-IMS can be used to characterize the nature of AUD-associated lipid biochemical abnormalities for correlation with lifetime exposures and WM degeneration, altered gene expression, and responses to abstinence or treatment.

Introduction

In adults, chronic alcohol abuse causes brain atrophy (Harper, 1982) with selective loss of white matter (WM) (de la Monte, 1988) and impairments in executive function (Chanraud et al., 2007). Degrees of WM atrophy correlate with maximum daily and lifetime alcohol exposures (de la Monte & Kril, 2014; Harper, Dixon, Sheedy, & Garrick, 2003; Sutherland et al., 2013). Neuroimaging studies showed that the corpus callosum is a vulnerable target of atrophy in people with alcohol use disorders (AUD) (Estruch et al., 1997; Pfefferbaum, Rosenbloom, Adalsteinsson, & Sullivan, 2007). Other notable targets of neurodegeneration in AUD include frontal, temporal, and cerebellar WM (de la Monte & Kril, 2014; Kril & Halliday, 1999; Phillips, Harper, & Kril, 1987). Diffusion tensor imaging studies predict that the underlying basis of atrophy is disruption of WM micro-structural integrity (Pfefferbaum, Adalsteinsson, & Sullivan, 2006; Schulte, Sullivan, Muller-Oehring, Adalsteinsson, & Pfefferbaum, 2005).

* Corresponding author. Pierre Galletti Research Building, Rhode Island Hospital, 55 Claverick Street, Room 419, Providence, RI 02903, USA.
E-mail address: Suzanne_DeLaMonte_MD@Brown.edu (S.M. de la Monte).

https://doi.org/10.1016/j.alcohol.2017.08.004
0741-8329/

Cerebral WM is largely composed of myelin, a lipid-rich membrane synthesized and maintained by oligodendrocytes. Wrapping of oligodendrocyte myelin sheaths around central nervous system (CNS) axons enables rapid and efficient neuroconductivity. Correspondingly, loss of myelin or impaired myelin homeostasis leads to deficits in CNS functions, including cognition. Major CNS WM lipids include cholesterol, glycosphingolipids, (i.e., cerebrosides galactosylceramide, galactocerebroside), sulfatides (sulfated galactocerebroside, sulfogalactosylceramide) and gangliosides, and phospholipids, consisting of glycerophospholipids [phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS) and plasmalogens] and sphingomyelin (Quarles, Macklin, & Morell, 2006). Sphingomyelin is composed of ceramide plus a phosphocholine or phosphoethanolamine polar head group (Quarles et al., 2006).

Abnormal metabolism and expression of phospholipids and sulfatides occur in a broad range of CNS diseases (Takahashi & Suzuki, 2012), including experimental alcohol-mediated WM degeneration (Roux et al., 2015; Yalcin, Nunez, Tong, & de la Monte, 2015). The mechanisms and consequences of aberrant myelin lipid expression are not well understood. However, some effects can be predicted based on specific functions of major lipid subtypes. Because membrane phospholipids regulate lipid rafts and receptor functions, their deficiencies could lead to impairments in intracellular signaling. Sulfatides, localized on the extracellular leaflet of myelin plasma membranes and synthesized by oligodendrocytes (Vos, Lopes-Cardozo, & Gadella, 1994) through sulfonation of galactocerebroside, regulate neuronal plasticity, memory, myelin maintenance, protein trafficking, adhesion, glial-axonal signaling, insulin secretion, and oligodendrocyte survival (Takahashi & Suzuki, 2012). Correspondingly, reductions in membrane sulfatide disrupt myelin sheath structure and function, and compromise neuronal conductivity (Kolesnick & Krönke, 1998). Sulfatide degradation via increased galactosylceramidase, sulfatidase, or aryl sulfatase activities yields ceramides (Eckhardt, 2008; Sundaram, Fan, & Lev, 1995; Vos et al., 1994) that promote neuroinflammation, reactive oxygen species formation, apoptosis, and dysregulated signaling through cell survival and metabolic pathways (Kolesnick & Krönke, 1998).

Despite abundant information about ethanol's adverse effects on WM, details about the biochemical nature of degeneration have not been well characterized due to the lack of suitable tools to efficiently study pathologic alterations in lipid-rich myelin. Fortunately, over the past several years, major advances in technology and computational science have facilitated extension of Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI-IMS) to human research. MALDI-IMS is used for *in situ* imaging of lipids, proteins, and adducts for correlation with histopathology and molecular pathology (*in situ* hybridization and immunohistochemistry) (Caprioli, Farmer, & Gile, 1997). Instruments equipped with an Nd:YAG Smartbeam laser enable time of flight (TOF; m/z) analysis for specific identification of molecules (Yalcin & de la Monte, 2015). For this study, we utilized MALDI-IMS to characterize AUD-associated alterations in frontal lobe WM lipid ion profiles in human postmortem brains.

Methods

Human subjects

The use of human subject tissue was approved by the Institutional Review Boards at the Rhode Island Hospital and University of Sydney. Postmortem formalin-fixed human adult brain tissue samples from 20 patients with AUD and 19 without CNS disease (controls) were obtained from the New South Wales Brain Tissue Resource Centre in Sydney, Australia. The mean ages, proportions of men and women, durations of alcohol exposure, high rates of regular tobacco use, and mean postmortem brain pH were similar in the control and AUD groups (Table 1). In contrast, the mean lifetime quantity of alcohol consumed was significantly greater, postmortem delay was significantly longer, and mean brain weight was significantly lower in the AUD group.

Sample preparation

At the initial brain dissection, one hemisphere was fixed for 3 weeks in 15% formalin and then embedded in agar to generate 3-mm interval sequential coronal slices that were stored in 10% formalin (Harper et al., 2003; Sutherland, Sheedy, & Kril, 2014). Two standardized blocks from the superior frontal gyrus (Brodmann Area 8/9; BA8/9) and the middle frontal gyrus (Brodmann Area 4; BA4) were dissected from each case and used in this study. Although BA8/9 is generally regarded as the frontal eye field, its functions are quite broad and diverse, including memory (short-term, spatial, semantic, perceptual, and recognizing emotions of others), language skills (verbal fluency), sustained attention, and executive functions (deductive and inductive reasoning, planning) (Abernathy, Chandler, & Woodward, 2010; Watanabe, 2017). In contrast, BA4, located just anterior to the central fissure, is the primary motor cortex and responsible for relaying voluntary motor functions along pyramidal pathways through the brainstem and spinal cord. BA8/9 was of interest because of its role in drug and alcohol addiction and well-documented adverse effects of alcohol abuse on the prefrontal cortex, including BA8/9 (Abernathy et al., 2010). Alcohol also affects the primary motor cortex by enhancing paired associative transcranial magnetic stimulation of long-term depression-like plasticity, enhancing intracortical inhibition, and suppressing intracortical facilitation (Srivanitchapoom et al., 2016; Ziemann, Lönnecker, & Paulus, 1995). Although the data suggest that the functional consequences of alcohol differ for the prefrontal and primary motor cortex, microarray studies showed that chronic alcohol misuse significantly alters myelin gene expression in both BA8/9 and BA4 (Mayfield et al., 2002).

Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS)

After thorough rinsing in phosphate-buffered saline (PBS) for 48 h at 4 °C, the formalin-fixed tissue blocks were blotted dry and a 6-mm diameter disc of cortex-free WM was excised using a disposable punch biopsy-coring tool. Color-coding and orientation of each specimen were achieved by microdot (1 μL) patterned labeling of the edges with surgical biopsy ink (MarginMarker; Vector Surgical, Waukesha, WI). The tissue disks were cryo-embedded in 2% sodium carboxymethylcellulose (CMC). Cryosections (8 μm) were thaw-mounted onto indium tin oxide (ITO) -coated slides (Delta Technologies, Loveland, CO) and coated by sublimation with 2,5-dehydroxybenzoic acid (DHB; Sigma–Aldrich Co, St. Louis, MO) as matrix (Angel, Spraggins, Baldwin, & Caprioli, 2012; Yalcin, Nunez, Cornett, & de la Monte, 2015; Yalcin, Nunez, Tong, Cornett, de la Monte, 2015). The sections were imaged in the negative ion mode using a reflectron geometry MALDI-time-of-flight (TOF)/TOF mass spectrometer (Ultraflextreme, Bruker Daltonics, Bremen, Germany), and analyzed by focusing a Smartbeam II Nd:YAG laser onto ~100 μm^2 areas of white matter (Jackson, Wang, & Woods, 2007; Yalcin, Nunez, Cornett, et al., 2015; Yalcin, Nunez, Tong, et al., 2015). Negative ion mode imaging is optimum for detecting sulfatides and most phospholipids. In contrast, ceramides, phosphotidylcholine, sphingomyelin, and cholesterol were not studied