Mechanical Tissue Resuscitation at the Site of Traumatic Brain Injuries Reduces the Volume of Injury and Hemorrhage in a Swine Model

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BACKGROUND: Traumatic brain injuries (TBIs) continue to be a devastating problem with limited treatment options. Previous research applying controlled vacuum to TBI in a rat model resulted in smaller injuries and more rapid recovery.

OBJECTIVE: To examine the effects of the application of a controlled vacuum (mechanical tissue resuscitation) to TBI in a large-animal model. The magnitude of vacuum, length of application, and length of delay between injury and the application of mechanical tissue resuscitation were investigated.

METHODS: Localized, controlled cortical injuries were created in swine. Vacuums of −50 and −100 mm Hg were compared. Mechanical tissue resuscitation for 3 or 5 days was compared. Delays of 0, 3, or 6 hours between the creation of the TBI and the initiation of mechanical tissue resuscitation were examined. Analysis included histological assessments, computed tomographic perfusion, and magnetic resonance imaging (T2, proton magnetic spectra).

RESULTS: A −100 mm Hg vacuum resulted in significantly smaller mean contused brain and hemorrhage volumes compared with −50 mm Hg and controls. Magnetic resonance spectra of treated animals returned to near baseline values. All 10 animals with 5-day mechanical tissue resuscitation treatment survived. Three of 6 animals treated for 3 days died after the discontinuation of treatment. A 3-hour delay resulted in similar results as immediate treatment. A 6-hour delay produced significant, but lesser responses.

CONCLUSION: Application of mechanical tissue resuscitation to TBI was efficacious in the large-animal model. Application of −100 mm Hg for 5 days resulted in significantly improved outcomes. Delays of up to 3 hours between injury and the initiation of treatment did not diminish the efficacy of the mechanical tissue resuscitation treatment.

KEY WORDS: Magnetic resonance imaging analysis, Mechanical tissue resuscitation, Subatmospheric pressure treatment, Swine model, Traumatic brain injury

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WHAT IS THIS BOX?
A QR Code is a matrix barcode readable by QR scanners, mobile phones with cameras, and smartphones. **The QR Code above links to Supplemental Digital Content from this article.**

Traumatic brain injuries are a devastating problem whose treatment remains an enigma. Over 1.7 million people sustain head or spinal cord injuries every year in the United States.[1] Approximately 52 000 of these patients will die, and an equal number will sustain permanent functional disability.[2] Following traumatic brain injury (TBI), impaired microcirculation in and around the injury results in the depletion of critical substrates such as oxygen, glucose, and adenosine triphosphate. Metabolites from the compromised cells around the initial injury accumulate in the interstitial and perivascular spaces. Accumulation of water in the injury penumbra results in further capillary compression, decreased perfusion, and progressive secondary injury defined as secondary neuronal degeneration.[3]

ABBREVIATIONS: BP, blood pressure; **CBF,** cerebral blood flow; **CCI,** controlled cortical injury; **EEG,** electroencephalogram; **ICP,** intracranial pressure; **TBI,** traumatic brain injury

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Our previous work in rodents demonstrated that the application of localized negative pressure to the area of injury produces mechanical tissue resuscitation of compromised cells.[4] Mechanical tissue resuscitation significantly modulated the concentration of metabolites and lactate in the area of injury, decreased water content and edema, decreased the volume of the resultant brain injury cavity, quantitatively improved ultimate neuronal survival, and improved the recovery of the animals treated. The primary goal of this study is to continue investigating the application of mechanical tissue resuscitation to prevent or attenuate the neurological sequelae of TBI.

Recently, swine have become widely used for large-animal neuroscience because their brains are gyrencephalic, similar to the human brain. Adult swine brains (approximately 180 g) are similar to larger nonhuman primates, such as the Rhesus monkey (approximately 100 g)[5] and baboons (190 g).[6] Swine are readily available, considerably less expensive than nonhuman primates, and straightforward to house. Brain development in swine is complete by 5 months, enabling the use of younger animals with adult-size brains. We therefore evolved our original rodent model for the study of mechanical tissue resuscitation in all aspects, including the application parameters and safety issues.

In wounds of the peripheral body, the application of negative pressure has been demonstrated to increase blood flow approximately 4-fold by laser Doppler measurement.[7] Changes in microvascular blood flow depend on the amount of negative pressure applied, the distance from the wound edge, and the type of tissue being treated.[8-10] In this study, we preliminarily examined blood flow as well as physical deformation of the brain parenchyma, electroencephalogram (EEG) changes, and behavioral changes when negative pressure was applied directly to the uninjured brain.

Following these basic studies, 3 sets of studies were then performed to determine:

1. The most effective level of negative pressure to the injured brain to effect an optimal response.
2. The optimal length of time that mechanical tissue resuscitation should be applied to the injured brain.
3. The effect of the time delay of treatment after the initial controlled cortical injury (CCI).

METHODS

Supplemental Methods

A more complete description of Methods, including equipment sources, may be found in the online supplemental materials (see Supplemental Methods, **Supplemental Digital Content 1**, http://links.lww.com/NEU/A623).

Animals

This study was approved by the Institutional Animal Care and Use Committee and followed Department of Health and Human Services guidelines. Female domestic swine (22-33 kg) were sedated, intubated, and anesthesia maintained with inhalation isoflurane.

Animals for Aim 1 were treated for 3 days, then euthanized 5 days later. For Aim 2, animals were treated for 3 or 5 days, then euthanized 5 to 7 days later. For Aim 3, animals were treated for 5 days, then euthanized 5 days later.

Uninjured Brain Blood Flow and Deformation

In 6 animals, a 3×4 cm craniectomy was performed and the dura was removed. A nonadherent synthetic matrix was placed on the brain surface and covered with a finely reticulated open cell matrix. An evacuation tube extended from the matrix to a computerized vacuum pump. An airtight seal was created by suturing closed the overlying skin. (Figure 1) Animals could move freely in their pens. The next day postsurgery intracranial pressure (ICP), EEG, and physiological parameters were measured by telemetry on awake animals to determine the residual effects of exposing to -50, -75, -100 or -125 mm Hg vacuum for 60 minutes. The following day, magnetic resonance (MR) images were taken during 30 minutes at the increasing vacuum levels followed by computed tomography (CT) perfusion studies.

Traumatic Brain Injury (CCI)

TBI studies were performed according to previous models.[11,12] Following a midline incision, a 17-mm defect was made in the right skull. The animal's head was placed in a stereotactic frame and a traumatic brain injury (CCI) was induced by a 12-mm-diameter pneumatic plunger (12 mm deep for 250 ms). Resultant injuries included dura lacerations, cerebrospinal fluid leaks, and brain contusions with hemorrhage. A sterile vacuum dressing filling the craniectomy defect was placed in treated, sham-treated, and non-treated controls. All animals were randomly assigned to their study groups after CCI.

ICP Monitoring

A 3.5-mm-diameter burr hole was made in the left skull, and ICP was monitored by inserting a PA-C40 pressure probe or a 1 probe of a dual-pressure D70-PCTP transmitter, with the second catheter probe placed in the left radial artery for continuous blood pressure (BP) measurement.[13] No interference from animal activity was apparent.

Two biopotential probes were connected to stainless screws across the craniotomy for EEG recording. The mean ICP, BP, temperature, heart rate (HR), and animal activity were collected from recorded data. EEG biopotential analysis was performed by NeuroScore (DSI).[14]

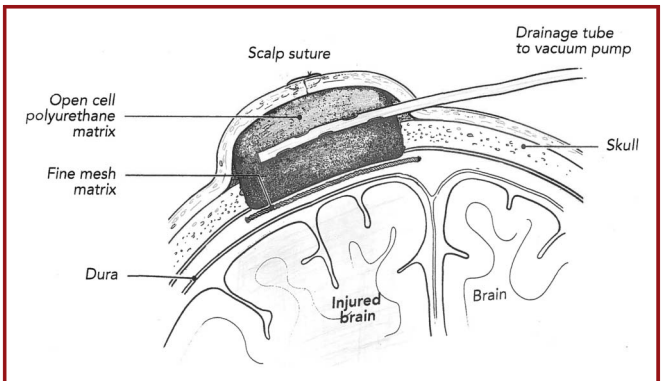

FIGURE 1. *Schematic of cross-section depicting placement of mechanical tissue resuscitation matrix over the area of the brain injured by a controlled cortical impact (CCI).*

CT Perfusion

CT perfusion imaging was started at the same time as the administration of a contrast bolus Omnipaque 350 (2 mL/kg).[12] The pericallosal artery was chosen as artery input and the superior sagittal sinus as venous outflow for TeraRecon analysis. The perfusion parameters including cerebral blood flow (CBF), cerebral blood volume, and mean transit time were calculated.

MRI Procedures

ICP and biopotential probes were removed. MRI was performed 3 days postinjury with a GE Signa EchoSpeed 1.5-T scanner for Aim 1, 5 days postinjury for Aim 2, and 5 days postinjury for Aim 3.[9,10]

MRI was performed for normal, noninjured swine with 3-D BRAVO procedures. All MRI measurements were performed on a TeraRecon workstation. Total contusion injured brain volumes were measured. Total hemorrhage area as hypointensity was measured in gradient echo images.

Proton magnetic resonance spectra were obtained postinjury from a 10-mm^3 voxel by using point-resolved spectroscopy.[11] All MR spectroscopy data were processed and analyzed by using a linear combination model.

Histology

Following euthanasia, the brain was immediately perfused with 4% paraformaldehyde. The brain was removed, postfixed, rinsed in phosphate-buffered saline, placed in 30% sucrose, then snap-frozen and stored at −80°C. Serial sections were cut and stained with hematoxylin and eosin. ImageJ software was used for analysis of areas of necrosis and hemorrhage.

Statistical Analysis

Values are reported as mean ± standard deviation. For ICP, neuroimaging analysis data, comparisons of groups, and time points were made using 2-way repeated-measures analysis of variance with Student-Newman-Keuls post hoc tests or Student *t* tests.

RESULTS

Flow Studies in Uninjured Brain

High-resolution 3-D images of brain showed no deformation of brain tissue or changes in brain size by TeraRecon analysis when tested levels of negative pressure were applied directly to the brain (Figure 2).

Telemetry revealed no detectable abnormalities in EEG at any pressure (Figure 2). There were no clinical seizures, rhythmic patterns of movement, or tonic postures. There were no changes in ICP or physiological responses after mechanical tissue resuscitation (see **Figures, Supplemental Digital Content 2 and 4,** http://links.lww.com/NEU/A624 and http://links.lww.com/NEU/A626). ICPs remained positive and stable at 2 to 7 mm Hg. ICP recordings revealed consistent rhythmic waveforms secondary to breathing regardless of the negative pressure applied (see **Figure, Supplemental Digital Content 3,** http://links.lww.com/NEU/A625). There was no statistical change in systemic BP or HR (see **Figure, Supplemental Digital Content 4,** http://links.lww.com/NEU/A626).

CBF was measured indirectly with CT perfusion in 5 continuous 5-mm-thick slices descending from the surface (Table 1). Negative pressure application increased CBF in intact swine brain. CBF increased an average of 25% with −50 mm Hg in all 5 slices. Blood flow increased an average of 34% with −75 mm Hg mechanical tissue resuscitation and was statistically different from baseline ($P < .05$). Increases of 37% (−100 mm Hg mechanical tissue resuscitation) and 56% (−125 mm Hg) were found. Ten minutes after cessation of −125 mm Hg mechanical tissue resuscitation, an increase in blood flow of 40% was noted in most layers with statistical significance. There

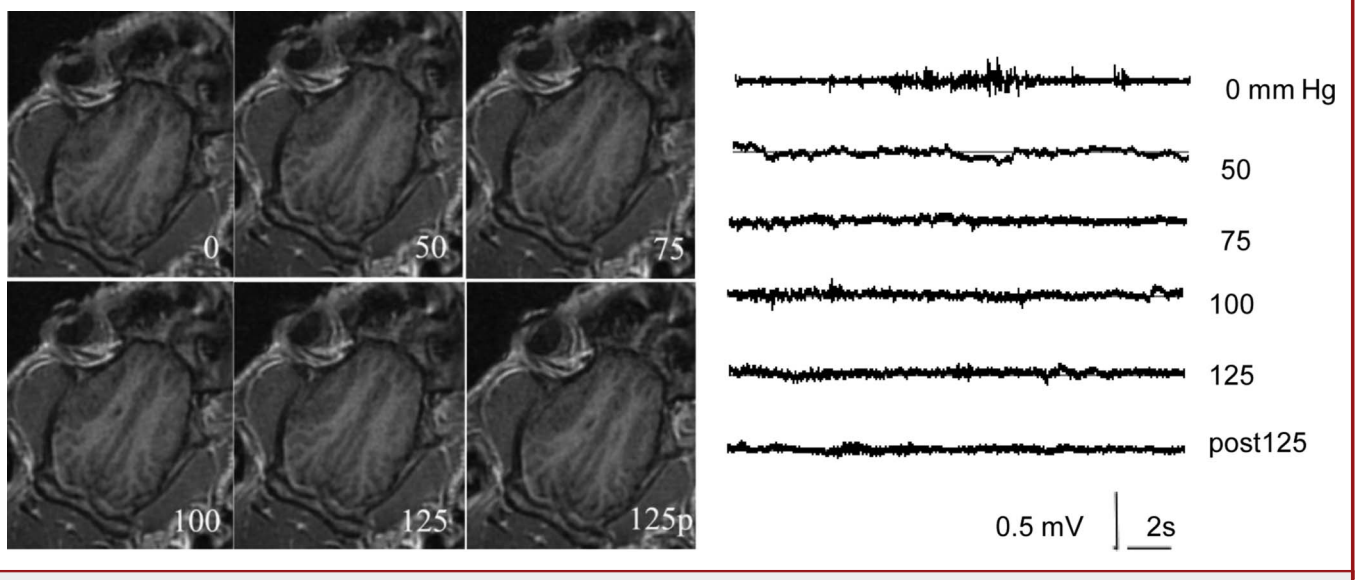

FIGURE 2. *Representative images of normal swine brain MRI BRAVO (A) and EEG (B) under different negative pressure applications over the brain. The negative pressures range from 0 to 125 mm Hg. The amplitude and duration of EEG are marked in the right bottom. EEG, electroencephalogram.*

TABLE 1. Cerebral Blood Perfusion Changes During Negative Pressure[a]

Neg. Pressure	Layer, 5 mm	CBF	CBV	MTT
0 mm Hg	1	75.2 ± 13.5	4.6 ± 0.2	3.9 ± 0.5
	2	76.8 ± 10.8	4.2 ± 0.2	3.6 ± 0.5
	3	82.0 ± 21.0	4.2 ± 0.5	3.5 ± 0.7
	4	89.8 ± 11.4	4.4 ± 0.2	3.4 ± 0.4
	5	96.7 ± 15.9	4.3 ± 0.4	3.2 ± 0.7
−50	1	93.1 ± 14.1	4.7 ± 0.2	3.4 ± 0.6
	2	94.8 ± 23.2	4.2 ± 0.2	3.0 ± 0.9
	3	109.8 ± 15.0	4.3 ± 0.1	2.7 ± 0.2
	4	105.3 ± 24.9	4.5 ± 0.2	3.1 ± 0.8
	5	123.4 ± 34.9	4.4 ± 0.3	3.1 ± 0.8
−75	1	94.6 ± 21.1	4.5 ± 0.4	3.2 ± 0.7
	2	102.1 ± 30.2	4.2 ± 0.2	2.9 ± 0.9
	3	120.8 ± 9.4[b]	4.4 ± 0.1	2.5 ± 0.2
	4	117.5 ± 24.5	4.4 ± 0.3	2.8 ± 0.8
	5	130.9 ± 37.1	4.4 ± 0.3	2.9 ± 0.7
−100	1	94.8 ± 29.8[b]	4.3 ± 0.5	3.1 ± 0.9
	2	116.6 ± 13.6[c]	4.3 ± 0.3	2.4 ± 0.4[b]
	3	123.7 ± 17.8	4.4 ± 0.3	2.4 ± 0.3
	4	113.5 ± 34.8[b]	4.4 ± 0.4	2.8 ± 0.9
	5	121.7 ± 36.8[b]	4.5 ± 0.1	2.4 ± 0.1
−125	1	110.3 ± 18.3[b]	4.9 ± 0.3	2.9 ± 0.5
	2	120.9 ± 29.4[b]	4.4 ± 0.3	2.5 ± 0.6[b]
	3	130.5 ± 27.4	4.6 ± 0.2	2.5 ± 0.5
	4	139.1 ± 34.8[b]	4.9 ± 0.2[b]	2.5 ± 0.6
	5	147.3 ± 36.2	4.9 ± 0.5	2.4 ± 0.5
Post −125	1	108.8 ± 4.2[c]	5.0 ± 0.5	3.0 ± 0.4
	2	109.9 ± 9.3[c]	4.5 ± 0.4	2.7 ± 0.3[b]
	3	121.7 ± 16.2	4.6 ± 0.1	2.7 ± 0.5
	4	118.7 ± 5.7[b]	4.4 ± 0.1	2.5 ± 0.2[b]
	5	129.3 ± 5.4[b]	4.7 ± 0.4	2.6 ± 0.5

[a]The CT perfusion measurements demonstrated relative cerebral blood flow (CBF, mL/100g tissue/min), cerebral blood volume (CBV, %), and mean transit time (MTT, seconds) in 5-mm-thick slices of uninjured brain tissue subjected to increasing negative pressures.
[b]$P < 0.05$.
[c]$P < 0.01$.

was no change in cerebral blood volume with different negative pressures except 1 layer treated at −125 mm Hg. Mean transit time tended to decrease following the application of mechanical tissue resuscitation: −13% with −50 mm Hg; −19% with −75 mm Hg; −26% with −100 mm Hg mechanical tissue resuscitation; −27% with −125 mm Hg; and −24% post −125 mm Hg mechanical tissue resuscitation treatments. Statistical differences of these trends were noted in several layers (Table 1).

Optimal Mechanical Tissue Resuscitation Level

Studies were performed on 30 animals divided into 4 groups: operated sham (n = 7); CCI, nontreated (n = 9); CCI with −100 mm Hg mechanical tissue resuscitation (n = 9); and CCI, with −50 mm Hg mechanical tissue resuscitation (n = 5). Animals were treated continuously for 72 hours.

The mean contused brain tissue volume as measured by MRI for animals in the −100 mm Hg group was significantly ($P < .01$) smaller than both the nontreated and the −50 mm Hg groups. There is no statistical significance between the nontreated and the −50 mm Hg groups (Figures 3 and 4).

The mean hemorrhage volume in −100 mm Hg animals was significantly smaller ($P < .01$) than both the nontreated and −50 mm groups. There was no statistical difference between the mean hemorrhage volume of the nontreated injured and the −50 mm Hg mechanical tissue resuscitation groups (Figures 5 and 6).

Histology demonstrated major neuronal tissue loss and intracerebral hemorrhage in nontreated CCI brains and confirmed that hypointense lesions seen on T2-weighted and hyperintense lesions on gradient echo MR images were hemosiderin deposits. The cortex, basal ganglia, and thalamus including the internal capsule, corpus callosum, portions of the lateral and third ventricle, as well as the hippocampus were affected. The total necrotic volume was

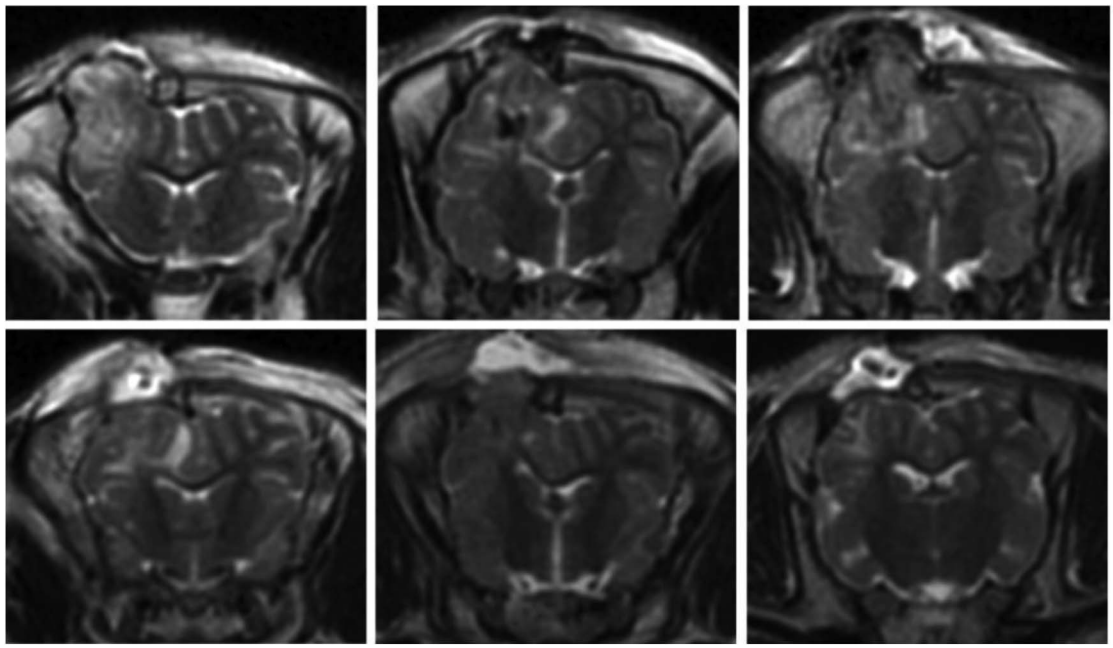

FIGURE 3. *T2-weighted MR images from a representative nontreated animal (**Top row**) and an animal treated with −100 mm Hg mechanical tissue resuscitation for 72 hours (**Bottom row**). The site of injury is the top left portion of the brain. Less edema and minimal herniation through the craniotomy site is present in the −100 mm Hg-treated animal.*

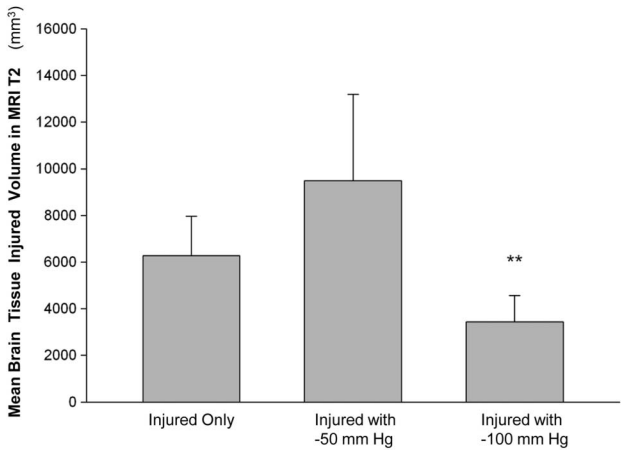

FIGURE 4. *The mean total brain tissue injury volumes for nontreated, −50 mm Hg, or −100 mm Hg mechanical tissue resuscitation as measured in T2-weighted MR images. Mechanical tissue resuscitation was applied for 3 days. Mean injury volumes were: 6590 ± 1760 mm³ for the nontreated group; 9490 ± 3710 mm³ for the −50 mm Hg mechanical tissue resuscitation group; and 3440 ± 1140 mm³ for the −100 mm Hg group. The injured brain tissue volume in mechanical tissue resuscitation −100 mm Hg group was significantly (**P < .01) smaller than in the nontreated group.*

1115.62 ± 482.77 mm³ in the untreated animals, 522.09 ± 381.43 mm³ in the −100 mm Hg mechanical tissue resuscitation treated group, and 1535.53 ± 526.17 mm³ in the −50 mm Hg mechanical tissue resuscitation treated group. The volume for the −100 mm Hg group was significantly less than for nontreated animals ($P < .05$) (Figure 7). Because of noncomplete perfusion, brains from only 3 of 5 swine in the −50 mm Hg mechanical tissue resuscitation group were analyzed. Less neuronal loss and hemorrhage in the injured area were observed after mechanical tissue resuscitation treatment.

Metabolic spectroscopy data of nontreated and −100 mm Hg treated animals showed changes from sham in amino acids and lactate following injury (Table 2). Significantly altered levels were found for sham vs nontreated groups for *N*-acetyl aspartate and total creatine ($P < .05$). Significant differences were found between treated and nontreated animals in *N*-acetyl aspartate ($P < .05$). Treatment with −100 mm Hg mechanical tissue resuscitation resulted in concentrations of most metabolites such as glutamine (Gln), glutamate (Glu), lactate, myoinositol (INS), taurine (Tau), and guanidinoacetate (Gau) reverting toward sham levels but without statistical significance.

Length of Treatment

Studies were performed on 25 animals that were divided into 3 groups: no treatment after CCI (n = 9), 3 days of −100 mm Hg mechanical tissue resuscitation treatment following CCI (n = 6),

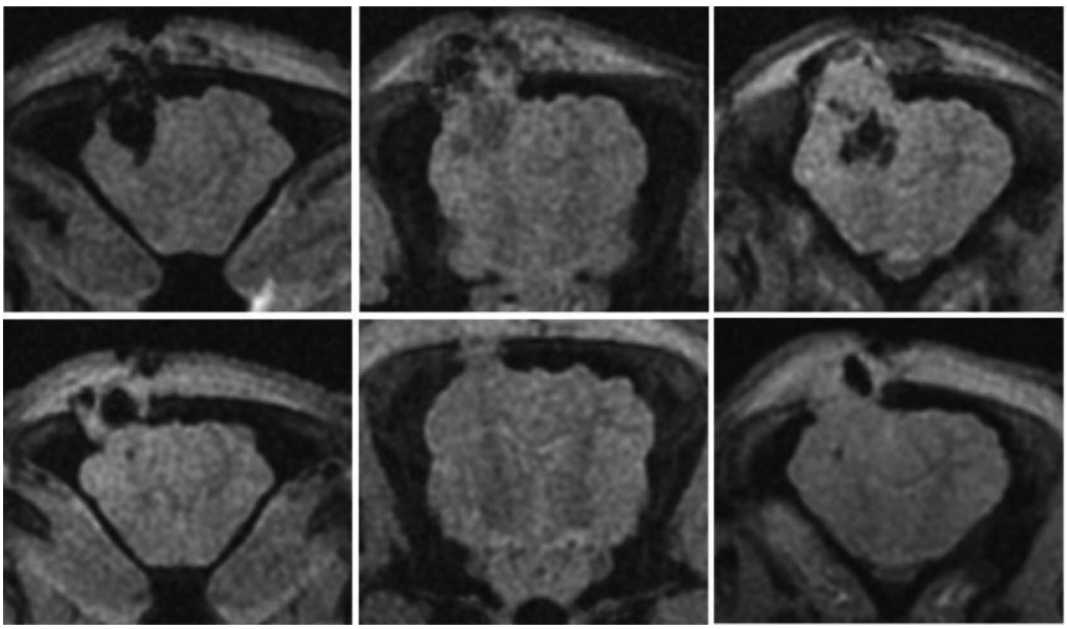

FIGURE 5. *Gradient echo MR images from a representative nontreated animal (**Top row**) and an animal treated with −100 mm Hg mechanical tissue resuscitation for 72 hours (**Bottom row**). Hemorrhage volumes for the −100 mm Hg treated animals were significantly less than for nontreated animals.*

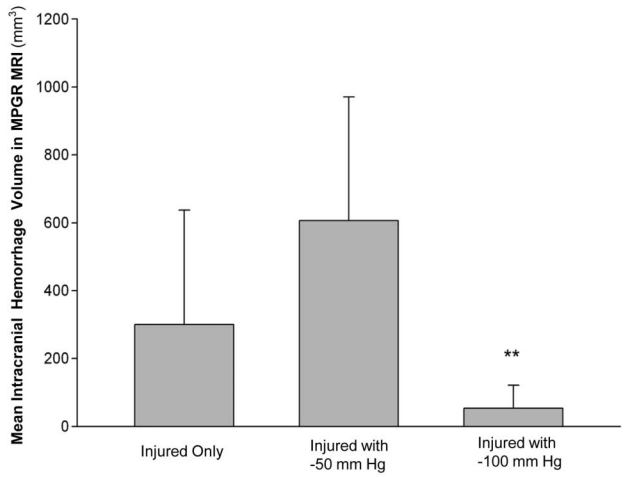

FIGURE 6. *The mean intracranial hemorrhage volumes measured in gradient echo MR images for nontreated, −50 mm Hg or −100 mm Hg mechanical tissue resuscitation groups of animals. Mean hemorrhage volumes were: 375.75 ± 348.9 mm³ for the nontreated group; 606.84 ± 364.05 mm³ for the −50 mm Hg mechanical tissue resuscitation group; and 53.31 ± 67.81 mm³ for the −100 mm Hg group. The hemorrhage volumes in mechanical tissue resuscitation −100 mm Hg group were significantly (**P < .01) smaller than in the nontreated groups.*

and 5 days of −100 mm Hg mechanical tissue resuscitation treatment following CCI (n = 10).

In the 3-day treatment group, there was no increase in ICP or change in physiological behavioral parameters, with normal appetite and activity during treatment. After cessation of treatment, animals became lethargic with occasional seizures and progressive loss of activity and appetite. Three of 6 animals died between day 3 and day 10.

In the 5-day treatment group, all animals maintained normal ICP, physiological, and behavioral parameters during and after the cessation of therapy (Figure 8). Less cerebral edema that resulted in brain herniation in the untreated group was seen in the treated group. All 10 animals survived until they were euthanized at day 10.

The volume of cerebral hemorrhage for animals treated for 5 days was (232 ± 68 mm³) significantly less ($P < .05$) than for animals in the nontreated control group (553 ± 100 mm³) (Table 3). The mean cerebral hemorrhage volume was 332 ± 122 mm³ in the nontreated 3-day group; this smaller volume indicates that the hemorrhage volume may increase between 3 and 5 days postinjury for unknown reasons.

Maximum Delay of Treatment Following CCI

Studies were performed on 32 swine divided into 4 groups: CCI with no treatment; CCI with no delay for treatment; CCI with 3-hour delay; and CCI with 6-hour delay. The injury volume for animals treated immediately was not significantly different

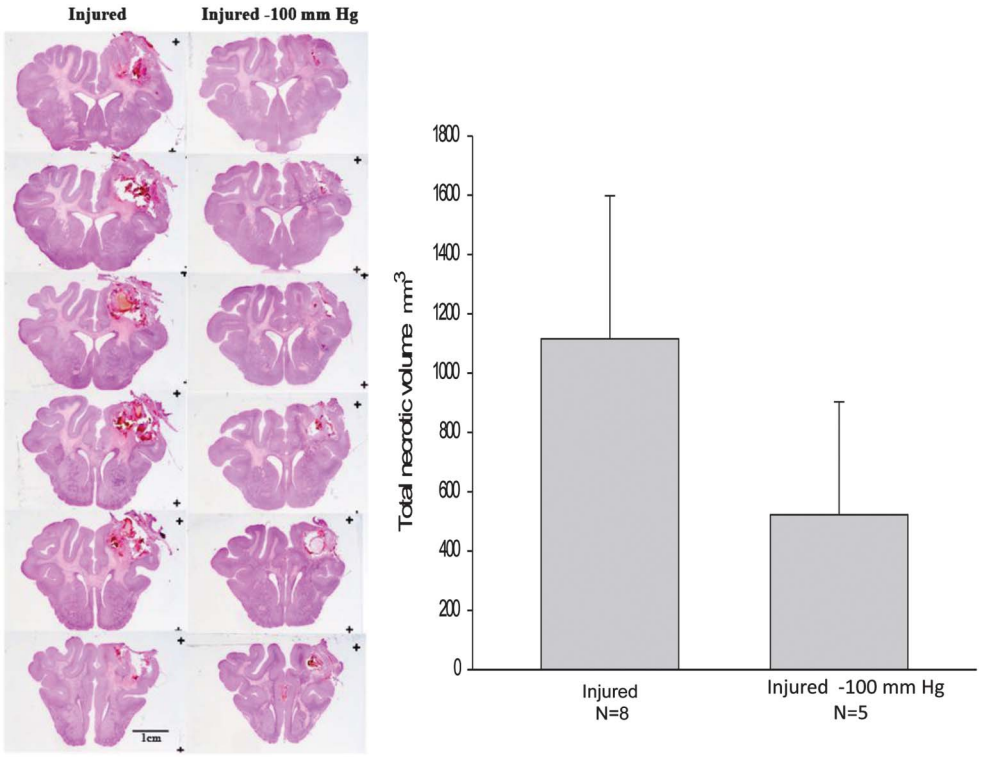

FIGURE 7. *Histological cross-sections were imaged, and the volume of necrosis was determined.* **Left**, *injured, nontreated brain slices 8 days postinjury.* **Right**, *CCI, treated (mechanical tissue resuscitation −100 mm Hg, 72 hours) brain slices 8 days postinjury. Slices are 3.2 mm apart through the center of CCI site. Hematoxylin and eosin. Magnification ×2. The volume of necrosis (mean ± standard deviation) for all treated animals was significantly (*$P < .05$*) smaller than the volume of necrosis for nontreated animals. CCI, controlled cortical impact.*

($P < .05$) than the volume for animals treated after a 3-hour delay. The injury volume of the 6-hour delay animals was not significantly larger than the areas of injury for the 0- and 3-hour delay animals (Table 3).

The volume for intracranial hemorrhage volume determined by MRI was, for all treated animals, significantly smaller than for nontreated animals. The hemorrhage for no delay and the 6-hour delay were similar, with the same degree of significance from the injured-only animals ($P < .05$). The volume of hemorrhage for the 3-hour delay was very significantly smaller ($P < .01$) than the nontreated volume. The volume of hemorrhage for each group of treated animals was not significantly different from each other (Table 3).

Necrotic brain volume by histological analysis for all treated animals was significantly ($P < .05$) smaller than the volume of necrosis for nontreated animals. The volume of necrosis for animals in the 3- and 6-hour delay group was very significantly ($P < .01$) smaller than the area of the nontreated animals. The area of necrosis between 0-hour delay and 3- or 6-hour delay was not statistically significant (Table 3).

DISCUSSION

TBI results in a primary area of injury surrounded by compromised cells that undergo secondary neuronal degeneration, resulting in a significantly larger area of ultimate neuronal death as well as progressive clinical pathology.[13-17] The progression of this pathology is well documented in the literature.[18-22]

A large number of pharmacological and surgical treatments have been recommended, but none has proven to be highly clinically successful.[23-27] Based on observations made in successfully treating peripheral wounds with negative pressure, the concept of mechanical tissue resuscitation evolved. Controlled negative pressure applied directly to injured tissue results in decreased edema, decreased interstitial pressure, and the return of more normal perfusion dynamics, thus creating a physiological environment more favorable to cell survival.[28-31] Burn studies have shown that compromised cells within the "zone of stasis" could be resuscitated and salvaged.[32]

Our previous TBI studies of traumatic brain injury utilizing a rat model showed that the application of negative pressure to the

TABLE 2. Local Brain Metabolites Concentrations From Sham, Injured and Treated Swine[a,b]

Metabolite, mM, Mean ± SD	Sham	Injured	Injured + −100 mm Hg
Cr	5.1 ± 0.6	2.8 ± 2.5	4.7 ± 0.4
Gln	2.0 ± 2.8	0 ± 0	3.0 ± 5.2
Glu	7.9 ± 2.7	5.1 ± 4.7	4.8 ± 4.7
Ins	6.3 ± 1.1	8.2 ± 8.0	4.7 ± 2.9
Lac	0.7 ± 1.3	6.3 ± 7.6	2.7 ± 2.1
NAA[c]	7.0 ± 1.2	2.4 ± 2.3	5.0 ± 1.8
NAAG	1.6 ± 1.1	1.6 ± 2.5	0.6 ± 1.3
Tau	0 ± 0	1.5 ± 2.9	0.8 ± 1.5
GPC	2.0 ± 0.3	1.2 ± 1.2	1.0 ± 0.9
PCh	0 ± 0	0.1 ± 0.4	0.6 ± 0.8
Gua	1.4 ± 1.4	14.6 ± 21.9	1.1 ± 2.4

[a]NAA, *N*-acetyl aspartate; GPC, glycerophosphocholine; PCh, phosphorylated choline.
[b]Metabolic spectral scan of excitatory amino acids and related mediators, comparing levels in control (sham) animals, injured but nontreated animals, and animals injured and treated with 100 mm Hg vacuum for 72 hours.
[c]$P < .05$.

injured cortex immediately after injury measurably decreased brain water content, the volume of contused brain, modulation of metabolite levels toward normal, more rapid recovery of function, and histological preservation of neural tissue.[4] This article more clearly defines the parameters for treatment of traumatic brain injuries with mechanical tissue resuscitation using swine for a more precise interpretation of data for potential scaling to humans.

Preliminary studies demonstrate that the application of negative pressure to the uninjured brain results in changes in blood flow similar to what has been previously documented in peripheral tissue.[7,9] Perfusion maps at multiple depths from the brain surface and increasing levels of negative pressure indicate this treatment can progressively increase CBF at all tested depths until a relative plateau is reached at −75 to −100 mm vacuum. This is a very rapid response in normal swine brain without measureable change of cerebral vessel bed or tissue anatomy. Importantly, no hypoperfusion is observed in brain tissue adjacent to the brain being treated.[5] Considering these data together, negative pressure does not seem to open more vascular beds in the brain, but may speed blood transit time to tissues. Increasing blood flow facilitates oxygenation, nutrient supply, and removal of waste products and has potential clinical therapeutic benefits.

Clinical monitoring and MRI anatomic studies demonstrated that our therapeutic range of vacuum could be applied to the brain without significantly distorting or compressing the brain or producing seizure while still increasing local blood flow.

Application of −100 mm Hg mechanical tissue resuscitation to the TBI emerged as superior to the application of −50 mm Hg mechanical tissue resuscitation. Animals treated at −100 mm Hg mechanical tissue resuscitation demonstrated almost halving of the contused brain volume by MRI ($P < .01$). The MRI findings were supported by histological examination. The animals treated with −100 mm Hg mechanical tissue resuscitation had significantly less necrosis, significantly less hemorrhage, and significantly less reactive gliosis seen on light microscopy (Figure 7). There was no significant difference between treatment with −50 mm Hg mechanical tissue resuscitation and no treatment.

An unexpected finding was that −100 mm Hg mechanical tissue resuscitation treatment significantly decreased the volume of hemorrhage in treated animals ($P < .01$). The −50 mm Hg mechanical tissue resuscitation, however, resulted in almost double the volume of hemorrhage over controls. Since there was no visible pressure deformation of the brain by MRI scans, it is difficult to postulate a tamponade effect of the matrix to the injured brain. The possibility that higher negative pressure improved removal of local obstructing edema vasoactive materials or cytokines that facilitate progressive hemorrhage may be postulated. Histological examination of the brain confirmed significantly less hemorrhage in −100 mm Hg mechanical tissue resuscitation animals.

MRI spectroanalysis confirmed our previous findings that negative pressure could ameliorate changes in metabolites in injured brain and return them to a more normal physiological level

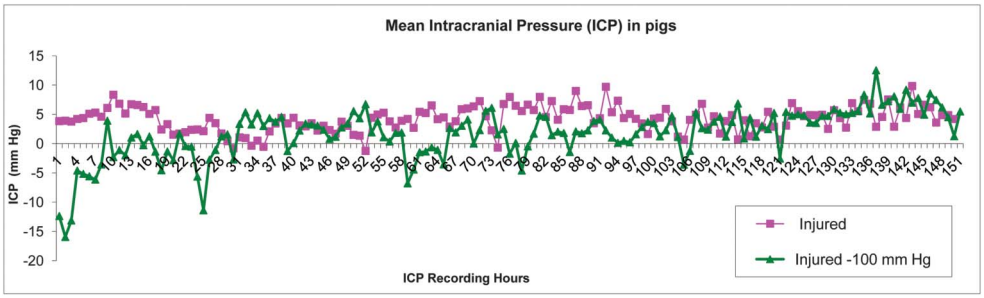

FIGURE 8. *The mean ICP recordings for 5 days in traumatic brain injury swine with/without mechanical tissue resuscitation treatments. The pink line is for injured swine (n = 9), and the green line is for injured with mechanical tissue resuscitation −100 mm Hg treatment (n = 7). ICP, intracranial pressure.*

TABLE 3. The Damaged Brain Volumes From MR Images and H&E Staining in Traumatic Brain Injured Swine[a,b]

Damaged Volumes, mm^3, Mean ± SD	Injured Only	No Delay Treated	3-hr Delay Treated	6-hr Delay Treated
Injured brain tissue volume; (T2 MR)	7900.7 ± 2248.8; n = 8	5621.5 ± 1435.5[c]; n = 8	5004.3 ± 2202.9[c]; n = 8	6919.4 ± 2038.2; n = 8
Intracranial hemorrhage volume; (GE MRI)	553.2 ± 283.4; n = 8	232.7 ± 194.5[c]; n = 8	102.9 ± 154.5[d]; n = 8	175.6 ± 229.9[c]; n = 8
Brain necrotic volume; (H&E staining)	985.8 ± 395.6; n = 8	388.2 ± 486.3[c]; n = 7	414.9 ± 341.9[d]; n = 8	338.2 ± 195.7[d]; n = 8

[a]SD, standard deviation; H&E, hematoxylin and eosin.
[b]Quantitative Table with data and sample sizes for the mean damaged brain volume measurements in MR images and H&E staining in traumatic brain injured swine. Animals were treated with 100 mm Hg vacuum for 5 days, with varying delays between the creation of injury and the application of the vacuum. The sample size for histological determination of the necrotic brain volume for the 0-hour delay group was 7 animals. All other groups contained 8 animals.
[c]$P < .05$ vs nontreated.
[d]$P < .01$ vs nontreated.

approaching sham control levels. CCI resulted in significantly altered levels for *N*-acetyl aspartate and total creatinine ($P < .05$) over baseline values. Treatment with −100 mm Hg mechanical tissue resuscitation shows statistically significant improvement in correcting abnormal levels of both ($P < .05$) and a definite trend, without statistical significance, in correction of other metabolites such as glutamine, glutamate, myoinositol, taurine, and guanidine acetate approaching sham nontraumatized brain. Additionally, lactate levels in animals treated with −100 mm Hg mechanical tissue resuscitation also trended baseline levels (Table 2).

Optimal Length of Treatment

Animals where mechanical tissue resuscitation was discontinued only 3 days after CCI exhibited loss of appetite, became lethargic, and developed seizures. We postulated that these changes were due to the recurrence of edema, decreased blood flow, and increased ICP. Posttreatment, half of the animals died before scheduled euthanasia. Unfortunately, brain specimens in the animals could not be processed for histology before degeneration occurred.

Treatment for 5 days with −100 mm Hg mechanical tissue resuscitation resulted in 100% survival of the animals with minimal physiological and behavioral abnormalities during and after treatment. One animal had a single brief seizure the day after mechanical tissue resuscitation was stopped. The MRI and histological studies in these animals demonstrated that volume of brain injury and hemorrhage were better controlled with at least 5 days of treatment. For reasons that are presently unknown, treatment less than 3 days appears deleterious with more bleeding and necrosis than controls. The events leading to decreased bleeding and less morbidity with longer treatment need further study.

Maximal Delay After CCI

A critical factor in translating this research to clinical practice was determining the interval between the CCI and the allowable delay in which mechanical tissue resuscitation could be therapeutic. Our previous experiments in rodents used mechanical tissue resuscitation immediately after the injury. We looked at 3 delay intervals after CCI: no delay, 3-hour delay, and 6-hour delay. MRI studies revealed that treatment 3 hours after injury was equally effective in decreasing the volume of brain tissue injury as applying the device immediately after injury ($P < .05$). Although there was a tendency for the 6-hour delay group to have increased the size of injury over the 3-hour delay, these changes were not statistically significant. Animals treated with the 6-hour delay still developed significantly less volume of injured brain on MRI than the untreated. These findings were confirmed on histological examination. Treatment following a delay of up to 6 hours after the initial injury resulted in a significant ($P < .01$) decrease in the total necrotic volume histologically, compared with untreated animals. Although there was a slight trend for the volume of histological brain necrosis to increase with increasing delay, differences between 0-, 3-, and 6-hour delay were not statistically significant. Overall, these data indicate that a 3- to 6-hour delay of treatment after CCI may still be therapeutically beneficial. This is compatible with triage and intervention times demonstrated by the military in the Iraq conflict.

After any of the treatment delay times tested, animals treated with −100 mm Hg had a significantly smaller volume of hemorrhage than nontreated animals. Delay of 3 hours dramatically decreased the volume of intracranial hemorrhage ($P < .01$). Given the dire consequences of an intraparenchymal hemorrhage after CCI, these observations further suggest the potential therapeutic efficacy of mechanical tissue resuscitation for TBI. It also suggests a potential role for mechanical tissue resuscitation for treatment of other conditions with associated brain hemorrhage such as subdural hematoma, stroke, and perhaps brain tumor resection.

CONCLUSION

These findings further define parameters that suggest the applicability of mechanical tissue resuscitation to clinical practice. The ability of the brain to tolerate −100 mm Hg of negative pressure over a focal area without the development of seizure or brain deformation while still increasing blood flow is encouraging. The ability of mechanical tissue resuscitation to achieve

meaningful reduction in loss of brain tissue and hemorrhage injury warrants further investigation. We are presently investigating the rate of negative pressure application and larger negative pressure at cycled rates, as well as determining the optimum treatment surface area relative to the volume of injury. Matrices placed into brain parenchyma as opposed to the brain surface are also under study. We hypothesize that recent biomaterial innovations such as absorbable matrix compositions in our laboratory will facilitate clinical use of mechanical tissue resuscitation with less invasive procedures.[33] Since mechanical tissue resuscitation is a purely mechanical treatment, its use in combination with other modalities such as pharmacological agents is attractive.

Disclosure

This study was supported by grant W81XWH-09-1-0437 from the US Army and by the Cheek Research Endowment of the Wake Forest University. A patent covering the technique described in this article has been issued to some of the authors (LA, MM, ST) and Wake Forest Health Services. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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COMMENTS

This article describes the intriguing application of a controlled vacuum wound care strategy to the treatment of cortical lesions produced by controlled cortical impact (CCI) traumatic brain injury (TBI) to swine. The currently reported project was performed as a logical follow-up in

a more humanlike gyrencephalic TBI paradigm to prior successful results in the rat CCI model. The mechanical tissue resuscitation (MTR) strategy consisted of placing a negative pressure wound dressing over the injured cortex and applying a controlled negative pressure. A very comprehensive set of experiments was performed to determine the optimal MTR dose (degree of vacuum), treatment duration, and treatment window. Also the effects of MTR were evaluated in uninjured swine on MRI measures of CBF. A pressure of −100 mm Hg applied for 5 days and initiated within 3 hours postinjury was found to be the most optimal to reduce lesion and hemorrhage volumes, as well as MR spectroscopy-measured metabolites. However, even with a treatment delay until 6 hours postinjury, a significant reduction in brain lesion volume was still observed. A therapeutic window of this magnitude reveals that this treatment should be clinically practical. The authors are taking a wonderfully careful and thorough approach to the development of MTR and indicate that they are in the process of investigating "the rate of negative pressure application, applying larger pressures at cycled rates and the optimal treatment surface area relative to the volume of injury." The results strongly suggest that MTR treatment is a promising approach for treating certain types of TBI involving subdural hematomas and/or hemorrhagic contusions as well as other brain disorders where focal hemorrhage and/or CBF compromise can occur, including "ischemic and hemorrhagic stroke and brain tumor resections." Last, it is pointed out in the article that this purely mechanical approach could possibly compliment pharmacological neuroprotective treatments. Since so many of the latter have yielded disappointing results in clinical trials, the combination of MTR with pharmacological neuroprotection may enhance the chance of finally seeing a significant beneficial effect of the latter. As someone whose career is devoted to neuroprotective drug discovery, that would be a wonderful eventuality.

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One of the fundamental problems confronting critical care providers and neurosurgeons is ameliorating the effects of secondary TBI and preventing brainstem compression during the first 10 days of trauma. There is every indication that maximum medical management is successful in controlling intracranial hypertension in approximately 85% of patients with severe diffuse traumatic brain injury. In addition, it is unlikely that MTR will be helpful in patients with ASDH and EDH who need urgent evacuation of intracranial bleed and patients who need decompressive craniectomy. There still remains a small percentage, but a significant group of patients with TBI who will have progressive hemorrhagic injury within the first 10 days of trauma and could be eligible for MTR application. These are lobar contusions that blossom and may require surgical intervention within the first week of injury. There are significant problems that MTR should resolve before application in human beings. Clinical feasibility, risk of infection, and the effect size must be addressed before initiating controlled clinical trials in human beings.

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