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Mass spectrometry imaging of rat brain lipid profile changes over time following traumatic brain injury

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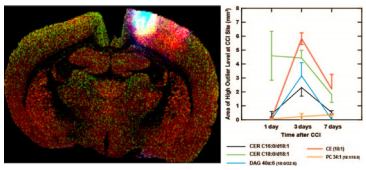
HIGHLIGHTS

- MSI allows the in situ mapping of molecules directly from tissues.
- Brain images showed changes in most lipids classes 3 days after TBI.
- Signaling lipids such as increase in ceramides increased as early as 1 day after the injury.
- More diffuse lipid changes are observed outside of the injured area.
- Ceramides, diacylglycerols and cholesteryl esters are good biomarkers for TBI.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Mild traumatic brain injury (TBI) is a common public health issue that may contribute to chronic degenerative disorders. Membrane lipids play a key role in tissue responses to injury, both as cell signals and as components of membrane structure and cell signaling. This study demonstrates the ability of high resolution mass spectrometry imaging (MSI) to assess sequences of responses of lipid species in a rat controlled cortical impact model for concussion.
New method: A matrix of implanted silver nanoparticles was implanted superficially in brain sections for matrix-assisted laser desorption (MALDI) imaging of 50 μm diameter microdomains across unfixed cryostat sections of rat brain. Ion-mobility time-of-flight MS was used to analyze and map changes over time in brain lipid composition in a rats after Controlled Cortical Impact (CCI) TBI.
Results: Brain MS images showed changes in sphingolipids near the CCI site, including increased ceramides and decreased sphingomyelins, accompanied by changes in glycerophospholipids and cholesterol derivatives. The kinetics differed for each lipid class; for example ceramides increased as early as 1 day after the injury whereas other lipids changes occurred between 3 and 7 days post injury.

Abbreviations: TBI, traumatic brain injury; MSI, mass spectrometry imaging; AgNPs, silver nanoparticles; CCI, controlled cortical impact; ROI, regions of interest; CER, ceramide; DAG, diacylglycerol; CE, cholesteryl ester; GALCER, galactosyl ceramide; PC, phosphatidylcholine; CHL, cholesterol; SM, sphingomyelin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; ST, sulfatide.

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Comparison with existing method(s): Silver nanoparticles MALDI matrix is a sensitive new tool for revealing previously undetectable cellular injury response and remodeling in neural, glial and vascular structure of the brain.
Conclusions: Lipid biochemical and structural changes after TBI could help highlighting molecules that can be used to determine the severity of such injuries as well as to evaluate the efficacy of potential treatments.

1. Introduction

Traumatic brain injury (TBI) is damage to the brain that temporarily or permanently impairs brain structure and functions. It is a serious public health problem and results in many permanent disabilities. The CDC reported that over 1.7 million people sustain TBI every year in the United States, which accounts for about a third (30.5%) of all injury-related deaths (Faul et al., 2010). Even non-lethal TBI causes a wide range of short and long term functional changes including sensory and cognitive impairments involving memory, movement, vision, hearing, as well as emotional dysfunctions, personality changes and depression (Rao and Lyketsos, 2000; Kushner, 1998). Moreover TBI increases risks of epilepsy (Agrawal et al., 2006) and other brain disorders with symptoms resembling Alzheimer's and Parkinson's diseases (National Institute of Neurological Disorders and Stroke, 2002). The pathophysiology of TBI involves two stages: (1) the primary injury occurring at impact resulting in direct tissue damage and impairment of cerebral blood flow and metabolism (Hardman and Manoukian, 2002), and (2) the secondary injury resulting from biochemical and pathological processes initiated by the mechanical damage (Hardman and Manoukian, 2002). Secondary injuries such as cerebral ischemia, intracranial hypertension, inflammation and edema are the leading causes of hospital deaths from a TBI etiology (Marshall et al., 1991).

When studying the brain, lipids are of particular interest because they account for more than 50% of its dry weight, are the major components of cell membranes, are repositories of chemical energy and play key roles in cell signaling and signal transduction (Fernandis and Wenk, 2007; Piomelli, 2005; Piomelli et al., 2007). The crucial role of lipids in brain physiology and cell signaling was demonstrated in neurologic disorders and several neurodegenerative diseases such as Alzheimer's, Parkinson's, and Niemann–Pick disease, in which the lipid metabolic pathways are disrupted (Sturley et al., 2004; Sharon et al., 2003; Cutler et al., 2004; Wenk, 2005). We previously studied a mild blast TBI model (Woods et al., 2013) and discovered a regionally specific accumulation of the ganglioside GM2 which plays a major role in cell recognition and signaling in the hippocampus, thalamus and hypothalamus. These changes were accompanied by depletion of ceramides, suggesting that lipids participate in both function and structure of neural membranes, and that disruption of lipid metabolism might be a key event in the pathophysiology of TBI (Adibhatla et al., 2006).

Driven by advances in mass spectrometry, the field of lipidomics has grown rapidly in the last two decades (Wenk, 2005, 2010). Direct tissue analysis/mass spectrometry imaging (MSI) allows both the characterization of lipid species and the mapping of their distribution in tissue (Jackson et al., 2005; Colsch and Woods, 2010; Delvolve et al., 2011). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is the most commonly used mass spectrometric technique for MSI (Woods and Jackson, 2006; Fernandez et al., 2011). Since MSI is an in situ method, there is no pre-analysis separation step needed before mass analysis. Although simple in concept, proper sample preparation is a key prerequisite for detecting analytes of interest by MSI. Recently, we developed a highly accurate and reproducible method for implanting silver nanoparticles (AgNPs) in tissue sections (Jackson et al., 2014). Stable and homogenous AgNPs matrix layers results in highly reproducible tissue implantation, thus yielding high quality images of the regional distribution of several lipid species in both heart and kidney tissue as well as in a mouse model of chronic alcohol abuse (Jackson et al., 2014; Muller et al., 2015; Roux et al., 2014). In the current study, a controlled cortical impact (CCI) injury model (Romine et al., 2014) in adult male rats were used to study the progression of the pathology resulting from TBI up to 7 days post injury. MSI with AgNPs was used to locate, analyze and study the changes in brain lipid composition at different time points.

2. Material and methods

2.1. Animals

All procedures were performed under protocols approved by the Uniformed Services University Animal Care and Use Committee. Adult male Sprague-Dawley rats, weighing 225–250 g at the start of the study, were kept in a 12/12 h light/dark cycle with access to food and water ad libitum. Animals were divided in 2 groups, controls (Sham) and Injured (CCI). The CCI group was subdivided into 3 sub-groups, reflecting the time elapsed after trauma before the animals were sacrifice (at day 1, 3 and 7). All groups, including Sham, contained 4 animals.

2.2. Controlled cortical impact

This method uses a rigid impactor to deliver mechanical energy to intact dura, exposed following a craniotomy (Romine et al., 2014) in rats anesthetized with isoflurane. The impact was centered 2.04 mm posterior to bregma and 3.0 mm left of the midline (Fig. 1) [−2.04 mm, Fig. 50 in Paxinos and Watson (2007) rat brain atlas]. The impactor had a 3 mm diameter flat tip and the parameters used for the impact were a depth of 2.0 mm, a velocity of 5 m/s and a latency of 2 ms. Control animals received no cortical impact strike, but otherwise received the same treatment as the CCI animals, including a craniotomy under isoflurane anesthesia. The animals were euthanized 1, 3 or 7 day post injury. Under ketamine/xylazine (100 mg/kg; 10 mg/kg) anesthesia, the chest of each rat was opened and the head perfused through a catheter placed in the ascending aorta with 50 to100 ml of phosphate buffered saline at room temperature, allowing blood to flush from the head through an opening in the superior vena cava. When the perfusate was largely clear of blood, the skull was carefully opened and the brain dissected. After removing meninges, each brain was rapidly frozen in a small beaker containing about 30 ml of cold isopentane pre-cooled by immersion of the beaker in solid CO_2, then removed, wrapped individually in aluminum foil and stored at −80 °C until sectioned. Coronal 18 μm sections through the area of the injury were cut using a cryostat (Leica Microsystems CM3050S, Bannockburn, IL) The specific location of individual sections was identified by comparisons of the morphology of major structures in relation to the Paxinos and Watson (2007)rat brain atlas.