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INFLAMMATION TRIGGERS PRODUCTION OF DIMETHYLSPHINGOSINE FROM OLIGODENDROCYTES

Y.-J. CHEN, [a,b,c†] **S. HILL,** [d,e†] **H. HUANG,** [d,e] **A. TARABOLETTI,** [d,e] **K. CHO,** [a,b,c] **R. GALLO,** [a,b,c] **M. MANCHESTER,** [f*] **L. P. SHRIVER** [d,e*] **AND G. J. PATTI** [a,b,c*]

[a] *Department of Chemistry, Washington University, St. Louis, MO 63130, United States*

[b] *Department of Genetics, Washington University, St. Louis, MO 63130, United States*

[c] *Department of Medicine, Washington University, St. Louis, MO 63130, United States*

[d] *Department of Chemistry, University of Akron, Akron, OH 44325, United States*

[e] *Department of Biology, University of Akron, Akron, OH 44325, United States*

[f] *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093, United States*

Abstract—Neuropathic pain is a chronic, refractory condition that arises after damage to the nervous system. We previously showed that an increased level of the endogenous metabolite N,N-dimethylsphingosine (DMS) in the central nervous system (CNS) is sufficient to induce neuropathic pain-like behavior in rats. However, several important questions remain. First, it has not yet been demonstrated that DMS is produced in humans and its value as a therapeutic target is therefore unknown. Second, the cell types within the CNS that produce DMS are currently unidentified. Here we provide evidence that DMS is present in human CNS tissue. We show that DMS levels increase in demyelinating lesions isolated from patients with multiple sclerosis, an autoimmune disease in which the majority of patients experience chronic pain. On the basis of these results, we hypothesized that oligodendrocytes may be a cellular source of DMS. We show that human oligodendrocytes produce DMS in culture and that the levels of DMS increase when oligodendrocytes are challenged with agents that damage white matter. These results suggest that damage to oligodendrocytes leads to increased DMS production which in turn drives inflammatory astrocyte responses involved in sensory neuron sensitization. Interruption of this pathway in patients may provide analgesia without the debilitating side effects that are commonly observed with other chronic pain therapies.

Key words: astrocytes, dimethylsphingosine, metabolomics, multiple sclerosis, neuropathic pain, oligodendrocytes.

INTRODUCTION

Neuropathic pain, a debilitating and widespread condition, is associated with significant medical care costs and lost worker productivity (Jay and Barkin, 2014). Most commonly, the disorder develops after injury to the peripheral or central nervous system (CNS). The mechanisms involved with the onset of neuropathic pain are complex and poorly characterized, but have been shown to involve both neurons and glial cells (Ducreux et al., 2006; Foley et al., 2012).

Current treatments for neuropathic pain rarely provide complete therapeutic relief and are associated with undesirable side effects such as addiction (Trescot et al., 2008; Vranken, 2012). A major limitation to developing better analgesics to treat neuropathic pain has been an incomplete understanding of the biochemical pathways involved in its pathogenesis. As such, we recently performed untargeted metabolomics to screen for potentially unknown molecules involved in the development of neuropathic pain (Patti et al., 2012). Mass spectrometry-based metabolomics enables thousands of small molecules to be profiled in an unbiased manner (Nordstrom and Lewensohn, 2009; Fei et al., 2014). The application of untargeted metabolomic technologies to a rat model of neuropathic pain led to the identification of increased levels of N,N-dimethylsphingosine (DMS) in the dorsal horn of animals suffering from peripheral nerve injury. It was also shown that intrathecal introduction of DMS into rats was sufficient to produce mechanical allodynia, a symptom of neuropathic pain in which subjects exhibit a pain response to a stimulus that is normally innocuous (Patti et al., 2012).

Although inhibition of DMS production is an interesting therapeutic target to be explored as a treatment for neuropathic pain, several important questions must be addressed. First, and most importantly, it is currently unknown if DMS is produced in human tissues. It has been hypothesized that DMS is a product of

*Corresponding authors. Address: Department of Chemistry, University of Akron, Akron, OH 44325, United States (L.P. Shriver). Address: Department of Chemistry, Washington University, St. Louis, MO 63130, United States (G.J. Patti).

E-mail addresses: mmanchester@ucsd.edu (M. Manchester), lshriver@uakron.edu (L. P. Shriver), gjpattij@wustl.edu (G. J. Patti).

† These authors contributed equally.

Abbreviations: CNS, central nervous system; DMEM, Dulbecco's Modified Eagle's Medium; DMS, dimethylsphingosine; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; IFN-γ, interferon-gamma; LPS, lipopolysaccharide; LC/MS, liquid chromatography/mass spectrometry; NAWM, normal appearing white matter; PBS, phosphate-buffered saline; QTOF, quadrupole time-of-flight; S1P, sphingosine-1-phosphate.

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sphingomyelin catabolism, but this has not been directly proven. In the sphingomyelin pathway, DMS is thought to be produced by methylation of sphingosine. However, the methyltransferase that may catalyze this reaction has not been identified. In the human genome, there are over 200 possible methyltransferases and many of their substrates have not yet been characterized (Petrossian and Clarke, 2011). Thus, evidence for production of DMS in human tissues cannot be obtained by simple analysis of the genome. An alternative approach, which is the one we applied here, is to perform mass spectrometry-based metabolomic analysis on human cells and tissues to measure DMS directly.

Multiple CNS cell types are involved in processing nociceptive input and have been implicated in the development of chronic pain symptoms. Currently, it is unknown which cell types produce DMS. The DMS that was detected in previous studies was identified in homogenized CNS tissue. This DMS could have arisen from neurons, astrocytes, microglia, and/or oligodendrocytes. Therefore, an important first step in advancing our understanding of the biology associated with DMS as well as facilitating its development as a new analgesic target is to identify cellular sources of DMS in the CNS.

In this work, we screened for DMS in the CNS tissue of human patients with multiple sclerosis by using mass spectrometry-based metabolomics. Multiple sclerosis is an autoimmune disease that results in demyelinating lesions in both the brain and spinal cord. Although multiple sclerosis is a heterogeneous disease with a varying clinical presentation, chronic pain is a common symptom that has been reported to occur in as many as 86% of patients (Stenager et al., 1995; Solaro et al., 2013). The cause of chronic pain in multiple sclerosis patients is unknown, but studies examining animal models of multiple sclerosis suggest that inflammation and glial cell activation present within plaques may promote abnormal firing of sensory neurons (Aicher et al., 2004; Olechowski et al., 2010; Lu et al., 2012). Our results provide a novel link between inflammation, demyelination, and chronic pain in the CNS of human patients and implicate oligodendrocytes as an important cell type in mediating abnormal pain responses through the action of the metabolite DMS.

EXPERIMENTAL PROCEDURES

Reagents

Ammonium acetate, ammonium hydroxide, and formic acid solutions were purchased from Sigma–Aldrich (St. Louis, MO, USA). Liquid chromatography/mass spectrometry (LC/MS) grade water, acetonitrile, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Chloroform (high-performance liquid chromatography (HPLC) Grade, $\geqslant$99.5%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Dul becco's Modified Eagle's Medium (DMEM), penicillin/streptomycin, phosphate-buffered saline (PBS), and fetal bovine serum (FBS) were purchased from Corning (Manassas, VA, USA). Trypsin–versene mixture was purchased from Lonza (Walkersville, MD, USA). Dimethylsphingosine (DMS) standards were purchased from Avanti (Alabaster, AL, USA) and Cayman Chemical (Ann Arbor, MI, USA).

Cell culture

The rat astrocyte cell line (CRL-2005) was purchased from American Type Culture Collection (Manassas, VA, USA). The Human Glial Oligodendrocytic (MO3.13) cell line was purchased from CELLutions Biosystems, Inc. (Burlington, Ontario, CA, USA). Cells were cultured in DMEM supplemented with 10% FBS, and 1% penicillin/streptomycin at 37 °C in 5% CO_2.

Cell treatment

For astrocyte cultures, 1×10^5 cells were plated per well in a 6-well plate and incubated overnight to allow the cells to adhere. Fresh culture medium was subsequently added and cells were cultured for another 24 h. Astrocytes were treated with 0.1 μM DMS (Cayman Chemical, Ann Arbor, MI, USA), 1 μg/mL lipopolysaccharide (LPS), or vehicle. After a 24-h treatment, cells were washed with PBS, metabolism was quenched with cold methanol, and collected by cell scraping. Cell pellets were obtained by centrifugation at $1000 \times g$ for 10 min. The cell pellets were either lyophilized or immediately extracted using the previously described procedures as detailed below (Nikolskiy et al., 2013).

Oligodendrocyte cultures were treated with the following after cell attachment: 1 mM cuprizone (Sigma–Aldrich) in 1% ethanol or vehicle containing 1% ethanol, 100 ng/mL interferon-gamma (IFN-γ) (R&D systems, Minneapolis, MN, USA), 100 ng/mL LPS (Sigma–Aldrich), or PBS. Oligodendrocytes were treated for 24 h and metabolites extracted with a modified Bligh Dyer method (Bligh and Dyer, 1959).

Multiple sclerosis tissue

De-identified human samples from multiple sclerosis patients were obtained from the Rocky Mountain Multiple Sclerosis Center Tissue Bank (Aurora, CO, USA) and approved by the Institutional Review Board at the University of California, San Diego. Tissue was examined by a neuropathologist and active plaques were identified based on histopathological evidence of T lymphocyte and macrophage infiltration. Adjacent normal appearing white matter (NAWM) was taken from the same patient and examined for the presence of intact myelin and lack of inflammatory cells.

Metabolite extraction

A methanol/acetone-based extraction was applied to astrocyte cell cultures and human multiple sclerosis tissue. A volume of 600 μL of cold (−20 °C) acetone was added to lyophilized cells or frozen tissue, vortexed for 30 s, and the sample incubated for 1 min in liquid nitrogen. The samples were thawed at room temperature and incubated in liquid nitrogen two more times prior to a 10-min sonication. After 1 h at −20 °C,