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Matrix-Assisted Laser Desorption Ionization Mapping of Lysophosphatidic Acid Changes after Traumatic Brain Injury and the Relationship to Cellular Pathology

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Lysophosphatidic acid (LPA) levels increase in the cerebrospinal fluid and blood within 24 hours after traumatic brain injury (TBI), indicating it may be a biomarker for subsequent cellular pathology. However, no data exist that document this association after TBI. We, therefore, acquired matrix-assisted laser desorption ionization imaging mass spectrometry data of LPA, major LPA metabolites, and hemoglobin from adult rat brains at 1 and 3 hours after controlled cortical impact injury. Data were semiquantitatively assessed by signal intensity analysis normalized to naïve rat brains acquired concurrently. Gray and white matter pathology was assessed on adjacent sections using immunohistochemistry for cell death, axonal injury, and intracellular LPA, to determine the spatiotemporal patterning of LPA corresponding to pathology. The results revealed significant increases in LPA and LPA precursors at 1 hour after injury and robust enhancement in LPA diffusively throughout the brain at 3 hours after injury. Voxel-wise analysis of LPA by matrix-assisted laser desorption ionization and β-amyloid precursor protein by immunohistochemistry in adjacent sections showed significant association, raising the possibility that LPA is linked to secondary axonal injury. Total LPA and metabolites were also present in remotely injured areas, including cerebellum and brain stem, and in particular thalamus, where intracellular LPA is associated with cell death. LPA may be a useful biomarker of cellular pathology after TBI. *(Am J Pathol 2018, 188: 1779–1793; https://doi.org/10.1016/j.ajpath.2018.05.005)*

Traumatic brain injury (TBI) affects nearly 1.7 million individuals each year, many of who sustain functional impairments from progressive axonal injury and neuronal death after trauma. Because of the complex pathology of TBI, there is no effective treatment to restore function in patients. The rapid onset of pathology and the complexity of the cellular response to TBI suggest that there are early signaling molecules involved in initiating the cascade of cellular events that promote functional impairment after trauma.[1–4] Lysophosphatidic acid (LPA) is one such molecule shown to be significantly involved in the pathology of central nervous system (CNS) injury.[5–7]

LPA and its metabolites act as potent cellular messengers[8] and are likely to be involved in much of the early pathologic processes after TBI, as indicated by overlapping data on the inflammatory cascade,[9] altered calcium homeostasis,[10,11] astrogliosis,[12,13] neurite retraction,[14,15] and cell death.[16] The parallel between the cellular

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responses to LPA and trauma-induced cellular pathologies suggests that LPA may be a useful biomarker for TBI. This is supported by a recent study demonstrating that LPA is significantly increased in the cerebrospinal fluid (CSF) 3 hours after injury in a mouse model of TBI as well as within 24 to 36 hours after injury in a subset of patients with severe TBI.[5] The CSF concentration of phospholipids related to LPA has also been shown to be significantly higher in patients with poor outcomes.[17] Activation of LPA receptor type 1 has been shown to contribute to the pathology of spinal cord injury.[18] Attenuating LPA signaling with anti-LPA antibodies has proved successful in improving recovery after a rodent model of TBI[5] and spinal cord injury,[6] suggesting that enhanced LPA production is involved in injury pathology after CNS trauma.

A comprehensive understanding of the contribution that LPA makes to TBI pathology is required to successfully address aberrant LPA signaling, including the source(s) of LPA changes, the spatial extent of LPA expression, and the duration in which aberrant LPA levels are present in the injured brain. Furthermore, it is also important to determine the effect of injury on accumulation of LPA and its metabolites in brain tissue, because LPA alone can initiate the production of more LPA within adjacent cells,[19,20] generating a feed-forward mechanism of LPA expression and signaling in the injured brain. Despite this knowledge, no study has determined if the levels of LPA actually change within the injury epicenter and regions distal to the injury site, and whether this change is associated with known markers of pathology. LPA and its associated phospholipids are essential for LPA metabolic regulation, cellular function, and signaling; thus, identifying the spatial-temporal disruption of LPA metabolic changes will be a crucial step in understanding the role of LPA in the pathogenesis of TBI. The results herein are to identify spatiotemporal LPA dysregulation within 3 hours of TBI in the rat brain.

Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) is a sensitive label-free approach capable of producing ion-density maps representing the distribution of a variety of analyses linked directly with tissue histopathology.[21–26] Using high-resolution MALDI IMS and immunohistochemistry (IHC), we determined whether changes in lipid distribution after controlled cortical impact (CCI) model correlate with markers of TBI pathology, such as axonal injury and necrosis. Brain tissue slices obtained from control and CCI rats at 1 and 3 hours after injury were analyzed via MALDI IMS. Modulation in the distribution of LPA was observed not only at the site of injury, but also in distal regions, suggesting that activated platelets within hemorrhagic areas of the brain are not the only source of enhanced LPA after injury. Using IHC, it was confirmed that increases of LPA in gray and white matter regions were closely associated with markers of neural degeneration and axonal damage, suggesting that early phospholipid changes may be useful biomarkers of pathology and longer-term functional impairments associated with axonal injury and cell death.

Materials and Methods

Experimental Protocol

Fresh frozen rat brains were collected from control animals or those undergoing injury, collected at 1 and 3 hours after CCI brain injury. Frozen brains ($n = 3$ per group) were sectioned and analyzed using MALDI IMS to visualize lipid distribution across the brains. Adjacent coronal sections were also collected for IHC.

Animals

Adult (3-month–old) male Sprague-Dawley rats (220 to 250 g) were purchased from Charles River Laboratories (Hollister, CA). All experiments were conducted in accordance with the University of California, Los Angeles, Chancellor's Animal Research Committee and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

CCI Injury

All rats were anesthetized with 3% to 4% isoflurane (2% maintenance) vaporized in oxygen flowing at 0.8 L/minute. After hair removal from the head and scalp and cleaning with betadine scrubs, the animal was positioned within a stereotaxic frame (Stoelting Co, Wood Dale, IL). A longitudinal skin incision was made, followed by a 6-mm–diameter craniotomy centered at 0.5 mm posterior to bregma and 1 mm lateral to the midline using a dental drill. Body temperature was maintained throughout the surgery using a thermostatically controlled heating pad (Harvard Apparatus, Holliston, MA). Cortical injury was performed with a flat 3-mm–diameter metal tip attached to an impactor rod and launched onto the dura using 20 ψ, and to a depth of 2 mm below the dura. The craniotomy was covered with a layer of nonbioreactive silicone elastomer (Kwik-Cast, Sarasota, FL) before suturing the wound closed.

MALDI IMS

Spectra Acquisition

After terminal pentobarbital anesthesia (50 mg/kg), rats were perfused with phosphate-buffered saline (0.1 mol/L) via transcardial perfusion to remove blood. Brains from injured and sham rats were collected at 1 and 3 hours after injury ($n = 3$ per group). Tissues were extracted and snap frozen under 2-methylbutane at −30°C, sectioned at 12 μm, and thaw mounted onto indium tin oxide–coated slides (Bruker Daltonics, Billerica, MA). Representative brain sections from all three groups ($n = 3$ sections per brain)