Reduction of Cerebral Edema via an Osmotic Transport Device Improves Functional Outcome after Traumatic Brain Injury in Mice

Devin W. McBride, Virginia Donovan, Mike S. Hsu, Andre Obenaus, V.G.J. Rodgers, and Devin K. Binder

Introduction

Traumatic brain injury (TBI) is the foremost cause of morbidity and mortality in persons under 45 years of age worldwide. Approximately 200,000 TBI victims in the United States require hospitalization annually, resulting in about 52,000 deaths [13]. TBI has a primary injury phase, caused by the direct external mechanical force, and a secondary injury phase, caused by a myriad of delayed deleterious physiological events. Secondary injury, characterized by cerebral edema, is a major contributor to the morbidity and mortality after TBI [10]. Cerebral edema, an increase in brain tissue water content, is classically identified as either vasogenic edema, water accumulation in the extracellular space after blood-brain barrier (BBB) disruption, or cytotoxic edema, intracellular water accumulation [10, 13].

Cerebral edema resulting from TBI includes both cytotoxic and vasogenic edema mechanisms. After TBI, glial cells swell due to shifts in the extracellular pH and ion concentrations, such as potassium, sodium, and chloride [10]. Vasogenic edema, caused by direct BBB injury, combines with the resulting cytotoxic edema. Ultimately, both types of edema cause a vicious cycle that can culminate in uncontrollable brain swelling, resulting in permanent brain damage or death.

In recent years, advances in diagnostic imaging allow for treatment and surgical intervention of severe brain edema to be performed more rapidly following TBI [3]. Severe TBI management requires a combinatorial approach of surgeries and therapies including osmotherapy, ventriculostomy, and decompressive craniectomy. Although these three treatments are standard practice for severe brain edema, even combinations of them may have limited success in treating patients [5, 16, 18]. Osmotherapy may be effective at acutely reducing intracranial pressure (ICP), but its disadvantages are clinical variability, transient duration of its effects, and possible deleterious systemic effects [2, 4, 8, 11, 17]. Ventriculostomy alone is often ineffective at reducing brain edema because removing the total volume of cerebrospinal fluid (CSF), approximately 150 ml for adults, only accounts for about 10 % of brain volume. Performing decompressive craniectomy surgery for severe TBI reduces ICP elevations, however, it does not treat cerebral edema directly. Recently, the therapeutic benefits of decompressive craniectomy have been debated; the DECRA trial results suggest that performing a decompressive craniectomy for diffuse TBI may not alter patient outcomes [5].

The ideal treatment for severe cerebral edema should be capable of directly removing water from injured tissue in a controlled manner that does cause harm to healthy tissue. An osmotic transport device (OTD) is one such intervention that directly extracts water from brain tissue via direct osmotherapy. We have developed an OTD using a hollow-fiber membrane module embedded in a hydratable material for osmotic therapy. Treatment by direct osmotherapy requires direct contact with the injured, edematous tissue for water extraction. An OTD was recently shown to enhance survival rate of water intoxicated mice [14] and reduce brain water

D.W. McBride
Department of Physiology and Pharmacology, Loma Linda University School of Medicine, Loma Linda, CA, USA

V. Donovan
Cell, Molecular and Developmental Biology Program, University of California, Riverside, CA, USA

M.S. Hsu • D.K. Binder, MD, PhD (✉)
Division of Biomedical Sciences, School of Medicine, University of California, 1247 Webber Hall, Riverside, CA 92521, USA
e-mail: dbinder@ucr.edu

A. Obenaus
Cell, Molecular and Developmental Biology Program, University of California, Riverside, CA, USA

Department of Pediatrics, Loma Linda University School of Medicine, Loma Linda, CA, USA

V.G.J. Rodgers
Department of Bioengineering, University of California, Riverside, CA, USA

R.L. Applegate et al. (eds.), *Brain Edema XVI: Translate Basic Science into Clinical Practice*, Acta Neurochirurgica Supplement, Vol. 121,
DOI 10.1007/978-3-319-18497-5_49, © Springer International Publishing Switzerland 2016

rapplegate@llu.edu

content in mice after TBI [15]. The purpose of this study was to evaluate the effect of direct osmotherapy using an OTD for improving neurobehavior in mice after TBI.

Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Loma Linda University. Adult female C57/BL6 mice, 10–12 weeks old, were used in all experiments.

Traumatic Brain Injury Model

Animals were anesthetized with 3 % isoflurane in O_2 (2.5 l/min initial, 1.5 l/min sustained). Anesthetized animals were placed into a standard rodent stereotactic frame, a midline skin incision was made, and a right-sided craniectomy (5 mm diameter) was performed (1 mm posterior to the bregma and 1 mm lateral to midline). TBI was induced via controlled cortical impact. Briefly, a 3-mm impactor tip was discharged at a 20° angle with a velocity of 5.0 m/s, a 200 ms dwell time, and an impact depth of 1.5 mm. After TBI, the craniectomy was left open, the skin was sutured, and the animal was allowed to recover.

Treatment

One day following TBI, animals were anesthetized with 3 % isoflurane in O_2 (2.5 l/min initial, 1.5 l/min sustained). Sutures were removed to expose the injury site. One group of animals ($n=3$) continued to be treated with a craniectomy for 2 more hours (craniectomy lasted 26 h in total). The other group of animals ($n=3$) was treated with a craniectomy and an OTD for 2 h (craniectomy for 24 h followed by 2 h of craniectomy plus OTD). After 2 h treatment, the craniectomy was sealed with bone wax, skin was sutured, and the animal was allowed to recover.

OTD treatment was previously described [14, 15]. Briefly, the lumen solution (350 g/l bovine serum albumin in artificial CSF at pH 7.4) was passed through hollow fibers (regenerated cellulose, 13 kDa molecular weight cut-off; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) embedded in an agar hydrogel (0.3 % agar in artificial CSF, pH 7.4) with a Reynolds number between 50 and 100.

Neurobehavior Testing

One day before surgical procedures, all animals were subjected to neurobehavioral testing. The foot fault test was used to evaluate sensorimotor response and proprioception. The beam balance test was used to evaluate proprioception and balance. Animals were also subjected to post-TBI neurobehavior testing 1 day after treatment (2 days post TBI). Additionally, naïve animals ($n=6$) were subjected to the neurobehavior tests.

A rectangular grid (3 ft × 1 ft, 0.6 in grid spacing, 7.5° from horizontal) was used for the foot fault test. Each animal was allowed to freely move on the grid for 60 s per trial and a total of two trials (30 min between each trial). Each trial was video recorded for post-test analysis. A foot fault was defined as the misplacement of a fore or hind limb, such that the paw fell completely through the bars. The number of hind limb foot faults per active time were analyzed by an investigator blinded to experimental conditions. Active time was defined as the time spent exploring the grid. Number of hind limb foot faults per active time is presented as the mean ± standard error of the mean (SEM). Intergroup comparisons were analyzed using one-way analysis of variance (ANOVA) with Tukey post hoc tests.

The beam balance test used a Plexiglas beam (2 ft × 0.2 in, 2 ft tall) to measure the time to fall, distance traveled, and number of turns. The animals were placed perpendicular to the beam in the center. Each animal was allowed to walk unrestricted in either direction for up to 60 s per trial (time to fall or 60 s, whichever came first) and a total of two trials (30 min between each trial). Each trial was video recorded for post-test analysis. The time to fall (s), distance traveled (recorded in 5 cm ticks), and number of turns (left, right, total) were analyzed by an investigator blinded to experimental conditions. The time to fall data for each trial was included in the analysis. The average distance traveled (between the two trials) was normalized to the average distance traveled in the pretest performance (pre-injury). The average number of turns was normalized to the average number of turns in the pretest performance for the number of left, right, and total turns. Each measure is presented as the mean ± SEM. Intergroup comparisons were analyzed using one-way ANOVA with Tukey post hoc tests for time to fall and ANOVA on ranks for normalized distance traveled and normalized number of turns (left, right, and total turns analyzed independently).

Results

Foot Fault Test

Naïve animals (Naïve, $n=6$) performing the foot fault test were able to traverse the grid having only 0.08 ± 0.018 hind limb foot faults per second. Injured animals treated with only a craniectomy (TBI + C, $n=3$) had a significantly higher number of hind limb foot faults per second (0.55 ± 0.294) compared with those of naïve animals ($p<0.05$). When TBI-injured animals are

rapplegate@llu.edu

treated with a craniectomy and an OTD (TBI+C+OTD, $n=3$), the number of hind limb foot faults per second (0.19±0.038) were reduced to a level comparable to those of naïve animals ($p>0.05$) but not statistically different from injured animals treated with craniectomy only ($p>0.05$) (Fig. 1).

Beam Balance Test

Naïve animals (Naïve, $n=6$) subjected to the beam balance test were all able to walk on the beam for the entire time allotted, so the time to fall was 60±0.0 s. However, injured animals treated with a craniectomy only (TBI+C, $n=3$) had more difficulty remaining on the beam (time to fall: 43±10.6 s); time to fall for injured animals treated with a craniectomy only was significantly lower than that of naïve animals ($p<0.05$). Treatment with a craniectomy plus an OTD (TBI+C+OTD) improved their proprioception (no animals fell, time to fall: 60±0.0 s) to that of the naïve group ($p>0.05$) (Fig. 2a).

In the 60 s given to naïve animals for walking on the beam, the distance traveled was 115±65 cm. When normalized to the baseline distance traveled for each animal, the naïve group had a normalized distance traveled of 1.0±0.08. Injured animals treated with a craniectomy only had a significantly lower normalized distance traveled (0.5±0.12) compared with naïve animals ($p<0.05$). But when injured animals are treated with a craniectomy and an OTD, the normalized distance traveled (1.2±0.25) was significantly higher than injured animals treated with only a craniectomy ($p<0.05$) and indistinguishable from naïve animals ($p>0.05$) (Fig. 2b).

The number of turns, normalized to naïve values, for the naïve animals were 1.0±0.26, 1.0±0.21, and 1.0±0.13 for the left, right, and total turns, respectively. Normalized number of turns for injured animals treated with a craniectomy only were 0.0±0.00, 0.3±0.16, and 0.2±0.11 for the left, right, and total turns, respectively. Normalized number of turns for injured animals treated with a craniectomy only were 1.0±0.58, 0.3±0.33, and 0.4±0.29 for the left, right, and total turns, respectively. Statistical significance was observed between the normalized number of turns for naïve animals and injured animals treated with craniectomy only for left and total turns ($p<0.05$). All other intergroup comparisons were not statistically significant ($p>0.05$) (Fig. 2c).

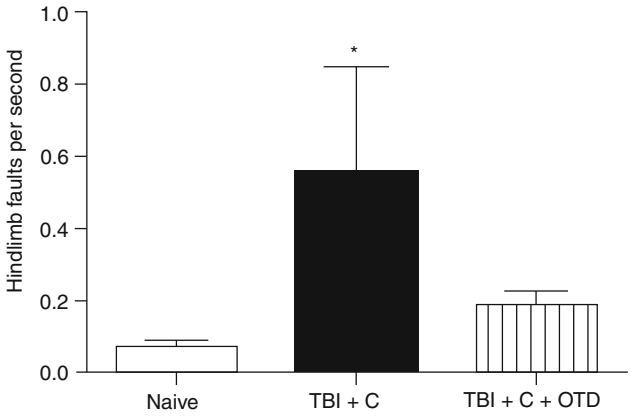

Fig. 1 Hind limb foot faults per second. Injured animals treated with a craniectomy only (*TBI + C*) have significantly more hind limb foot faults per second than naïve animals (Naïve). Injured animals treated with a craniectomy plus OTD (*TBI + C + OTD*) have a reduced number of hind limb foot faults per second, such that the foot faults are comparable (not statistically significant) to the number of foot faults per second by naïve animals. * $p<0.05$ vs Naïve

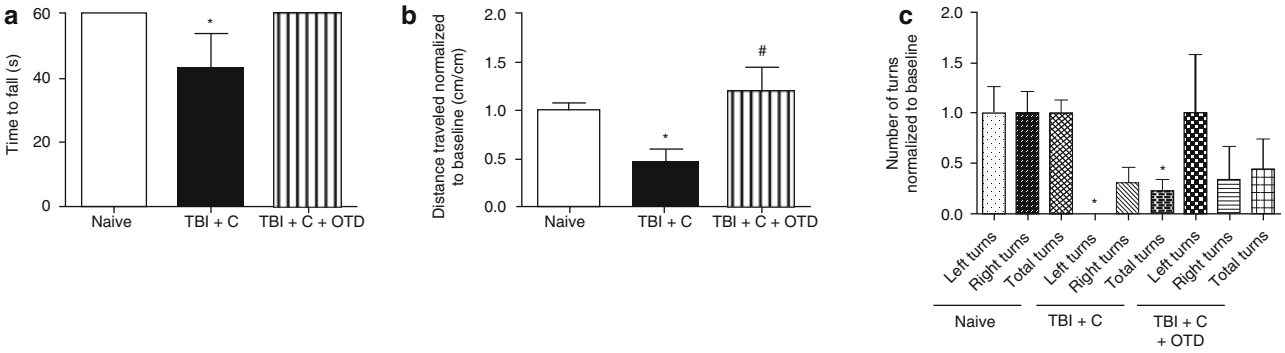

Fig. 2 Beam balance test. (**a**) Time to fall (s) was significantly lower for injured animals treated with a craniectomy (*TBI + C*) compared with naïve animals (Naïve). Injured animals treated with a craniectomy plus OTD (*TBI + C + OTD*) had a time to fall similar to naïve animals. (**b**) Distance traveled (normalized to the pretest baseline distance traveled, cm/cm) was significantly reduced in injured animals treated with a craniectomy only (*TBI + C*) compared with naïve animals. Treatment with a craniectomy plus OTD (*TBI + C + OTD*) significantly improved the distance traveled compared with animals treated with a craniectomy only. (**c**) Number of turns (normalized to the pretest baseline values) was significantly reduced in injured animals treated with a craniectomy only (*TBI + C*) for the number of left and total turns. Injured animals treated with a craniectomy plus OTD (*TBI + C + OTD*) improved the number of turns such that no significant difference was observed between naïve animals for the number of left and total turns. No difference was observed between the numbers of right turns between any of the groups. * $p<0.05$ vs Naïve, # $p<0.05$ vs TBI+C

rapplegate@llu.edu

Discussion

The effect of craniectomy treatment for severe TBI on patient outcomes has been debated. Experimental evidence for the therapeutic benefits, including functional recovery, for craniectomy treatment of severe TBI in animal models has been thoroughly examined and primarily indicates that craniectomy is neuroprotective [6, 20]. However, the study by Floyd et al. [6] examined the effect of craniectomy position and found that slight differences in the position of the craniectomy can produce changes in cognitive performance in a rat TBI model. The results of Floyd et al. were mimicked in the study by Lee et al. [12], which also observed a correlation between the location of craniectomy and functional recovery. Clinical reports are also inconsistent on the effect of craniectomy and patient outcome; whereas several reports observed improved patient outcome after decompressive craniectomy [1, 9, 19], the HeADDFIRST [7] and DECRA [5] clinical trials found conflicting and/or inconclusive results of craniectomy treatment on patient outcome. While we wait for the results of the RESCUEicp trial, the need for a treatment of cerebral edema remains of the utmost importance. An OTD has been shown to improve survival rates of water intoxicated mice [14] and reduce brain edema in mice with TBI [15]. Herein, we hypothesized that direct osmotherapy via an OTD is more neuroprotective than craniectomy alone, improving functional outcomes after TBI in mice.

The neurological function of mice after TBI was examined via testing of sensorimotor, proprioception, and balance. The foot fault test found TBI to be associated with functional deficits in the hind limbs of injured animals treated with a craniectomy only compared with those of naïve animals. When injured animals are treated with a craniectomy and an OTD, the number of hind limb faults decreased such that it was not statistically different from the naïve animals. The beam balance test identified that TBI was associated with balance (time to fall) and motor deficits (reduced distance traveled and turning) in animals treated with a craniectomy only compared with naïve animals. When injured animals are treated with a craniectomy plus an OTD, balance is improved and motor deficits are reduced. These tests supported our hypothesis that craniectomy plus OTD (combined therapy) improves functional outcome better than craniectomy alone and further support the therapeutic benefits of direct osmotherapy.

Although the efficacy of surgical decompressive craniectomy for brain swelling treatment is debated [5], in the most severe cases of cerebral edema it is still performed because it reduces elevated ICP to prevent to occurrence of transtentorial herniation. However, decompressive craniectomy does not directly treat cerebral edema. Yet, direct osmotherapy via an OTD is capable of direct water removal and reduction of brain swelling [15]. The combination of a craniectomy and an OTD is more advantageous than a craniectomy only inasmuch as, not only will ICP be reduced, but water will be directly removed from edematous tissue.

In conclusion, a craniectomy plus an OTD is capable of providing more functional recovery compared with craniectomy alone. Future studies are needed to examine the effect of the craniectomy plus OTD treatment on long-term functional recovery, including sensorimotor, anxiety, and memory neurobehavior tests.

Acknowledgments This work was supported by National Institutes of Health K08 grant NS-059674 (DKB). Devin McBride was supported by a National Science Foundation IGERT Video Bioinformatics Fellowship (Grant DGE 0903667).

Disclosure This device and its applications are described in a patent application titled Compositions and Methods for Reducing Edema (# 20,130,115,267) submitted by the authors and the University of California, Riverside on November 1, 2012. There is no commercial support at this time. The authors have no conflicts of interest to report.

References

1. Adamo MA, Drazin D, Waldman JB (2009) Decompressive craniectomy and postoperative complication management in infants and toddlers with severe traumatic brain injuries. J Neurosurg Pediatr 3:334–339
2. Bingaman WE, Frank JI (1995) Malignant cerebral edema and intracranial hypertension. Neurol Crit Care 13:479–509
3. Bullock MR, Povlishock JT, Carney NA, Ghajar J, Bratton SL, Chestnut RM, McConnell Hammond FF, Harris OA, Hartl R, Manley GT, Nemecek A, Newell DW, Rosenthal G, Schouten J, Shutter L, Timmons SD, Ullman JS, Videtta W, Wilberger JE, Wright DW (2007) Guidelines for the management of severe traumatic brain injury. J Neurotrauma 24:S1–S106
4. Castillo LB, Bugedo GA, Paranhos JL (2009) Mannitol or hypertonic saline for intracranial hypertension? A point of view. Crit Care Resusc 11:151–154
5. Cooper DJ, Rosenfeld JV, Murray L, Arabi YM, Davies AR, D'Urso P, Kossman T, Ponsford J, Seppelt I, Reilly P, Wolfe R, DECRA Trial Investigators, Australian and New Zealand Intensive Care Society Clinical Trials Group (2011) Decompressive craniectomy in diffuse traumatic brain injury. N Engl J Med 364:1493–1502
6. Floyd CL, Golden KM, Black RT, Hamm RJ, Lyeth BG (2002) Craniectomy position affects Morris water maze performance and hippocampal cell loss after parasagittal fluid percussion. J Neurotrauma 19:303–316
7. Frank JI, Schumm LP, Wroblewski K, Chyatte D, Rosengart AJ, Kordeck C, Thisted RA (2014) Hemicraniectomy and durotomy upon deterioration from infarction-related swelling trial: randomized pilot clinical trial. Stroke 45:781–787
8. Hariri RJ (1994) Cerebral edema. Neurosurg Clin N Am 5:687–706
9. Howard JL, Cipolle MD, Anderson M, Sabella V, Shollenberger D, Li PM, Pasquale MD (2008) Outcome after decompressive craniectomy for the treatment of severe traumatic brain injury. J Trauma 65:380–385; discussion 385–386

rapplegate@llu.edu

10. Kettenmann H, Ransom BR (2005) Neuroglia. Oxford University Press, London
11. Keyrouz SG, Dhar R, Diringer MN (2008) Variation in osmotic response to sustained mannitol administration. Neurocrit Care 9:204–209
12. Lee DH, Hong SH, Kim SK, Lee CS, Phi JH, Cho BK, Wang KC (2009) Reproducible and persistent weakness in adult rats after surgical resection of motor cortex: evaluation with limb placement test. Childs Nerv Syst 25:1547–1553
13. Marmarou A (2003) Pathophysiology of traumatic brain edema: current concepts. Acta Neurochir Suppl 86:7–10
14. McBride DW, Hsu MS, Rodgers VGJ, Binder DK (2012) Improved survival following cerebral edema using a novel hollow fiber-hydrogel device. J Neurosurg 116:1389–1394
15. McBride DW, Szu JI, Hale C, Hsu MS, Rodgers VGJ, Binder DK (2014) Reduction of cerebral edema after traumatic brain injury using an osmotic transport device. J Neurotrauma 31:1948–1954
16. Rabinstein AA (2006) Treatment of cerebral edema. Neurologist 12:59–73
17. Suarez JI, Qureshi AI, Bhardwaj A, Williams MA, Schnitzer MS, Mirski M, Hanley DF, Ulatowski JA (1998) Treatment of refractory intracranial hypertension with 23.4% saline. Crit Care Med 26:1118–1122
18. Timofeev I, Dahyot-Fizelier C, Keong N, Norje J, Al-Rawi PG, Czosnyka M, Menon DK, Kirkpatrick PJ, Gupta AK, Hutchinson PJ (2009) Ventriculostomy for control of raised ICP in acute traumatic brain injury. Acta Neurochir Suppl 102:99–104
19. Vahedi K, Vicaut E, Mateo J, Kurtz A, Orabi M, Guichard JP, Boutron C, Couvreur G, Rouanet F, Touze E, Guillon B, Carpentier A, Yelnik A, George B, Payen D, Bousser MG (2007) Sequential-design, multicenter, randomized, controlled trial of early decompressive craniectomy in malignant middle cerebral artery infarction (DECIMAL Trial). Stroke 38:2506–2517
20. Zweckberger K, Stoffel M, Baethmann A, Plesnila N (2003) Effect of decompression craniotomy on increase of contusion volume and functional outcome after controlled cortical impact in mice. J Neurotrauma 20:1307–1314

rapplegate@llu.edu