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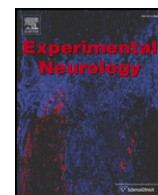

Regular Article

Blocking leukotriene synthesis attenuates the pathophysiology of traumatic brain injury and associated cognitive deficits

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ABSTRACT

Neuroinflammation is a component of secondary injury following traumatic brain injury (TBI) that can persist beyond the acute phase. Leukotrienes are potent, pro-inflammatory lipid mediators generated from membrane phospholipids. In the absence of injury, leukotrienes are undetectable in the brain, but after trauma they are rapidly synthesized by a transcellular event involving infiltrating neutrophils and endogenous brain cells. Here, we investigate the efficacy of MK-886, an inhibitor of 5-lipoxygenase activating protein (FLAP), in blocking leukotriene synthesis, secondary brain damage, synaptic dysfunction, and cognitive impairments after TBI. Male Sprague Dawley rats (9–11 weeks) received either MK-886 or vehicle after they were subjected to unilateral moderate fluid percussion injury (FPI) to assess the potential clinical use of FLAP inhibitors for TBI. MK-886 was also administered before FPI to determine the preventative potential of FLAP inhibitors. MK-886 given before or after injury significantly blocked the production of leukotrienes, measured by reverse-phase liquid chromatography coupled to tandem mass spectrometry (RP LC–MS/MS), and brain edema, measured by T2-weighted magnetic resonance imaging (MRI). MK-886 significantly attenuated blood–brain barrier disruption in the CA1 hippocampal region and deficits in long-term potentiation (LTP) at CA1 hippocampal synapses. The prevention of FPI-induced synaptic dysfunction by MK-886 was accompanied by fewer deficits in post-injury spatial learning and memory performance in the radial arm water maze (RAWM). These results indicate that leukotrienes contribute significantly to secondary brain injury and subsequent cognitive deficits. FLAP inhibitors represent a novel anti-inflammatory approach for treating human TBI that is feasible for both intervention and prevention of brain injury and neurologic deficits.

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Introduction

Accumulating evidence of neurodegenerative pathology and progressive neurological dysfunction following repetitive concussion in high-impact sports (Jordan, 2013; McKee et al., 2013; Smith et al., 2013) and the rising number of TBI cases in war veterans exposed to explosive blasts (Taber et al., 2006; Warden, 2006) has increased public awareness of TBI. An estimated 1.7 million people in the United States suffer a TBI each year, but this estimate only includes injuries for which medical care is sought (Faul et al., 2010; Langlois et al., 2006). Because of this, TBI is considered a 'silent epidemic' as many mild TBI cases are unrecognized and unreported, and the magnitude of morbidity and mortality associated with these injuries has been largely underestimated. Advances in life support procedures have decreased the mortality rate of TBI, but many patients still face life-long physical and cognitive disabilities (Selassie et al., 2008). In the past several years, there has been increased interest in the diagnosis of mild TBI through advanced neuroimaging techniques and the use of plasma biomarkers. However, the development of drugs for blocking the detrimental consequences of TBI is lagging behind.

The pathophysiology of TBI is complex and heterogeneous. The primary injury at the time of trauma activates multiple pathways that lead to secondary injury days to weeks later (Gennarelli, 1993; Kochanek et al., 2009). The primary injury can present as any combination of skull fractures, intracranial hematomas, lacerations, contusions, and/or penetrating wounds. Secondary injury results from the activation of multiple pathways that lead to altered ionic balance, BBB permeability, edema, increased intracranial pressure, oxidative stress, neuronal cell

Abbreviations: 5-LO, 5-lipoxygenase; BBB, blood–brain barrier; EB, Evan's blue; fEPSP, field excitatory post-synaptic potential; FLAP, 5-lipoxygenase activating protein; FPI, fluid percussion injury; Gd, gadolinium-diethylenetriamine pentaacetic acid; LTP, long-term potentiation; RAWM, radial arm water maze; RP LC–MS/MS, reverse-phase liquid chromatography coupled to tandem mass spectrometry; TBI, traumatic brain injury.

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death, and eventual neurologic impairment (Barkhoudarian et al., 2011). At the time of BBB disruption a neuroinflammatory response is activated that can persist for several weeks following TBI (Morganti-Kossmann et al., 2007; Shlosberg et al., 2010). This disruption results from mechanical shearing of blood vessels at the time of injury and/or chemically-mediated signaling cascades resulting in increased BBB permeability (Morganti-Kossmann et al., 2007; Schmidt et al., 2005; Shlosberg et al., 2010). Infiltrating peripheral immune cells (i.e. leukocytes) activate resident astrocytes and microglia, which initiates pro-inflammatory signaling pathways that contribute to further BBB breakdown and brain edema (Morganti-Kossmann et al., 2007; Schmidt et al., 2005; Streit et al., 2004).

Leukotrienes are potent bioactive lipids that are important mediators of inflammation (Murphy et al., 1979). Leukotriene biosynthesis is initiated by mechanical injury to cells or by calcium entry, which releases arachidonic acid (AA) from membrane glycerophospholipids (Folco and Murphy, 2006). The enzymatic action of 5-LO and FLAP converts AA into leukotriene A_4 (LTA_4). LTA_4 is quickly converted to LTB_4 by LTA_4-hydrolase or to LTC_4 by LTC_4-synthase. LTC_4 can then be converted to LTD_4 and LTE_4, and these three LTs (LTC_4, LTD_4, LTE_4) are collectively known as the cysteinyl-leukotrienes. The actions of cysteinyl leukotrienes have been studied primarily in the context of asthma where they are known to induce vascular permeability, extravasation of large molecules, stimulation of cytokine release, and contraction of bronchial smooth muscle (Boyce, 2007).

Leukotrienes are undetectable in the healthy brain (Farias et al., 2009). However, our laboratory has demonstrated that leukotrienes are rapidly produced after TBI by a transcellular mechanism involving infiltrating neutrophils and endogenous brain cells (Farias et al., 2007, 2009). To explore the role of leukotrienes in TBI and the clinical potential of using FLAP inhibitors, we investigated the efficacy of a commercially available FLAP inhibitor, MK-886, in reducing injury-induced leukotriene production, edema, BBB disruption, as well as hippocampal-related synaptic dysfunction and cognitive deficits. Our findings have important implications for treating human TBI and suggest that development of FLAP inhibitors for use in TBI is feasible for both intervention when administered shortly after TBI and prevention when administered to “at risk” individuals prior to potential brain trauma.

Materials and methods

Animals

Adult male Sprague Dawley rats (9–11 weeks old, 250–300 g; Harlan Laboratories) were housed individually in temperature- and light-controlled housing with free access to food and water ad libitum. All procedures as described were performed under protocols approved by the University of Colorado Institutional Animal Care and Use Committee and in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. A total of 134 animals were used in this study.

Lateral fluid percussion injury

Craniotomy and FPI were performed using a previously validated and published procedure (Farias et al., 2009; Frey et al., 2009). Briefly, animals were anesthetized with 3–5% isoflurane (IsoSol, VEDCO Inc., St. Joseph, MO) via nose cone and mounted on a stereotaxic head frame. A 3 mm craniotomy was created and centered at 3 mm caudal to bregma and 3.5 mm left of the sagittal suture, keeping the exposed dura intact. One steel support screw was embedded in the skull on the contralateral side. A Luer-Lock hub (inside diameter of 3.5 mm) was centered over the craniotomy and bonded to the skull with cyanoacrylate adhesive and capped. Dental acrylic (Snap, Parkell, Inc., Edgewood, NJ) was poured around the hub and screw. After the acrylic hardened, antibiotic ointment was applied around the cap, and animals were returned to their cages. The next day (15–20 h later) animals were anesthetized with isoflurane in an induction chamber, immediately connected to the FPI apparatus, and received a 20 msec pulse of pressurized sterile saline (2.7 atm, moderate severity impact) on the intact dural surface before awakening from anesthesia. Sham-injured animals underwent craniotomy and were anesthetized and connected to the FPI apparatus, but they did not receive the fluid pulse. All animals received a subcutaneous injection of the analgesic, buprenorphine (0.05 mg/kg; Buprenex), prior to craniotomy, and subsequent injections every 12 h for two days. Moistened food pellets were provided after injury, and all animals were monitored daily for well-being and weight changes.

Administration of MK-886 and vehicle

MK-886 was prepared at a concentration of 2.5 mg/ml, dissolved in DMSO and then diluted with 0.9% saline to 10% DMSO. Animals were briefly anesthetized with 3–3.5% isoflurane and either MK-886 (6 mg/kg) or vehicle was administered intravenously (IV) by tail vein. All animals were allowed to wake before undergoing additional procedures.

Extraction of rat brain lipids

Cortical and hippocampal regions from ipsilateral and contralateral hemispheres were collected in 4 ml of 80% methanol, homogenized with a Dounce homogenizer, and internal standards were added to the homogenates. Protein content was measured using BCA protein assay to normalize lipid levels to the amount of tissue. Samples were centrifuged and the supernatant was collected. Samples were diluted to a final methanol concentration of lower than 15% and then the lipids were extracted using a solid phase extraction cartridge (Strata C18-E, 100 mg/1 ml, Phenomenex, Torrance, CA). The eluate (1 ml of methanol) was dried down and reconstituted in 70 μl of HPLC solvent A (8.3 mM acetic acid buffered to pH 5.7 with NH_4OH) + 20 ml of solvent B (acetonitrile/methanol, 65/35, v/v).

Measurement of brain leukotrienes by RP LC–MS/MS

An aliquot of each sample (35 μl) was injected into an HPLC system and subjected to reverse-phase chromatography using a C18 (Columbus 150 × 1 mm, 5 μm, Phenomenex) column eluted at a flow rate of 50 μl/min with a linear gradient from 25% to 100% of mobile phase B. Solvent B was increased from 25% to 85% by 24 min, to 100% by 26 min, and held at 100% for a further 12 min. The HPLC effluent was directly connected to the electrospray source of a triple quadrupole mass spectrometer (Sciex API 2000, PE-Sciex, Thornhill, Ontario, Canada) and mass spectrometric analyses were performed in the negative ion mode using multiple reaction monitoring (MRM) of the specific transitions, m/z 624 → 272 for LTC_4, m/z 495 → 177 for LTD_4, m/z 335 → 195 for LTB_4, m/z 339 → 197 for d4-LTB_4, and m/z 629 → 277 for d5-LTC_4. Quantitation was performed using a standard isotope dilution curve as previously described (Farias et al., 2007) with reference leukotriene standards and stable isotope analogs (Cayman Chemical, Ann Arbor, MI).

MRI acquisition

All MRI studies were performed in the University of Colorado Animal Imaging Shared Resource (AISR) facility. Animals underwent MRI imaging at 72 h after injury, using T2-weighted sequences. For all MRIs, the rats were anesthetized with 2.5% isoflurane. Scans were done using a 4.7 T Bruker PharmaScan, and a quadrature birdcage coil (inner diameter of 38 mm), tuned to the ^{1}H frequency of 200.27 MHz, was used for RF transmission and reception. T2-weighted axial MR scans were acquired using a RARE (rapid acquisition with relaxation enhancement) sequence with the following parameters: FOV: 4.6 cm; TE/TR: 32/5000 msec; slice thickness = 1.20 mm; interslice distance = 1.20 mm (no gap); number

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