Early Microstructural and Metabolic Changes Following Controlled Cortical Impact Injury in rat: A Magnetic Resonance Imaging and Spectroscopy Study

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Abstract

Understanding tissue alterations at an early stage following traumatic brain injury (TBI) is critical for injury management and limiting severe consequences from secondary injury. We investigated the early microstructural and metabolic profiles using in vivo diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H MRS) at 2 and 4 hours following a controlled cortical impact injury in the rat brain using a 7.0 Tesla animal MRI system and compared to baseline. Significant decrease in mean diffusivity (MD) and increased fractional anisotropy (FA) was found near the impact site (hippocampus and bilateral thalamus; p<0.05) immediately following TBI suggesting cytotoxic edema. Although the DTI parameters largely normalized on the contralateral side by four hours, a large inter-individual variation was observed with a trend towards recovery of MD and FA in the ipsilateral hippocampus and a sustained elevation of FA in the ipsilateral thalamus (p<0.05). Significant reduction in ratios of N-acetylaspartate (NAA, p=0.0002), glutamate (p=0.0006), myo-inositol (Ins, p=0.04), phosphocholine and glycerophosphocholine (PCh+GPC, p=0.03), and taurine (Tau, p=0.009) were observed ipsilateral to the injury as early as 2 hours, while glutamine concentration increased marginally (p=0.07). These metabolic alterations remained sustained over four hours after TBI. Significant reductions of Ins (p=0.024), and Tau (p=0.013) and marginal reduction of NAA (p=0.06) were also observed at four hours on the contralateral side following TBI. Overall our findings suggest significant microstructural and metabolic alterations as early as two hours following injury. The tendency towards normalization at four hours from the DTI data and no further metabolic changes at four hours from MRS suggest an optimal temporal window of about 3 hours for interventions that might limit secondary damage to the brain. Results indicate that
early assessment of TBI patients using DTI and MRS may provide valuable information on the available treatment window to limit secondary brain damage.

**Keywords:** traumatic brain injury; MR spectroscopy; diffusion tensor imaging; controlled cortical impact; early changes
**Introduction**

Traumatic brain injury is a major cause of death and disability worldwide (Langlois et al., 2006; Maas et al., 2008). TBI occurs when an external mechanical force or pressure force (as in the case of blast injury) traumatically injures the brain. The primary injury is characterized by acute biochemical and cellular changes that contribute to continuing neuronal damage and lead to permanent or temporary impairment of physical, cognitive, emotional, and behavioral functions. The biochemical and cellular changes in neurons and glia after TBI are complex and dynamic. The pathophysiology typically begins with mechanical trauma to the brain (the primary injury), followed rapidly by increased vascular permeability, altered ionic balance, oxidative stress, excitotoxic damage, inflammation, and mitochondrial dysfunction leading to further cell death and injury (the secondary injury) (Yang et al., 1985; Ishige et al., 1988; Hovda et al., 1991; Kawamata et al., 1995; Xiong et al., 1997; Lenzlinger et al., 2001; Morganti-Kossmann et al., 2002; Roberson et al., 2006).

Diffusion tensor imaging (DTI) and $^1$H MRS have recently emerged as powerful approaches for characterizing the microstructural and metabolic responses after TBI. Studies have shown that diffusion-weighted imaging (DWI) and DTI, are highly sensitive to tissue microstructure change and axonal damage associated with TBI (Arfanakis et al., 2002; Huisman et al., 2003; Bazarian et al., 2007; Mac Donald et al., 2007a,b; Rutgers et al., 2007; Mayer et al., 2010). In addition to quantifying the average diffusion coefficient of water in the brain, DTI measurements can determine the preferential direction of water diffusion within axons. This information can be used to generate and visualize both normal and abnormal white matter fiber tracts. DTI parameters include mean diffusivity (MD), which measures the average water diffusion within the brain tissue, and fractional anisotropy (FA) which measures the degree of
diffusion anisotropy present within axons. The values of FA range from 0 to 1, with highly organized white matter tracts exhibiting higher anisotropy compared to the rest of the brain tissue because of the directionally restrictive structure of bundled axons. Alterations of MD reflect pathological changes in the brain tissue due to changes in the diffusion characteristics of the intra- and extra-cellular water compartments, including restricted diffusion and water exchange across permeable boundaries (Gass et al., 2002). Change in the FA is indicative of the structural integrity of the tissue. More specifically, the FA change is due to the disproportional change in the diffusion along the neuronal axons (axial diffusivity, $\lambda_a$) and the diffusion perpendicular to the axons (radial diffusivity, $\lambda_r$). Axial diffusivity is believed to be sensitive to axonal integrity and radial diffusivity reflects myelin integrity (Song et al., 2002, 2003). Previous TBI studies on both humans and animals have shown altered MD (Alsop et al., 1996; Liu et al., 1999; Huisman et al., 2003; Mamere et al., 2009), and FA in white matter regions (Arfanakis et al., 2002; Huisman et al., 2004, Wilde et al., 2006; Mac Donald et al., 2007a,b; Wozniak et al., 2007; Bazarian et al., 2007; Mayer et al., 2010) anywhere from 24 hours to several days after the injury.

In contrast to the structural information offered by DTI regarding brain integrity after TBI, high resolution in vivo proton magnetic resonance spectroscopy ($^1$H MRS) provides complementary information and assesses metabolic irregularities following the injury. Several of the metabolites detected by $^1$H MRS are highly sensitive to the pathology that contributes to TBI, including hypoxia or ischemia, bioenergetic dysfunction, and inflammation. A majority of the clinical TBI studies have consistently found decreases in the neuronal marker, N-acetylaspartate (NAA) in the brains of TBI patients (Ross et al., 1998, Macmillan et al., 2002, Govindaraju et al., 2004, Marino et al., 2007, Govind et al., 2010]. A decrease in NAA (or NAA to total creatine
ratio (NAA/tCr), NAA to choline ratio (NAA/Cho)) has been observed within the first 24 hours of injury (Ross et al., 1998, Holshouser et al., 1997, 2000), and can remain depressed as long as 8 days after TBI (Condon et al., 1998, Brooks et al., 2000, Ross and Bluml 2001, Holshouser et al., 2005). While reports of changes in other cerebral metabolites have been less consistent (Friedman et al., 1999, Brooks et al., 2000, Garnett et al., 2000, Ross et al., 2001, Shutter et al., 2004, Marino et al., 2007, Yoon et al., 2005, Yeo et al., 2006, Choe et al., 1995, Cecil et al., 1998, Wild et al., 1999), a few studies on human TBI have shown that the changes in NAA, lactate (Lac), and choline (Cho) are predictive of neurologic outcome 1-25 days pos-injury (Ross et al., 1998; Friedman, 1998, 1999; Brooks et al., 2000; Garnett et al., 2000; Signoretti et al., 2002). However, none of these clinical studies addressed the very early changes following TBI.

MRS studies on animal models of TBI have improved our understanding of the metabolic events underlying the injury process and have supported the interpretation of clinical MRS data. As shown in human TBI studies, the metabolic changes in TBI brain may occur over weeks to months following TBI, and these changes may persist for several years post-injury in humans (Ross et al., 1998; Friedman 1999; Brooks et al., 2000). Previous in vivo $^1$H MRS studies in various rat model injuries also indicated a time evolution of TBI (Schuhmann et al., 2003; Vagnozzi et al., 2007; Lescot et al., 2010). Schuhmann et al. (2003) showed that tCr, NAA, glutamate (Glu), and Cho concentrations significantly decreased during the first 24 hour, and then started to increase at 7 days in a controlled cortical impact (CCI) model. At the same time, Lac increased and reached its peak at 7 days after TBI. In a combined DWI and $^1$H MRS study on a lateral fluid percussion injury model, Lescot et al. (2010) reported low ADC values in the brain which correlated with decreased NAA/tCr and increased Lac level at 24 hours after TBI. However, understanding the microstructural and neurochemical changes at very early stages
following injury may help in elucidating molecular pathophysiology and determining the time window available for treatment. Further, previous studies have paid very little attention to changes in the brain tissue far removed from injury, for example in the contralateral hemisphere.

Experimental models of animal TBI are useful for understanding the cerebral microstructure and metabolic mechanisms of brain cell death and neurologic impairment that occur in the various forms and intensity of human TBI (Dixon et al., 1988; Lighthall et al., 1989; Gennarelli, 1994; Park et al., 1999; Finnie and Blumbergs, 2002; Morales et al, 2005). The CCI rat model of TBI uses a known impact interface and a measurable, controllable impact velocity and cortical compression and translates to a repeatable injury with little variability that can be studied over time (Dixon et al., 1988; Lighthall et al., 1989; Meaney et al., 1994, Hall et al., 2005). The features of this model include constant injury reproduction with cortical contusion and distal axonal injury that exhibit cognitive, memory, and motor deficits mimicking human TBI (Dixon et al., 1991; Chen et al., 1996; Tang et al., 1997). We report here for the first time the acute cerebral microstructural and metabolic changes following CCI starting at 2 hours using DTI and $^1$H MRS in vivo using a 7.0 Tesla scanner.

Materials and Methods

CCI TBI Model

Adult male Sprague-Dawley rats (n=8, 250-350 grams) were subjected to left parietal controlled cortical impact injury (Robertson et al., 2006). TBI was performed using the controlled cortical impact device (Pittsburgh Precision Instruments, Pittsburgh, PA) as previously described (Dixon et al., 1991). Briefly, after being initially anesthetized with 4% isoflurane, the rat was maintained at 2% isoflurane, and the left parietal bone was exposed via a midline incision after positioning it in a stereotactic frame. A high-speed dental drill (Henry
Schein, Melville, NY) was used to perform a left-sided craniotomy that was centered 3.5 mm posterior and 4 mm lateral to bregma. A 5 mm round impactor tip was accelerated to 5 m/sec with a vertical deformation depth of 1.0 mm and impact duration of 50 ms consistent with mild injury. The bone flap was immediately replaced with dental acrylic and the scalp incision was closed with silk. After the surgery, the animal was allowed to recover for about 1.5 hours after which it was transported to MRI for the 2 and 4 hour imaging session. The experimental protocol was approved by the Committee for the Welfare of Laboratory Animals of the University of Maryland.

In vivo DTI and $^1$H MRS

All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner (Bruker Biospin MRI GmbH, Germany). The scanner is equipped with a BGA12S gradient system capable of producing 400 mT/m pulse gradients in each of the three orthogonal axes and interfaced to a Bruker Paravision 5.0 console. A Bruker four-element $^1$H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. The rat was anesthetized in an animal chamber using a gas mixture of O$_2$ (1 L/min) and isoflurane (3%; IsoFlo, Abbot Laboratories, North Chicago, IL). Animal was then placed prone in a Bruker animal bed and the RF coil was positioned and fixed over the brain of the animal. The animal bed was moved to the center of the magnet and the isoflurane level was changed to 1.5% and maintained at this level for the remainder of the experiment. At all times during the experiment, the animals were under 1-2% isoflurane anesthesia and 1 L/min oxygen administration. A MR compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, USA) was used to monitor the animal respiration rate and body temperature. The animal body
temperature was maintained at 36-37°C using a warm water circulation. The total duration of the MR imaging and spectroscopic experiment was approximately 2 hours at each time point. The animal was under continuous anesthesia from the start of the 2 hour imaging session until the completion of the 4 hour imaging session.

A three-slice (axial, mid-sagittal, and coronal) scout using fast low angle shot magnetic resonance imaging (FLASH, Frahm et al. 1986; Haase et al. 1986) was obtained to localize the rat brain. A fast shimming procedure (Fastmap) was used to improve the \( B_0 \) homogeneity within a region of the object (Gruetter et al., 1993). Both proton density- and \( T_2 \)-weighted images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence (Hennig et al., 1986) with repetition time/effective echo time (TR/TE\(_{\text{eff1}}\)/TE\(_{\text{eff2}}\)) = 5500/18.9/56.8 ms, echo train length = 4, matrix size = 256 x 256, slice thickness = 1 mm, number of averages = 2, in both axial (field of view (FOV)) = 3.0 x 3.0 cm\(^2\), number of slices = 24 ) in the coronal plane, and the axial (FOV = 3.0 x 3.2 cm\(^2\), number of slices = 16 ) plane for anatomic reference.

Diffusion tensor images were acquired with single shot spin-echo echo-planar imaging (EPI) sequence in the coronal plane. Diffusion sensitive gradients were applied in 30 non-collinear directions at \( b = 1000 \text{ s/mm}^2 \). Five additional images at \( b = 0 \text{ s/mm}^2 \) were also acquired. The acquisition parameters were FOV of 3.0 x 3.0 cm\(^2\) at a matrix resolution of 128 x 128, TR/TE of 6000/50 ms, slice thickness of 1 mm for a total of 24 slices, and two averages and covered the same area as the coronal structural acquisitions. Each rat was scanned at 3 time points: before the injury, 2 hours, and 4 hours after TBI.

For \(^1\)H MRS, adjustments of all first- and second-order shims over the voxel of interest were accomplished with the Fastmap procedure. At a TE of 20 ms, the shimming procedure
routinely resulted in line-widths of 7-9 Hz of the single 1H metabolite resonance (0.023-0.03 ppm). This allowed for a good separation of the glutamate (2.35 ppm) and glutamine (2.45 ppm) peaks (see Fig. 3). The water signal was suppressed by variable power radiofrequency (RF) pulses with optimized relaxation delays (VAPOR, Tkác et al., 1999). Outer volume suppression combined with point-resolved spectroscopy (PRESS) sequence (Price and Arata, 1996) from a 3 x 3 x 3 mm³ voxel was used for signal acquisition, with TR/TE = 2500/20 ms, spectral bandwidth = 4 kHz, number of data points = 2048, number of averages = 300. The voxel covered the immediate pericontusional zone, all layers of the hippocampus, and the superior thalamic structures. MRS data were acquired immediately following the DTI acquisition at each time point in both the pericontusional and the corresponding contralateral voxels (Fig. 3).

Data Processing

Maps of MD and FA were generated offline, using FDT (FMRIB’s Diffusion Toolbox, Oxford, UK). Regions of interest (ROIs) were drawn manually on three contiguous slices using ImageJ v1.38x (Wayne Rasband, NIH, Bethesda, MD). Regional measures of MD, FA, $\lambda_a$ (axial diffusivity, $\lambda_a = \lambda_1$) and $\lambda_r$ (radial diffusivity, $\lambda_r = (\lambda_2 + \lambda_3)/2$) values were obtained from the corpus callosum (CC) and both the ipsilateral and contralateral side of the injury from the hippocampus (hip_ips, hip_con), thalamus (tha_ips, tha_con), cortex (cor_ips, cor_con), the olfactory (of_ips, of_con), fimbria of the hippocampus (fi_ips, fi_con) as illustrated in Figure 1.

$^1$H MRS data was fitted using the LC-Model package (Provencher, 2001), and only metabolites with standard deviations (SD) % ≤ 20 were included for further analysis. Comparisons of the DTI and MRS parameters were performed for each region of the two hemispheres (uninjured and injured) and at each time point using one way repeated analysis of
variance (ANOVA) followed by paired t-tests adjusted for multiple comparisons using Bonferroni correction. Statistical significance was defined as $P < 0.05$.

**Results**

**DTI and $^1H$ MRS at 2 hours after injury**

The $T_2$-weighted MR images demonstrated distinct, but heterogeneous lesion at the location of the injury in the left cortical region of the brain at 2 hours following CCI (Fig.1A, and 1B, yellow lines). Although the parameters for CCI injury induction were the same for all the animals, we observed some variability in the extent of bleed and edema formation between individual animals (Mclntosh et al., 1989; Smith et al., 1997; Immonen et al., 2009). Despite the presence of blood products at the site of the injury, the quality of the DT images was not compromised as exemplified by the FA and MD images shown in Figure 1 D&E respectively.

Figure 2 shows the average MD, FA, $\lambda_a$ and $\lambda_r$ values from the 11 ROIs shown in Figure 1. In the ipsilateral side, the most obvious alterations in the DTI parameters were in the cortical region where all the four DTI parameters were altered significantly. In this region, the MD ($p = 0.004$), $\lambda_a$ ($p = 0.018$), and $\lambda_r$ ($p = 0.002$) were significantly decreased while FA ($p = 0.002$) increased. The ipsilateral hippocampus also underwent significant changes where the MD ($p = 0.01$), $\lambda_a$ ($p = 0.02$) and $\lambda_r$ ($p = 0.009$) were significantly decreased. A significant increase in FA ($p = 0.003$) for the ipsilateral thalamus was observed that was mainly driven by a decrease in $\lambda_r$ ($p = 0.067$). The olfactory region, a remote area from the location of the injury in the cortex showed a marginal decrease in $\lambda_a$ ($p = 0.062$). It should be noted that the two white matter dominated regions which are close to the injury site, the corpus callosum and fimbria of the
hippocampus showed little alterations in the DTI parameters at 2 hours following injury. Brain regions contralateral to the injury also experienced significant changes in DTI parameters including a significant increase in FA in the thalamus (p=0.029), marginal decrease in $\lambda_a$ in the olfactory region (p=0.055), and significant decrease in $\lambda_r$ in the hippocampus (p=0.04).

Coronal anatomic images along with the spectroscopic voxel locations in the pericontusional and contralateral region along with the corresponding spectra from an animal are shown in Figure 3. The in vivo $^1$H spectra demonstrate excellent spectral resolution and sensitivity both at the pericontusional zone and the contralateral sides. At 2 hours after injury, the metabolites in the pericontusional zone, including Glu/tCr (p = 0.0006), PCh+GPC/tCr (p = 0.03), Ins/tCr (p=0.04), NAA/tCr (p=0.0002), and Tau/tCr (0.009) were significantly reduced compared to the baseline while Gln/tCr was marginally increased (p = 0.07; Fig. 4). Contralateral zone also exhibited several biochemical changes after TBI but the changes were milder compared to the pericontusional region (Fig. 5). There was a significant reduction in NAA/tCr (a 6.1 % reduction in contralateral zone vs. a 29.4 % reduction in the pericontusional zone).

**DTI and $^1$H MRS at 4 hours after injury**

At 4 hours after injury, the DTI parameters near the pericontusional cortical region maintained the same levels as at 2 hours after TBI (Fig. 2). While a marginal recovery of MD (p = 0.054) and $\lambda_r$ (p = 0.07) was seen in the ipsilateral hippocampus in comparison to the 2 hour time point, overall these parameters were still reduced compared to the baseline. A further increase in the FA (p =0.04) and a reduction in $\lambda_r$ (p = 0.02) was observed in the ipsilateral thalamus compared to the baseline. Axial diffusivity, $\lambda_a$ which was decreased at 2 hour point normalized to baseline levels in the olfactory region. The DTI parameters for corpus callosum
did not undergo any further change compared to the 2 hour point. However, the fimbria of the hippocampus showed significant decrease in both the MD ($p = 0.02$) and $\lambda_c$ ($p = 0.01$) compared to the baseline. On the contralateral side, the DTI parameters demonstrated a recovery to the baseline level by 4 hours.

Most of the metabolites including Glu/tCr ($p = 0.008$), PCh+GPC/tCr ($p = 0.009$), Ins/tCr ($p = 0.01$), NAA/tCr ($p = 0.0002$), and Tau/tCr ($p = 0.0039$) continued to be depressed at the 4 hours following injury compared to the baseline (Fig. 4). In addition, a significant reduction of GABA/tCr ($p = 0.02$) was observed by this time compared to the baseline (Fig. 4). The Gln/tCr ratio exhibited more variability compared to the levels observed at 2 hours (Fig. 4). Despite these few changes compared to baseline, no significant changes were noted in the ratios of GABA/tCr, Gln/tCr, Glu/tCr, Ins/tCr, NAA/tCr, Tau/tCr, and PCh+GPC/tCr, between 2 and 4 hours following TBI, which indicates that most significant metabolic alterations occur within 2 hours after the injury in the pericontusional zone. In the contralateral side, significant alterations of Ins/tCr ($p = 0.024$) and Tau/tCr ($p = 0.013$) were observed in the hippocampus. Although, the changes in these metabolites were not as sizeable as in the pericontusional zone, the reductions of Ins/tCr (a 14.8 % reduction in contralateral zone vs. a 16.6 % reduction in the pericontusional zone) and Tau/tCr (a 15.2 % reduction in contralateral zone vs. a 12.6 % reduction in the pericontusional zone) were statistically significant at 4 hours.

**Discussion**

Both experimental and human studies have shown the existence of a temporal window of metabolic vulnerability of TBI (Vagnozi et al., 2007; Tavazzi et al., 2007). However, these experiments were based on repeat concussions on animals which concluded that repeat injuries
within 3 days would lead to profound changes in mitochondrial-related mechanism in animal models which could be easily detected based on reduction in NAA. While these are very important studies to understand the effects of repeat TBI’s, we hypothesized that a knowledge of the very early changes in the metabolic profiles and microstructural changes following a single impact injury, will have a profound impact on effective management of the injury in the acute stage and lead to the development of neuroprotective agents to reverse or contain the damage from the injury. Because of the sensitivity of DTI and $^1$H MRS to microstructural and metabolic changes in vivo, we choose to examine the early changes in the parameters derived from these techniques using a reproducible model of TBI.

We observed decreased MD, $\lambda_a$, $\lambda_r$, and an increased FA as early as 2 hours in a variety of regions immediately following TBI consistent with cytotoxic edema and inflammatory response to the injury. These results agree with the findings from the human TBI studies performed during the acute stage both at the whole brain and regional level (Bazarian et al., 2007; Wilde et al., 2008; Chu et al., 2010; Buki and Povlishock, 2006; Mayer et al., 2010). As expected, ipsilateral cortex was most affected due to direct impact, followed by ipsilateral hippocampus, olfactory, thalamus and fimbria of the hippocampus. Consistent with the findings by Mac Donald et al. (2007ab), we found that the DTI parameters of the contralateral hippocampus was also altered but to a lesser degree compared to the ipsilateral hippocampus. We also report here for the first time, the involvement of the contralateral olfactory cortex and the thalamus suggesting that the injury may lead to disruption in olfaction and executive function (Halbauer et al., 2009; Sigurdardottir et al., 2010; Onyszchuk et al., 2009). In these regions, a decrease in axial diffusivity was accompanied by a stronger decrease in radial diffusivity that contributed to a decrease in MD and an increase in FA. Mayer et al., (2010) found increased FA in a
prospective study of mild TBI patients in the semi-acute stage and argued that the mechanical forces from TBI result in the stretching of axons and related structures, which alters the function of the gated ion channels resulting in changes in water homeostasis. Other factors such as high viscosity from cell debris and elevated lipid content within area of necrosis, decreased water content within myelin sheaths can also limit water diffusivity (Peled S., 2007). Taken together, our findings of increased FA and decreases in MD, $\lambda_a$, and $\lambda_r$ suggests reduced extracellular space and highly restricted water diffusion consistent with cytotoxic edema.

$^1$H MRS, which covered all layers of the hippocampus and the superior thalamic structures revealed reductions of Tau/tCr and Ins/tCr. Both Tau and Ins are one of the many organic osmolytes that are regulated in the brain and are believed to be located primarily in glia and absent in the neurons (Verbalis and Gullans, 1991; Lang et al., 1998; Dutton et al., 1991; Brand et al., 1993). Histopathological evidence of astrocyte damage in cortex and hippocampus has been reported in rats as early as 30 minutes following TBI (Zhao et al., 2003). Current understanding holds that the brain tissue looses a broad range of organic compounds following swelling, including amino acids (Verbalis and Gullans, 1991; Law, 1996; Kimelberg and Mongin, 1998) and amino acid derivatives, such as Tau and NAA (Sanchez-Olea et al., 1993, 1996; Olson and Kimelberg, 1995; Taylor et al., 1995; Sager et al., 1997; Phillips et al., 1998). Clinical studies have reported decreased levels of various metabolites, most notably Ins, in a number of patients on whom plasma osmolarity was lowered (Haussinger et al., 1994; Videen et al., 1995; Cooper and Wyatt, 2000). The reductions of Tau and Ins found in this study may reflect local hyponatremia leading to lowering of intracellular sodium and gain of water resulting in hypo-osmolality in the vicinity of the impact at the very early stage following injury (Arieff,
In such situations it has been shown that Ins and Tau are reduced in brain tissue (Bothwell et al., 2001; Silver et al., 2006). It should be noted that in addition to Tau and Ins, NAA, Glu and GABA are also osmotically active molecules (Bothwell et al., 2001). To date most clinical and experimental animal studies have largely shown an increase in Ins following injury. However the increase in Ins was observed in the sub-acute to chronic stages following TBI and these changes have been attributed to osmolality change due to increased astrocytic activity (Zhao et al., 2003; Schuhmann et al., 2003). Schuhmann et al., (2003) observed a decrease of up to 31% for both Ins and Tau during the first 24 hours that eventually rose above the baseline by about 31% and 44% respectively by seven days following CCI. Although the early decreases in Ins and Tau were not explained, the authors interpreted the late increase in Ins to be due to increased glial content/glial proliferation. Overall the changes in these metabolites most likely reflect the local tissue osmolality at the very early stages following TBI.

The considerably dynamic decrease in the NAA/tCr during the first 4 hours after TBI in this study agrees with the observations from other groups using similar approaches (Schuhmann et al., 2003). We found that the most severe drop of NAA occurred at 2-4 hours after the injury in the pericontusional zone. In addition, a much smaller but statistically significant decrease of NAA was observed in the contralateral side suggesting a global NAA disturbance. Although sharp decrease of NAA immediately after TBI has not yet been fully understood, it may due to impaired NAA synthesis in the mitochondria (De Stefano et al., 1995; Bates et al., 1996; Schuhmann et al., 2003; Signoretti et al., 2008; Lescot et al., 2010). The nervous system-specific metabolite NAA is synthesized from aspartate and acetyl-coenzyme A, relies on ATP, through the action of L-aspartate N-acetyltransferase in mitochondria, or through the cleaving of N-
acetyl-aspartyl-glutamate by N-acetylated-α-linked-amino dipeptidase, along with glutamate (Baslow, 2003). Therefore, the synthesis and catabolism of NAA is related to mitochondrial integrity. Our previous mitochondrial respiration study (Robertson et al., 2006) has showed mitochondrial dysfunction as early as 1 hour after CCI TBI in the pericontusional zone. Several investigations have furnished strong evidence to support the view that initial NAA reduction reflects dysfunctional neurons suffering energetic impairment after TBI (Signoretti et al., 2001; Schuhmann et al., 2003).

The decrease of Glu/tCr in close proximity to the injury at the early stage of the CCI agreed with a similar study by Schumann et al. (2003). In addition, we found a local reduction of GABA and an accumulation of Gln. At a TE of 20 ms and at a field strength of 7 Tesla, the resonance of Glu (2.35 ppm) and Gln (2.45 ppm) were well resolved yielding reliable quantification of the metabolites with the coefficient of variation for these metabolites as low as 4-12% for Glu and 8-16% for Gln as reported from LC-Model’s processing of spectra. The injury-induced alterations in concentrations of the excitatory neurotransmitter Glu and the inhibitory neurotransmitter GABA may indicate an imbalance in excitatory and inhibitory activity in the hippocampal region at the very early stage of TBI, and therefore may further contribute to the neurological dysfunction caused by TBI. At present, it is thought that the neurotransmission process is completed through the Glu-Gln cycle. The cycle begins with the release of Glu from presynaptic terminals to transport primarily to astrocytes, where it is converted to Gln via the Gln synthetase pathway. The Gln is released back to the neurons, where Glu is regenerated via phosphate-dependent glutaminase, a mitochondrial enzyme. GABA is synthesized by decarboxylation of Glu by glutamic acid decarboxylase. It is possible that the mitochondrial dysfunction caused by the very early stage of TBI may affect the glutaminase
activity leading to depletion of Glu, with a compensatory reduction of GABA and an accumulation of Gln. Further studies with electrophysiological correlation may provide more insight into the disruption of the Glu-Gln cycle.

The significant decrease of PCh+GPC/tCr at 4 hours after TBI also agrees with other reports (Schuhmann et al., 2003; Viant et al., 2005). As a metabolic marker of myelin and cellular membrane density and integrity, i.e. phospholipid synthesis and degradation, the decrease of PCh+GPC in the initial stage of the trauma is possibly a result of membrane degradation in the hippocampus area. Histopathological studies have shown evidence of astrocyte damage in hippocampus in rats as early as 30 minutes following TBI (Zhao et al., 2003).

The current study did not find a significant increase in the levels of lactate in the mild CCI TBI model. Although, most of the TBI rats did not show significantly elevated Lac levels, one out of the eight rats exhibited a dramatic increase of Lac coupled with more severe drop in NAA and Glu although the conventional MRI showed similar level of injury as other rats. While the discrepancy in this one rat is unclear, the presence of Lac has been related to multiple factors, including the increased energy demand to restoring the ionic balance (Kawamata et al., 1995), and the disordered mitochondrial dysfunction (Yang et al., 1985; Ishige et al., 1988; Unterberg et al., 1988) at the initial stage of the injury. Future studies with histological verification will need to be performed in order to understand the subtle changes that may be responsible for the ischemic conditions far from the injury as evidenced from increased lactate from the hippocampus and the thalamic region.

In the current study, the $^1$H MRS data was normalized to the resonance intensity of tCr, as this is present relatively equally in all brain cells and tends to be stable (Arnold and Matthews
1996). However, Schumann et al. (2003) observed a decrease in tCr concentration during the first 24 hour in a similar experimental condition as used in this study. While absolute quantification of tCr was not possible with our data, a reduction in the concentration of tCr would imply that the observed reductions in concentration of various metabolites (except for Gln) are an underestimate and hence the changes more significant, which does not alter the overall conclusion from this study. Although it is possible that reduction in concentrations of metabolites may be a result of increased creatine, to our knowledge there are no studies that have reported an increase in the concentration of Cr following TBI. Nevertheless, this underscores the importance of monitoring the absolute concentrations of the metabolites for an unbiased estimate. Taken together, these findings of this study indicate that the knowledge of alterations in cerebral metabolites and microstructural changes as early as two hours post-injury by MRS and DTI respectively have the potential to have an impact on the management of TBI patients.

Despite dramatic improvements in the management of TBI, to date there is no effective treatment available to patients, and morbidity and mortality remain high (Hall et al., 2010, Meyer et al., 2010, Stein et al., 2011). At present, there are no FDA-approved pharmacological therapies for acute treatment of TBI patients (Hall et al. 2010). The majority of posttraumatic neurodegeneration is due to secondary pathochemical and pathophysiological cascades that occur during the first few minutes, hours or days following the injury which exacerbate the damaging effects of the primary injury. The recently reported clinical trials on acute TBI patients used a 4 hours or longer window of treatment (Hall et al., 2010, Stein et al., 2011) which may not be optimum given that we see changes as early as two hours in the rat model. Although both DTI and MRS have been studied at the sub-acute and chronic stages among the TBI population, the combined use of these techniques during the early and sub-acute stages has been limited. Early
evaluation followed by sub-acute stage evaluation using these techniques may provide insights into the progression of the pathophysiology from the injury that may provide insights into the optimum time window for treatment and also provide the much needed predictive value in determining outcomes. Given that the combined use of MRS and DTI is sensitive in detecting damage in areas that conventional MRI techniques deem to be normal, they may be very helpful in the early evaluation of patients whose CT and conventional MRI are occult when the clinical status of the patient dictates otherwise. Of particular note is that the DTI and MRS data can complement each other as they assess different aspects of brain parenchyma and appear to be more sensitive compared to the conventional MR imaging techniques used to assess trauma and therefore can be very helpful in the assessment of novel therapeutic strategies (Tollard et al., 2009; Signoretti et al., 2008).

Conclusion

This study for the first time demonstrates that the combination of information from $^1$H MRS and DTI can detect changes in metabolic and microstructural changes in vivo using as early as two hours following CCI in rat brain. The microstructural and neurochemical changes were observed within 2 hours following injury in the cortex, hippocampus and the thalamus. In addition, changes in the microstructural environment and neurochemistry extended beyond the site of the injury to the contralateral hippocampus and the thalamus. The tendency towards normalization of tissue changes as indicated by DTI and no further metabolic changes at 4 hours as determined by MRS indicates the existence of a temporal window of about 2-3 hours for planning interventions that might limit secondary damage to the brain.
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Author Disclosure Statement

No competing financial interests exist.
References


104. Wozniak J.R., Krach L., Ward E., Mueller B.A., Muetzel R., Schneebelen S.,
Neuropsychol. 22, 555-568.

Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury.
J. Neurotrauma. 14, 23-34.

metabolite levels following mild experimental head injury in the cat. J Neurosurg. 63,
617-621.

brain injured patients in correlation with functional status by localized $^1$H-MR

140-152.
Figure legends:

Fig. 1. Representative MR images of a rat at 2 hour after TBI showing the extent of the injury (white contours) and the specific ROIs (black contours). A. T$_2$-weighted axial slice. B. T$_2$-weighted coronal slices showing the placement of the ROIs. C. coronal FA and MD maps. ROI 1 and 2 = hippocampus, 3 and 4 = thalamus, 5 and 6= cortex, 7 and 8 = olfactory, 9 and 10 = fimbria of hippocampus, and 11= corpus callosum.

Fig. 2. Regional MD, FA, $\lambda_a$, and $\lambda_r$ values at 2 hour and 4 hour after TBI for hippocampus ipsilateral (hip_ips) and contralateral (hip_con), thalamus ipsilateral (tha_ips) and contralateral (tha_con), cortex ipsilateral (cor_ips) and cortex contralateral (cor_con), olfactory ipsilateral (of_ips) and contralateral (of_con), fimbria of hippocampus ipsilateral (fi_ips) and fimbria of hippocampus contralateral (fi_con), and corpus callosum (cc). Data are expressed as mean ± standard deviation. “+” indicates p < 0.05. “*” indicates p < 0.01. The cross marks indicates a significant difference between 2 and 4 hours.

Fig. 3. Localized in vivo $^1$H spectra and corresponding voxel location depicted on the anatomic image of a TBI rat at 2 and 4 hours after injury on both the pericontusional and contralateral sides. $\gamma$-aminobutyric acid (GABA), creatine (Cr), glutamate (Glu), glutamine (Gln), glycerophosphorylcholine (GPC), lactate (Lac), myo-inositol (Ins), N-acetylaspartate (NAA), phosphocreatine (PCr), phosphorylcholine (PCh), and taurine (Tau). M1, M2, M3 and M4 are macromolecules.
Fig. 4. Comparison of the neuro-metabolic levels in the pericontusional zone before injury (baseline), at 2 hours, and at 4 hours after injury in CCI TBI rat brains. Data are expressed as mean ± standard deviation. “+” indicates p < 0.05. “*” indicates p < 0.01, “#” indicates p < 0.001.

Fig. 5. Comparison of the neuro-metabolic levels in the contralateral zone before injury (baseline), at 2 hours, and at 4 hours after injury in CCI TBI rat brain. Data are expressed as mean ± standard deviation. “+” indicates p < 0.05. “*” indicates p < 0.01, “#” indicates p < 0.001.