Intracranial biomechanics following cortical contusion in live rats

Laboratory investigation

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Object. The goal of this study was to examine the mechanical properties of living rat intracranial contents and corresponding brain structural alterations following parietal cerebral cortex contusion.

Methods. After being anesthetized, young adult rats were subjected to parietal craniotomy followed by cortical contusion using a calibrated weight-drop method. Magnetic resonance imaging was used to visualize the contusion. At the site of contusion, instrumented force-controlled indentation was performed 2 hours to 21 days later on the intact dural surface. The force-deformation (stress-strain) relationship was used to calculate elastic (indentation modulus) and strain changes over time, and constant hold or cyclic stress was used to evaluate viscoelastic deformation. These measurements were followed by histological studies.

Results. At contusion sites, the indentation modulus was significantly decreased at 1–3 days and tended to be above control values at 21 days. Multicycle indentation showed that the brain tended to accumulate more strain (an indicator of viscosity) by 1 day after the contusion. Imaging and histological studies showed local edema and hemorrhage at 6 hours to 3 days and accumulation of reactive astrocytes, which began at 3 days and was pronounced by 21 days.

Conclusions. The viscoelastic properties of living rat brain change following contusion. Initially, edema and tissue necrosis occur, and the brain becomes less elastic and less viscous. Later, along with undergoing reactive astroglial changes, the brain tends to become stiffer than normal. These quantitative data, which are related to the physical changes in the brain following trauma and which reflect subjective impressions upon palpation, will be useful for understanding emerging diagnostic tools such as magnetic resonance elastography.

Key Words • traumatic brain injury • contusion • viscoelasticity • edema • magnetic resonance imaging • animal model • rat

Following brain contusion, neurosurgeons intuitively know that there are changes in the tactile properties of intracranial contents, including the brain. The dura mater over the swollen brain feels tense, while damaged brain tissue may feel soft. Objective measures of these mechanical properties might help in clinical decisions related to intracranial pressure monitoring or the decision to decompress by large craniotomy or tissue removal.

Abbreviations used in this paper: GFAP = glial fibrillary acidic protein; MRE = magnetic resonance elastography.

Emerging technologies such as magnetic resonance elastography (MRE), which would allow noninvasive measurement of these mechanical parameters, require basic data about the physical properties of injured brain tissue to facilitate interpretation. The brain has material properties that are described as nonlinear viscoelastic—that is, spring- and fluid-like qualities coexist, and depending on the time course of an applied physical force, the relative contribution of each differs. Biomechanical analyses of traumatized brain have for decades focused on understanding how the normal brain responds to physical impacts in experimental situations.
mechanical properties of the brain in living animals following injury were examined in a single experiment. Two days after cryogenic brain injury in dogs, an increase in brain elasticity was documented.\(^1\,^2\)

We recently developed a technique for measuring intracranial viscoelastic parameters in the living rat.\(^3\,^4\) With the dura closed, responses to mechanical indentation are determined by the brain tissue immediately under the test device, as well as the rest of the intracranial contents.\(^5\)\(^6\) We have shown that changes in mechanical indentation response from birth to 6 months depend on water content and on tissue protein properties.\(^7\,^8\) We have also shown that acute hydrocephalus is associated with only small changes.\(^9\)

The purposes of this study were to examine the mechanical properties of living rat intracranial contents following cortical contusion and to correlate these properties with the histological features of the brain tissue. To create brain contusions, we used a method that involves dropping a weight onto the exposed cerebral dura.\(^10\) This method is considered to have features similar to those seen in human traumatic brain injury. It is very reproducible, unlike some of the closed skull methods.\(^11\) We hypothesized that intracranial contents display alterations in viscoelastic deformability following contusion and during healing of the contusion.

### Methods

#### Animal Preparation

The University of Manitoba Animal Care and Use Committee approved animal experiments in accordance with the guidelines of the Canadian Council on Animal Care. One hundred locally bred male Sprague-Dawley young adult rats (20 in the pilot group, 68 in the injured group, and 12 in the sham group; age 56 days; weight 350–400 g) were maintained on a cycle of 12 hours of light followed by 12 hours of darkness, with free access to water and pellet food. Spontaneously breathing rats were anesthetized with 1.5% isoflurane in oxygen. All rats underwent craniotomy under aseptic conditions using a surgical microscope. The scalp was shaved and cleansed with chlorhexidine and povidone-iodine wash. The scalp and periosteum were opened, and a right parietal craniotomy (5 mm in diameter, 2 mm lateral from the sagittal sinus, and 2–3 mm posterior from coronal suture) was performed with a fine drill, leaving the dura intact. This procedure allows collective measurement of the mechanical properties of the intracranial contents.\(^27\)\(^30\)\(^31\)

#### Mechanical Brain Injury Device

The heads of 88 anesthetized rats (20 in the pilot group and 68 in the injured group) were then positioned in a stereotactic head holder (David Kopf Instruments). A weight-drop device affixed to the stereotactic frame was modified from that previously described.\(^10\) A sterile stainless-steel cylindrical tube (length 45 cm; inner diameter 0.8 cm) was manufactured with a 4-mm opening at one end. The coaxial impacter (weight 4 g) inside the tube has a 4-mm-diameter contact surface and rests on a circumferential sponge that allows a maximum travel of 2 mm. The sterile impacter was positioned in the vertical plane in contact with the intact dura, flush with the parietal cerebrum. In pilot studies on 20 rats, 3 weights (10, 20, and 40 g) dropped from 3 heights (range of impact velocity 0.44–2.76 m/sec) onto the impacter were tested. The most consistent cortical contusion, spanning the full thickness of the cerebrum (shown by histological examination), was achieved with the 20-g weight dropped from a height of 25 cm. Bench testing of the device on a force sensor with temporal resolution of 10 Hz showed that the impact velocity in this condition is 2.21 m/sec (kinetic energy 0.05 J) and the duration of deformation (dwell time) is 0.1 sec. Following cortical contusion, the scalp incision was closed with polypropylene suture, and the rats were allowed to recover with free access to water and pellet food. They were weighed daily and monitored in an open field setting for evidence of neurological deficits. Criteria for euthanasia were inability to ambulate or loss of more than 10% of body weight. The sham group was subjected to craniotomy but not weight drop.

#### Magnetic Resonance Imaging

Rats underwent MRI (21-cm bore, 7-T Bruker magnet; Bruker Biospec/3 imaging software; isoflurane anesthesia) within 2 hours before sacrifice. Coronal T2-weighted images were acquired using a 1-mm slice thickness. Details have been published previously.\(^26\)

#### Indentation Testing

Sixty-eight injured rats were studied at 2 (n = 11), 6 (n = 11), and 24 (n = 10) hours or 3 (n = 11), 7 (n = 13), and 21 (n = 12) days after brain contusion. Sham rats were studied at 2 hours (n = 6) or 21 days (n = 6) after craniotomy. After MRI, anesthetized rats were positioned on a warming blanket in the custom fabricated head holder whose stage is affixed to the open platform microhardness indentation tester (CSM Instruments SA).\(^27\) The scalp incision was reopened, and the site of the parietal craniotomy was exposed. Twenty-one days after contusion or sham procedure, adhesions between the dura and epicranium had developed; these were separated to free the dura at the edge of the craniotomy site. Instrumented indentation of the intracranial contents was performed with a custom-made axisymmetric “flat punch” indenter (radius 2 mm). The indenter was advanced over 30 seconds to achieve a set force that, depending on the condition of the brain, resulted in a deformation of 1.0–1.5 mm in depth. The relationship between the applied force (stress) and the evoked deformation (strain) as a function of time was recorded in 3 variations: 1) load and immediate unload was used to calculate the indentation modulus (E<sub>IT</sub>), which is largely a measure of elastic rebound following withdrawal of the deforming force; 2) load and hold at a constant force for 60 seconds was used to measure progressive viscoelastic deformation expressed as a percentage change in the strain, during the hold; and 3) repeated application and partial (50%) removal of the force in 5 cycles (multicycle) was used to measure viscoelastic deformation expressed as a percentage change in the strain, recorded as percentage...
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increase of the intracranial content deformation between the first and final cycles. The details and validations of these tests appear in prior publications. A minimum recovery period of 15 minutes was allowed between tests. The measured epidural brain temperature at the end of indentation was never lower than 35.4°C.

Evans Blue Injections

Following completion of mechanical testing, while the rat was still under anesthesia, we cannulated the right femoral vein to inject Evans blue dye (0.5 ml of 1% solution in 0.9% NaCl, [Sigma Chemical Co.]) in order to assess integrity of the blood-brain barrier (BBB). Ten minutes later, anesthetized rats were killed by exsanguination; ice-cold phosphate-buffered saline followed by 100 ml of 10% formalin was then perfused through the heart.

Histology and Immunohistochemistry

The heads were further fixed for 2 days before the brains were removed from the skulls. Each brain was sliced into 2-mm coronal slices, photographed, dehydrated, and processed for paraffin embedding. Serial sections were cut at 6-μm thickness, and every 10th section was stained with H & E. At the center of the contusion, the presence of reactive astrocytes was demonstrated using immunohistochemistry to detect glial fibrillary acidic protein (GFAP). Activated microglia were labeled with Giffonia simplicifolia B4 isoelectin. The approximate size of the contusion was determined by measuring the maximum depth and breadth of either petechial hemorrhages in the acute stages (up to 7 days) or reactive glial changes in the subacute stages (21 days).

Statistical Comparisons

Statistical assessment involved performing ANOVA with the Dunnett test, followed by post hoc intergroup comparisons using the Tukey-Kramer test (n = 7–11 per group) (JMP 10.01 statistics software, SAS Institute, Inc.). The 2-hour and 21-day sham control groups had no differences in any measured parameter; therefore, they were combined into a single control group. All data are reported as mean ± SEM.

Results

Almost all contused animals (n = 68) had brief periods of apnea (< 5 seconds) following the weight drop. Five rats had apnea lasting longer than 30 seconds, at which time we resuscitated them by decreasing the inhaled isoflurane, increasing oxygenation, and applying external chest massage (no more than 30 seconds). Four rats died at the time of injury from persistent apnea. In the postcontusion period, the rats exhibited mild weight loss because of reduced feeding for 2–3 days (mean body weight 375.3 ± 5.7 g for control rats vs 361.4 ± 6.1 g for contused rats; p = 0.06, paired t-test). Subsequently all rats gained weight well (for example, at 21 days the mean control weight was 441.8 ± 19.3 g and the mean contused weight was 444.7 ± 15.0 g). With the exception of a single rat that had mild contralateral weakness (hindleg dragging) for 2 days, all rats exhibited a normal level of consciousness, ambulation within the box cage, and no obvious focal deficits. Ten rats were eliminated from the study or analysis due to atypically small contusion, development of subdural hematoma, or meningeal disruption with CSF leak, and 14 rats were eliminated because of technical problems with the testing data. Forty contused rats and 12 sham rats were used for the final analysis. Coronal T2-weighted MR images acquired 2 hours after injury showed no obvious changes (for example, there was no increase in T2 signal indicative of increased water content). One to 3 days after cortical contusion, there was bulging of the brain into the craniotomy site and blurring of the interface between the cerebral cortex, underlying white matter, and subjacent hippocampus. By 7 days, brain swelling was reduced, and abnormal T2 brightness had dissipated (Fig. 1).

Viscoelastic Properties

Rats tolerated the indentation testing well. The load-unload test revealed a decline of intracranial indentation modulus (EIT), indicating a decrease in elasticity, evident 1 day after contusion and reaching a minimum of 57% of the control value (p < 0.05) at 3 days. Subsequently, the EIT rebounded and by 21 days was 152% of the control value, although this increase was not statistically significant (Fig. 2). Viscoelastic deformation during a 60-second application of constant stress had increased to 132% of the control value at 2 hours after contusion and gradually declined, reaching the control value by 21 days, but the change was not statistically significant (not shown). Viscoelastic deformation during the multicycle load-unload test progressively increased beginning at 6 hours and reached a statistically significant value of 193% of the control value at 1 day, after which there was a return to the control value (Fig. 3).

Macroscopic and Microscopic Features

Sham-operated rats subjected to indentation tests exhibited only very small blood collections (< 20 μm thick) on the external surface of the dura. Evans blue was evident only in the vicinity of the choroid plexus. The injured rats had a 4- to 5-mm-diameter contusion on the right parietal cortex. Initially, the tissue was hyperemic and bulged slightly. Subarachnoid hemorrhage (≤ 50 μm thick) and intracortical petechial hemorrhages were evident from 2 hours to 3 days. Evans blue dye staining was evident in the vicinity of hemorrhage and occasionally in the cerebral cortex lateral to the contusion. Yellowish discoloration due to hemosiderin formation became apparent at 7 days. Focal brain atrophy with a slightly sunken surface was evident at 21 days (Fig. 1). Microscopically, early contusions (6 hours to 3 days) were characterized by pyknotic neurons, swollen (edematous) cells, and scattered petechial hemorrhages in the cortex and underlying white matter. By 7 days, the cellular edema and blood collections had diminished, and macrophages were apparent. Small irregular cavities and regions of neuron loss tapering from the brain surface were apparent by 21
Fig. 1. Magnetic resonance images and the gross and microscopic appearances of the brains of a sham control rat that underwent craniotomy only and of rats at 1 and 21 days after contusion. A: T2-weighted MR images. At 1 day, the interface between the parietal cortex, white matter, and hippocampus is blurred due to edema (arrow). At 21 days, the cortex is sunken due to atrophy (arrow). B: Photographs of the dorsal surface of the rat brains at corresponding time points. At 1 day there is considerable discoloration due to petechial hemorrhages at the site of contusion, and at 21 days the cortical surface is sunken (arrow). C and E: Coronal slices through control and 21-day-contused rat brain. D: Bar graph showing the calculated relative area of brain injury at different time points. The maximal damage area did not statistically significantly differ between the 2-hour and the 21-day contusion groups. F: Photomicrograph showing GFAP immunohistochemistry of control rat cerebral cortex. Only scattered GFAP immunoreactive astrocytes (dark stained cells, arrow) are apparent in the subpial region. Original magnification ×100. G: Photomicrograph showing the contused cortex 3 days after injury. There is focal pallor due to edema and an accumulation of macrophages (arrow). H & E, original magnification ×400. H: Photomicrograph showing GFAP immunohistochemistry of rat cerebral cortex 21 days after contusion. Reactive astrocytes (dark stain, arrow) span the cortex. Original magnification ×100.
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The maximum contusion area (in mm$^2$) calculated on coronal slices was not statistically significantly different between the survival times (Fig. 1D). Hypertrophic reactive astrocytes, demonstrated by increased GFAP immunoreactivity, appeared at 3 days; increased GFAP expression persisted for 21 days at the contusion site. Griffonia simplicifolia B4 lectin labeling revealed reactive microglia by 1 day and macrophages by 7 days. The spatial distribution was the same as that of the reactive astrocytes. In sham controls, there was no evidence of contusion, although slight microglial activation was apparent under the craniotomy site.

Discussion

The focal cortical trauma created by weight drop in this experiment resembles brain contusion associated with depressed skull fracture, because the brain tissue is transiently deformed rather than simply impacted by the inner surface of the skull.\textsuperscript{10,23} We observed an evolution of the contusion-related histological features similar to those described in more detail previously.\textsuperscript{7,28} In previous rat experiments, others have shown that intra- and extracellular edema formation peaks from 12 hours to 2 days following contusion,\textsuperscript{3,15} which corresponds to a peak in the intracranial pressure.\textsuperscript{9}
is applied, and viscosity (the resistance to gradual deformation). Brain tissue is complex and behaves as a compressible, nonlinear viscoelastic, fluid-saturated, porous material. The viscous and elastic components contribute to measured values differently depending on the rate of force application. Because brain tissue is complex, measuring its physical properties separately is not easy; therefore, mechanical tests are used to infer the complex properties from relatively simple measurements.

Using our recently developed method for measuring biomechanical properties of the intracranial contents of living rats, we tested the hypothesis that mechanical properties of intracranial contents change as the brain composition changes following contusion injury. Our results show decreased elastic rebound from 1 to 3 days following contusion. Given that the elastic behavior of brain tissue is largely a function of the cell structure, this change corresponds well to the timing of proteolytic tissue destruction following contusion, which begins at 24 hours.

We also observed increased gradual deformation of intracranial contents in the force-decay and multicyle tests. Reduced viscosity beginning 2–6 hours after contusion and reaching a minimum at 3 days correlates well with the temporal changes in edema and tissue degeneration. Extracellular edema fluid can readily flow between the cells, and supratentorial CSF can shift to other compartments during application of the deforming forces. In the subsequent recovery phase, edema resolves, macrophages clear the tissue debris, and reactive astrocytes increase. Despite the presence of fluid-filled cavities due to cortical necrosis and atrophy, there was a tendency for the brain to be more elastic, which was indicated by an indentation modulus greater than the control value at 21 days. A likely explanation is that reactive astrocytes contribute to a change in the mechanics of surviving brain tissue. At the individual astrocyte level, atomic force microscopy analysis showed that reactive changes in the cytoskeleton, including increased GFAP content, are associated with increased stiffness (that is, increased elasticity) of individual cells.

Although the testing method used here offers a very high degree of precision, the numeric values cannot be considered as accurate material property constants. The measured values are intimately related to the details of the testing method. Owing to the complexity of the experimental setup, mechanical testing of living brain is seldom performed. Aoaygi and coworkers used technically similar, but less precise, flat indentation testing through the open dura to measure changes in the dog brain following injury by freezing. They calculated the elastic (Young’s) modulus and the viscosity coefficient from a relaxation response (force change following application and hold of a 2-mm-deep indentation). Two days after freeze injury, the elastic modulus, which in our model system was shown to correlate well with the indentation modulus, had decreased by 50%. In another study, after cryogenic brain injury in cats, brain samples were removed and tested by indentation. Tissue elasticity had decreased by 43% and fluidity had increased by 376% at 1 day after injury. In a study by Shafieian et al., following fluid percussion brain injury in rats, postmortem analysis of brainstem tissue showed a significant reduction in the instantaneous elastic force needed to deform the tissue. All of these results are very similar to our current results and generally support the idea that contused brain tissue is less elastic and more fluid (less viscous) than normal tissue.

Shortcomings of our study include the absence of direct brain water measurements. We chose not to measure the amount of water in the brain because the indentation test itself might have changed the water content. Another possible study limitation is that small sample sizes may have precluded reaching statistically significant changes in some of the tests; however, the trends do correspond well to the biological findings recorded in other studies of experimental brain injury. Transient apnea must be considered as a factor that has the potential for aggravating contusion injury; however, because it occurred consistently, we do not consider it a complicating factor. The most important considerations are technical. We strove to preserve the integrity of the meninges during the injury and during the mechanical testing. By doing so, we avoided variations in CSF efflux that might occur, but it meant that we measured not only the mechanical factors of the brain tissue but also those of other intracranial contents. Meningeal adhesions and fibrosis that had developed by day 21 might have contributed to the apparent increase in the elastic response of the intracranial content. We have not attempted to apply to our data any complex mathematical models such as quasi-linear viscoelasticity.

The practical value of our data lies in the relative changes in mechanical properties and the extent to which they might aid in the understanding of technologies applied to the human situation. An example of one such technology is handheld mechanical testing devices, which can be used to obtain quantitative data from palpation. When one of these devices was applied to the skin of head injury victims who had undergone decompressive craniotomy, stiffness (measured as shear elastic modulus) increased, which correlated well with increased intracranial pressure and brain swelling. Such technology and information might also be important for the development of sensors for robotic neurosurgery. Noninvasive measurements of elastic properties allow “palpation” of tissues not normally exposed. Magnetic resonance elastography, which measures displacement caused by propagating shear waves, has been applied to humans for the study of hydrocephalus and brain tumors.

In an MRE experiment, researchers subjected adult rats to cortical impact injury immediately and up to 28 days before imaging, which was performed on excised brains which had been frozen and later thawed; they found a 23%–32% reduction in “stiffness” (similar to elasticity) of the injured cortex. Using a similar imaging approach, the same group of investigators recently conducted in vivo MRE on mice subjected to craniotomy and controlled cortical impact injury followed by closure of the calvaria. The injured mice along with controls were imaged prior to injury and repeatedly at 6 hours, 24 hours, 7 days, and 28 days. In the region of impact, the researchers observed decreases in the stiffness (by 24% or 29%), depending on whether the injury was 0.5
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Changes in the elastic and viscous mechanical properties of the intracranial compartment following brain contusion in living rats correspond to the accumulation of edema and subsequent tissue necrosis and reactive changes. Quantification of the relative changes in the mechanical properties of intracranial contents will be useful for the eventual application of emerging diagnostic techniques such as MRE.

Conclusions

or 0.75 mm deep), storage modulus, and loss modulus at 6 hours, with gradual normalization by 7–28 days. Their numeric values are not directly comparable to our data because their dynamic analysis involved using an oscillatory force. However, the time scale of changes is similar. Another novel MRE method eliminates the requirement for external vibrations by measuring the brain motions generated from blood vessel pulsations but has not yet been applied to brain injury situations. When MRE becomes applicable to physiologically unstable patients such as brain trauma victims, it will be important to have data such as those presented herein.

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Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Authors contributions to the study and manuscript preparation include the following. Conception and design: Del Bigio. Acquisition of data: Alfasi, Shulyakov. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript for external vibrations by measuring the brain motions generated from blood vessel pulsations but has not yet been applied to brain injury situations. When MRE becomes applicable to physiologically unstable patients such as brain trauma victims, it will be important to have data such as those presented herein.

Conclusions

Changes in the elastic and viscous mechanical properties of the intracranial compartment following brain contusion in living rats correspond to the accumulation of edema and subsequent tissue necrosis and reactive changes. Quantification of the relative changes in the mechanical properties of intracranial contents will be useful for the eventual application of emerging diagnostic techniques such as MRE.

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