Diffusion-Weighted Imaging Differentiates Ischemic Tissue From Traumatized Tissue

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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 bulk diffusion in traumatized brain. The present study sought to establish whether diffusion-weighted MRI would be similarly effective in predicting outcome after traumatic brain injury.

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.

Results  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 noninjured hemisphere, but only in the transverse plane in the x direction. In contrast, induction of diffuse, nonfocal ischemia by unilateral carotid ligation resulted in scattered regions of hyperintensity with a significant (P<.001) decrease in relative diffusion coefficient as early as 1 hour after ligation compared with the noninjured hemisphere. This decrease exhibited no directionality.

Conclusions  We conclude that traumatic brain injury results in an increased water diffusion distance with the directionality 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

Characterization of early metabolic events after traumatic brain injury permits the development of therapies targeted at attenuating these pathophysiological changes and subsequently improving outcome. A variety of factors have been identified as contributing to 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.

Received May 7, 1993; final revision received November 5, 1993; accepted December 6, 1993.

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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 percussion-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.24 Animals subjected to fluid percussion-induced brain injury were placed in a stereotaxic holder and the skull exposed by removing the skin and muscle. A 4-mm craniotomy was performed over the left parietal cortex, and a female Leur-loc fitting 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 percussion injury as previously described in detail elsewhere.25 Briefly, animals were connected to the fluid percussion injury device via the female Leur-loc fitting cemented in place over the exposed parietal cortex. Initiation of a pressure pulse within the saline-filled reservoir of the fluid percussion 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 parts in 1000 across the whole head. A standard T2-weighted coronal image was then obtained to align the location of the desired transverse slice. In those animals subjected to traumatic brain injury, the transverse slice was aligned to an external standard located adjacent to the trauma site. Care was taken such that the chemical shift of the water in the external sample was the same as brain tissue, thus ensuring that there was no chemical shift artifact in the apparent position of the sample in the image. Subsequent transverse images were obtained with a modified spin-echo pulse sequence with unipolar diffusion gradients before and after the refocusing pulse, according to the method of Stejskal and Tanner.26 A typical image was obtained with the following acquisition parameters: field of view, 6x6 cm; slice thickness, 2.5 mm; read direction resolution, 256 points zero-filled to 512; phase-encode direction, 64 points zero-filled to 512; repetition time (TR), 2 seconds; echo time (TE), 256 milliseconds; b values, 0, 544, and 1110 s·mm-2 (diffusion gradients =0, 2.8, and 4.0 G·cm-1·s-1, respectively; Δ=15 milliseconds; δ=38 milliseconds); diffusion gradients unipolar either along the read or phase-encode directions; and two scans acquired per phase-encoding step. To minimize the signal losses caused by the long TE (resulting in part from the long δ and Δ times required to produce large b values using the maximum 4-G·cm-1·s-1 gradient available), it was necessary to minimize the acquisition time by selecting a maximum read gradient strength and relatively large field of view. For this application, the loss of spatial resolution in the images due to the reduction in the acquisition time to 4 milliseconds was minimal. Images were obtained for the three b values selected at 1, 2, and 4 hours after ischemia or after trauma. Apparent diffusion coefficients (ADC) were determined according to the method described by Le Bihan and colleagues.27 In brief, integrals of 0.5×0.5-mm voxels were obtained across the image and for each b value in the series. ADC values were then calculated for each voxel ADC (x,y,z) using the relation

\[
ADC(x,y,z)=\ln[S_0(x,y,z)/S_1(x,y,z)]/b_1-b_0
\]

where \(S_0(x,y,z)\) is the signal intensity at \(b=b_0\), and \(S_1(x,y,z)\) the signal intensity at \(b=b_1\). The b values were calculated according to

\[
b = \gamma G^2 \delta (\Delta - \delta/3)
\]

where \(\gamma\) is the gyromagnetic ratio; \(G\) is the diffusion gradient strength; \(\delta\) is the diffusion gradient duration; and \(\Delta\) is the separation of the leading edges of the two diffusion gradient envelopes. A qualitative ADC distribution image throughout the whole brain was then obtained by dividing image data sets obtained at different b values, in accord with \(ADC\) being proportional to \(S_0/S_1\) as described above.

Data Analysis

All data are expressed as mean±SE. Comparisons across groups were done by ANOVA followed by individual Student Newman-Kuels to determine significance.

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 Mosley et al.\cite{1} 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.\cite{2,3} Furthermore, there was no directionality 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 $1.20 \pm 0.10 \times 10^{-3}$ mm$^2$/s for cortical gray matter and $0.45 \pm 0.05 \times 10^{-3}$ mm$^2$/s for hippocampal formation (Table). These values are in excellent agreement with previously reported values in cat and rat brain.\cite{4,5} 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 = $1.05 \pm 0.05$) 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.\cite{6,7} 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 in large part account for the increase in image intensity in these regions, with minimal contributions from microscopic brain pulsations and temperature.\cite{8}

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.\cite{9,10} Traumatic brain injury results in focal blood flow reductions of less than 50%. This value does not approach thresholds reported for energy failure.\cite{11,12} Furthermore, magnetic resonance spectroscopy studies have shown that there is no energy failure after traumatic brain injury of moderate severity.\cite{13} 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.\cite{14} Previous studies have shown that traumatic brain injury results in protein extravasation in the injured cortex,\cite{15,16} with an associated increase in tissue water content. The resulting tissue pressure gradient would therefore cause movement of extracellular fluid by bulk flow.\cite{17} 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.\cite{18}

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.
Similarly, the water diffusion path length of the hippocampal formation had increased by 9%. This implies that edema formation in the injured cortical gray matter was four times greater that 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 noninjured 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 imposed 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

This study was supported in part by an Australian National Health and Medical Research Council grant (Dr Vink) and grants from the US National Institutes of Health (RO1 NS27849) and Centers for Disease Control (R49 CCR306634) (Dr Faden). We thank Dr J.P. Headrick for helpful comments.

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### 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 precede changes in any other MRI parameter studied to date and demonstrate a relatively large (40% to 80%) decline in the translational motion of water.

In the preceding article by Hanstock and colleagues, we have seen the completely opposite response. Here, the diffusion of water increased after moderate trauma in the presence of a time-dependent field gradient. *J Cereb Blood Flow Metab.* 1987;7:394-402.

There is currently no direct evidence that cytotoxic edema is a significant mechanism responsible for the decline in water diffusion. Zhong and colleagues have shown a significant decline in water diffusion ($\approx 18\%$) during bicuculline-induced status epilepticus in rats, a condition during which blood flow is significantly increased and alterations in ATP levels are minimal.

There is also currently no direct evidence that cytotoxic edema is a significant mechanism responsible for the decline in water diffusion after cerebral ischemia; at this time it is merely a convenient explanation. In fact, the group that first proposed this as an underlying mechanism, it is now clear that these results are discussed on the basis of a need for energy failure leading to cytotoxic edema as a prerequisite for a decrease in water diffusion.

Although it is tempting to point at energy failure either from reduced blood flow or enzyme inhibition as an underlying mechanism, it is now clear that these conditions are not required for a decline in water diffusion. Zhong and colleagues have shown a significant decline in water diffusion ($\approx 18\%$) during bicuculline-induced status epilepticus in rats, a condition during which blood flow is significantly increased and alterations in ATP levels are minimal.

There is also currently no direct evidence that cytotoxic edema is a significant mechanism responsible for the decline in water diffusion after cerebral ischemia; at this time it is merely a convenient explanation. In fact, the group that first proposed this as a mechanism has recently published evidence to the contrary. Sevick et al. measured water diffusion coefficients in combination with acute hyponatremia in rats as a model of cytotoxic brain edema. The decline in water diffusion measured under these conditions was only 8%, which is 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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Stroke. 1994;25:843-848
doi: 10.1161/01.STR.25.4.843

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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