Ameliorating Spinal Cord Injury in an Animal Model With Mechanical Tissue Resuscitation

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BACKGROUND: Traumatic spinal cord injury (SCI) is a major worldwide cause of mortality and disability with limited treatment options. Previous research applying controlled negative pressure to traumatic brain injury in rat and swine models resulted in smaller injuries and more rapid recovery.

OBJECTIVE: To examine the effects of the application of a controlled vacuum (mechanical tissue resuscitation [MTR]) to SCI in a rat model under several magnitudes of vacuum.

METHODS: Controlled contusion SCIs were created in rats. Vacuums of −50 and −75 mm Hg were compared. Analysis included open-field locomotor performance, magnetic resonance imaging (in vivo T2, ex vivo diffusion tensor imaging and fiber tractography), and histological assessments.

RESULTS: MTR treatment significantly improved the locomotor recovery from a Basso, Beattie, and Bresnahan score of 7.8 ± 1.9 to 11.4 ± 1.2 and 10.7 ± 1.9 at −50- and −75-mm Hg pressures, respectively, 4 weeks after injury. Both pressures also reduced fluid accumulations > 10% by T2-imaging in SCI sites. The mean fiber number and mean fiber length were greater across injured sites after MTR treatment, especially with treatment with −50 mm Hg. Myelin volume was increased significantly by 60% in the group treated with −50 mm Hg.

CONCLUSION: MTR of SCI in a rat model is effective in reducing edema in the injured cord, preserving myelin survival, and improving the rate and quantity of functional recovery.

KEY WORDS: BBB score, Diffusion tensor imaging, Magnetic resonance imaging analysis, Mechanical tissue resuscitation, Spinal cord injury, Subatmospheric pressure treatment

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Spinal cord injury (SCI) is a major worldwide cause of mortality and disability. Trauma accounts for 70% of SCIs. Lifelong medical complications for individuals who survive spinal cord injuries are well documented.[1-3] SCI initiates a series of pathological events after the initial direct neuronal damage that may extend or compound the injury. These include secondary injuries of hemorrhage, metabolite abnormalities, compromised-cell death, tissue loss, the formation of cystic cavities, and ultimately scar and tissue distortion. Although there are no universally accepted treatments for SCI, the need to initiate early treatment is generally accepted to prevent extension of the injury. Early management of SCI includes the prevention of progressive spinal cord trauma, maintenance of blood flow and oxygenation, relief of spinal cord compression, and stabilization of the involved vertebrae.[4] A wide variety of pharmacological interventions have been proposed to directly treat neural tissue, but none has proved universally successful for routine clinical implementation. These therapies include early methylprednisolone therapy,[5,6] hypothermia induction,[7,8] and lithium administration.[9] Multiple therapies involving stem cell grafting are also undergoing clinical trials.[10-12]

ABBREVIATIONS: BBB, Basso, Beattie, and Bresnahan; **DTI,** diffusion tensor imaging; **FA,** fractional anisotropy; **MTR,** mechanical tissue resuscitation; **MTR50,** mechanical tissue resuscitation with 50-mm Hg subatmospheric pressure; **MTR75,** mechanical tissue resuscitation with 75-mm Hg subatmospheric pressure; **ROI,** region of interest; **SCI,** spinal cord injury

Mechanobiology is the science of effecting biological change in living tissues by the application of mechanical forces. Although standard pharmacological therapies attempt to block or modify biochemical processes to achieve a therapy goal, mechanical tissue resuscitation (MTR) uses the application of physical forces to effect change in tissue in which survival has been compromised or impaired. MTR uses a controlled negative pressure over a period of time on the treated tissues and their surroundings to produce a physiological state that is more compatible with survival of cells that have been compromised. This form of treatment is especially valuable in terminally differentiated tissue such as brain and heart where regrowth of functional tissue is not possible. MTR has been demonstrated to reduce tissue injury in models of myocardial infarction,[13,14] traumatic brain injury,[15,16] and burns.[17] This protection appears to be the result of increased blood flow,[13,15,18] decreased edema, cytokine modulation, and inhibition of apoptosis.[13,16]

MTR with controlled subatmospheric pressure on central nervous system tissue has been demonstrated to reduce edema, to normalize metabolites, to preserve neural cells from secondary injury, to increase cerebral blood flow, and to decrease intracranial hemorrhage, resulting in decreased volume of the ultimate injury in animal models.[15,16] This study was initiated to investigate whether MTR could improve recovery from traumatic SCI in a rat model.

METHODS

SCI Model and Treatment Procedures

All surgical procedures and animal care issues for this study were approved by the Institutional Animal Care and Use Committee and followed Department of Health and Human Services guidelines. Long-Evans rats that were 77 days old were used. Anesthesia was induced with isoflurane, and the animals were warmed with a heating pad. The contusion SCIs were made with the New York University impactor device (MASCIS Impactor, Piscataway, New Jersey). The anesthetized rats underwent a laminectomy to expose the spinal cord between T9 and T10. The vertebral column was stabilized by clamping the spine processes of vertebra T8 and T11. The impactor rod was slowly lowered until it contacted the dura, and then the impactor rod was raised to a height of 25 mm and released onto the exposed cord by free weight drop to produce the contusion. Dural tears and contusions of the dura were often seen. Impact parameters, including degree of cord compression, velocity, time, and height of weight drop, were recorded.[6]

After surgery, animals were housed individually in cages containing soft bedding and additional environmental enrichment. Cefazolin was administered intramuscularly daily for 7 days. The animals were checked twice daily, and their bladders were emptied by the Crede method as required.

Animals were divided into 4 groups. One group (sham) had the spinal cord exposed as described, but no contusion was created. For 3 groups, the spinal cord was exposed and an impaction contusion was created as described. One group received no treatment after impaction injury. The 2 treated groups had either 50 mm Hg (MTR50) or 75 mm Hg (MTR75) subatmospheric pressure applied continuously for 5 days with a fenestrated PLLA/PGA shell placed over the cord to prevent compression. For the treated groups, a polyvinyl alcohol foam vacuum dressing was trimmed to the size of the surgical defect and placed over the PLLA/PGA shell that tented the cord. The evacuation tube was tunneled under the skin and exited distantly. The incision was sutured closed, and the site was covered with an adhesive film dressing to ensure an airtight seal of the wound and the tube evacuation site. The evacuation tube was protected and attached to computerized vacuum pump. The neurological deficits, histopathological changes, and magnetic resonance imaging (MRI) findings were studied in treated animals compared with sham and nontreated animals.

Open-Field Locomotor Performance

Open-field locomotor performance was assessed and scored according to the 21-point Basso, Beattie, and Bresnahan (BBB) locomotor rating scale.[19] This nonlinear scale was developed to measure multiple criteria for functional outcomes after SCI in rats. The total BBB score is used to quantify the functional recovery of an animal after experimental SCI. A naturally recovering reflex in the hind limbs of SCI rats allows hind-limb stepping without direct brain stimulation and regaining some ability to ambulate within the weeks after injury. SCI rats generally improve for the first week or 2, so later BBB evaluations are more indicative of the extent of permanent damage. We scored BBB for 4 weeks to evaluate MTR treatments from their behavioral outcomes in SCI.

MRI Procedures

MRI is an important tool for SCI diagnosis and evaluation. T1- and T2-weighted images are generally used to assess the severity of the injury and to identify many of the associated pathological changes, including edema, hemorrhage, atrophy, and cyst/syrinx formation, especially in the acute period. Edema is seen on T2 sequences as a hyperintensity of the signal; hemorrhage is seen as a hypointensity. We measured T2 signal density in the epicenter of the SCI (2 mm) by TeraRecon (version 4.4.8.36) and compared it with the mean density of whole cord.

An initial spinal cord MRI was performed 24 hours after injury with a horizontal 7-T magnet (Bruker BioSpin, Billerica, Massachusetts) scanner with a 72-mm single channel volume radiofrequency coil. T2-weighted images were acquired with a multiple slice–multiple echo with the following parameters: repetition time = 1123.25 milliseconds, echo time = 35 milliseconds, number of excitations = 4, field of view = 21, matrix size = 256 × 256, and slice thickness = 1 mm. A reference cylinder of distilled water was placed adjacent to the animal as a control for T2 density measurement. Spin-echo T1-weighted images were acquired with a fast low-angle shot sequence with the following parameters: repetition time = 325.35 milliseconds, echo time = 5.40 milliseconds, number of excitations = 8, field of view = 21, matrix size = 256 × 256, and slice thickness = 1 mm.[20]

After the final behavioral tests at 4 weeks after injury, rats were deeply anesthetized and transcardially perfused with cold phosphate-buffered saline followed by 4% paraformaldehyde in phosphate-buffered saline. The vertebral column was excised and postfixed in 4% paraformaldehyde for 48 hours at 4°C. The vertebral columns were washed extensively in phosphate-buffered saline. Ex vivo MRI scans were performed to avoid artifacts from heartbeat and breathe motions. Diffusion tensor imaging (DTI) was performed on the same 7-T MR system with the following DTI–spin-echo imaging parameters: repetition time = 9000 milliseconds, echo time = 29.7 milliseconds, thickness = 0.50 mm over 6-mm length of spinal column, field of view 23, and matrix size = 128 × 128.

All spinal cords were assessed by MRI for size, signal characteristics, hemorrhage and cystic formation, degree of enhancement, location, etc.

T1 and T2 images of the spinal cord 24 hours after injury were observed and measured in TeraRecon (version 4.4.8.36). The T2 signal density was measured in all axial entire cords. For DTI analysis, the original MRI data were converted and processed in MedINRIA (version 1.9.4). The DTI track module was loaded, and fiber tracking procedures were performed. Regions of interest (ROIs) were manually outlined in the entire spinal cord at the epicenter (least cross-sectional area) from the spinal cord image every 0.5 mm, which includes the dorsal, ventral, and lateral funiculus. The variables fractional anisotropy (FA), relative anisotropy, Lambda1, Lambda2, Lambda3, apparent diffusion coefficient, linear coefficient, planar coefficient, spherical coefficient, and volume ratio were measured in the same thresholds for all samples. Compared with DTI parameters, the fiber count by fiber tractography is a more advanced method based on original DTI data in multiple continuous images. The predominant direction of the diffusion in each image is extracted for directional information as fiber tractography. Their relative parameters, including fiber counting and length and volume of the intact fiber passing through the epicenter of the spinal cord, were calculated.[21-23] For fiber tractography, the parameters such as FA threshold 1, FA threshold 2, smoothness, minimum length, and sampling were kept the same for all spinal cord measurements.

Method for Quantitative Fiber Tractography

MediNRIA software was used for fiber tractography as developed by Dr Don Gage in the Department of Radiology and Biomedical Engineering of Wake Forest University Health Sciences. After the color FA image of the SCI was computerized, the axial full-screen mode was used to draw ROIs on the cross-sectional image as described above for DTI analysis. As described for DTI analysis, images were analyzed every 0.5 mm for a total of 6 mm starting from immediately cranial from the injury, through the injured region, and further caudally. Identification of the cord within the ROI allows analysis of material specifically within the ROI. Objects outside the ROI such as vessels and tracts of fascia are excluded from analysis. The programming allows the analysis and bundling of sequential sections/images of individual and groups of fibers to determine a variety of parameters, including number of fibers, volume of fibers, and minimum, maximum, and mean length of fibers. The fiber tractography video can be watched on YouTube (https://www.youtube.com/watch?v=YWvxZ7bIyAQ).

Histological Examination

After ex vivo MRI scans, the spinal cords of the rats were removed from the column and embedded in paraffin. Paraffin blocks contained the portion of the cord spanning from noninjured cord through the contusion epicenter to noninjured cord tissue distally. Two serial cross sections (10-μm thickness) were collected at 0.5-mm intervals both caudal and cephalad and from the epicenter of the contusion to the cord. A mean of these 5 sections was then reported. Sections were stained with hematoxylin/eosin and Luxol fast blue (myelin stain) for histopathological analysis. Luxol fast blue–cresyl echt violet stain kit (American MasterTech, Lodi, California) was used to stain myelin sheaths and Nissl substances in the spinal cord.[21,24] Both Luxol fast blue and cresyl echt violet produced blue to turquoise in myelin fibers, dark blue in Nissl substances, and blue in nuclei.

For quantitative analysis of tissue damage, all cross-sectional fast blue staining images from light microscopy, including the injury epicenter, were analyzed with ImageJ software (National Institutes of Health, Bethesda, Maryland) by investigators blinded to the experimental treatment. The preserved blue myelin fiber area in each section was determined quantitatively with an internal ruler, and total volume in the SCI epicenter was calculated from continuous sections. Histological examination with hematoxylin/eosin staining was used to determine neuronal tissue survival, cavity formation, local reactions of microglia and macrophages, and infiltration of neutrophils and leukocytes.

Statistical Analysis

All data are expressed as mean ± SD and were analyzed with a commercially available computer program (SigmaStat, Systat Software, San Jose, California). For each rat, BBB scores from each hind limb were averaged to yield 1 score per test session. BBB scores, spinal DTI variables, and mean fast blue tissue volume in the epicenter in the MTR50, MTR75, and injured-only groups were compared by use of analysis of variance with the Holm-Sidak or Dune method for comparison. Differences were considered statistically significant at $P < .05$.

RESULTS

Animals in the sham group showed no functional deficits. As demonstrated in Figure 1, functional recovery is significantly improved over nontreated animals at all measured points from week 1 through 4 when MTR50 (n = 11) is administered. Animals treated at MTR75 demonstrated better improvement than nontreated animals and were statistically different ($P \leq .01$) at day 3 and weeks 3 and 4 after injury. There was no statistical difference between the MTR50- and MTR75-treated groups, although BBB scores were consistently greater in the MTR50 group.

MRI Studies

MTR treatment reduced edema in the injured spinal cord when analyzed by MRI. For contused nontreated animals, the T2 signal indicated that edema increased 22% over controls in 24 hours after injury (n = 11). For both treated groups, the T2 signals increased only 9% to 10% when the animals were treated with MTR50 (n = 10) and MTR75 (n = 8). There are statistically significant differences between the nontreated and both MTR-treated groups ($P < .05$). Less edema is evident in impacted areas in treated spinal cord, indicating that this treatment can reduce or prevent the accumulation of fluid in and adjacent to the site of injury.

DTI Analysis

MTR-treated animals preserved more intact nerve fibers in the area of SCI than untreated animals. DTI, used to examine pathway integrity after traumatic SCIs, has been shown to have good correlation with anatomical and behavioral observations (Figure 2A).[23,25] The axial slice that demonstrated the smallest cross-sectional area of the spinal cord was defined as the epicenter in DTI analysis with MedINRIA. Results from the statistical analysis of the differences in the variables and fiber count values in the epicenter of injured spinal cords are shown in the Table. There were significant differences between the injured-only

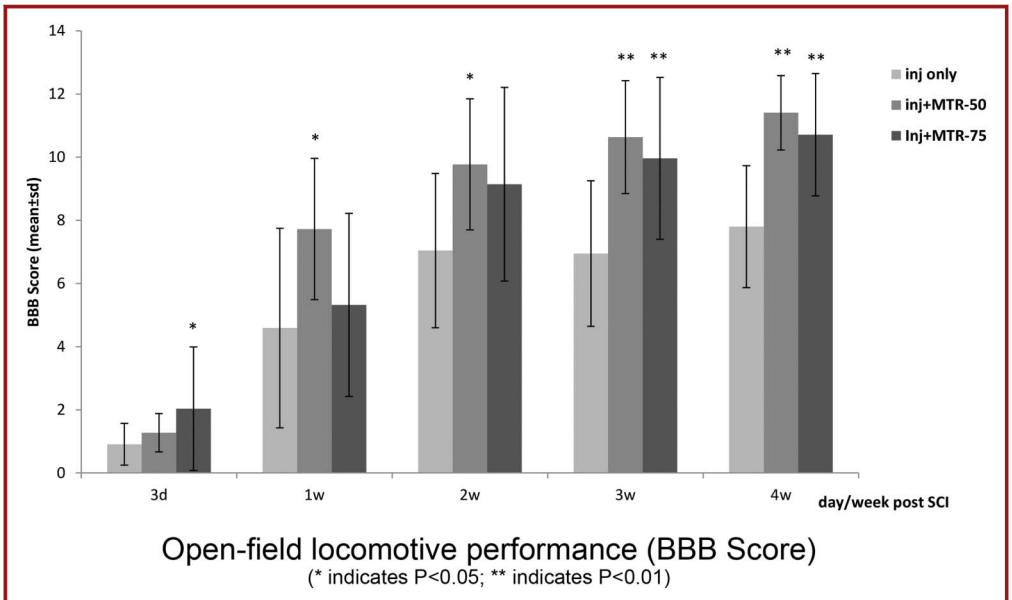

FIGURE 1. *Mean Basso, Beattie, and Bresnahan (BBB) scores of rats with spinal cord injuries (SCIs) treated with/without mechanical tissue resuscitation (MTR) at 50-mm Hg (MTR50) and 75-mm Hg (MTR75) subatmospheric pressure from 3 days to 4 weeks after injury (Inj). The BBB score in the MTR50 group was significantly greater than in the nontreated controls from week 1 to 4 after SCI, and the BBB score in the MTR75 group was significantly higher at 3 days and later at weeks 3 and 4 after SCI. *P < .05 and ** P < .01 difference from SCI-only group.*

group (n = 10) and sham group (n = 5) for many variables, including FA, relative anisotropy, linear coefficient, spherical coefficient, and volume ratio ($P < .01$; listed in the Table), indicating that disruption of nerve tracts occurred with the injury. There are apparent trends of changes of these variables in the MTR50 (n = 10) and MTR75 (n = 12) groups compared with the sham group. Although there are no statistically significant differences between the injured nontreated group and the MTR-treated groups, there are apparent trends in the variables both in the MTR50 (n = 10) and MTR75 (n = 12) groups, suggesting the benefit of MTR.

There is a significant decrease in the fiber counts in the 4 weeks after injury for the nontreated group (mean fiber number, 77.7) compared with the sham group (mean, 840; $P < .01$). Fewer than 10% of fibers structurally survived after injury in our SCI model. When treated with MTR50 or MTR75, more than twice as many fibers survived compared with nontreated levels. There is a significant increase in fiber survival numbers for the MTR50 group (mean, 169.8) compared with the injured, nontreated group ($P < .05$), but the increase is less pronounced in the MTR75-treated group (mean, 159.4; $P = .07$; Figure 2B). The mean length of the fibers was much shorter in the injured, nontreated group (mean fiber length, 2.0 mm) compared with the sham group (mean length, 5.2 mm; $P < .001$; Figure 2C). When treated with MTR50, the mean fiber length increased significantly to 3.1 mm ($P < .05$ vs injured, nontreated group). MTR75 treatment also increased the mean fiber length to 3.0 mm, with significance not quite achieved ($P = .05$ vs injured, nontreated group). The normal fiber volume was 34.824 mm^3 in the sham group spinal cords. The fiber volume increased significantly from a mean of 4.27 mm^3 in the injured, nontreated group to 7.756 mm^3 in the MTR50 group ($P < .05$). MTR75 treatment also increased the fiber volume to 8.552 mm^3, without statistical significance ($P = .07$; Figure 2D).

Histological Examination

MTR-treated injuries demonstrated preservation of more intact myelination after injury. Luxol fast blue was used to stain myelin in SCI rats treated with MTR50 and MTR75. The total blue area was measured in the epicenter of the SCI, and the total intact myelin volume was calculated in samples 2 mm from the epicenter (Figure 3A). Because of the differences in staining intensity of white matter among samples, the optical density was not measured. Four weeks after SCI, the total intact myelin volume was significantly reduced from 5.622 mm^3 in the sham group to 1.701 mm^3 in the injured, nontreated group ($P < .01$). When treated with MTR50, myelin volume was significantly less reduced to 2.72 mm^3 ($P < .05$), whereas MTR75 treatment revealed a myelin volume of 2.32 mm^3, without statistical significance ($P = .19$; Figure 3B).

Hematoxylin and eosin examination showed extensive pathological changes in the epicenter, including cell death, tissue loss, the formation of cystic cavities, and tissue distortion (Figure 3C).

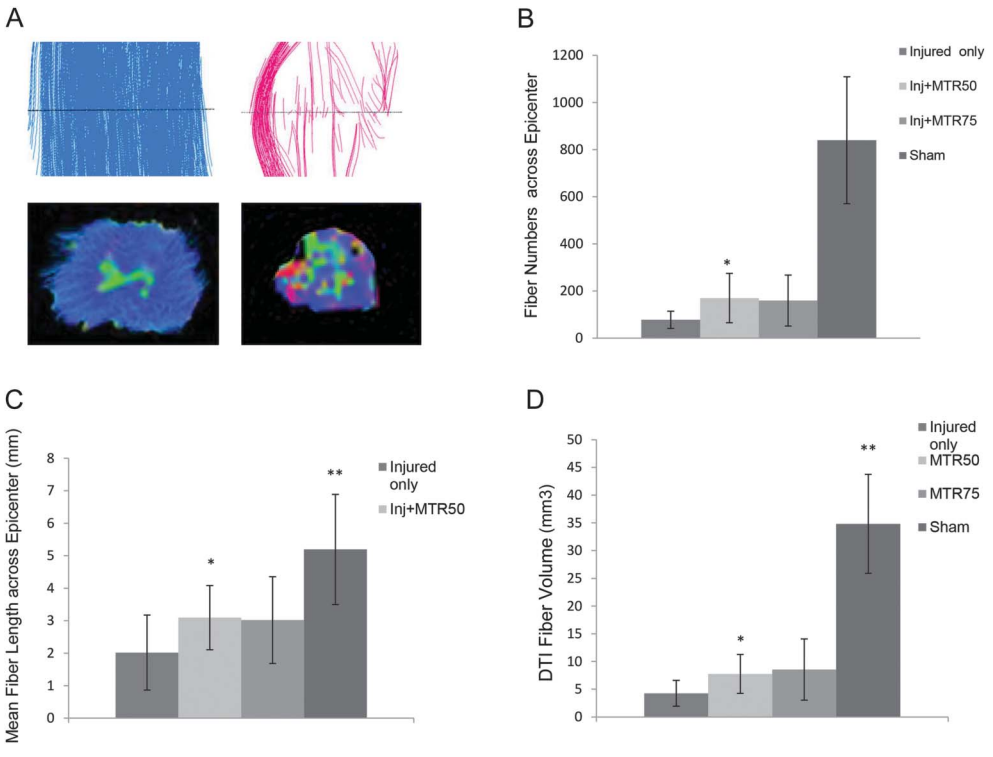

FIGURE 2. *The sample images and fiber extraction variables of fiber tractography in a rat spinal cord injury (SCI) model.* **A**, *fibers extracted from spared white matter tracts in sham and injured (Inj) spinal cords. Blue fibers are used for sham spinal cords in axial (***A**, *bottom left) and lateral views (***A**, *upper left). Pink fibers represent residual intact fibers in the axial view (***A**, *bottom right) and lateral view (***A**, *upper right) of the epicenter of the SCI. Bundles in dorsal funiculus and tracts around after the SCI.* **B**, *relative fiber numbers crossing through the epicenter of the SCI and sham spinal cord.* **C**, *relative fiber length (mm) crossing through the epicenter of the SCI and sham spinal cord.* **D**, *relative fiber volume (mm³) measured from the epicenter of SCI and sham spinal cord (n = 10 for injured only and Inj + mechanical tissue resuscitation [MTR] at 50-mm Hg subatmospheric pressure [MTR50], n = 12 for Inj + MTR at 75-mm Hg subatmospheric pressure [MTR75], and n = 5 for sham group). DTI, diffusion tensor imaging.*

DISCUSSION

Traumatic SCI consists of both primary and secondary injuries. Gray matter and neurons are damaged and axons are disrupted in the initial insult. Blood vessels usually leak or rupture, producing hemorrhage into cord tissue. Within minutes after injury, the spinal cord swells at the injury level, impairing local blood flow and delivery of oxygen and nutrients. Systemic blood pressure decreases as a result of spinal shock, which further compromises the cord. Pathophysiological changes, including increased local edema, decreased blood flow, the release of excitatory and toxic amino acids, lipid hydrolysis, free radical release, lactate accumulation, and activated inflammatory immune system responses, result in secondary injury and bidirectional expansion of the area of the insult. Histological and MRI analyses have identified edema and hemorrhage in the early stages followed by tissue damage leading to early inflammation, cavitation with cyst formation, white matter tract demyelination, and proximal and distal wallerian degeneration of axons. Most current therapeutic treatments are directed toward minimizing the deleterious effects of early trauma inflammation with the hope of reducing loss of axon function while maintaining structural integrity.

The application of subatmospheric pressure has revolutionized the treatment of a wide variety of soft tissue injuries and conditions of increased interstitial pressure. The induced pressure gradient between the vacuum dressing and the injured tissues results in controlled, sustained flow of fluid and soluble mediators from the compromised injured tissue into the device, thus decreasing interstitial pressure and resulting in increased blood flow.[13] Removal of factors detrimental to healing, including toxins, venom, and chemotherapeutic agents, has been demonstrated.[26] Application of subatmospheric pressure to crush-injured muscle directly removes myoglobin from the traumatized area, preventing its entry into the systemic circulation and preventing eventual damage to the kidney.[27] In tissues with the potential to develop granulation tissue, prolonged application of negative pressure has been shown to increase mitosis, to decrease wound size, and to promote healing.

TABLE. Diffusion Tensor Imaging Analysis[a]

DTI	Injured Only	Injured + MTR50	Injured + MTR75	Sham
FA	0.33 ± 0.08	0.37 ± 0.09	0.38 ± 0.06	0.55 ± 0.05[b]
Relative anisotropy	0.12 ± 0.02	0.11 ± 0.02	0.12 ± 0.02	0.21 ± 0.01[b]
Lambda1	0.37 ± 0.10	0.40 ± 0.05	0.31 ± 0.06	0.37 ± 0.04
Lambda2	0.29 ± 0.09	0.32 ± 0.05	0.23 ± 0.07	0.21 ± 0.03
Lambda3	0.23 ± 0.09	0.26 ± 0.05	0.17 ± 0.06	0.15 ± 0.03
Apparent diffusion coefficient	0.88 ± 0.28	0.98 ± 0.142	0.72 ± 0.17	0.72 ± 0.09
Linear coefficient	0.11 ± 0.03	0.11 ± 0.02	0.12 ± 0.03	0.28 ± 0.02[b]
Planar coefficient	0.17 ± 0.03	0.16 ± 0.02	0.19 ± 0.04	0.18 ± 0.02
Spherical coefficient	0.71 ± 0.07	0.74 ± 0.04	0.66 ± 0.10	0.54 ± 0.03[b]
Volume ratio	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.02 ± 0.0[b]

[a]DTI, diffusion tensor imaging; FA, fractional anisotropy; MTR50, mechanical tissue resuscitation with 50-mm Hg subatmospheric pressure; MTR75, mechanical tissue resuscitation with 75-mm Hg subatmospheric pressure. Measurements are from DTI analysis in the epicenter of the spinal cord with spinal cord injury treated with/without MTR50 and MTR75. Values reported are mean ± SD of each injury region.
[b]Indicates significant differences between the injured-only group and sham group.

Our previous studies in animal models of traumatic brain injury have demonstrated that both the structure and function of terminally differentiated neural tissue in and surrounding an injury can be preserved with MTR treatment administered for short periods of time. MTR reduced T2 signal intensity in T2-wighted MRIs in the early stage of traumatic brain injury, normalized metabolites in injured brain areas, improved functional recovery in behavior tests, and preserved more neuronal tissues in histological examinations. Computed tomography perfusion studies in pig brains indicated that application of subatmospheric pressure could increase cerebral blood flow and reduce mean transit time.[15,16] This concept is extended here to injuries of the extracranial central nervous system, the spinal cord.

Preliminary research demonstrated that direct sponge contact on the dura for subatmospheric pressure application in rat SCI model did not significantly improve functional recovery after injury. Computed tomography studies demonstrated that the sponge became compressed to the cord as vacuum was applied, resulting in further cord compression and impairment. A rigid, fenestrated polyglycolic construct (PLLA/PGA) was abutted on the laminectomy site interposed between the cord and the dressing material to prevent this compression. This screen allowed negative

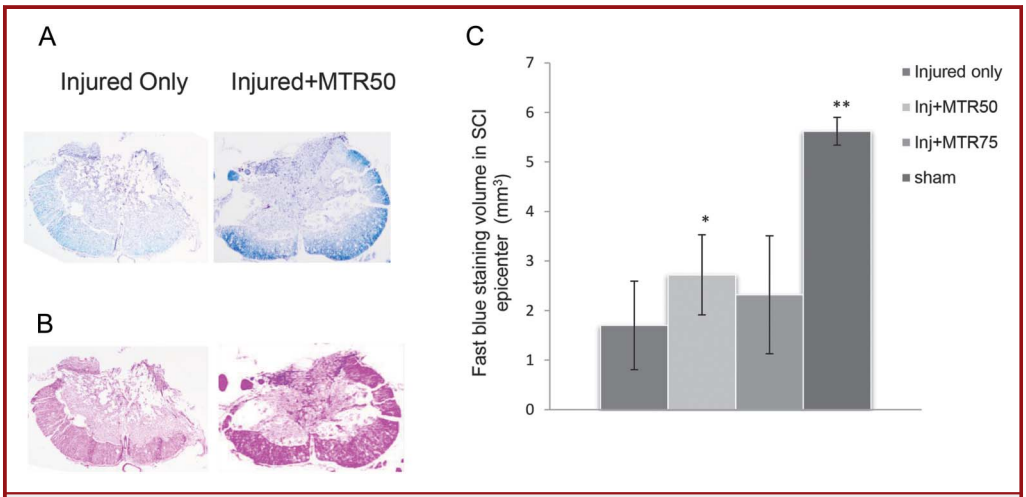

FIGURE 3. *Luxol fast blue and hematoxylin and eosin (H&E) examinations of epicenter in rats with spinal cord injury (SCI) treated with mechanical tissue resuscitation (MTR).* **A**, *representative Luxol fast blue–positive staining in the epicenter of an SCI rat treated with MTR at 50-mm Hg subatmospheric pressure (MTR50) or injured only 4 weeks after SCI.* **B**, *SCI rats treated with MTR50 exhibit a significantly higher volume of Luxol fast blue staining than SCI injured–only rats at the epicenter of the spinal cord (in 2-mm length;* $P < .05$; *n = 10 for injured only and Inj + MTR50, n = 12 for Inj + MTR at 75-mm Hg subatmospheric pressure [MTR75], and n = 5 for sham group).* **C**, *representative hematoxylin and eosin staining in the epicenter of an SCI rat treated with MTR50 or injured only 4 weeks after SCI.*

pressure to be applied to the cord without contact compression directly on the cord (Figure 4A and 4B).

MRI studies 24 hours after injury demonstrated that the amount of edema in the cord was approximately halved by the application MTR50 and MTR75 (Figure 4C). We postulate, on the basis of our previous brain studies, that this lessening of edema results in better blood flow and removal of potentially detrimental metabolites. Because of the small size of the rat spinal cord, MRI quantification of metabolites, as performed in our previous swine brain models, could not be performed.

Assessment of locomotor function in rat SCI is through the BBB rating scale.[19] Significant improvements in BBB scores were found from week 1 to 4 after SCI after treatment with MTR50 and from week 3 to 4 after MTR75 treatment. BBB scores clearly demonstrated measureable overall neurological recovery from SCI when the injury was treated with MTR at either vacuum level.

Because myelin is essential for the conduction of neurological impulses, the initial loss of myelin and subsequent demyelination process play a major role in the loss of motor and sensory function and poor recovery after SCI. Preservation of the myelin sheaths of the surviving fiber tracts could therefore contribute significantly to functional preservation and recovery of patients with SCI. Because it is difficult to directly assess the demyelination and remyelination progress by noninvasive techniques, in vivo indirect assessment by DTI of MRI was used. DTI is the only available technique capable of measuring molecular diffusion of water in vivo. Molecular diffusion is a process in which molecules move along random paths. Diffusion of water molecules in tissue is affected by the presence of cellular structures that provide barriers to free movement and thus exhibit a directional dependence. These diffusion tensors are measured in separate directions in space and provide information about tissue microstructure.[28,29] DTI thus has been used to characterize highly organized tissues like brain and spinal cord with parallel bundles of white matter axons. The diffusion anisotropy is affected by the state of both myelin and axonal structure when white matter is damaged in injured brain and spinal cord in animal models.[23,30,31]

The major parameter in DTI variables is the FA value, which has been reported to be significantly decreased in the epicenter of injured spinal cord compared with noninjured cord in humans and in animal models.[23,31,32] Lower FA values indicate decreased anisotropic water diffusion in damaged spinal cord resulting from pathological changes such as axonal degeneration, demyelination, hemorrhage, ischemic infarction, edema, and cystic formation during the injury process. Our DTI results 4 weeks after primary injury showed that FA values in the epicenter decreased significantly in injured rats compared with sham rats. It strongly

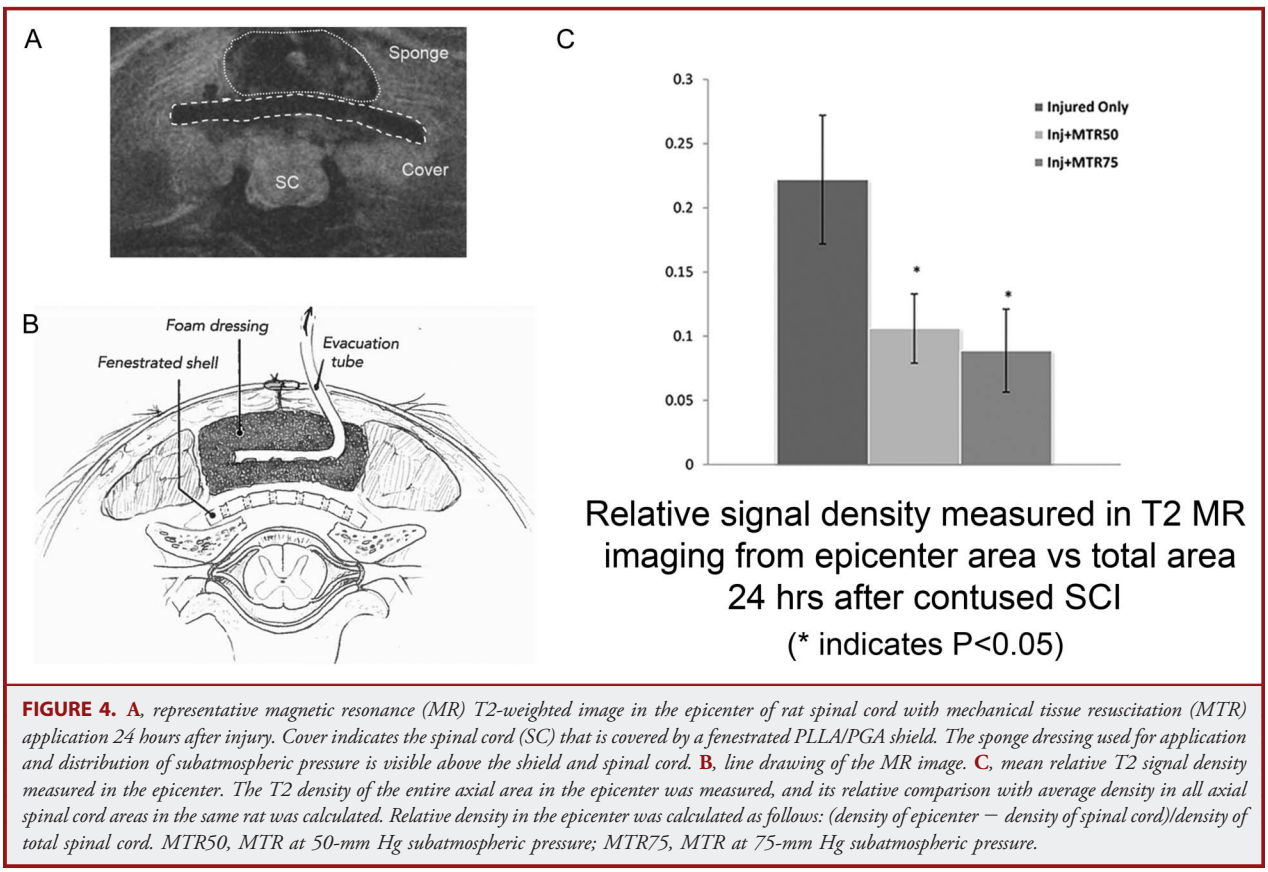

FIGURE 4. **A**, *representative magnetic resonance (MR) T2-weighted image in the epicenter of rat spinal cord with mechanical tissue resuscitation (MTR) application 24 hours after injury. Cover indicates the spinal cord (SC) that is covered by a fenestrated PLLA/PGA shield. The sponge dressing used for application and distribution of subatmospheric pressure is visible above the shield and spinal cord.* **B**, *line drawing of the MR image.* **C**, *mean relative T2 signal density measured in the epicenter. The T2 density of the entire axial area in the epicenter was measured, and its relative comparison with average density in all axial spinal cord areas in the same rat was calculated. Relative density in the epicenter was calculated as follows: (density of epicenter − density of spinal cord)/density of total spinal cord. MTR50, MTR at 50-mm Hg subatmospheric pressure; MTR75, MTR at 75-mm Hg subatmospheric pressure.*

indicated that the spinal cord in the injury epicenter had significantly lost most of its sensory-motor pathways and few white matter tract structures remained intact. Although our results did not show significant differences in FA between the cords of animals in the injured-only group and the MTR-treated groups, there were trends of higher FA for the MTR-treated groups, indicating that less damage resulted in the treated group. The absence of statistically significant results may be due to slight differences in the ROI. In our study, ROIs were drawn manually around the entire spinal cord in the epicenter of SCI, including gray and white matter. White matter is more anisotropic; gray matter is more isotropic. To avoid any bias in the ROI drawing from the boundary line between white matter and gray matter, the entire spinal cord was selected in all our subjects to make the results comparable. Thus, the diffusion values extracted from our ROIs were the averaged results of gray and white matters. The technique used may not be sensitive enough for monitoring and evaluating residual white matter fibers in our treated animals. Another major factor is the response of astrocytes in SCI because their processes also influence water diffusion coefficients. Thus, astrocyte hypertrophy in the white matter may further influence the FA results in ways not related to spinal cord pathways.[22,33]

Recent studies have used fiber tractography to assess the connectivity within specific brain areas and spinal cords.[34-39] Fiber tractography from DTI measurements is a technique that uses specialized tracing algorithms to obtain a 3-dimensional reconstruction of white matter tracts in the brain and spinal cord. These studies have counted the total number of fibers reconstructed from MRI. In the present study, we introduced this method to counter the potential bias of differences in the FA values when the entire epicenter was chosen as a ROI and to avoid interface from astrocytic reactions in SCI. The total number of fibers was reconstructed from imaging under the same parameters for all samples by the MedINRIA software. It uses the tract statistics function to evaluate the number of fiber projections occupied by reconstructed fibers. The number of fiber projections is the number of reconstructed streamlines that penetrated the ROI. Thus, this is a semiquantitative number limited by the spatial resolution and the technique applied in this study, and it is not a true number of axons passing through the ROI. From our results, there is a significant difference in fiber counting, mean fiber length, and fiber volume between injured and sham rats and a significant difference between injured and MTR50-treated rats, which indicates the fiber tractography may be more sensitive than FA values in assessing SCI recovery.

The small size of the rat spinal cord may be the reason why no differences were seen in the results of application of the 55-mm Hg vacuum vs the 75-mm Hg vacuum. Either vacuum level may have had a global effect on the rat spinal cord. In our previous study of traumatic brain injury in swine, there was a difference in vacuum levels with the focal application of differing vacuum levels to the much larger swine brain. In the swine traumatic brain injury study, a delay in application of the vacuum to the site of injury of 3 hours provided the same results as immediate application of the vacuum to the site of injury. A 6-hour delay resulted in significant but lesser results. In this rat study, the vacuum was applied immediately after injury creation. Ongoing studies are examining the effect of delayed application of vacuum to a spinal cord contusion in the rat model. If a "working window" is identified in which treatment can still be efficacious after a delay between injury creation and treatment initiation, then translation of this technique to a larger animal model and ultimately humans is possible.

CONCLUSION

MTR of SCI in a rat model is effective in reducing edema in the injured cord, preserving myelin survival, and improving the rate and quantity of functional recovery. DTI studies of the injured cord, although not statistically significant probably because of limitations of the model, show trends indicating that MTR may improve neural length and volume in the treated area. Semi-quantitative fiber tractology studies further suggest the efficacy of treating SCI with MTR. Because there are no treatments at this time that are universally accepted, further studies in larger models are indicated. As a purely mechanical treatment, the possibility of using MTR with currently used treatments such as drug and hypothermia warrants further investigation.

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