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A brief report on MRI investigation of experimental traumatic brain injury

Timothy Q. Duong*, Lora T. Watts

Research Imaging Institute, Departments of Cellular and Structure Biology and Ophthalmology, University of Texas Health Science Center, South Texas Veterans Health Care System, San Antonio, TX, USA

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Abstract

Traumatic brain injury is a major cause of death and disability. This is a brief report based on a symposium presentation to the *2014 Chinese Neurotrauma Association Meeting* in San Francisco, USA. It covers the work from our laboratory in applying multimodal MRI to study experimental traumatic brain injury in rats with comparisons made to behavioral tests and histology. MRI protocols include structural, perfusion, manganese-enhanced, diffusion-tensor MRI, and MRI of blood-brain barrier integrity and cerebrovascular reactivity.

Key Words: *MRI; traumatic brain injury; magnetic resonance imaging; diffusion tensor imaging; cerebral blood flow*

Correspondence to:
Timothy Q. Duong, Ph.D., duongt@uthscsa.edu.

orcid:
0000-0001-6403-2827 (Timothy Q. Duong)

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Introduction

Traumatic brain injury (TBI) is a major cause of death and disability, affecting 3.2 to 5.7 million in the United States, with an annual cost exceeding $60 billion in the civilian population (Coronado et al., 2012). TBI is a contributing factor to about a third of all injury-related deaths in the United States. In addition, over 270,000 U.S. Service Members have been diagnosed with TBI since the beginning of the global war on terrorism and is considered the signature injury of the Iraq and Afghanistan wars (Norman et al., 2013; Cifu et al., 2014; Pugh et al., 2014). Many more remain undiagnosed or underreported. Moreover, TBI increases susceptibility to suicide, post-traumatic stress disorder, and chronic pain, among other comorbidities, with negative long-term effects on quality of life (Levin and Diaz-Arrastia, 2015).

The initial direct mechanical damage in TBI is followed by a cascade of secondary damage that include impaired cerebral blood flow and oxygen delivery, cerebrovascular autoregulation, and metabolic dysfunction. Ischemia-like events (such as membrane depolarization, ion dysregulation, oxidative stress, excitotoxicity, inflammation, among others) subsequently lead to apoptotic and necrotic cell death. The cascades of secondary brain injury offer many potential targets for therapeutic intervention.

Multimodal MRI offers a means to non-invasively image anatomical, physiological and functional changes associated with brain injury. T_2 anatomical MRI allows for the visualization of gross morphological and microstructural damage, as well as brain edema. Diffusion-weighted imaging is used to detect ischemic brain injury (Moseley et al., 1990). Diffusion tensor imaging provides a means to track axonal fibers associated with TBI and diffuse-axonal injury. Fractional anisotropy measures white matter and structural integrity (Rutgers et al., 2008). Cerebral blood flow has also been studied in both humans and animals after TBI using techniques such as laser Doppler Flowmetry, SPECT, PET and MRI, although they are sparse by comparison to structural MRI. These approaches have contributed substantially toward improved understanding of TBI. Non-invasive MRI has the potential to be used to improve diagnosis, staging of injury, and monitor injury progression and treatment effects.

In this brief report, we describe the work from our laboratory, where we have applied multimodal MRI to study experimental TBI in rats. This paper starts by describing the TBI animal model used, then the MRI protocols utilized including structural MRI, perfusion, manganese-enhanced MRI, MRI of blood-brain barrier integrity, diffusion-tensor imaging, and cerebrovascular reactivity. Comparisons were made with behavioral tests and histology. This report is based on a symposium presentation to the *2014 Chinese Neurotrauma Association Meeting* held in San Francisco, USA.

Controlled Cortical Impact (CCI) Model

Rodent models have been widely utilized to study TBI. The most popular models currently utilized to study TBI include the CCI, fluid percussion, acceleration-impact or weight drop, Marmarou, Feeney, and blast injury models (see reviews) (Cernak, 2005; Xiong et al., 2013). The common areas of impact included somatosensory/motor, auditory, parietal, and visual cortices. Outcomes and lesion sizes are highly variable due to the use of different experimental models and the use of different injury parameters (Cernak, 2005; Xiong et al., 2013). There have only been a few quantitative multimodal MRI studies of animal models of TBI with behavioral and

histological correlation.

In our experimental TBI model, we used the open-skull CCI model. TBI was generated by creating a 5 mm craniotomy over the left S1 cortex in rats, exposing the dura matter. The intact dura was impacted using a pneumatic controlled cortical impactor (3 mm tip, 5 m/s, 250 μs dwell time, 1 mm depth) (Watts et al., 2014; Long et al., 2015a). Following the impact, the craniotomy was sealed with bone wax, the incision was sutured closed and the animal was moved to the MRI scanner for imaging. Blood pressure, arterial oxygen saturation, heart and respiration rates were within normal physiological range unless otherwise perturbed (*i.e.*, by 5% CO_2).

T_2 and Diffusion-Tensor MRI

Long et al. (2014) utilized quantitative multi-parameteric MRI to report longitudinal T_2 and diffusion-tensor changes in the cortex and underlying corpus callosum using a mild open-skull, CCI model of TBI in rats from 3 hours to 14 days after TBI. The impact was applied over the left primary forelimb somatosensory (S1FL) cortex. MRI measures were compared to longitudinal behavioral measurements using the foot fault and asymmetry tests. Further MRI defined lesion volume was also compared with end-point histology using Fluro Jade and Nissl staining. This study had several notable findings. First, within the S1FL impacted cortex we found that at 3 hours after TBI, T_2 increased while fractional anisotropy (FA) decreased. Subsequently, these values gradually returned toward normal by day 14. Within the same region, apparent diffusion coefficient (ADC) values increased acutely (by 3 hours) and were found to be highest 2 days post-injury with a gradual return toward normal at day 14. During further assessment of the corpus callosum directly underneath the S1FL cortex, the authors found that from 3 hours up to 2 days post-injury, FA decreased but returned to normal at days 7 and 14. In contrast, T_2 and ADC values were found to be normal throughout all time points explored. The authors also found heterogeneous hyper- and hypointense T_2 map intensities that likely indicate the presence of hemorrhage, although the authors did not verify this with histological assessments. The temporal pattern of lesion volume defined by abnormal T_2, ADC, and FA was similar across time points with the peak lesion volume occurring around day 2 and then returning toward normal by day 14. When the lesion volumes were compared with behavioral outcomes measured by the foot fault and asymmetry tests, it was determined that the temporal profiles of lesion volumes were consistent with behavioral scores assessed. Long et al. (2014) also demonstrated that at 14 days post-TBI, there was substantial tissue recovery detected by MRI, which suggests that MRI could either reflect true tissue recovery or reabsorption of edema. Histological analysis of neurodegeneration using Fluro Jade staining and morphological changes of neurons using Nissl staining was performed 14 days post-TBI. Histological assessment revealed a small cavitation and significant neuronal degeneration surrounding the cavitation in the S1FL cortex. The authors speculate that the observed improvement of behavioral scores supports the notion that both functional recovery and/or functional compensation may be involved.

Cerebral Blood Flow (CBF) MRI

In a separate study, Long et al. (2015b) investigated the effects of perturbed CBF and cerebrovascular reactivity (CR) on relaxation time constant (T_2), ADC, FA and behavioral scores at 1 and 3 hours, 2, 7 and 14 days post-TBI in rats using the same model described in the previous section. In this study, the authors found that acutely (1–3 hours post-TBI) there were substantial perfusion deficits within and surrounding the impacted area. However, CBF was not affected by TBI at all time points. Interestingly, we found that the abnormal areas of CBF and CR were larger than those of the T_2, ADC and FA abnormalities. Furthermore, there were substantial heterogeneous contrasts found across time points. In the impact core, there were acute CBF reductions followed by increased CBF (up to 2.5 times of normal) by day 2, and a return towards normal by day 14. In contrast, in the tissue surrounding the impact, the authors reported hypoperfusion on days 0 and 2. CR in the impact core in response to 5% CO_2 inhalation was negative at 1 and 3 hours, became the most severe on day 2 but gradually returned toward normal at later time points. The authors also detected T_2, ADC, and FA abnormalities within the impact core on day 0, peaked on day 2, and pseudonormalized by day 14. T_2 determined lesion volumes consistently peaked on day 2 and were temporally correlated with functional outcome measures using the forelimb-asymmetry and foot-fault scores.

Blood-Brain Barrier (BBB) MRI

Li et al. (2014) developed and employed an MRI technique to measure BBB disruption, a common occurrence following TBI. Dynamic contrast enhanced MRI can longitudinally measure the transport coefficient K^{trans} which reflects BBB permeability. K^{trans} measurements however are not widely used in TBI studies because it is generally considered to be noisy and possesses low spatial resolution. We improved spatiotemporal resolution and signal sensitivity of K^{trans} MRI in rats by using a high-sensitivity surface transceiver coil. To overcome the signal drop off profile of the surface coil, a prescan module was used to map the flip angle (B1 field) and magnetization (M0) distributions. A series of T_1-weighted gradient echo images were acquired and fitted to the extended Kety model with reversible or irreversible leakage, and the best model was selected using F-statistics. We applied this method to study the rat brain 1 hour following controlled cortical impact (mild to moderate TBI), and observed clear depiction of the BBB damage around the impact regions, which matched that outlined by Evans Blue extravasation. Unlike the relatively uniform T_2 contrast showing cerebral edema, K^{trans} showed a pronounced heterogeneous spatial profile in and around the impacted region, displaying a non-linear relationship with T_2. This improved K^{trans} MRI method is also compatible with the use of high-sensitivity surface coil and the high-contrast two-coil arterial spin-labeling

method for cerebral blood flow measurement, enabling a more comprehensive investigation of the pathophysiology in TBI.

Manganese-enhanced MRI (MEMRI)

Calcium plays an important role in normal cell physiology and can be perturbed during the secondary injury cascade following a TBI which causes further cellular damage and can lead to cell death. Talley Watts et al. (2015) employed MEMRI to investigate its applicability to study experimental TBI using the CCI model in rats. MEMRI is based on the ability of the manganese ion to act as a calcium analog and a MRI contrast agent by becoming trapped inside cells with a particularly long half-life. In this study, the authors compared conventional T_2 MRI with sensorimotor behavioral outcomes, and immunohistology for glial fibrillary acidic protein expression. The T_1-weighted MEMRI images revealed hyperintensity in the impact area at 1–3 hours, hypointensity on day 2. By days 7 and 14, the study found markedly hypointense areas within the impacted area that were surrounded by an area of hyperintensity. These findings were in contrast to the vehicle group, which did not show a biphasic profile. The authors also found that in the hyperacute phase, the area of hyperintense T_1-weighted MEMRI was larger than that of T_2 MRI. Due to the heterogeneous contrasts detected, glial fibrillary acidic protein staining was performed in the same animals and revealed that the MEMRI signal void in the impact core and the hyperintense area surrounding the core corresponded to tissue cavitation and reactive gliosis, respectively. In comparison to the findings using T_1-weighted images, T_2 MRI showed little contrast in the impact core at 2 hours. On day 2, the T_2 map detected hyperintense areas within the impacted area that likely indicate the presence of vasogenic edema. In some animals this hyperintensity remained but pseudo-normalized in others on days 7 and/or 14. Behavioral deficits peaked on day 2 as was found in the previously described studies above. The primary conclusion from this study was that MEMRI allows for the early detection of excitotoxic injury in the hyperacute phase that precedes vasogenic edema formation. Furthermore, in the subacute phase, MEMRI detected contrast was found to be consistent with tissue cavitation and the presence of reactive gliosis. MEMRI offers novel contrasts of biological processes that provide complementary information to conventional MRI in TBI.

Conclusions

Multimodal MRI offers the means to non-invasively image anatomical, physiological and functional changes associated with TBI longitudinally. These approaches have contributed substantially toward improved understanding of TBI and will continue to grow. Future studies will include repeated closed-skull TBI, chronic TBI, functional changes after rehabilitation, as well as other MR techniques (such as resting-state fMRI and spectroscopic imaging) to study TBI.

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