Diffusion-Weighted Imaging Differentiates Ischemic Tissue From Traumatized Tissue

Christopher C. Hanstock, PhD; Alan I. Faden, MD; M. Robin Bendall, DSc; Robert Vink, PhD

Background and Purpose Diffusion-weighted magnetic resonance imaging (MRI) has been shown to be particularly effective in detecting early (0 to 4 hours) pathophysiological changes indicative of the development of this irreversible brain damage after a traumatic event, including alterations in energy metabolism,1,2 ion homeostasis,3-4 amino acids,5-6 oxygen free radicals,7-8 and endogenous opioids,9 among others. In addition, traumatic brain injury may also result in local reductions in cerebral blood flow. Previous studies have demonstrated that moderate brain injury results in local reductions in blood flow that do not approach ischemic thresholds,10-12 whereas more severe traumatic brain injury is complicated by pathophysiological changes indicative of focal ischemia.13 Because the fundamental biochemistry of brain trauma and that of brain ischemia differ in terms of energy conservation, pH homeostasis, and edema development,14-16 it would therefore be advantageous to distinguish at an early stage traumatic injuries with ischemic phenomena from those traumatic brain injuries that have no focal ischemia so that therapy can be appropriately targeted.

Methods Diffusion-weighted MRI images and T2-weighted MRI images were obtained over 4 hours after either moderate fluid percussion-induced traumatic brain injury or unilateral carotid ligation in rats. Diffusion-weighted MRI images of traumatic brain injury demonstrated focal regions of image hypointensity as early as 1 hour after trauma. The relative diffusion coefficient in these hypointense regions was significantly increased (P < .005) by 4 hours after trauma compared with the uninjured hemisphere, but only in the transverse plane in the x direction. In contrast, induction of diffuse, nonfocal ischemia complicated by pathophysiological changes indicative of bulk flow of extracellular fluid toward the lateral ventricles (vasogenic edema). In contrast, the decreased water diffusion distance with no apparent directionality observed in ischemia is most likely indicative of cytotoxic edema. Diffusion-weighted MRI therefore has the potential to differentiate cases of traumatic brain injury with no focal ischemia from those instances of traumatic brain injury in which focal ischemia is a complication. (Stroke. 1994;25:843-848.)

Key Words • brain edema • brain injuries • magnetic resonance imaging • rats

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Magnetic resonance imaging (MRI) has been successfully used to monitor pathophysiological changes after brain and spinal cord injury.17,18 In particular, the use of T2-weighted MRI sequences permits the noninvasive visualization of edema development that is commonly associated with ischemic phenomena. Unfortunately, these changes in the T2-weighted MRI scans do not become apparent until several hours after the traumatic or ischemic event. A variation of standard MRI techniques known as diffusion-weighted MRI has recently been described,19,20 which permits early detection of metabolic changes associated with edema development.21 By monitoring the diffusion of water in brain after an ischemic insult, diffusion-weighted MRI has been shown to be a useful technique for the enhancement of contrast when compared with standard T2-weighted images.19,21-23 This enhanced contrast reflects early (0 to 2 hours) edema development as extracellular water moves into the intracellular space (cytotoxic edema). The region of ischemic tissue injury detected by diffusion-weighted MRI has been correlated to histopathological findings at later time points, and the movement of the water into the intracellular space is thought to be associated with energy depletion.21,22 The purpose of this study was to determine whether diffusion-weighted MRI could similarly predict outcome after moderate fluid percussion-induced traumatic brain injury in rats. We report that traumatic and ischemic injury result in different forms of edema (vasogenic versus cytotoxic) that can be distinguished by diffusion-weighted MRI as early as 1 hour after the event.
Materials and Methods

Animal Preparation
All studies were conducted in accordance with the Australian National Health and Medical Research Council and US National Institutes of Health guidelines on the care and use of animals in research as approved by the James Cook University Experimentation Ethics Committee. Male Sprague-Dawley rats (weight, 300 to 350 g; n = 18) were initially anesthetized with sodium pentobarbital (60 mg/kg IP) so that a femoral catheter could be implanted for continuous infusion of anesthetic (sodium pentobarbital, 15 mg/kg per hour). Animals were then randomly divided into three groups and subjected to either unilateral carotid artery ligation (n = 3) or fluid perfusion-induced traumatic brain injury (n = 9) or served as controls (n = 6).

Animals subjected to unilateral carotid artery ligation had a silk suture placed around the right carotid artery. The artery was then ligated 1 hour after induction of anesthesia. This form of injury in the rat greatly reduces cerebral blood flow without preventing flow. Nonetheless, the substantial increase in brain free fatty acids and the compromised mitochondrial function under these conditions indicate localized ischemia.

Animals subjected to fluid perfusion-induced brain injury were placed in a stereotaxic holder and the skull exposed by removing the skin and muscles. A 4-mm craniotomy was performed over the left parietal cortex, and a femoral arterial catheter was fixed over the craniotomy with dental cement. One hour after induction of anesthesia, animals were subjected to moderate (2.6 ± 0.2 atm) fluid perfusion injury as previously described in detail elsewhere.

Briefly, animals were connected to the fluid perfusion injury device via the femoral Leur-loc fitting cemented in place over the exposed parietal cortex. Initiation of a pressure pulse within the saline-filled reservoir of the fluid perfusion device resulted in rapid and brief injection of pressurized saline into the closed cranial cavity of the rat. The pressure pulse was recorded by transducer and stored on an oscilloscope.

Magnetic Resonance Imaging
All images were obtained using a Varian 7-T horizontal bore magnet with a 15-cm clear bore. A 6-cm-diameter saddle coil providing a reasonably uniform radio frequency field throughout the entire rat brain was used for data acquisition. After placing the animal in the center of the magnet bore, static magnetic field homogeneity was optimized to typical values of 0.2 to 0.3 ppm. A standard T₂-weighted spin-echo pulse sequence with unipolar diffusion gradients was then obtained such that the chemical shift of the water in the region on the brain surface directly under the skin. Relative to the uninjured right hemisphere, the injured left (L) hemisphere shows significant regions of hypointensity where the normally blue sections of brain have become black and the normally green sections of brain (the hippocampal formation and cortical gray matter) have become blue. The site and direction of the fluid perfusion injury pulse are shown with an arrow.

![Image](http://stroke.ahajournals.org/)

**Results**

After moderate traumatic brain injury, T₂-weighted images obtained between 1 and 4 hours after trauma did not show any regions of altered signal intensity in the injured (left) hemisphere compared with uninjured
(right hemisphere) tissue. In contrast, diffusion-weighted images obtained as early as 60 minutes after trauma demonstrated a decrease in signal intensity in the injured hemisphere compared with the uninjured hemisphere. This hypointensity was particularly apparent in our qualitative diffusion-weighted images in which we divided the maximum b value image from the minimum b value image (Fig 1). This technique is similar to that used by Moseley et al21 and emphasizes the apparent diffusion contribution to the images. Any change in signal intensity in the diffusion-weighted image would therefore be enhanced. The contrasts observed after traumatic brain injury were only visible when the diffusion gradient was applied along the phase-encode direction (x plane in transverse images) as opposed to the read direction.

After right unilateral carotid ligation, T2-weighted images showed no relative contrast between the hemispheres between 1 and 4 hours after ligation. In contrast, qualitative diffusion-weighted images demonstrated clear hyperintense ischemic regions in the right hemisphere as early as 60 minutes after ligation (Fig 2). This enhanced contrast in ischemic tissue using diffusion-weighted imaging is similar to that reported previously.21-22 Furthermore, there was no directional influence with respect to the intensity changes observed in the carotid ligation group.

To determine the quantitative nature of these differences, diffusion coefficients were calculated using equations 1 and 2 for uninjured, traumatized, and ischemic brain. In control animals, apparent diffusion coefficients were 1.20±0.10x10^-3 mm^2/s for cortical gray matter and 0.45±0.05x10^-3 mm^2/s for hippocampal formation (Table). These values are in excellent agreement with previously reported values in cat and rat brain.21-22 Traumatic brain injury resulted in a significant increase (P<.005) in the relative diffusion coefficient (injury ADC/control ADC) in cortical gray matter and hippocampal formation by 4 hours after trauma (Table). This increased diffusion coefficient was detected in cortical gray matter as early as 1 hour after trauma (ADC = 1.05±0.05) but was not significant at this time compared with the uninjured hemisphere. The increase in the relative diffusion coefficient was limited to the injury site and was not detected in either the adjacent uninjured tissue in the same hemisphere or in the contralateral hemisphere.

In ischemic tissue there was a significant decrease (P<.001) in the relative diffusion coefficients (ischemic ADC/control ADC) in both cortical gray matter and hippocampal formation by 1 hour after ligation (Table). There were no further significant changes in these values between 1 and 4 hours. No changes in the coefficients were detected in the uninjured left hemisphere, suggesting that the unilateral carotid ligation in the rat induced perturbations in water diffusion that were limited to the ipsilateral hemisphere.

Discussion

Previous studies of brain ischemia have demonstrated that diffusion-weighted MRI is a valuable tool for the detection and localization of early metabolic abnormalities associated with profound reductions in blood flow.21,22 Using diffusion-weighted MRI, ischemic tissue in these images is characterized by a hyperintensity that is thought to represent the onset of cytotoxic edema. As water protons enter the intracellular compartment from the extracellular space, the distance that they can diffuse is restricted by the cell membrane. This restriction in diffusion path length is thought to be in large part account for the increase in image intensity in these regions, with minimal contributions from microscopic brain pulsations and temperature.22

In the present study we have demonstrated that after moderate traumatic brain injury, diffusion-weighted MRI images do not develop regions of hyperintensity during the first 4 hours after trauma. Indeed, the opposite occurred, with regions of hypointensity appearing in the injured cortex and hippocampus. This indicates that in the immediate 4-hour period after trauma, there appears to be no movement of extracellular fluid into the cells. We can conclude that any reductions in blood flow observed at this level of injury are therefore not of a magnitude sufficient to cause metabolic failure and associated influx of water as the transmembrane ATPase pumps fail. This observation is consistent with previous studies of moderate brain trauma. Yamakami and McIntosh21-22 and DeWitt and colleagues10,11 have demonstrated that moderate traumatic brain injuries result in focal blood flow reductions of less than 50%. This value does not approach threshold values reported for energy failure.28,29 Furthermore, magnetic resonance spectroscopy studies have shown that there is no energy failure after traumatic brain injury of moderate severity.30 Our results with diffusion-weighted MRI are consistent with these independent observations.

The increase in apparent diffusion coefficients after moderate brain trauma suggests that there is an increase in diffusion path length of water. An increase in the volume of the extracellular fluid may account for this observation. Furthermore, the directionality of the diffusion coefficients suggests that there is some form of directional bulk flow. Both of these observations are consistent with vasogenic edema formation.31 Previous studies have shown that traumatic brain injury results in protein extravasation in the injured cortex,23,32 with an associated increase in tissue water content.33 The resulting tissue pressure gradient would therefore cause movement of extracellular fluid by bulk flow.34 The directionality observed in our studies is indicative of bulk flow and suggests that this flow is toward the lateral ventricles away from the injured regions of higher pressure.35

The apparent diffusion coefficients calculated from the diffusion-weighted images indicate that in cortical gray matter, the water diffusion path length had increased by 36% at 4 hours after moderate trauma.

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**Relative Diffusion Coefficients for Ischemic and Traumatically Injured Rat Brain**

<table>
<thead>
<tr>
<th></th>
<th>Ischemia/Injury</th>
<th>Injury/Control</th>
<th>Injury/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical gray matter</td>
<td>0.67±0.04*</td>
<td>1.36±0.08†</td>
<td>1.07±0.02</td>
</tr>
<tr>
<td>Hippocampal formation</td>
<td>0.75±0.02</td>
<td>1.09±0.03†</td>
<td>0.97±0.01</td>
</tr>
</tbody>
</table>

*P<.001 vs injury/control; †P<.005 vs injury/Control.

Injury indicates injured brain tissue; Injury, uninjured brain tissue in injured hemisphere.
Similarly, the water diffusion path length of the hippocampal formation increased by 9%. This implies that edema formation in the injured cortical gray matter was four times greater than that observed in the hippocampus. This is consistent with results reported by Soares et al., who demonstrated that edema formation after moderate lateral fluid percussion brain injury in rats is three times greater in the injured cortex than that observed in the ipsilateral hippocampus. However, unlike the findings of Soares et al, we find that this difference is apparent by 4 hours after trauma. This is more likely a reflection of the increased resolution and sensitivity of the noninvasive MRI technique compared with invasive techniques. Furthermore, diffusion-weighted MRI indicates that this vasogenic edema formation commences in the first hour after trauma, unlike previous reports that suggest that vasogenic edema may take hours or even days to develop.

The increase in the apparent diffusion coefficient was limited to the injured tissue and did not appear in adjacent uninjured tissue or in the contralateral hemisphere. This localization of moderate traumatic injury is consistent with the observation that alterations in monovalent ions and the divalent cation magnesium are restricted to injured tissue in this model of head injury. The advantage with diffusion-weighted MRI is that the alterations are detected noninvasively and can be detected within the first few hours after trauma.

Our results with unilateral common carotid ligation are consistent with previous studies using diffusion-weighted MRI to characterize ischemia. An increased signal intensity in the injured tissue is apparent as early as 1 hour after the event. This increased signal intensity is thought to be due to a decreased water diffusion path length being imaged as the fluid moves from the extracellular space to the intracellular space, indicative of cytotoxic edema formation. It is of interest that unilateral common carotid ligation does in fact result in scattered regions of cytotoxic edema. This suggests that while the method may be a poor model for reproducible, focal ischemia, it is an excellent model for the production of localized ischemic regions nonuniformly distributed throughout the brain and involving cortical and hippocampal regions. This unpredictable pattern of injury is perhaps more in keeping with the profile seen in more severe cases of clinical traumatic brain injury.

In conclusion, the lack of hyperintensity in traumatized brain compared with the clear and sustained hyperintensity in ischemic tissue suggests that diffusion-weighted MRI may be used to differentiate between moderate traumatic brain injury and more severe traumatic brain injury with ischemic complications. While more severe injury levels are thought to cause more profound reductions in flow that approach ischemic thresholds, moderate injury does not produce such reductions in flow that may be expected to result in cytotoxic edema. In contrast, moderate traumatic brain injury results in vasogenic edema formation; as such, diffusion-weighted MRI may therefore be expected to identify regions of abnormal metabolism on the basis of this difference in edema formation.

Acknowledgments

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References


**Editorial Comment**

Magnetic resonance imaging (MRI) techniques have recently been developed that are capable of producing images with contrast based on the translational motion (diffusion) of water. Until recently, diffusion-weighted imaging has mainly been used to study the effects of cerebral ischemia. This is probably because changes in water diffusion measured after the onset of cerebral ischemia have been far less than the 40% to 60% decline reported by this group and others after cerebral ischemia.

The present work has added another piece to the puzzle in our understanding of changes in water diffusion resulting from various pathologies. However, a specific mechanism that explains these observations remains to be determined. It would be interesting to compare the results presented here with those published by our group, which have demonstrated a temporal relationship between the increase in water diffusion measured at later times after cerebral ischemia with histological evidence of cell membrane disruption.

In this regard, we look forward to further reports from Hanstock and colleagues that include histopathological assessment of the injured tissue in an attempt to shed more light on this subject.

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**References**


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