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Bolus tracer delivery measured by MRI confirms edema without blood-brain barrier permeability in diffuse traumatic brain injury

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Summary

Introduction. Previous studies have shown that edema formation after diffuse traumatic brain injury (TBI) with secondary insult is cytotoxic and not vasogenic. This assumption is based on observations of reduced apparent diffusion coefficient (ADC) and lack of significant accumulation of intravascular tracer in brain tissue. However, ADC reduction does not exclude vasogenic edema, and intravascular tracer can only accumulate when it reaches the tissue and is not perfusion limited. This study aims to confirm tissue delivery of intravascular tracer and lack of BBB opening during a phase of rapid brain swelling after diffuse TBI.

Methods. Rats were exposed to either TBI using the impact acceleration model combined with 30 minutes of hypoxia and hypotension, or sham injury. At 2 or 4 hours after injury, ADC and tissue water content were assessed using MRI. Gd-DTPA was given followed by a combination of rapid T2 imaging (60 seconds) and T1 imaging (30 minutes). Signal intensity changes were analyzed to determine a bolus effect (dynamic susceptibility contrast) and longer term tissue accumulation of Gd-DTPA.

Results. Mean increase in cortical water content on the left was 0.8% at 2 hours, 2.1% at 4 hours; on the right it was 0.5% at 2 hours and 1.7% at 4 hours ($p < 0.05$). Mean ADC reduction over 4 hours was 0.04×10^{-3} mm^2/s on the left and 0.06×10^{-3} mm^2/s on the right. Kinetic analysis of signal intensity changes after Gd-DTPA showed no significant difference in inward transfer coefficient (BBB permeability) between sham injury and 2 or 4 hours post-injury. T2 imaging showed consistent tissue delivery of a bolus of Gd-DTPA to the tissue at 2 and 4 hours post-injury, comparable to sham animals.

Conclusions. Progressive cerebral edema formation after diffuse TBI occurred during ADC reduction and without continued BBB permeability. Tissue delivery of Gd-DTPA was confirmed, verifying that lack of tracer accumulation is due to an intact BBB and not to limited perfusion.

Keywords: Magnetic resonance imaging; brain edema; blood-brain barrier; traumatic brain injury.

Introduction

The role of blood-brain barrier (BBB) damage in posttraumatic brain swelling is not well understood. Recent studies have highlighted the importance of a cellular swelling process in edema formation after injury, as assessed by measurement of the apparent diffusion coefficient (ADC) of water [1, 2]. Other studies have suggested a permissive role for BBB damage [3]. Experimental studies of diffuse traumatic brain injury (TBI), in comparison to focal injury, have not demonstrated more than transient opening of the BBB. There are several methodological limitations in studies of the BBB. Of special relevance to TBI is the problem of flow-limited diffusion; if intravascular tracer cannot reach the tissue because of low blood flow, then BBB damage may be underestimated or undetected.

Magnetic resonance imaging (MRI) techniques are useful for assessing BBB damage after injury because it also provides other information such as ADC and degree of tissue water content concurrently over multiple time-points. However, visual assessment of signal intensity changes with intravascular administration of gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) may underestimate tissue accumulation. Therefore, numerical assessment of signal intensity changes is recommended. In addition, bolus delivery of Gd-DTPA to tissue has been shown to generate a rapid and transient drop in signal intensity on T2-weighted images [7], the so-called dynamic susceptibility contrast phenomenon.

The aim of this study was to evaluate BBB damage in experimental diffuse TBI using MRI with Gd-DTPA, and to demonstrate tracer delivery to the tissue definitively in order to rule out underestimation of BBB damage due to flow-limited diffusion. A second goal of the study was to demonstrate progressive brain

swelling in the absence of prolonged BBB opening, thereby confirming that posttraumatic brain swelling is cellular and not vasogenic in origin.

Materials and methods

In this study, experimental diffuse TBI was combined with a secondary insult of hypoxia and hypotension. BBB damage was assessed by serial measures of tissue T1-weighted contrast change after intravascular administration of Gd-DTPA. Tissue delivery of tracer was demonstrated using T2 imaging immediately after bolus administration. Tissue water content was assessed from the calculation of absolute values of T1 for the tissue. The ADC was measured at the same time as tissue water content.

All animals received humane care in compliance with the Guide for the Care & Use of Laboratory Animals (National Research Council, National Academy Press, Washington D.C., 1996). Adult-male Sprague-Dawley rats (350 to 380 g; n = 16) were exposed to the impact acceleration model of diffuse brain injury [5] using a weight of 450 g over 2 m. Injury was combined with hypoxia and hypotension. Sham-injured animals (n = 6) underwent all procedures except for the impact of the weight or the imposition of secondary insult. Secondary insults of hypoxia and hypotension were applied by reduction of FiO_2 to 12%, resulting in arterial PO_2 levels of 30 to 40 mmHg, and arterial blood pressures of 30 to 40 mmHg. Secondary insults were initiated immediately after injury and maintained for 30 minutes.

At either 2 hours (n = 9) or 4 hours (n = 7) after injury, animals were placed in a 2.35 T, 40 cm bore magnet (Biospec, Bruker Instruments, Billerica, MA). Initial measures of tissue ADC and water content were made, followed by baseline T1 and T2 images according to methods described below. All images were obtained from a 3 mm thick slice, positioned 7.5 mm caudal to the anterior pole of the cerebrum. Following baseline imaging, each animal was infused with an intravenous bolus of 0.2 mmol/kg Gd-DTPA (Omniscan, Nycomed, Wayne, PA). Sixty seconds of rapid T2 imaging were performed for description of the signal intensity change due to dynamic susceptibility, followed by 30 minutes of T1 imaging to assess longer-term Gd-DTPA accumulation assessed by changes in T1 signal intensity.

Changes in T1 or T2 signal intensity were used to calculate Gd-DTPA concentration in the bolus and in the tissue, respectively, for regions of interest. Conversions to concentrations were based on previously defined calibration curves derived from known standards. The time course of Gd-DTPA accumulation acquired from the T1 images was then subjected to kinetic analysis using a previously described kinetic model [6] in order to derive features of BBB permeability. Concentration profiles were fitted to a derived equation using a non-linear least squares algorithm (Levenberg-Marquardt).

Mean arterial blood pressure was assessed continuously from the time of injury. BBB permeability parameters were compared with measured changes in tissue water and ADC values. Statistical significance was assessed using ANOVA, with appropriate post hoc tests (Fisher least significant difference, Newmann-Keuls) and p-values less than 0.05 were considered significant.

MRI measurements

At the time of assessment, animals were placed in a 2.35 T, 40 cm bore magnet (Bruker Instruments) equipped with a 12 cm inner diameter actively shielded gradient insert. RF excitation and reception were performed using a 4.5 cm helmet coil. In order to minimize any macroscopic motion artifacts, the rat's head was rigidly supported with a specially designed stereotactic device, including both ear and mouth supports mounted inside a Plexiglas cylinder. For evaluation of Gd-DTPA accumulation secondary to BBB damage, serial T1 images were obtained from a 3 mm thick slice, 7.5 mm caudal to the frontal pole. Imaging parameters used were TR = 700 ms, TE = 22 ms, FOV 4 cm^2, with a 64 × 64 matrix.

To demonstrate tissue delivery of Gd-DTPA, T2 imaging was used as described above. An example of a T2 MRI sequence with sufficient TR is the gradient echo method, acquired with TR/TE values of 27/20 ms, respectively, using a matrix size of 32 × 32 pixels and a 4 cm^2 FOV. These parameters generated an image acquisition time of 1000 msec. Images were repeated sequentially at maximum speed in order to provide TR of 1 second.

ADC measurements were performed using a 2-dimensional spin echo imaging technique (diffusion weighted imaging) appropriately modified to include diffusion-sensitizing gradients along the readout (horizontal) direction with a duration of 4 ms and a gradient separation of 20 ms. Each dataset consisted of a single coronal slice (3 mm thick) positioned 7.5 mm caudal to the frontal pole imaged with a 64 × 64 matrix using a TR/TE of 1500/33 ms and FOV 4 cm^2. Diffusion weighing factors, or *b* values, of 10, 340, 670, and 1000 s/mm^2 were used (maximum gradient strength of 23 G/cm). Pure ADC maps were calculated for each slice from the diffusion-weighted images using a pixel-by-pixel 3-parameter least squares fit to the magnitude image data. The effect of the frequency encoding gradients was included in the ADC calculations.

The concept of utilizing MRI for measuring brain water is based on laboratory and clinical studies directed toward noninvasive monitoring of brain edema formation and resolution [4]. Briefly, pure T1 maps are generated and then converted to water maps by means of the following equation:

$$\frac{1}{W} = 0.907 + \frac{0.407}{T1}$$

T1 is the measured T1 value of the tissue expressed in seconds and W is the tissue water content measured in gm H_2O/gm tissue.

Results

Figures 1a–c show profiles of tissue tracer concentration over time for 1 minute after tracer injection in sham animals, 2 and 4 hours after injury. There are no appreciable differences between the profiles; therefore, comparable quantities of tracer are delivered to the tissue in the injured animals compared with sham animals. Figures 1d–f show the profile of tracer concentration change in the tissue over 30 minutes after injection, based on T1W imaging. Figure 1f is taken from muscle, and represents the accumulation of Gd-DTPA in a tissue without a blood-brain barrier. Gd-DTPA follows a characteristic wash-in and wash-out profile. In contrast, there is minimal Gd-DTPA accumulation over 30 minutes in either the left (Fig. 1d) or right (Fig. 1e) cortex of 2-hour and 4-hour injured animals, consistent with either an impermeable BBB or severe flow-limited diffusion.

Application of the concentration-time data to a pre-

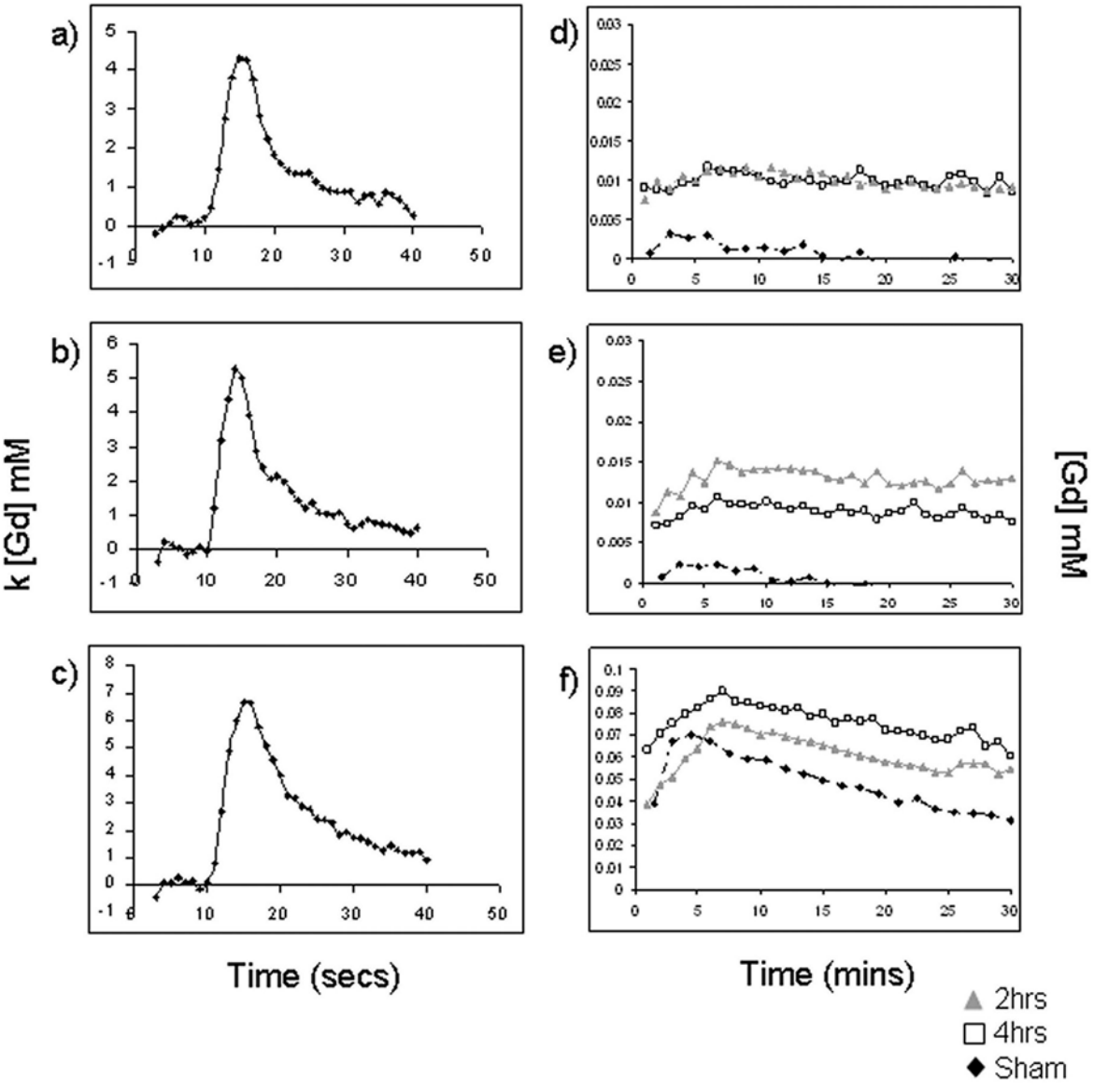

Fig. 1. Concentration time curves for initial bolus delivery of Gd-DTPA to the tissue (a–c) derived from signal intensity change due to dynamic susceptibility. Concentration time curves for Gd-DTPA accumulation over 30 minutes after infusion (d–f) showing longer term accumulation of tracer derived from T1W signal intensity changes under sham conditions, 2 and 4 hours after injury, (d) left cortex, (e) right cortex, (f) paraspinal muscle

viously published kinetic model [6] was performed. The derived parameter D_1, according to this model, is the product of the extracellular volume (Ve), peak plasma tracer concentration (A_0), and K_1, the inward transfer coefficient. Because Ve and A_0 are considered to be relatively constant, changes in D_1 are thought to reflect changes in K_1 closely. Table 1 shows the mean calculated D_1 values (uM/min) for each group in the left and right cortex. They are not different and do not appear to be influenced by trauma with secondary insult or time after trauma. Table 1 also shows the tissue water content and ADC values obtained in sham animals and at 2 and 4 hours after injury. At 2 hours after injury, tissue water content rose from 79.5% to 80.3% in the left hemisphere, and from 79.3% to 79.8% in the right hemisphere. Tissue water content continued to rise by 4 hours after injury to 81.6% and 81.0% in the left and right hemispheres, respectively. The rise in tissue water content was accompanied by a steady decline in ADC from 0.66 ($\times 10^{-3}$ mm^2/sec) to 0.62 in the left hemisphere and from 0.70 to 0.64 in the right hemisphere.

Table 1. *Comparison between mean values (±SD) for D1 (uM/min), tissue water content (%) and ADC (×10^{-3} mm^2/sec) values in each experimental group.*

	Left			Right		
	Sham	2 hours	4 hours	Sham	2 hours	4 hours
D1	1.6 ± 1.0	2.0 ± 1.0	1.0 ± 0.2	2.0 ± 2.0	2.0 ± 2.0	1.0 ± 0.3
Tissue Water	79.5 ± 0.7	80.3 ± 1.4	81.6 ± 1.9	79.3 ± 0.9	79.9 ± 1.2	81.0 ± 2.5
ADC	0.66 ± 0.03	0.64 ± 0.09	0.62 ± 0.17	0.70 ± 0.06	0.65 ± 0.09	0.64 ± 0.16

D1 is the derived parameter from kinetic analysis of the gadolinium-diethylenetriamine pentaacetic acid concentration time-curves and is closely related to the inward transfer coefficient; D1 represents blood-brain barrier permeability. Tissue water is derived from tissue T1 data and is expressed as a percentage. ADC is the apparent diffusion coefficient of water. Reduction of apparent diffusion coefficient is associated with intracellular water accumulation.

Conclusions

The contribution of cytotoxic and vasogenic edema to posttraumatic cerebral swelling is not entirely clear. Recent studies have highlighted the importance of cytotoxic swelling [1, 2], and possibly a permissive role of BBB damage [3]. Although BBB damage has been well documented experimentally in models of focal contusion, nothing more than a transient opening has been demonstrated in experimental models of diffuse injury. In order to be sure that measures of BBB damage are not flawed, it is necessary to demonstrate delivery of tracer to the tissue.

Using rapid T2 imaging, this study confirms that an intravascular bolus of Gd-DTPA reaches the brain in rodents with diffuse TBI. The study also demonstrates that there is minimal accumulation of Gd-DTPA over 30 minutes of circulation time, consistent with an intact BBB. This was confirmed by application of a kinetic model that demonstrated no difference in BBB permeability between sham animals and animals 2 hours and 4 hours after injury. Despite the lack of BBB permeability, the injured brains showed progressive accumulation of water associated with ADC reduction up to 4 hours after injury.

For the first time, this study demonstrates progressive edema formation associated with ADC reduction and without BBB permeability in the context of confirmed tracer delivery, with all measures obtained at the same time in the same experimental subject. Our data confirms the dominant role of cellular swelling after TBI. However, further studies will be needed to characterize better the nature of edema associated with diffuse TBI.

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