A New Method for Modulating Traumatic Brain Injury With Mechanical Tissue Resuscitation

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BACKGROUND: Traumatic brain injuries remain a treatment enigma with devastating late results. As terminally differentiated tissue, the brain retains little capacity to regenerate, making early attempts to preserve brain cells after brain injury essential.
OBJECTIVE: To resuscitate damaged tissue by modulating edema, soluble cytokines, and metabolic products in the "halo" of damaged tissue around the area of central injury that progressively becomes compromised. By re-equilibrating the zone of injury milieu, it is postulated neurons in this area will survive and function.
METHODS: Mechanical tissue resuscitation used localized, controlled, subatmospheric pressure directly to the area of controlled cortical impact injury and was compared with untreated injured controls and with sham surgery in a rat model. Functional outcome, T2 magnetic resonance imaging hyperintense volume, magnetic resonance imaging spectroscopy metabolite measurement, tissue water content, injury cavity area, and cortical volume were compared.
RESULTS: There were significant differences between mechanical tissue resuscitation treated and untreated groups in levels of myoinositol, *N*-acetylaspartate, and creatine. Treated animals had significantly less tissue swelling and density than the untreated animals. Nonviable brain tissue areas were smaller in treated animals than in untreated animals. Treated animals performed better than untreated animals in functional tests. Histological analysis showed the remaining viable ipsilateral cerebral area was 58% greater for treated animals than for untreated animals, and the cavity for treated animals was 95% smaller than for untreated animals 1 month after injury.
CONCLUSION: Mechanical tissue resuscitation with controlled subatmospheric pressure can significantly modulate levels of excitatory amino acids and lactate in traumatic brain injury, decrease the water content and volume of injured brain, improve neuronal survival, and speed functional recovery.

KEY WORDS: Subatmospheric pressure treatment, Tissue resuscitation, Traumatic brain injury

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Traumatic brain injuries (TBIs) consist of both primary injury in which tissue is irreversibly damaged from the insult and secondary injury in which cells progressively die because of pathological cascades that culminate from cerebral edema, decreased blood flow, and hypoxia. These cascades include the release of excitatory amino acids and toxic amino acids, as well as accumulation of lactate and increases in water content.

ABBREVIATIONS: CCI, controlled cortical impact; **GABA,** γ-aminobutyric acid; **INS,** myoinositol; **MRS,** magnetic resonance spectroscopy; **MTR,** mechanical tissue resuscitation; **NAA,** *N*-acetylaspartate; **TBI,** traumatic brain injury

Two types of brain edema are thought to contribute: vasogenic edema caused by plasma proteins released when the blood-brain barrier is damaged and cytotoxic brain edema secondary to autodestructive mediation characterized by intracellular water accumulation.[1,2] Edema caused by secondary injury progresses over 24 to 72 hours, resulting in the "talk and die" phenomena.[3] This is postulated to be related to widespread hemispheric disruption of perivascular fluid movements in the brain initiated by a focal edematous cortical lesion.[4]

After injury, the expanding volume of the brain is confined within the cranium and dura so the pressure within the brain must increase, similar to

other compartment syndromes. As cerebral vascular pressure increases above systemic pressure, further ischemia and hypoxia of tissue result with increasing neural loss and worse functional outcomes.[5-8]

Secondary apoptotic or necrotic death as a sequela to the release of, for example, excitatory amino acids and buildup of lactate[9-11] also contributes to ultimate cell death. The release of excitatory amino acids such as glutamate and aspartate disturbs ion homeostasis and increases energy demand and lactate production.[12,13] Microdialysis studies have demonstrated lactate levels up to 50 times normal, which may last as long as 4 days post-injury.[14,15] This increase in lactate reflects increased energy demand and is inversely related to patient outcome.[14,16,17] Lactate production contributes directly to apoptotic neuronal cell death.[13,18] Elevated levels of glutamate have been shown to be correlated with increased levels of lactate.[16]

Disruption of the blood-brain barrier spills intravascular proteins into the central nervous system interstitium and allows migration of neutrophils into the brain tissue.[19] This results in inflammation with release of inflammatory mediators including cytokines and adhesion molecules.[19-23] Initiation of a variety of cascades results along with release of excitatory amino acids, ion shifts, release of proteases, oxygen radicals, complement proteins, and other immune mediators that cause additional neuronal death through concomitant activation of the neuroinflammation cascade.[19,24]

A wide variety of pharmacological interventions have been proposed to clinically treat TBIs, but none have proven to be highly successful.[19,20,25-27] As a nonspecific alternative, decompressive craniectomy has been performed on patients with TBI and increased intracranial pressure that is refractory to medical measures. Although 1 study showed that passive decompressive treatment increased survival and quality of life, another showed no improvement in outcome despite lowering intracranial pressure.[28]

Cerebral mechanical tissue resuscitation (MTR) is based on the authors' extensive experience with negative pressure used to treat cutaneous wounds over the past 15 years. Modulating the area of injury mechanically with negative pressure restores a more physiological environment that favors cellular survival. The differential pressure between the applied vacuum dressing and the injured tissue causes fluid to flow out of the treated tissue. This results in decreased edema and interstitial pressure and the reestablishment of more normal fluid hemodynamics that optimize wound healing. Negative pressure wound therapy has been applied with great success to a wide variety of soft-tissue injuries and conditions of increased pressure elsewhere in the body.[29-31] Within the fluid that is removed are cytokines and other soluble factors associated with inflammation and healing.[32,33] The application of subatmospheric pressure to crush injuries has been shown to remove myoglobin, preventing entry into the systemic circulation and preventing eventual damage to the kidneys.[34] Controlled negative pressure applied to deep partial-thickness burns is effective in decreasing the progression of burn injury into the zone of stasis, thus salvaging considerable tissue that would otherwise die.[35]

This study seeks to ameliorate TBI by controlled localized MTR. In this study, MTR is achieved by application of controlled, localized subatmospheric pressure directly to dura over the site of brain injury in an attempt to decrease edema, remove soluble mediators, and improve ultimate neuron survival and function. As a mechanical procedure, it does not preclude the use of standard simultaneous pharmacological treatment.

METHODS

Animals

Male adult Sprague-Dawley rats weighing 355 ± 15 g were used in this study. All surgical procedures and animal care issues were approved by the Institutional Animal Care and Use Committee and followed Department of Health and Human Services guidelines. The TBI was induced with a controlled cortical impact (CCI) device (Model AMS 201; AmScien Instruments, Richmond, Virginia). Rats were anesthetized with 3.5% isoflurane and maintained with 2% isoflurane during the preparation and the impact procedure. A midline incision was made over the skull, and an 8-mm diameter burr hole made between bregma and lambda (1 mm lateral to midline). The 6-mm diameter impactor tip was centered in the craniectomy site perpendicular to the exposed surface of the brain. Gas pressure of 60 psi was used, giving an impact velocity of approximately 2.7 m/s and duration of approximately 250 milliseconds to produce a brain deformation of 2 mm during the impact.[35-37] Impact resulted in injury to the underlying brain with occasional, inconsistent, small lacerations of the dura.

All study animals were divided into 3 groups. Sham controls underwent craniectomy, placement of an 80- to 150-μm pore open cell foam matrix and closure of the scalp without application of subatmospheric pressure. The untreated group underwent craniectomy, impaction injury, placement of the matrix, and closure of the wound with no application of subatmospheric pressure. The treatment group underwent craniectomy, impaction injury, and placement of the matrix and continuous application of 25 mm Hg of subatmospheric pressure for 72 hours. In these experiments, the matrix was placed directly on the impacted dura and contused brain. The matrix was held in place, and a controlled sealed wound was created by suturing closed the overlying skin. The matrix was connected by a flexible silicone tube to a vacuum pump and a constant subatmospheric pressure of 25 mm Hg was applied continuously. Animals could move freely in the cage during treatment. The pressure of 25 mm Hg was used based on its efficacy of providing negative pressure to the treatment area without eventration of the brain.

T2-Weighted Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy

All magnetic resonance imaging (MRI) was performed 24 hours after injury using a 7-T horizontal bore magnet (Bruker Biospin, Billerica, Massachusetts). Radiofrequency signal transmission and reception were performed with a 38-mm ID Litzcage volume coil (Doty Scientific, Columbia, South Carolina). Animals were anesthetized during imaging with isoflurane by inhalation.

High-resolution T2-weighted axial scout images were planned based on the triplane localizer images. The T2-weighted images were acquired in an anatomically coronal orientation using a RARE 8 pulse sequence.

From the T2-weighted images, brain tissue volume and density of CCI injured, MTR subatmospheric pressure treated, and sham rats were measured by ImageJ Software (National Institutes of Health, Bethesda, Maryland). The dorsal third ventricle was the reference for all measurements. A total of six 1-mm sections from the contusion sites were measured. Total contusion injured brain areas were measured on all coronal T2-weighted MRI as the sum of all injury areas in both groups. The injured area was identified and traced as the hyperintense region ipsilateral to the injured site on T2-weighted MRI.

A cubic voxel (5-mm length of each edge) for shimming was positioned to include as much of the injury site as possible using the Fastmap method. A point resolved spectroscopy pulse sequence voxel was placed in the same position as the shim voxel, but the size of the point resolved spectroscopy pulse voxel was decreased to 4 mm on each edge. One spectrum was acquired with no water suppression for use as a reference when quantifying metabolite concentrations. A second spectrum was acquired using variable pulse power and optimized relaxation delays water suppression. All magnetic resonance spectroscopy (MRS) data were processed and analyzed using a linear combination model.[38-41]

To calibrate absolute metabolite concentrations, the "replace-and-match" method was used.[42] A phantom with known metabolic concentrations was scanned using the same MRS protocol used to scan the live rats in same radiofrequency coil. The MRS data from the calibration phantom was processed using a linear combination model with the same basis set used to analyze the in vivo data and to derive a conversion factor for this system and protocol. That conversion factor was then used to convert the in vivo linear combination model metabolite concentrations to institutional units that are close to millimoles per liter.

Tissue Water Content Measurement

Brain tissues were dissected 48 hours after brain injury with wet and dry weights measured using 3 rats per group. Rats were anesthetized, decapitated, and brain tissues were collected. These tissues included contused cortex and adjacent hippocampus and the ipsilateral, non-contused side. Each section was placed in preweighed containers that were capped to prevent evaporation. The containers were reweighed to the nearest 0.01 mg and opened to dry at 60°C with 0.3-atm pressure for 48 hours. After drying, the samples were reweighed. Tissue water content was measured in the 3 groups by wet weight minus dry weight divided by wet weight resulting in a percentage.[43,44]

Behavioral tests were performed with investigators blinded to the treatment conditions of each animal.

All functional tests were recorded before brain injury and then 24, 48, and 72 hours and 5, 7, 9, 11, 16, 21, and 31 days after brain injury.[45-47]

Rotarod Test

Locomotor behavior was assessed by a rotarod test performed on a Rotemex-5 device (Columbus Instruments, Columbus, Ohio). Rats were trained on the rotarod device 1 week before surgery. The turn speed started at 8 rpm and gradually increased to 27 rpm. Trials were halted if the rat fell off or hung on for 2 consecutive turns. Duration of staying on the rotarod was recorded for 3 trials. The maximum time was 120 seconds.[48]

Balance Beam Test

Vestibulomotor function was evaluated on the balance beam, a narrow elevated wooden beam 30.5 cm long, 1.5 cm wide, and 27 cm from the bench top. Rats were placed on the beam and allowed to remain for 60 seconds. Rats had been pretrained before surgery. The duration of time the rats remained on the beam was recorded for a maximum of 60 seconds.[49-52]

Cylinder Test

To test for asymmetry of forelimb use after brain injury, rats were placed in a transparent cylinder (20 cm in diameter, 45 cm high). Forelimb movements were recorded for 5 minutes. "Wall exploration" is the initial placement of a forelimb on the wall and contact during subsequent lateral movements. Independent forelimb use is expressed by percentage (number of contacts with contralateral forelimb + one half of both)/(ipsilateral forelimb use + contralateral forelimb use + both)·100. This test system tends to find that rats use the forelimb contralateral to the lesion less frequently.[25,51,52]

Histology and Immunohistochemistry

After surgery and behavior testing, the rats used for histological analysis were anesthetized and perfused with heparinized saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were then post fixed overnight at 4°C and kept at −80°C. Coronal sections of injured area were cut into 15-μm sections, mounted, and kept frozen until use. Sections were collected every 1 mm from bregma 2 to bregma 12 according to the rat brain stereotaxic atlas of Paxions and Watson.

Serial sections from each rat were immunostained with a mouse monoclonal antibody against neuron-specific protein NeuN. After blocking, antibody NeuN was diluted in 2% normal serum and incubated over sections for 1 to 2 hours. After washing with phosphate-buffered saline, the slides were incubated with secondary antibody against mouse IgG conjugated with horseradish peroxidase for 1 hour. The slides were visualized with diaminobenzidine (DAB substrate kit, Vector Laboratories, Burlingame, California) after washing with phosphate-buffered saline.[53,54] From images of the stained brains of sham, untreated, and treated animals examined 1 month post-injury, the remaining ipsilateral cerebral cortical areas were measured as was the area of the cavity resulting from the injury.

Statistical Analysis

All study animals had been divided into 3 groups as previously discussed. All data are presented as mean ± SD. Continuous variables compared across groups in sham controls, impacted rats, and impacted plus controlled subatmospheric pressure–treated rats were examined using a 1-way analysis of variance with SAS software (SAS Institute, Cary, North Carolina). Multiple comparisons between groups were obtained using the Bonferroni correction.

RESULTS

Imaging

Figure 1 illustrates typical serial 1-mm T2-weighted magnetic resonance images of the right side cortex and hippocampal area in the 3 groups. Extensive hyperintensities are visible in the untreated animal (Figure 1B) as opposed to the sham (Figure 1A). Amelioration of the hyperintensity and overall volume is evident when comparing untreated (Figure 1B) with treated (Figure 1C). Table 1 reveals changes in brain tissue volume and integrated tissue density in the respective areas at 24 hours post-injury. Twenty-four hours after CCI, untreated ipsilateral injured brain tissues demonstrated significant increases in volume and

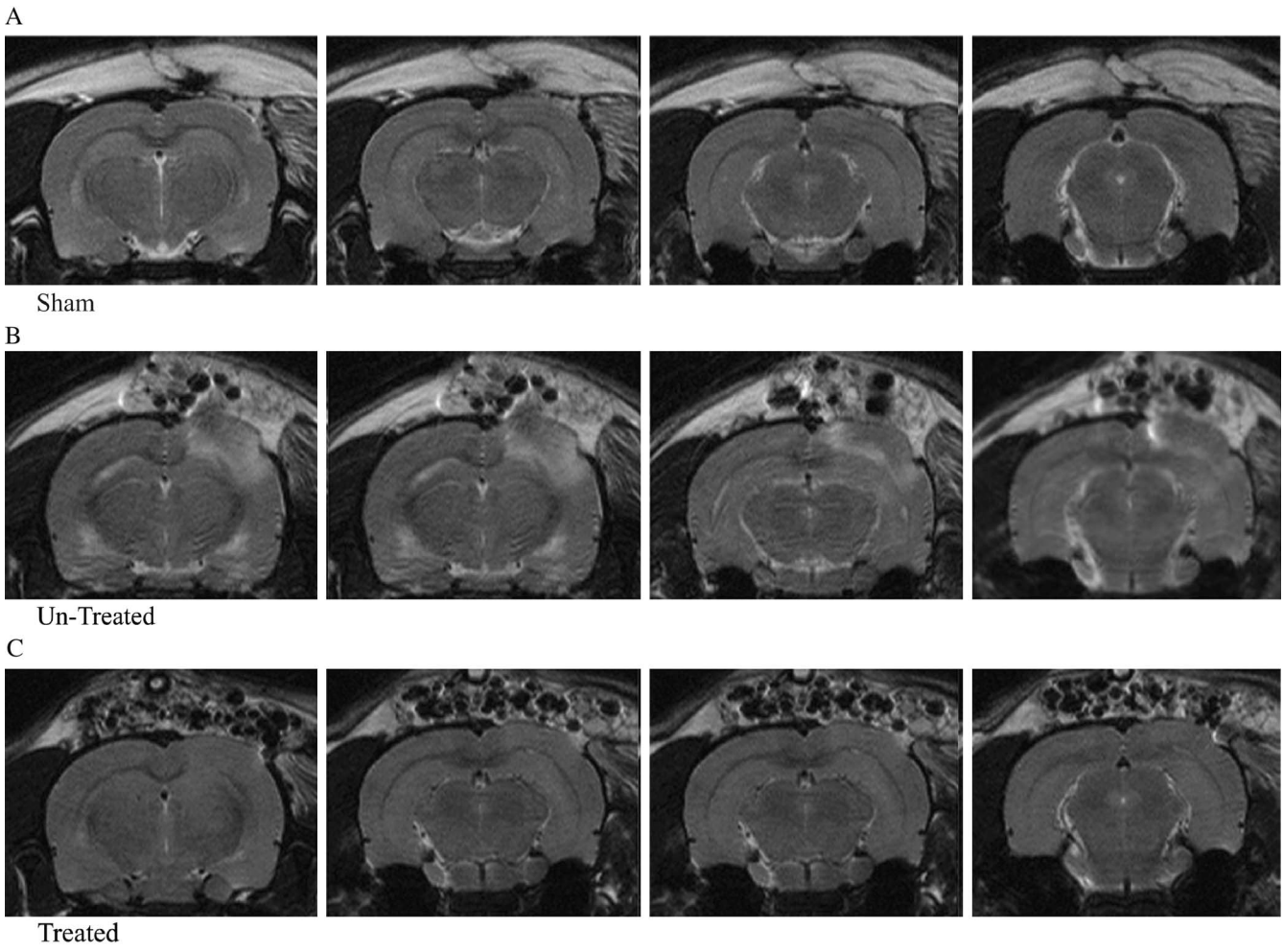


FIGURE 1. *Continuous T2-weighted magnetic resonance images obtained from rat brain 24 hours after controlled cortical impact (CCI) injury.* **A**, *from nonimpacted sham surgery rat.* **B**, *from CCI-injured, untreated animal showing the hyperintensities in the right side cortex and hippocampus area. After mechanical tissue resuscitation treatment (*C*), fewer hyperintensive signals are seen in the CCI-injured area compared with the untreated animals (*B*). The distance between slices in each panel was 1 mm.*

tissue water content swelling over sham ($P < .001$ and $P < .001$, respectively). The injured brain tissue demonstrated significantly less volume and water content after MTR treatment compared with the untreated injured animals ($P < .01$). Brain tissue volume and water content demonstrated no statistical difference in treated animals and sham animals, suggesting an amelioration of injury and more normal brain hemodynamics. Also, the measurement of hyperintense areas in T2-weighted images indicated MTR treatment (Figure 1C) decreased contused brain tissue areas significantly compared with CCI untreated animals (Figure 1B) ($P < 0.01$).

Ipsilateral Brain Tissue Volume, Water Density, and Contused Tissue Area on T2-Weighted MRI

Analysis of T2-weighted images (Figures 1 and 2, Table 1) showed a significant difference in tissue volume ($P < .001$) and density ($P < .001$) between the sham animals (A) and the untreated impacted animals (B), with untreated animals exhibiting a higher tissue volume and density (Table 1). Treated animals had significantly less tissue swelling ($P < .01$) and density ($P < .001$) than the untreated animals. There was no significant difference in tissue volume seen between treated and sham animals. Also, contused brain tissue areas are much smaller in MTR-treated animals than in untreated impacted animals ($P < .01$).

Tissue Water Content Weighted Measurement

The physical measurement of water content of brain tissue showed a significant ($P < .001$) decrease in mean local tissue water content for treated animals (80 ± 0.09%) vs untreated animals (83 ± 0.31%). Sham animals (79 ± 0.42%) had significantly less ($P < .001$) water content than untreated animals

TABLE 1. Measurements of Ipsilateral Brain Tissue Area[a]

	Ipsilateral Tissue Area (Pixels), Mean ± SD	Tissue Integrated Density ($\times 10^3$), Mean ± SD	Contused Tissue Area (Pixels), Mean ± SD
(A) Sham surgery, n = 7	104 ± 10.8	994.85 ± 107.84	N/A
(B) CCI untreated, n = 13	130 ± 7.3	1386.2 ± 117.17	137 ± 40.6
(C) CCI, MTR treated, n = 10	113 ± 16.4	1152.07 ± 166.22	88.8 ± 19.3

[a]N/A, not available; CCI, controlled cortical impact; MTR, mechanical tissue resuscitation. Measurements of ipsilateral brain tissue area, integrated density in injured areas, and contused brain tissue areas from T2-weighted magnetic resonance imaging 24 hours after CCI. Ipsilateral local brain tissues swelled and expanded significantly 24 hours after CCI brain injury (B) ($P < .01$ in CCI rats vs sham rats). After MTR treatment, the injured brain tissue volume was significantly less compared with untreated animals (B) ($P < .01$). There is no significant difference in volume in treated animals compared with sham animals. On T2-weighted magnetic resonance imaging, the untreated injured brain tissues (B) showed higher integrated density compared with sham animals (A) ($P < .01$), indicating more tissue water content. MTR treatment (C) reduced tissue water content significantly compared with CCI untreated animals (B) ($P < .01$). Treated groups were treated 24 hours post-impaction. Treated animals (C) continued to have a significantly higher tissue integrated density than sham animals ($P < .05$) The measurement of hyperintensive areas on T2-weighted magnetic resonance imaging showed that MTR treatment (C) decreased contused brain tissue areas significantly compared with CCI untreated animals (B) ($P < .01$).

(83 ± 0.31%) There were insignificant ($P < .05$) differences between sham animals and treated animals.

Metabolite Concentrations on MRS

Analysis of single-voxel spectroscopy data (Figure 3, Table 2) of sham vs untreated and treated groups of animals showed changes in amino acid concentrations and lactate levels. Significantly altered levels were found for untreated vs sham groups for glutamate ($P < .01$), myoinositol ($P < .05$), *N*-acetylaspartate (NAA) ($P < .01$), and total creatine ($P = .1$) (Table 3). Treatment with MTR resulted in concentrations of these amino acids approaching baseline levels. There was a difference in metabolite levels between treated and untreated animals. Significant differences in levels of myoinositol ($P < .05$) were found, with levels of *NAA* ($P = .09$) and creatine ($P = .09$) approaching significance. The differences between sham and treated groups were not statistically different.

Behavior Tests

Rotarod

A total of 9 rats were in the untreated group, 9 rats in the treated group, and 6 rats in the sham group. There was a statistical difference in performance through all but 1 test point between animals in the sham and untreated groups for 16 days ($P < .05$). Treated animals performed statistically better than untreated animals for the first 5 days after injury. After day 5, there was no significant difference between treated and untreated animals.

Untreated animals exhibited a significant ($P < .001$) decrease in ability to stay on the rod at all times after CCI (Figure 4). Treated animals also showed a significant decrease at postinjury

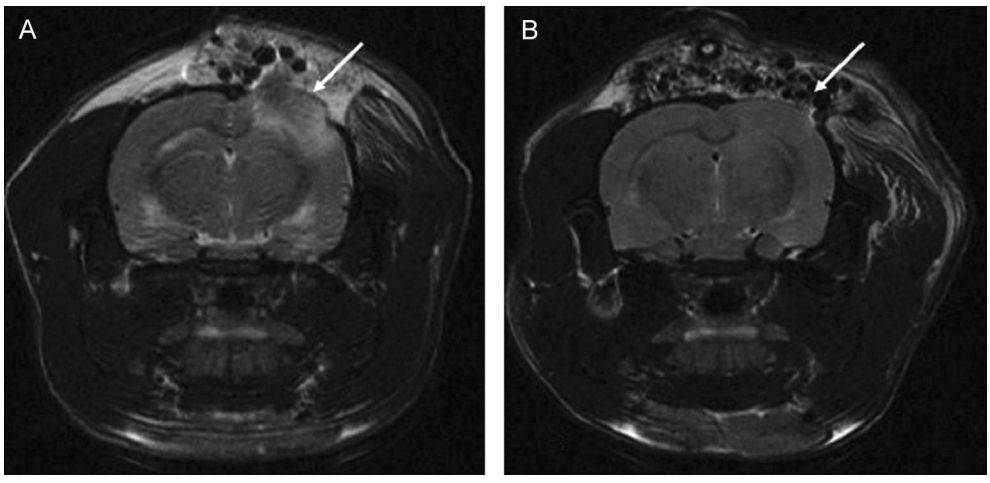


FIGURE 2. *Higher magnification of the T2-weighted magnetic resonance images in Figure 1.* **A**, *an untreated animal with greater water content than a treated animal (***B***). Arrows highlight areas of injury.*

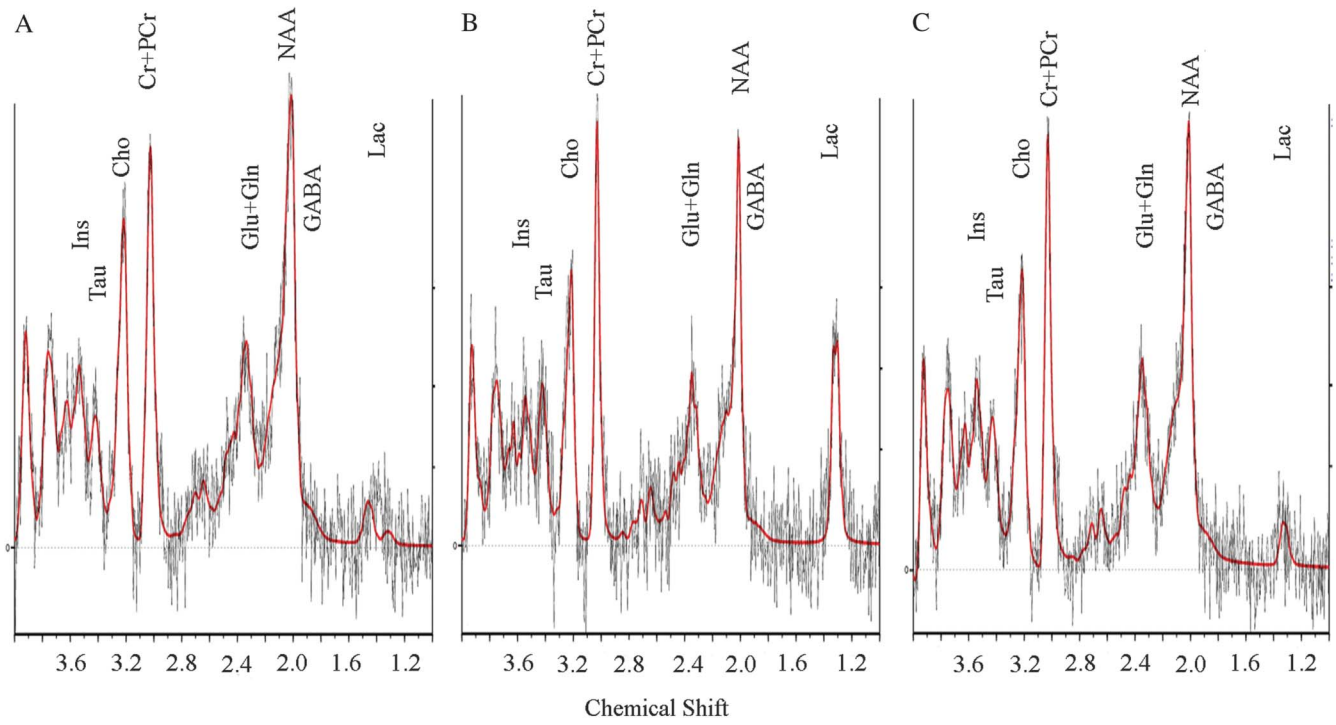


FIGURE 3. *Illustration of single-voxel magnetic resonance spectrum obtained from brains of sham surgery (A), untreated (B), controlled cortical impact–injured and mechanical tissue resuscitation–treated (C) animals. The metabolites are labeled as Ins (myoinositol), Tau (taurine), Cho (choline-containing compounds), Cr+PCr (creatine and phosphorus creatine), Glu+Gln (glutamate and glutamine), NAA (N-acetylaspartate), GABA (γ-aminobutyric acid), and Lac (lactate).*

day 1, but by postinjury day 2 had nearly recovered to sham levels. Treated animals exhibited significantly restored ability to stay on the rod compared with untreated animals. All animals showed a decrease in ability to stay on the rod 3 weeks after the start of testing, probably reflective of weight gain.

Beam Balance Test

Similar to the rotarod test results, untreated animals exhibited a significant ($P < .05$) decrease in ability to stay on the beam after injury compared with sham animals (Figure 5). Treated animals exhibited a nonsignificant decrease on postinjury day 1, but had recovered by postinjury day 2 to levels similar to those of sham animals. Treated animals performed better than untreated animals for the first 5 days after CCI ($P < .05$). There were no significant differences between the 2 groups after day 5.

Cylinder Test

Untreated animals exhibited a significant ($P < .05$) decrease in wall exploration symmetry after injury for 9 days (Figure 6). Treated animals exhibited a slight, nonsignificant decrease in symmetry, but had returned to levels similar to those of sham animals by postinjury day 3. Treated animals exhibited a significant increase in symmetry compared with untreated animals before day 16, after which the difference was not significant.

Histology and Immunohistochemistry

Within 72 hours post-injury, a significant increase in the number of cells undergoing neuronal degradation and death was noted. Treated animals demonstrated fewer such cells (Figure 7).

At 1 month post-injury, the remaining viable ipsilateral cerebral areas were significantly ($P < .01$) greater for sham animals than untreated animals (Figure 8). The remaining ipsilateral cortical area for treated animals was significantly greater ($P < .01$) than for untreated animals, but less than sham animals ($P = .15$) (Figure 9). The mean area of the cavity resulting from the CCI for untreated animals was significantly ($P < .05$) larger than in sham animals. The cavity for treated animals was significantly ($P < .01$) smaller than for untreated animals and was not significantly different from sham animals (Figure 10).

DISCUSSION

TBI extent is determined by the force and duration of impact and the specific location of brain to which it is applied. Earlier reports[55-57] demonstrated posttraumatic histological findings of cortical and hippocampal neuronal loss, brain tissue cavitations, and ventricle enlargement. Similar findings were seen in all of our untreated control animals. Moderate swelling of the contused brain and dura into the craniectomy site was noted in the

TABLE 2. Metabolite Concentrations[a]

	GABA, Mean ± SD	Glu, Mean ± SD	Gln, Mean ± SD	INS, Mean ± SD	NAA, Mean ± SD	Tau, Mean ± SD	Total Cr, Mean ± SD	Lac, Mean ± SD
Sham surgery	5.88 ± 1.67, n = 6	17.83 ± 3.13, n = 9	9.49 ± 1.32, n = 9	11.42 ± 1.41, n = 9	12.09 ± 2.58, n = 9	6.72 ± 1.66, n = 9	12.51 ± 1.51, n = 9	2.08 ± 2.66, n = 5
CCI untreated	4.84 ± 1.47, n = 4	13.20 ± 2.22, n = 12	8.63 ± 1.66, n = 12	8.89 ± 1.74, n = 12	8.58 ± 1.89, n = 12	6.73 ± 1.97, n = 12	10.70 ± 1.69, n = 12	7.23 ± 2.41, n = 10
CCI treated, 24 h	5.04 ± 0.89, n = 11	16.03 ± 3.29, n = 12	8.95 ± 1.53, n = 12	11.28 ± 2.27, n = 12	10.79 ± 2.36, n = 12	8.06 ± 1.38, n = 12	12.39 ± 1.90, n = 12	4.36 ± 2.47, n = 9

[a]GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; INS, myoinositol; NAA, *N*-acetylaspartate; Tau, taurine; Cr, creatine; Lac, lactate. Metabolite concentrations (millimoles) and data variations (mean ± SD) 24 hours after injury in ipsilateral controlled cortical impact–injured brains with/without mechanical tissue resuscitation treatment and sham surgery animals by magnetic resonance spectra analysis. n indicates the numbers of animals for each metabolite qualified for magnetic resonance spectra data.

untreated group. However, the degree of swelling was not sufficient to produce strangulation at the craniectomy and did not appear to contribute to the ultimate lesion size.

Application of controlled negative pressure for 48 hours directly to the injured cortex immediately after injury resulted in significant amelioration of the degree and extent of the ultimate pathological damage. Application of MTR to injured brain resulted in measurably decreased brain water content, contused tissue area, and brain volume; re-equilibration of metabolites to levels approximating sham controls; speedier functional recovery by 3 different tests; and ultimate preserved neural tissue. This study suggests that with this technique, traumatically injured brain tissue within the halo of injury can be resuscitated and salvaged. Importantly, this technique does not preclude the simultaneous use of other modalities such as mannitol, hypertonic saline, and neuroprotective strategies such as antioxidants.

Toxic cerebral edema causes rapid depletion of substrates such as oxygen, glucose, and adenosine triphosphate. Microcirculation adjacent to the initial injury demonstrates impaired vascular reactivity soon after injury, exacerbating vasogenic edema by allowing osmotic molecules and radicals to enter the contused brain area.[25,26] Cellular metabolites from surviving cells in the damaged area cannot be efficiently removed and accumulate in the interstitial and perivascular spaces. This results in the accumulation of more water in the contused area around the impact, resulting in further capillary compression, decreased cerebral perfusion, microthrombi, and other secondary injury. This sequence is probably responsible for the development of the traumatic penumbra.[58-64]

Sections of the cerebral injury showed that water content increased 83% at 48 hours after CCI in the untreated group. These results were similar to those of previous reports[50] but differed from others.[65-69] Forty-eight hours after injury, untreated animals had accumulated significantly more local water content than the sham brains ($P < .001$), indicating a significant disruption of brain hemodynamics. Treatment of animals with MTR resulted in significantly less local water than in the untreated group at 48 hours ($P < .001$). Water content of the treated group was not statistically different from that in animals undergoing sham surgery, suggesting that the system had successfully extracted a significant amount of water from the injured brain, returning it to a more homeostatic level.

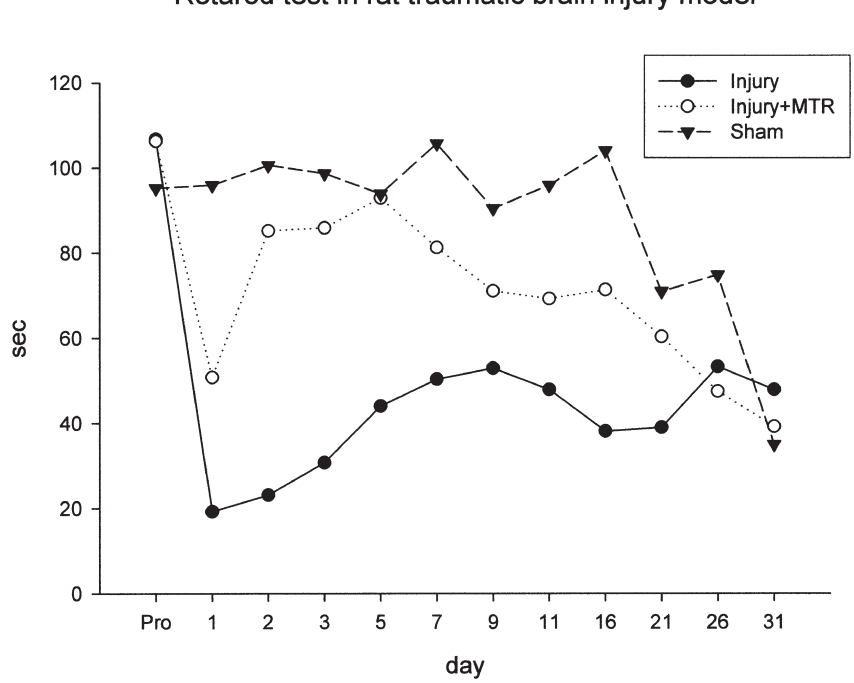
T2-weighted MRI confirmed that signal hyperintensity adjacent to the contused area was acute edema and that the volume of untreated contused brain tissue expands significantly in the first 24 hours after CCI. The area of the contused tissue demonstrated cerebral edema at 6 hours after injury that was maximal at 24 hours, confirming previous reports.[70-73] When rats were treated with a subatmospheric pressure of 25 mm Hg for 24 hours, there was a significant decrease in the contused brain tissue volume and water content on T2-weighted MRI compared with untreated animals. Fluid extracted by the MTR device during the treatment approximated 2 to 6 mL over 72 hours and was usually a yellow serum.

TABLE 3. Brain Metabolite Statistics[a]

	Glu	INS	NAA	Total Cr
CCI untreated vs sham surgery	0.002[b]	0.006[b]	0.002[b]	0.029[c]
CCI untreated vs CCI injured, MTR treated	0.030[c]	0.007[b]	0.03[c]	0.033[c]
Sham surgery vs CCI injured, MTR treated	0.191	0.862	0.228	0.888

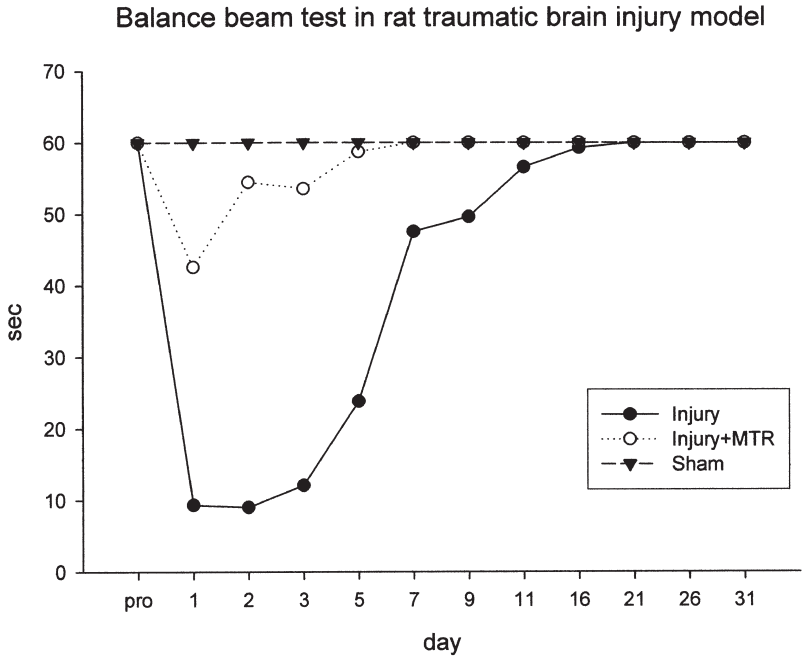
[a]Glu, glutamate; INS, myoinositol; NAA, *N*-acetylaspartate; Cr, creatine; CCI, controlled cortical impact; MTR, mechanical tissue resuscitation. Statistical *P* values for each brain metabolites after comparisons in sham surgery, CCI injured, untreated animals, and CCI injured + MTR-treated animals by analysis of variance. The difference between levels in sham and treated animals are not significantly different.
[b]$P < .01$.
[c]$P < .05$.

These results suggest that a negative pressure gradient is applied by the treatment device to brain tissue and the lymphatic-like perivascular space. Drainage within the perivascular space is attributed to the physical pulsation of the cerebral arterioles. In cerebral edema, this pulsation gradient is decreased, probably diminishing the overall efficiency of pumping in the perivascular space. Creation of a pressure gradient with subatmospheric pressure is postulated to re-establish and possibly facilitate this drainage, allowing for evacuation of edema, lowering of local tissue pressure, and an ultimate increase in local blood supply with possible rescue of damaged neuronal cells.



	day 1	day2	day 3	day 5	day 7	day 9	day 11	day 16
Sham vs treated	+	-	-	-	-	-	-	-
Sham vs non-treated	+	+	+	+	+	-	+	+
Treated vs non-treated	+	+	+	+	-	-	-	-

FIGURE 4. *Rotarod performances in controlled cortical impact–injured, untreated, and MTR-treated animals, and sham surgery animals. Significant differences in an analysis of variance at* $P < .05$ *level for rotarod testing. + indicates significantly different; − indicated not significantly different. The* P *value of each group comparison was determined by the Dunn or Holm-Sidak method. Pro, baseline, presurgery training values.*



	day 1	day 2	day 3	day 5	day 7	day 9	day 11	day 16
Sham vs treated	+	-	-	-	-	-	-	-
Sham vs non-treated	+	+	+	+	+	-	+	+
Treated vs non-treated	+	+	+	+	-	-	-	-

FIGURE 5. *Balance beam tests in injured, untreated, and mechanical tissue resuscitation (MTR)–treated animals and sham surgery animals. Significant differences in an analysis of variance at* P $<$ *.05 level for rotarod testing. + indicates significantly different; − indicated not significantly different. The* P *value of each group comparison was determined by the Dunn or Holm-Sidak method. Pro, baseline, presurgery training values.*

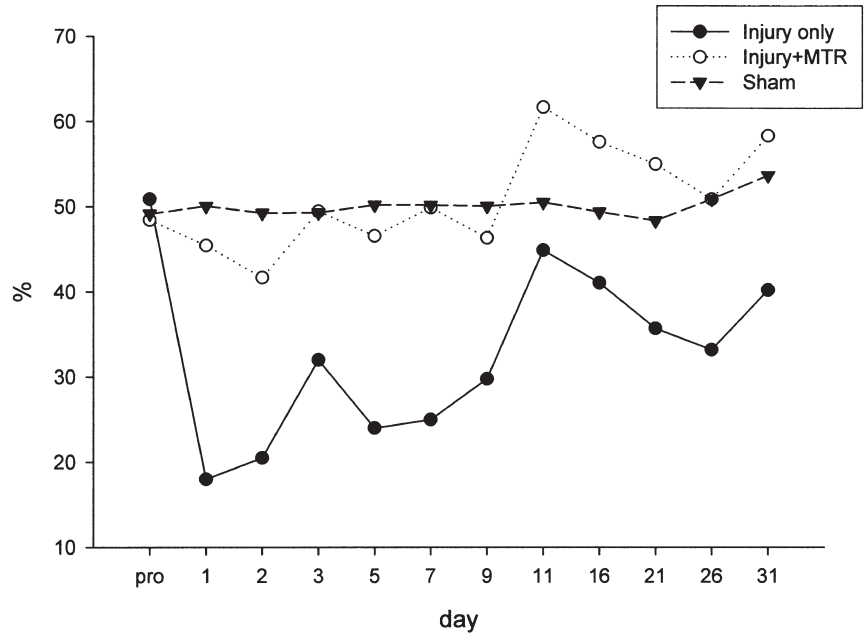
Cerebral Metabolite Modulation

TBIs result in dramatic change in cerebral metabolites. Proton MRS is a simple, noninvasive, and reliable measurement for specific areas within the traumatized brain tissue. Metabolites that are molecular markers for neural function in the injury site may be helpful in predicting the extent of injury and outcome. Neural markers such as NAA, astrocyte markers such as myoinositol (INS), neurotransmitters such as glutamate and glutamine, and energy metabolites such as creatine phosphocreatine, and lactate can be accurately delineated by this technique (Table 2). Twenty-four hours after injury, magnetic resonance spectrum analysis revealed detectable decreases in concentrations of NAA, glutamate, glutamine, INS, and total creatine. These findings are compatible with anatomic and functional impairment of brain tissue after injury compared with sham injury animals. Simultaneously, lactate levels almost tripled 24 hours after injury, as previously reported (Table 3).[40]

Quantitative analysis of INS, choline compounds, creatine/phosphocreatine, glutamate, glutamine, NAA, γ-aminobutyric acid (GABA), and lactate demonstrated that MTR was able to return concentrations of these mediators to levels not statistically different from sham levels. Only in the case of taurine did MTR-treated animals have concentrations that differed from those of sham animals. The normalization of metabolite concentrations by MTR indicates a salutary change that has the potential to meaningfully decrease the extent of brain injury.

INS has been found to increase in astrocytosis, reactive gliosis, or inflammation.[18] Previous observations found that INS initially decreased after TBI, but increased after the first week.[40] In our study, INS concentration decreased 24 hours post-injury, probably an indication of astrocyte loss. Modulation with MTR resulted in significantly less decrease in concentration compared with untreated cases ($P < .01$). Concentrations in the treated group were not statistically different from sham surgery–treated animals ($P = -.86$), indicating that a relative re-equilibration had been achieved that may indicate protection of viable astrocytes by MTR.

Lactate levels in untreated animals increased more than 340% over sham animals. When treated with MTR, the lactate level was



	day 1	day 2	day 3	day 5	day 7	day 9	day 11	day 16
Sham vs treated	-	-	-	-	-	-	-	-
Sham vs non-treated	+	+	+	+	+	+	-	-
Treated vs non-treated	+	+	+	+	+	+	+	-

FIGURE 6. *Results of cylinder tests in untreated animals, mechanical tissue resuscitation–treated animals, and sham surgery animals. Significant differences in an analysis of variance at* P $<$ *.05 level for rotarod testing. + indicates significantly different; − indicated not significantly different. The* P *value of each group comparison was determined by the Dunn or Holm-Sidak method. Pro, baseline, presurgery training values.*

approximately halved compared with untreated animals. Because of the wide SDs, statistical significance was not achieved. The results suggest that accumulation of lactate can be ameliorated to more tolerable levels with MTR.

NAA is localized in neuron cytoplasm that is thought to be involved in water homeostasis.[74] It is considered a neuronal marker whose concentration correlates with neuron and axonal loss and is an outcome predictor in TBI patients.[25,75-77] Untreated control animals demonstrated a significant decrease in NAA after trauma compared with sham surgery ($P < .01$). Significantly increased levels of NAA were measured after MTR treatment compared with untreated animals ($P = .01$). Levels of NAA in treated animals were not significantly different from those of uninjured sham animals ($P = .68$), again indicating amelioration of the injury.

Glutamate is a widely distributed excitatory neurotransmitter in the central nervous system. Glutamine levels in untreated animals decreased significantly compared with sham controls ($P < .01$), indicating altered function of traumatized neurons. In the MTR-treated group, glutamine levels improved significantly ($P < .01$) over untreated group concentrations to levels that were not significantly different from sham controls. We postulate that this indicates that neurons in the MTR-treated group are functionally more normal than those in the untreated group.

Total creatine is a critical component in maintaining neural energy homeostasis. Impaired energy metabolism plays a role in the neuronal death cascade,[78] and the degree of decrease in creatine correlates with the severity of the brain injury.[79] Creatine levels in untreated animals decreased 14.7% compared with sham animals ($P = .1$). In the MTR group, creatine levels improved over the untreated group to levels ($P = .09$) that were nearly identical to those of the sham group. Improved cellular energy homeostasis may contribute to the better functional testing

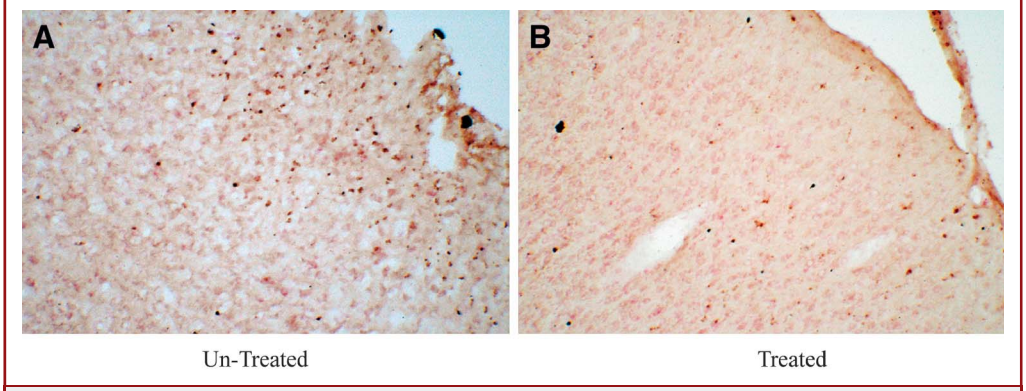


FIGURE 7. *Immunohistochemical analysis of neuronal degradation and death was performed by staining for nitrotyrosine on brain samples harvested 72 hours after impaction. Treated group (B) was exposed to subatmospheric pressure for the entire 72 hours. The number of degrading and dead cells (in dark spots) is significantly less in the untreated samples (A).*

results in the treated group as well as preservation of neuronal tissue on histological examination.

GABA is the most widespread inhibitory neurotransmitter in the brain. It is thought to suppress cellular metabolism in ischemic brain regions that lack oxygen and glucose, thus protecting cells from unfavorable surroundings.[15] GABA levels were decreased in untreated animals compared with shams. Application of MTR results in increases in GABA concentrations that approached sham levels. Although statistical significance of GABA levels was not reached, the trend seen with MTR treatment suggests that a more favorable clinical milieu may be approached.

Functional Tests

Three behavioral/functional tests were used in this study to determine and compare motor impairment in each group. All 3 functional tests demonstrated impairment secondary to TBI and assessed the speed of recovery after CCI. In each test, there was a significant decline in function after CCI with a gradual recovery in all groups after a period of time. The application of MTR in all 3 groups resulted in a decrease in the maximal loss of function as well as more rapid recovery from the injury (Figures 4, 5, and 6). These tests all showed altered motor behavior that normalized after time.

The rotarod and cylinder tests appear to be more reliable than the beam balance test when correlated with the final histological findings. The CCI-injured rats remained on the rod for a shorter period of time compared with the sham group from day 2 to day 21 ($P < .05$). They also demonstrated 4-limb use asymmetry during the first 9 days of the cylinder test compared with sham animals ($P < .05$). In contrast, animals treated with MTR were able to stay on the rotarod for significantly longer periods of time from day 2 through day 5 of the rotarod testing compared with untreated animals ($P < .05$). Treated animals demonstrated less 4-limb use asymmetry from day 1 on the cylinder test and more stability in the balance beam test. Differences between the treated and untreated groups were obvious until day 5 post-injury in the balance beam test; day 7 in the rotarod test, and day 11 in the cylinder test; thus, the functional differences were only transient.

Histology

Brain tissue was harvested from the 3 groups at 31 days after the injury for histological examination. Figure 8 demonstrates areas of the injury. Untreated animals demonstrated extensive cortical tissue loss and cavitation of the injured areas. Animals treated with MTR demonstrated significant preservation of more cortical structure and less cavitary formation. The statistical analysis shown in Figure 9 reveals that untreated animals had significant loss of cortical tissue compared with sham animals ($P < .001$). The animals treated with MTR, however, demonstrated that a significant amount of cortical tissue was preserved, resulting in statistical improvement compared with injured animals ($P < .01$). There is a nonsignificant loss of cortical tissue when treated animals compared with sham animals after 72 hours ($P = .15$), indicating that cell salvage was not complete and suggesting that the effects of MTR are on the halo around the primary, irreversible necrotic injury.

These histological studies demonstrate that the application of controlled subatmospheric pressure after CCI results in changes that permit survival of brain tissue that would normally die in the zone of injury and the penumbra surrounding the zone of injury. These studies reveal that this treatment facilitates significant but not total survival of injured brain tissue.

CONCLUSION

This study demonstrates that MTR by immediate local application of controlled subatmospheric pressure after TBI can significantly modulate metabolite and lactate concentrations in the area of injury, decrease water content and volume of injured brain, speed the improvement of function, and quantitatively improve

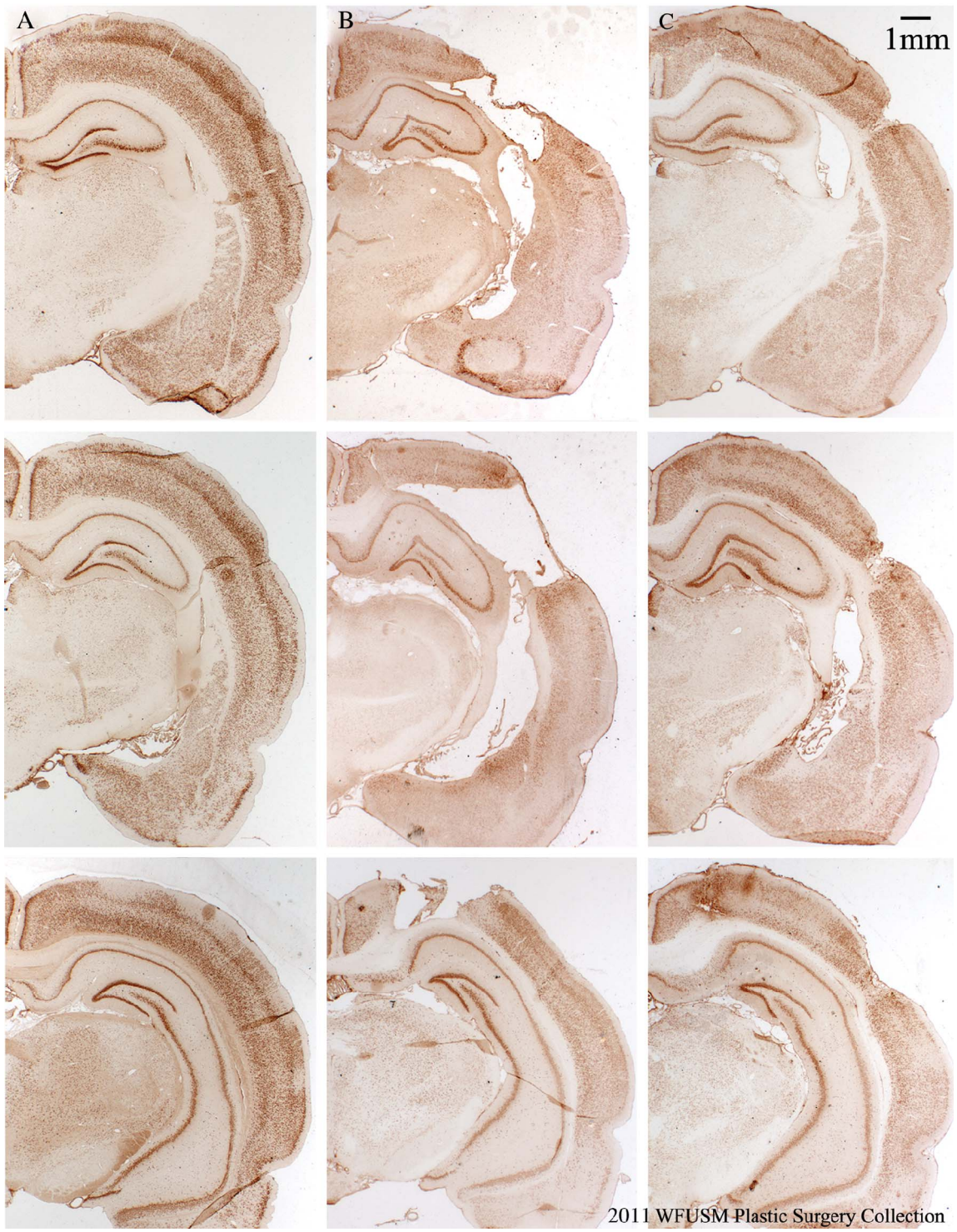


FIGURE 8. *Sections harvested 1 month after controlled cortical impact (CCI). The representative images of coronal sections on ipsilateral rat brain immunostaining corresponding to different distances to the bregma (top row at −3.5 mm, middle row at −4.5 mm, and bottom row at −5.5 mm).* **A**, *sham brain.* **B**, *untreated.* **C**, *treated. The CCI induced injured site cortical tissue loss and cavity formed in injured and surrounding areas. Mechanical tissue resuscitation treatment reduced the CCI-injured cortical tissue loss and cavity formation.*

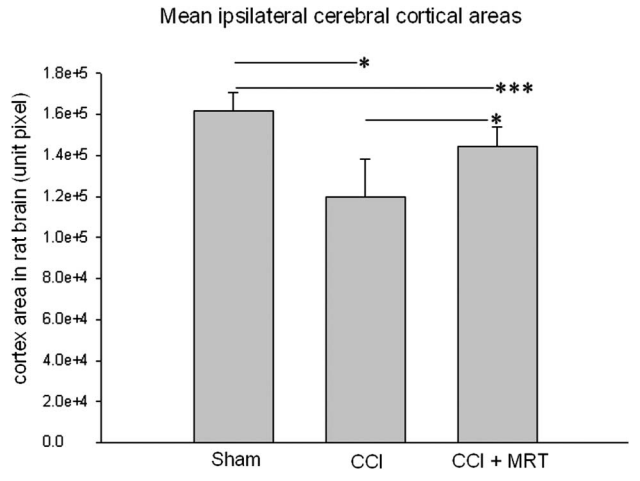


FIGURE 9. *The mean cortical areas in sham animals, controlled cortical impact (CCI)–injured untreated animals, and CCI-injured mechanical tissue resuscitation (MTR)–treated animals. Plotted is the mean ± SD, indicating the remaining cortical area per section in each group. The CCI untreated injury induced cortical tissue loss significantly more than sham animals at 1 month post-CCI (*P < .01). After MTR treatment, the cortical area is significantly larger than for untreated animals (**P < .01), but still smaller than sham group (***P < .05).*

ultimate neuronal survival. The technique is straightforward and potentially targets multiple pathological pathways. These promising early results in small animals with this technique warrant further investigation, refinement, and application.

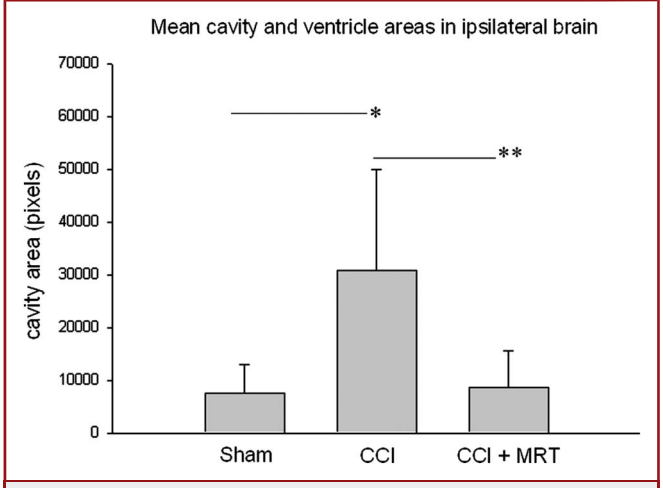


FIGURE 10. *The mean cavity area in sham, controlled cortical impact (CCI)–injured magnetic resonance spectra analysis untreated, and CCI-injured mechanical tissue resuscitation (MTR)–treated animals. Plotted is the mean ± SD, indicating the cavity area per ipsilateral brain section in each group. In the CCI untreated animals, a significantly larger cavity or enlarged ventricle developed compared with sham animals (*P < .05). After MTR treatment, a significantly smaller cavity was formed 31 days after CCI (**P < .05). There was no difference between treated animals and sham animals.*

Disclosures

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