Non-Invasive Clinical Imaging of Cerebral Metabolism Following Traumatic Brain Injury Using ^{13}C Magnetic Resonance Spectroscopy

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Background

Despite the decline in fatal TBI incidence in recent years, TBI morbidity remains a public health challenge and is the leading cause of disability in the United States [1,2,4]. Residual impairments have been estimated to occur in 67% to 100% of TBI survivors, with cognitive impairments being the most disabling sequelae. [3,4] In addition, TBI has emerged as one of the most significant health risks to military personnel related to the wars in Iraq and Afghanistan [3] and an important medical concern due to potential long-term physical, cognitive and functional disabilities [5]. To combat these effects, new research is needed to identify mechanisms of injury that will lead to potential targets for therapeutic interventions that improve neurological outcome.

One promising area of research is the cerebral metabolic dysfunction following TBI. Studies of post-traumatic cerebral metabolism have shown that CMRglc decreases for a period of days, weeks or months after injury with the duration and degree of hypometabolism correlating to level of consciousness and a strong predictor of long-term neurological outcome [6,7,8]. However, specific changes in intermediary carbohydrate metabolic pathways have not yet been identified. In addition, the role of astrocyte metabolism in the post-injury metabolism has not been studied. This study uses in vivo ^{13}C MRS at 3T, a novel method in the clinical study of TBI, to non-invasively study the metabolic fate and flux of glucose (metabolized in both neurons and astrocytes) and acetate (metabolized in astrocytes) through metabolic pathways during the hypometabolic period. In addition, we will study the association of decreased cerebral perfusion with changes in glucose and acetate metabolism following TBI, as changes in cerebral perfusion could profoundly affect cerebral metabolism by limiting substrate availability.

Cellular Mechanisms Affecting Cerebral Metabolism

While, mitochondrial dysfunction has been proposed as a key factor contributing to the hypometabolic response following injury, the down regulation and/or inhibition of enzymes or cofactors associated with intermediary metabolism may also decrease glucose metabolism following TBI. For example, increased production of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and hydroxyl radical following TBI [9,10] could inactivate pyruvate dehydrogenase (PDH), due to its sensitivity to ROS [11,12]. Impaired PDH activity after TBI would limit the flux of substrates into the TCA cycle, decreasing oxidative metabolism and increasing the flux of substrate through anaplerotic pathways as a way of supplying needed TCA cycle intermediates to affected cells.

Astrocyte Metabolism and Intracellular Metabolic Compartmentation Following TBI

Still unclear is the role of metabolic coupling between astrocytes and neurons following TBI. It has long been recognized that astrocytes play a pivotal role in meeting the energy requirements of neurons through various metabolic pathways, including the glutamate-glutamine cycle which links the exchange of neurotransmitter glutamate (Glu) and glutamine (Gln) between glutamatergic neurons and astrocytes [13]. Moreover, the net synthesis of Glu in neurons requires a flux of TCA cycle intermediates, notably Gln, from astrocytes [14] as neurons lack the capacity to generate TCA cycle intermediates. The net synthesis of TCA cycle intermediates, Glu and Gln depends upon the entry of pyruvate via an anaplerotic pathway into the TCA cycle. In the brain this is exclusively achieved by pyruvate carboxylase (PC), an astrocyte specific enzyme [15,16]. Numerous in vitro studies have shown that astrocytes supply TCA cycle substrates to neurons during periods of glucose and/or oxygen deprivation [17,18,19], suggesting that astrocytes may play an even greater nutritional role for neurons in the injured state. Given the essential role of neuron-astrocyte metabolic coupling in normal brain, a greater appreciation of the effect of TBI on metabolic coupling is an important and necessary contribution to understanding of metabolic dysfunction following TBI. Moreover, the identification of novel metabolic therapies that target astrocytes may prove important in limiting neurological injury. In

this study, we will use [1, 2 $^{13}C_2$] acetate as an astrocyte specific metabolic substrate together with [1-^{13}C] glucose to study injury-induced changes in neuron-astrocyte metabolic coupling.

Decreased Cerebral Perfusion Following TBI

Decreased cerebral perfusion following TBI is associated with reductions in the utilization of glucose which could profoundly affect cerebral glucose metabolism by limiting substrate availability. In this study, we will use MR dynamic susceptibility contrast perfusion weighted imaging (DSC-PWI) to measure changes in cerebral perfusion in the hypometabolic period following TBI. DSC-PWI can provide a measure of tissue perfusion similar to results found using more traditional methods of CBF determination, with the advantage of relative higher spatial resolution, the absence of need for radioactive isotopes, and can be combined with other MRI studies in one evaluation.

Tracking the Metabolic Fate of Glucose: ^{13}C-MR spectroscopy

In order to determine how glucose is being metabolized, ^{13}C MRS offers an attractive, novel method to study alterations in multiple enzyme complexes following a TBI. The primary advantage of this technique arises from its ability to distinguish ^{13}C incorporation into multiple metabolites as well as into the specific carbon positions within the same metabolite, resulting in a detailed analysis of the metabolic fate of ^{13}C label [20,21,22]. The relative ^{13}C enrichment at each carbon position and the ratios between isotopomers of Glu and Gln gives additional information regarding enzyme usage and metabolic compartmentation [23,24,25,26]. The acquisition of ^{13}C spectra over time enables the dynamic measurement and calculation of in vivo rates of flux through metabolic pathways, an important technique to study in vivo cerebral metabolism. To our knowledge, the use of ^{13}C glucose together with ^{13}C acetate to measure metabolic flux simultaneously has not been performed in either the normal or injured human brain. In this study, we will use of [1-^{13}C] glucose together with [1, 2 $^{13}C_2$] acetate to measure changes in both neuronal and astrocyte metabolism in both TBI and control subjects.

Significance

Detailed knowledge of the metabolic alterations following TBI will provide a significant advancement to our understanding of the hypometabolic response to TBI, which is key information for the future development and testing of novel therapeutic interventions that by-pass or compensate for the metabolic dysfunction.

Study Objectives

The goals of this inter-departmental study are: 1) the clinical application of in vivo ^{13}C MRS at 3T to identify specific metabolic alterations following TBI and 2) determine if changes in cerebral perfusion effect metabolism by limiting metabolic substrate flow into the brain following TBI.

Hypothesis

We hypothesize that following TBI, metabolic pathways are altered causing an incomplete oxidative of glucose in neurons and astrocytes resulting in a decrease in cerebral metabolism.

Research Design and Methods

Imaging Protocol Overview

All control and TBI subjects will undergo an identical MRS/MRI protocol and neurological exam. MRS/MRI will be performed on a 3T Siemens TIM Trio system equipped with a second radiofrequency channel (Siemens Medical Solutions, Erlangen, Germany) using a specially designed and manufactured ^{1}H-^{13}C dual tuned volume head coil (Advanced Imaging Research, Cleveland,

OH). Sagittal 3D T2 weighted (T2WI) and 3D T1 weighted (T1WI) MR images, reformatted in axial and coronal planes, will be obtained for image co-registration and segmentation. The 2D DSC-PWI sequence will be used to determine cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), and the cerebral metabolic rate of oxygen ($CMRO_2$). Detailed sequence description and image processing methods are explained below.

TABLE 1. Imaging Parameters for 3.0T Siemens TIM Trio Scanner

Pulse Sequence	Plane	TR (ms)	TE (ms)	Slice Thickness (mm)	FOV (mm^2)	Time (min)	NA	Note
T2 SPC	3D sagittal	3200	458	1	290x232	4:30	1	matrix = 320x319x320, iPAT=2; ETL=139; BW=744Hz/px
T1 MP-RAGE	3D sagittal	1950	2.26	1	256x224	3:54	1	FA=90º, matrix = 256x256x246, BW=200Hz/px, iPAT=2
DSC-PWI	2D axial	1700	40	5 (20% gap)	220x220	1:30	1	Matrix=128x100, measures = 50, FA=90º, fat saturation, BW=1860Hz/px, Echo spacing =.72
ISIS-DEPT ^{13}C MRS	2D axial	2500			160x200	60:00	24	Waltz16 ^{1}H decoupling, FA=45º, outer volume suppression, 200μs rectangular excitation pulse, voxel size = 7x5x6xcm^3
TOTAL IMAGING TIME: 70 minutes plus shimming time								

In vivo ^{13}C MRS

[1-^{13}C] glucose and [1, 2-$^{13}C_2$] acetate infusion protocol

To reduce the complicating effects of endogenous glucose or the conversion of acetate to glucose in the liver, both control and TBI subjects will undergo a hyperinsulemic euglycemic glucose clamp via insulin infusion ($60mU \cdot m^{-2} \cdot min^{-1}$) in conjunction with a variable infusion of 20% dextrose solution. Following the insulin clamp, 30 ml of 100% isotopically enriched [1-^{13}C] glucose and [1, 2-$^{13}C_2$] acetate (Cambridge Isotope Laboratories, Andover, MA, USA) in a 20% w/v solution will be infused for 10 minutes followed by a constant infusion ($3mg \cdot kg^{-1} \cdot min^{-1}$) of 30% isotopically enriched [1-^{13}C] glucose and [1, 2-$^{13}C_2$] acetate. This infusion protocol results in normoglycemic plasma glucose levels of ~ 5.3 mmol/L[28], which will be maintained for the duration of the ^{13}C MRS experiment. Blood samples (0.5ml) will be drawn in duplicate at 5-minute intervals for the immediate measurement of plasma glucose level using an Accu-Chek glucose meter and for measuring plasma glucose and acetate isotopic enrichment by high-resolution ^{1}H and ^{13}C NMR spectroscopy.

ISIS-DEPT ^{13}C MRS sequence

Using the T2WI images, a 7 x 5 x 6 cm^3 volume covering portions of the fronto-parietal lobe will be positioned, avoiding areas of obvious contusion. Direct detection, localized in vivo ^{13}C MRS spectra will be acquired using a gradient-spoiled semi-adiabatic distortionless enhanced polarization transfer (DEPT) sequence (TR = 2.5 sec, interpulse delay = 3.8 ms, 45º flip angle, 128 acquisitions and WALTZ-16 decoupling applied during the acquisition) with three-dimensional image selected in vivo spectroscopy (ISIS) localization and outer volume saturation [27,28]. Spectra will be post-processed off-line using NUTS data processing software (Acorn NMR Inc., Livermore, CA). All relevant peaks will be identified and assigned by comparison to previously published values [21,22,23], integrated, and the relative amount of ^{13}C incorporation into Glu and Gln will be calculated. To calculate the various flux

parameters, a two-compartment model of flow from [1-^{13}C] glucose and [1, 2 $^{13}C_2$] acetate will be implemented in CWave software using modifications of previously published equations [27].

^{13}C MRS voxel tissue composition

In vivo MRS does not have the spatial resolution or the sensitivity to eliminate partial volume effects of different tissue types. Using an automatic MRS voxel segmentation program developed in our laboratory incorporating routines from MATLAB (Version 7.0.4, MathWorks, Natick, MA, USA) and SPM5 (The Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, London, England), the relative gray matter and white matter tissue components will be calculated and applied as correction to factors to the ^{13}C enrichment curves.

Plasma NMR spectroscopy

The peripheral metabolism of infused ^{13}C labeled substrates is a confounding factor when measuring metabolism in the brain. Acetate metabolism is known to occur in the liver, resulting in the synthesis of glucose by gluconeogenesis [30]. In the brain, ^{13}C labeled glucose produced in the liver would lead to isotopomer labeling patterns similar to the metabolism of [1, 2-$^{13}C_2$] acetate resulting in a potential source of error in our experiments. As a result, the fractional ^{13}C enrichment of all plasma glucose isotopomers will be calculated by ^{13}C and ^{1}H high resolution NMR using a Bruker 600 MHz spectrometer to correct brain isotopomer enrichment levels for the contribution of peripheral metabolism.

DSC-Perfusion Weighted imaging

A 2D single shot gradient echo echo-planar imaging (EPI) sequence will be used to obtain DSC_PWI using the following parameters: TR/TE = 1700/40 ms, FA = 90º, slice thickness = 5mm, matrix size = 128^2, and 50 measures. Gadolinium-DPTA (Gd-DTPA) contrast (0.1 mmol/kg) will be administered intravenously at the completion of the 5th scan. The resultant images are transferred to an offline program where CBV, CBF and MTT maps will be generated for each slice. To calculate $CMRO_2$, an arterial input function is chosen from the M1 section of the middle cerebral artery for each subject. A least squares exponential fitting is used to minimize secondary circulation; a singular value decomposition deconvolution is then used to obtain estimates of CBF and CBV.

To determine the effect of substrate limitation on glucose metabolism the position of the MRS voxel will be overlaid on CBF, CBV, and $CMRO_2$ maps using an automatic image overlay program developed in our laboratory incorporating routines from MATLAB (Version 7.0.4, MathWorks, Natick, MA, USA). The relative CBF (ml/100g/min), CBV (ml/100g), and $CMRO_2$ (ml/100g/min) within the region of the MRS voxel will then be compared to the final ^{13}C Glu and Gln enrichment pools to determine if substrate limitation correlates with metabolite enrichment.

Clinical Chart Review

Additional Clinical Information including: age, gender, demographic data, medical history, cause of brain injury, GCS score (initial, admission, and lowest post-resuscitation), Abbreviated Injury Score (AIS), pupillary reaction at admission, presence of associated injuries, laboratory (BUN, creatinine, alcohol and drug screening) and radiographic data, number of days after insult, length of patient's unconsciousness, length of post-traumatic amnesia (PTA), evidence of hypoxia, duration of ventilatory support, time to follow commands, medication regimen, and duration of stay in the ICU will be recorded from the patients' chart.

Neurological Assessment

Neurological assessment will consist of a standardized examination of neurologic function as listed in Table 2 by a neurologist or intensivist on the day of admission and the day of the MRS/MRI study.

Table 2: Neurologic examination

1. GCS score (initial, admission, post-resuscitation)	7. Motor tone and strength
2. Abbreviated Injury Score (AIS)	8. Deep tendon reflexes
3. Length of unconsciousness	9. Sensory function (touch, pain, temperature)
4. Duration of PTA	10. Cerebellar and basal ganglia function and gait
5. Time until able to follow commands	11. GOS score
6. Cranial nerve examination	12. Length of stay in ICU

Enrollment

Subjects with severe brain trauma will be recruited from admissions to the medical, surgical, or neurological services of LLUMC, which is a Level 1 Trauma Center verified by the American College of Surgeons.

TBI Patient enrollment

Only patients that are hemodynamically stable will be targeted for enrollment and the MRS/MRI studies will be ordered only after scheduled repairs for traumatic injuries have taken place. TBI participants will undergo the studies depending on clinical status as determined by the intensivist, Dr. Dorotta, attending neurosurgeon or neurologist. Sedation and mechanical ventilation will be continued and monitored for all patients during transport and during the studies by Critical Care nursing and respiratory therapy staff according to hospital policies. Once a potential candidate has been identified, Dr. Bartnik Olson will interview the candidate and/or family members, screen for inclusion/exclusion criteria, and enroll the candidate by obtaining the properly signed consent. The specific inclusion and exclusion criteria are:

TBI Patient Inclusion Criteria:

- Patients will be at least 18 years of age without gender or ethnic restrictions.
- Severe accidental TBI defined as the lowest post-resuscitation GCS $\leq$ 8 prior to administration of sedatives or paralytics.
- Eligibility for MRI per routine screening checklist.

TBI Patient Exclusion Criteria:

- History of neurosurgical intervention, excluding the placement of ventriculostomy shunt
- History of a prior known brain injury with associated loss of consciousness.
- History of a known neurological disorder prior to qualifying injury.
- History of psychiatric disorder.
- History of diabetes or current unstable serum glucose level.
- Renal insufficiency or known history of kidney disease.
- Known contraindication to MRI such as, pacemaker, pregnancy, and/or other non-MR compatible implanted device.

Ten (10) severe TBI patients will have the MRS/MRI study between 3 and 7 days post-injury to target the hypometabolic period.

Control Subject enrollment

Five (5) age/sex matched normal volunteers will be recruited as control subjects during the first year of the study. Control subjects will be recruited from Loma Linda University and/or Medical Center

staff, student or Resident populations. The specific inclusion and exclusion criteria for the control subjects are:

Control Subject Inclusion Criteria:

- At least 18 years of age without gender or ethnic restrictions.
- Eligibility for MRI per routine screening checklist.

Control Subject Exclusion Criteria:

- MRI Department staff or subordinate of project Investigator.
- History of neurosurgical intervention, excluding the placement of ventriculostomy shunt.
- History of a prior known brain injury with associated loss of consciousness.
- History of a known neurological disorder.
- History of psychiatric disorder.
- History of diabetes or current unstable serum glucose level.
- Renal insufficiency or known history of kidney disease.
- Previous allergic reaction to gadolinium MR contrast.
- Known contraindication to MRI such as, pacemaker, pregnancy, and/or other non-MR compatible implanted device.

Personnel

In addition to the principal investigator and co-investigators, research assistants or student may perform chart review and data base entry.

Statistical Analysis

The data analysis will emphasize descriptive and graphical statistics. Descriptive statistics used to detect statistically significant differences between control and TBI subjects will include means and associated 95% confidence intervals, one-way ANOVA with Scheffe post-hoc analysis, and Pearson correlation coefficients to identify possible correlations between metabolic changes and/or perfusion changes with neurological status.

Security

The study principal investigator will keep all information obtained from the study in a locked filling cabinet and password protected database. A study number will replace control and subject names and any PHI will be removed.

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