Inhibition of Myosin Light-Chain Kinase Attenuates Cerebral Edema after Traumatic Brain Injury in Postnatal Mice

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Abstract

Traumatic brain injury (TBI) in children less than 8 years of age leads to decline in intelligence and executive functioning. Neurological outcomes after TBI correlate to development of cerebral edema, which affect survival rates after TBI. It has been shown that myosin light-chain kinase (MLCK) increases cerebral edema and that pretreatment with an MLCK inhibitor (ML-7) reduces cerebral edema. The aim of this study was to determine whether inhibition of MLCK after TBI in postnatal day 24 (PND-24) mice would prevent breakdown of the blood–brain barrier (BBB) and development of cerebral edema and improve neurological outcome. We used a closed head injury model of TBI. ML-7 or saline treatment was administered at 4 h and every 24 h until sacrifice or 5 days after TBI. Mice were sacrificed at 24 h, 48 h, and 72 h and 7 days after impact. Mice treated with ML-7 after TBI had decreased levels of MLCK-expressing cells (20.7 ± 4.8 vs. 149.3 ± 40.6), less albumin extravasation (28.3 ± 11.2 vs. $116.2 \pm 60.7 \text{ mm}^2$) into surrounding parenchymal tissue, less Evans Blue extravasation (339 ± 314 vs. $4017 \pm 560 \text{ ng/g}$), and showed a significant difference in wet/dry weight ratio (1.9 ± 0.07 vs. $2.2 \pm 0.05 \text{ g}$), compared to saline-treated groups. Treatment with ML-7 also resulted in preserved neurological function measured by the wire hang test (57 vs. 21 sec) and two-object novel recognition test (old vs. new, 10.5 touches). We concluded that inhibition of MLCK reduces cerebral edema and preserves neurological function in PND-24 mice.

Key words: BBB; brain injury; closed head injury model; edema; mice

Introduction

TRAUMATIC BRAIN INJURY (TBI) is often described as the leading cause of disability in children. Each year, an estimated 3000 children die from TBI, 29,000 are hospitalized, and 400,000 are treated in hospital emergency departments in the United States.¹ Children with TBI are known to suffer a variety of cognitive and behavioral issues, including a loss in developmental milestones and difficulty achieving new ones, change in personality, decline in adaptive functioning, problems in school, and decreased participation in social activities.^{2,3} Injured children less than 8 years of age may be especially vulnerable to declines in intelligence and executive functioning, causing difficulty in achieving their full potential and resulting in loss of future revenues.^{4–6}

TBI is a complex injury that leads to a cascade of events that result in compromise of the cognitive and physical functioning of the body. The primary injury is followed by a secondary injury, which extends the area of involved brain, worsening the initial injury. A number of mechanisms have been identified as inducers of secondary injury development. These include an increase in

inflammatory mediators, free radical damage, thrombosis, macromolecule extravasation, and an increase in water channels.^{7–11} Importantly, all of these mechanisms lead to the development of cerebral edema. Compromise of blood–brain barrier (BBB) function leads to cerebral edema and is a primary determinant of survival after TBI. Understanding the mechanisms regulating the BBB under normal conditions, and compromise after TBI, may hold the key to identifying targets for biomarkers and potential treatment for TBI. In the long term, understanding these mechanisms will advance the development of therapies for preventing post-TBI cerebral edema, thereby reducing secondary injury, improving survival and neurologic outcome, and, ultimately, reducing the cost to society.

Increased expression of myosin light-chain kinase (MLCK) correlates with increased cerebral edema subsequent to ethanol metabolism in a cell model of BMVECs (bovine brain microvascular endothelial cells).¹² Pretreatment with an inhibitor of MLCK has been shown to reduce cerebral edema after controlled cortical impact in a mouse model; however, the effects of treatment with an MLCK inhibitor after TBI are not known.¹³ Here, we examined the

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effects of treatment with an MLCK inhibitor on cerebral edema and neurological functions after TBI in postnatal day 24 (PND-24) mice, a development state similar to a 7- to 8-year-old child.¹⁴

Methods

Animals

All experiments were performed in accord with the National Institutes of Health (NIH) publication, *Guide for Care and Use of Laboratory Animals*. The institutional animal care and use committee at the Louisiana State University Health Sciences Center (New Orleans, LA) approved all experimental procedures. C57BL6 male mice at PND-24, and weighing 10–12 g, were used for these experiments.

Model of closed head injury

Mice were anesthetized with 1% avertin (2,2,2 tribromethanol and tertiary amyl alcohol, intraperitoneally [i.p.] at 20 μ L/g; Sigma-Aldrich, St. Louis, MO) and then noninvasively mechanically ventilated (Hugo Sachs Elektronik, March-Hugstetten, Germany) using an oral/nasal mask. Core temperature was monitored during surgery using a rectal probe (IT-4; Physitemp, Clifton, NJ) and also maintained at 36.8–37.2°C by surface heating and cooling after impact, using a heating pad under the home cage, until mice were able to maintain their own body temperature. Mice were subjected to closed-skull TBI using a stereotactically guided electromagnetic compression device with minor modification from a previously described method.¹⁵ In brief, a mid-line sagittal scalp incision was made and the periosteum reflected to reveal the appropriate landmarks. Immediately caudal to the bregma at 0.10 mm, a right lateral closed-skull impact was delivered by an electromagnetic impactor (Leica Microsystems, Rockford, IL) using a 3.0-mm steel-tip impounder at a controlled velocity (3.0 ± 0.2 m/sec), impact depth (2.25 mm), and dwell time (0.01 sec).

Treatment and experimental protocol

Mice were treated with an inhibitor of MLCK, ML-7 1-[5-Iodonaphthalene-1-sulfonyl]-1H-hexahydro-1,4-diazepine hydrochloride; 1 mg/kg, i.p.; Sigma-Aldrich or comparable volume of 0.9% normal saline at 4 h after TBI and daily over the course of 5 days.^{13,16} All animals were randomly assigned to saline versus ML-7 groups, and the investigator was blinded to treatment and experimental groups.

Assessment of BBB leakage by MLCK and albumin immunohistochemistry

After TBI, mice were treated with ML-7 or saline ($n=6$ in each group), sacrificed at 24, 48, and 72 h and 7 days, and immunostaining for MLCK and albumin was performed at each time point. Animals were anesthetized with 1% avertin (100 μ L/g) and perfused through the left ventricle with chilled phosphate-buffered saline (PBS), followed by perfusion with 4% paraformaldehyde. Brains were removed, immersed in 4% paraformaldehyde for 3 days at 4°C and then cryopreserved in 20% sucrose in PBS at 4°C before preparing 20- μ m-thick sections on a cryostat. Serial sections extending from bregma 0 to –3.46 mm were prepared. Every fourth section was mounted and used for analysis. For each of the antibodies (Abs), four sections were used in sequence. The sequence of brains for Ab processing was the same for each brain. Figure 1 shows a section of brain with section 1 represent-

ing the impact site and extravasation measured from sections 1 to 5.

All sections were rinsed in PBS, incubated in 1% hydrogen peroxide, rinsed in PBS again, and placed in a blocking solution (10% normal swine serum in PBS; Vector Laboratories, Burlingame, CA) for 1 h before incubating with respective primary Ab: Albumin (1:1000), rabbit polyclonal (Wako Chemicals USA Inc., Richmond, VA) MLCK (1:150), a rabbit polyclonal immunoglobulin G (IgG; Abgent Inc., San Diego, CA), and NeuN (1:200), a mouse monoclonal (Chemicon, Temecula, CA), overnight at room temperature. Negative control sections were incubated in normal serum or PBS in place of primary Ab. Sections were then rinsed in PBS and incubated for 1 h at room temperature with the appropriate biotinylated or fluorescent secondary Ab at 1:200 dilution.

MLCK was detected using fluorescein-isothiocyanate-conjugated donkey anti-mouse IgG (1:150; Jackson ImmunoResearch Laboratories Inc., West Grove, PA). NueN was detected using a Texas Red-conjugated mouse anti-rabbit IgG (1:200; Millipore, Billerica, MA), and 4',6-diamidino-2-phenylindole (DAPI)-conjugated donkey anti-mouse IgG (100 ng/mL; Invitrogen, Eugene, OR).¹⁷ Four brain sections were analyzed and averaged per animal using Deconvolution Axioplain Z imaging software with Sensicam QE and Slidebook 5.0. Fluorescence of MLCK was measured using ImageJ (NIH, Bethesda, MD) quantification modifications on 20- μ m-thick sections from 10 $\times$ photographs. The number of MLCK-expressing cells was counted.

Albumin was measured by quantifying extravasation of albumin into brain parenchyma, as previously described.¹⁸ Albumin was detected using a Vectastain Elite ABC immunodetection kit and diaminobenzidine substrate (Vector Laboratories). Albumin-stained tissue was counterstained with cresyl violet (Sigma-Aldrich). Four brain sections from each brain were analyzed and averaged per animal using MCID basic software (Version 7.0; InterFocus Imaging Ltd., Linton, UK). Area of albumin extravasation was measured and compared to the total brain area to determine the area of injury.

Assessment of BBB leakage by Evans Blue extravasation

After TBI, mice were treated with ML-7 or saline ($n=6$ in each group) and sacrificed at 24, 48, and 72 h and 7 days after impact. Evans Blue (EB) dye (2%, 10 μ L/g, i.p.; Alpha Aesar, Ward Hill, MA, and Fisher Scientific, Fairlawn, NJ) was injected in mice 4 h before sacrifice, as previously described.¹⁹ Mice were then anesthetized and perfused with saline. Whole brains were placed in tubes and immediately submerged in liquid nitrogen, then stored in a –80°C freezer until processing. Brain samples from the cortex and striatum (below the impact site of 0.0 mm bregma to –3.46 mm [Fig. 1] and just to the right lateral of the sagittal suture) were excised, weighed, sonicated in formamide (Sigma Aldrich), and then incubated overnight at 37°C. Samples were centrifuged, and the supernatant was aliquoted to a 96-well plate. Fluorescence was measured using an excitation of 620 nm and emission of 680 nm with a Spectramax M5 (Molecular Devices, Sunnyvale, CA). Fluorescence was analyzed by using SoftMax[®] Pro software (Version 5.4; Analytical Technologies, Lexington, KY).

Assessment of brain edema

Mice were treated with ML-7 or saline ($n=6$ in each group), sacrificed at 24, 48, and 72 h and 7 days after TBI, and wet/dry weights were measured at each time point. Brains were removed,

FIG. 1. Coronal brain section (bregma level -1.70 mm) showing location of TBI impact (section 1) and areas of measurement for MLCK expression and albumin extravasation (sections 1–5).

weighed, and then placed at 60°C for 3 days. Dried brains were then reweighed, and the ratio of wet to dry was calculated as previously described.¹⁸

Assessment of behavior

Mice were treated with ML-7 or saline ($n=6$ in each group), and the wire hang and two-object novel recognition tests were conducted on day 7.

Wire hang test

Mice were tested for motor strength and control on day 7 to avoid effects of cellular changes and cerebral edema noted at earlier time points. Hang times (latency to fall) for each treatment group were averaged. In brief, mice were individually placed on a wire cage lid until footing was established. The cage lid was then inverted, and the time to fall was recorded with a 2-min maximum testing time. Animals that were able to climb to the topside were awarded the maximum 2-min score. The test was terminated at 2 min for those mice that neither climbed topside nor fell.²⁰

Two-object novel recognition test

This test is used to evaluate cognition, specifically recognition memory. Mice were habituated to the testing room in their home cage for 24 h. On day 1, mice were individually habituated to a black box for 10 min each. On day 2, mice were placed individually in the black box with two identical objects for 10 min each. Four h later, mice were placed individually in the black box with one familiar (old) object and one novel (new) object.²¹ Each mouse was videotaped for 10 min, and the number of touches per object was recorded. Touches to the old object were compared to touches to the new object (nose touch = 1 point; two paws on object = 2 points; all

four paws on top of object = 3 points). Points were tallied and touches to old versus new objects were compared.²²

Statistical analysis

Values are expressed as mean $\pm$ standard error of the mean (SEM) for each group. Tests for normality were performed for each data set. One-way analysis of variance (ANOVA; or Kruskal-Wallis' test for nonparametric analysis) was performed to compare three or more groups. Bonferroni's multiple comparison procedure (or Dunn's procedure for nonparametric analysis) was used for post-hoc analysis. The t -test with Mann-Whitney's test for post-hoc analysis was used for two groups. Two-way ordinary ANOVA with Tukey's test for multiple comparisons was used for time and treatment analysis. Significance was defined as $p < 0.05$ for all tests. All data were reported out as mean $\pm$ SEM. Prism software (Version 5.0; GraphPad Software, Inc., San Diego, CA) was used for statistical analyses. All analyses were performed by an investigator blinded to treatment groups and time points.

Results

MLCK expression

MLCK was measured by counting fluorescently stained MLCK cells under $10\times$ magnification. Saline treatment (Fig. 2A) had significantly increased levels of MLCK expression by 87% at 24 h, by 80% at 48 h, and by 76% at 72 h, compared to ML-7-treated mice (Fig. 2b). There was no significant trend on day 7 (44.83 ± 15.5 vs. 19.83 ± 3.9). MLCK was up-regulated with a peak point of up-regulation at 24 h postsaline, compared to ML-7 treatment ($p < 0.001$ and $p < 0.01$). Time course was not significant ($p = 0.6208$; Fig. 2C, graph).

Albumin expression

At 24 h after TBI, albumin infiltration of saline-treated (Fig. 2D) mice was significantly higher than ML-7-treated mice (6.7 ± 2.0 vs. $1.8 \pm 0.9 \text{ mm}^2$, respectively; Fig. 2E). This significant difference continued through 48 h (9.0 ± 1.5 vs. $1.2 \pm 0.3 \text{ mm}^2$) and peaked at 72 h (116.2 ± 60.7 vs. $28.3 \pm 11.2 \text{ mm}^2$). After 72 h, the area of albumin infiltration began to return to baseline and continued to decline through day 7 (4.6 ± 1.4 vs. $2.2 \pm 0.3 \text{ mm}^2$). For albumin extravasation ($p < 0.001$, $p < 0.0001$, and $p < 0.01$), time course was not significant ($p = 0.8840$; Fig. 2F, graph).

EB extravasation

The amount of EB in the brain parenchyma (ng/g) that leaked from the vascular space into the brain parenchyma initially was similar in saline and ML-7-treated mice at 24 h (311 ± 65 vs.

$323 \pm 62 \text{ mm}^2$). EB extravasation was significantly increased in saline treatment (Fig. 2G) at 48 h (2911 ± 10 vs. $75 \pm 46 \text{ mm}^2$) and 72 h (4017 ± 560 vs. $339 \pm 313 \text{ mm}^2$) and began approaching baseline by day 7 (2487 ± 927 vs. $428 \pm 299 \text{ mm}^2$), compared to ML-7-treated mice (Fig. 2H). For EB content ($p < 0.0001$ and $p < 0.01$), time course was not significant ($p = 0.7288$; Fig. 2I, graph).

Brain edema

Mice treated with saline had significantly higher wet/dry weight ratio only at 72 h (2.2 ± 0.05 vs. $1.94 \pm 0.07 \text{ mm}^2$), compared to the ML-7 group (Fig. 2J) graph. There were no intergroup differences in development of cerebral edema at 24 h (2.03 ± 0.20 vs. $1.96 \pm 0.03 \text{ mm}^2$) and 48 h (2.05 ± 0.03 vs. $1.98 \pm 0.03 \text{ mm}^2$) and on day 7 (2.17 ± 0.06 vs. $2.17 \pm 0.08 \text{ mm}^2$). Time course was not significant ($p = 0.9509$).

FIG. 2. MLCK expression, albumin extravasation, Evans Blue content, and brain edema after TBI. MLCK expression in saline- (A) and ML-7-treated (B) mice. Positive MLCK cells (C). MLCK expression is down-regulated with the peak difference at 24 h in ML-7-treated mice, compared to saline treatment ($*p < 0.001$; $**p < 0.01$). MLCK (yellow/green fluorescence), DAPI nuclear stain (blue), and NeuN neuronal stain (red). Albumin immunostaining in saline- (D) and ML-7-treated (E) mice. (F) Albumin extravasation is decreased in ML-7-treated mice, when compared to saline-treated mice, with a peak difference at 72 h and begins to return to baseline by day 7 ($***p < 0.001$; $**p < 0.0001$; $*p < 0.01$). Albumin (brown stain); scale bars, $25 \mu\text{m}$. Evans Blue representative brain sections from saline- (G) and ML-7-treated mice (H). The amount of Evans Blue content (I) was decreased by ML-7 treatment, compared to saline treatment, until 72 h and then began returning to baseline by day 7 ($***p < 0.0001$; $**p < 0.01$). (J) Wet/dry weight in saline- and ML-7-treated mice. Wet/dry weight ratios were similar in both saline- and ML-7-treated mice until 72 h when ML-7-treated mice significantly reduced wet/dry ratio, compared to saline-treated mice ($*p < 0.05$). Data are mean $\pm$ standard error of the mean, as measured by two-way analysis of variance; $n = 6$ per group. MLCK, myosin light-chain kinase; TBI, traumatic brain injury; DAPI, 4',6-diamidino-2-phenylindole. Color image is available online at www.liebertpub.com/neu

Motor and cognitive testing

Wire hang test. Mice treated with either saline or ML-7 were tested for motor control on day 7. Hang times for each treatment group were averaged. Saline-treated mice had significantly lower hang times, compared to ML-7-treated mice (21 vs. 57 sec, respectively; Fig. 3A).

Two-object novel recognition test. The ability to differentiate a new from an old object was investigated in mice treated with either saline or ML-7 on day 7. Saline-treated mice were unable to distinguish the new from the old object (old vs. new, 2.66 touches). Mice treated with ML-7 were significantly more likely to investigate the new object, compared to the old object (old vs. new, 10.50 touches; Fig. 3B).

Discussion

Our results corroborate previous findings that MLCK plays a pivotal role in barrier dysfunction after TBI. The results of this study show that inhibition of MLCK (1) reduced MLCK expression, (2) reduced infiltration of albumin into parenchymal tissue, (3) inhibited EB extravasation, (4) reduced cerebral edema, and (5) preserved cognitive and motor function after TBI in PND-24 mice.

TBI is well known to cause the development of cerebral edema, and despite the often late onset of cerebral edema, it is still one of the main determinants of neurological outcome after TBI.²³ Diffuse cerebral edema develops more commonly in children than adults after TBI.²⁴ Children who develop diffuse cerebral edema have a higher mortality rate (53%) than children who do not develop cerebral edema (16%) and adults who develop cerebral edema (46%).²⁵ An immature rat model of closed head injury using a weight drop showed that young rats, when compared to adult rats, had similar neurological injury patterns and edema patterns, but

required one quarter of the weight to produce a similar injury to the adult rat.²⁴

Cerebral edema develops from two forms of edema: vasogenic and cytotoxic. Vasogenic edema results from increased BBB permeability and apoptotic cell death.^{10,26,27} It is caused by dysfunction in the autoregulation of brain perfusion and paracellular transport as a result of disruption of the tight and adherens junctions.^{28,29} Cytotoxic edema results from a shift of water content from the extra- to the intracellular compartments.¹⁰

MLCK is responsible for stimulating the cascade of cytoskeletal changes that lead to disruption of the junctions in endothelial and epithelial cells.³⁰ MLCK has two main isoforms: the long form (210 kDa) and the short form (130 kDa). Both forms of MLCK are regulated by calcium calmodulin and are responsible for phosphorylation of myosin light chain (MLC), leading to cell contraction. MLCK210 is the prominent form of MLCK in endothelial cells and nonmuscle cells and possesses an amino-terminal extension that enables its interaction with the actin cytoskeleton, compared with MLCK130.³ MLCK is present in multiple cell types, including cerebral vasculature endothelial cells, neurons, astrocytes, and pericytes.^{32–36} However, the specific role of different MLCK isoforms in the different cell types in the brain remains to be determined.

ML-7 has been known to inhibit MLCK. Inhibition of MLCK prevents junctional disruption and paracellular transport of fluid and macromolecules, thus minimizing BBB fluid shifts and disruption of osmotic gradients. This allows cellular energy pathways to function longer, preventing accumulation of intracellular Ca^{2+} and toxic intracellular metabolites. Without the development of the cytotoxic changes, inflammatory mediators are minimized, allowing time for recovery from the ischemia reperfusion suffered after TBI.

Studies in multiple organs, such as lungs, skin, intestines, and brains, support the idea that phosphorylation of MLC by MLCK is a

FIG. 3. Wire hang and two object novel recognition tests. (A) Wire hang ability of saline-treated mice (black bars) was compared to wire hang ability of ML-7-treated mice (white bars); latency to fall from wire cage lid was compared for both groups. Saline-treated mice fell in less than 20s, whereas ML-7-treated mice were able to maintain grip for over 60s (* $p < 0.001$). (B) Two-object novel recognition test after TBI. Touches to an old object recognized by mice were compared to touches to a new object. Saline-treated mice (black bars) investigated both objects equally. ML-7-treated TBI mice recognized the new object as novel, compared to saline-treated mice (white bars; * $p < 0.01$). TBI, traumatic brain injury.

key step in disrupting the endothelial barrier leading to increased vascular permeability.^{13,18,37–32} Here, we show that MLCK is up-regulated after TBI, peaking in the first 24 h. Up-regulation of MLCK results in endothelial cell contraction and disruption of the tight junctions, occludin 5, and claudin in bovine brain microvascular endothelial cells exposed to ethanol.³⁸ This allows albumin to extravasate from the microvasculature into the brain parenchyma.²⁷ Here, we show that albumin extravasates after TBI, peaking at 72 h. This time frame would suggest that up-regulation of MLCK was the precipitating event leading to albumin extravasation. We have previously shown that as albumin extravasates from the vasculature, it interacts with astrocytes and stimulates up-regulation of MLCK in both the 210 and 130 isoforms in astrocytes.¹⁵ Albumin also stimulates uptake of Ca^{2+} in astrocytes through the IP_3R (inositol trisphosphate receptor) by an IP_3R -gated intracellular Ca^{2+} release, which stimulates MLCK phosphorylation.^{36,41} This uptake of calcium and stimulation of MLCK completes the disruption of the BBB and allows macromolecules to move into the brain parenchyma, perpetuating the cyclic disruption of the BBB.

Here, we show that inhibition of MLCK at a clinically relevant time point—4 h after TBI—was able to reduce up-regulation of MLCK. This reduction in up-regulation of MLCK led to (1) improved integrity of the BBB, as measured by the decreased expression of MLCK in ML-7-treated TBI mice over saline-treated TBI mice, and (2) a decrease in infiltration of albumin in the ML-7-treated mice, compared to the saline-treated mice.

EB, an accepted measure of barrier dysfunction, supports this self-stimulating cycle of BBB disruption by continuing to extravasate, peaking at 72 h and slowly trending toward insignificance by day 7. Inhibition of MLCK by ML-7 also reduced the extravasation of EB from the vasculature into the brain parenchyma, indicating less cerebral edema. This is supported by our wet/dry weight measurements that showed that ML-7-treated mice had less cerebral edema at 72 h, which is considered to be the peak of edema development after TBI. This supports a decrease in the vasogenic component of cerebral edema.

However, cerebral edema can also be induced by cytotoxic edema that can occur after TBI. It develops in response to an osmotic gradient that develops after failure of adenosine-triphosphate-dependent Na^+/K^+ pumps as a result of ischemia that develops after TBI. Cytotoxic edema adversely affects cellular function by altering the metabolism of the cells after TBI.⁴² Aquaporins, matrix metalloproteinases, inflammatory mediators, and oxidative stress have all been implicated in the development of the cytotoxic cerebral edema that occurs after TBI.^{8,11,43–48} It would be reasonable to think that MLCK plays a role in the development of cytotoxic edema as well as vasogenic edema. The common thread between each of these mechanisms and MLCK is calcium calmodulin; however, how MLCK interacts with any of these pathways after TBI has yet to be determined.

Clinically, cerebral edema correlates with neurologic outcomes after TBI. To further support the idea of MLCK as a treatment target for BBB dysfunction after TBI and ultimately improve cognitive and motor outcomes, we tested mice on the wire hang and two-object novel recognition tests. Mice treated with ML-7 after TBI had preserved motor function, as shown by the ability to maintain a grip on the wires for 2 min, compared to saline-treated mice. This implies preservation of the motor cortex in ML-7-treated mice, compared to saline-treated mice. To test their ability to perform executive functioning, we used a new object to test the recognition ability of the mice. We showed here that mice treated with ML-7 had improved ability to recognize new objects, com-

pared to saline-treated mice. This shows that they retained the ability to recognize new objects, which would imply they have the ability to form new memories (this ability was not tested on these mice). These two behavior tests focused on a small part of the cognitive and functional ability of the brain, but they support the theory that decreased cerebral edema, as inhibited by ML-7, leads to improved neurological outcomes. Ultimately, this reduction in cerebral edema translated to improved motor and cognitive function. Recently, other models of TBI also showed that decrease in cerebral edema leads to improved neurologic outcome. Recent data support a causal relation between hypoxia-inducible factor/aquaporin-mediated cerebral edema, secondary neuronal injury, and tertiary behavioral deficits post-closed head TBI.⁴⁹ Postcortical control impact of TBI salidroside improved long-term behavioral and histological outcomes and reduced brain edema and apoptosis after TBI, at least partially, by the phosphoinositide 3-kinase/protein kinase B signaling pathway.⁵⁰ These recent works, along with the slight decrease in cerebral edema, as shown in our wet/dry weights at 72 h, support the idea that both vasogenic and cytotoxic edema are detrimental to neurological outcome and that prevention of both may be necessary to functional neurological recovery after TBI.

Conclusions

In conclusion, our studies suggest that inhibition of MLCK reduces cerebral edema, preserves neurological function in postnatal mice, and might prove beneficial in treating TBI patients. Given that cerebral edema is a late complication of TBI, there is plenty of opportunity to intervene before its onset. Understanding the mechanisms that lead to cerebral edema has the potential to result in clinical treatments that may help reduce swelling, preserve brain tissue, and preserve neurological function. Until more is known about all the pathways invoked by TBI, treatment of TBI probably will not progress beyond what has currently existed for more than the past 20 years.⁵¹ A better understanding of these mechanisms could potentially lead to development of treatments that could be useful in reducing swelling, preserving brain tissue, and preserving neurological function in TBI patients.

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Author Disclosure Statement

No competing financial interests exist.

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