Short Term Mild Traumatic Brain Injury Mechanisms and Pathogenesis Studied in a Göttingen Minipig Model

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ABSTRACT
Traumatic brain injury (TBI) is a persistent problem with an estimated 1.7 million occurrences annually in the United States, accounting for 30.5% of all injury-related deaths. This study seeks to answer many of the unknowns surrounding mild TBI regarding the mechanisms and pathogenesis involved. The current study will ultimately produce graded injury metrics to relate translational and rotational head accelerations to levels of axonal damage and predict functional outcome in patients with TBI. The first phase of this study focuses on determining a relationship between short term neuronal damage as assessed using magnetic resonance spectroscopy (MRS) and immunohistochemistry (IHC) using a Göttingen minipig model. Two injury devices were designed and fabricated for this study: one imparts a repeatable rotational impact (combined rotation and translation), while the other imparts a repeatable purely translational impact. The minipigs undergo baseline MR scans (7T Bruker MR scanner) prior to injury, immediately post-injury, and twenty-four hours post injury, at which point the minipig brains are perfused and harvested for IHC. MRS is carried out on a voxel placed in the genu of the corpus callosum. Metabolites of interest include glutamate, N-acetylaspartate, choline, myoInositol, and lactate. To map the changes in metabolite levels seen with MRS to brain injury confirmed by IHC, two staining methods were used; light neurofilament, and heavy neurofilament for labeling axonal injury. The animals tested so far include: 1-hr survival rotation injury (n=3), 24-hr survival rotation injury (n=8), 24-hr linear injury (n=1), 1-hr survival sham control (n=1), and 24 hr survival sham controls (n=2). Preliminary results of IHC show that there is consistent neurofilament staining present in all rotationally injured animals dropped from ≥15°. Metabolite trends in these animals suggest increases in NAA and glutamate and a decrease in NAAG 24 hours after injury. Once the mapping of neuronal damage to metabolite concentration changes is completed, longitudinal development of injury can be characterized by metabolic changes observed with MRS at different time points, and related to input head kinematics. High-speed biplane x-ray studies looking at head kinematics, brain response, and brain injury will support the development of an FE model of the minipig brain. Once validated, the model can be used in conjunction with an FE model of the human head (e.g. SIMon) to scale the results and develop graded injury metrics for prediction of brain injury in the human.
INTRODUCTION

Traumatic brain injury is a leading cause of death and disability in the United States, accounting for 30.5% of all injury-related deaths (CDC, 2010), even though a wide range of research has been dedicated to the understanding, intervention, and prevention. Fluid percussion (Lindgren and Rinder, 1966), inertial rotation (Unterharnscheidt and Higgins, 1969), controlled cortical impact (Lighthall, 1988), and weight drop (Marmarou et al., 1994) are some of the classic experimental brain injury models. There are limitations associated with these models, with one example being the animal model used. Rodent models have been commonly used to study traumatic brain injury, but they have dissimilar brain morphology compared to humans, and much smaller brain than humans. Inertial rotation methods expose the animal to a non-impact based biphasic acceleration pulse (Gennarelli et al., 1982; Meaney et al., 1993). Clinically, the majority of reported TBIs result from a direct impact (CDC, 2010), and it is not known if resulting injury is due to either the starting or stopping phase, or both. Importantly, consideration of the temporal component (via pulse either duration or angular speed) is required when examining angular kinematics with respect to injury (Ommaya et al., 1971).

This current study seeks to gain a better understanding of the mechanisms surrounding traumatic brain injury by using a new injury model. This first phase of the project focuses on determining a direct relationship between measured input kinematics, short term neuronal damage as assessed using magnetic resonance spectroscopy (MRS), and immunohistochemistry in the Göttingen minipig model.

METHODS

Fifteen female Göttingen minipigs (Marshall Bioresources, North Rose, NY) (~5 months, 10 kg) were divided into five experimental groups: 1 hour survival rotational injury (n=3), 1 hour survival control (n=1), 24 hour rotational injury (n=8), 24 hour linear injury (n=1), and 24 hour control animals (n=2). The animal protocol was approved by the Wake Forest University Baptist Medical Center’s Animal Care and Use Committee.

Animals were initially sedated with an oral dose of midazolam (1 mg/kg) before being anesthetized with 3-5% isoflurane for induction with 1.5-2% maintenance. Intravenous fluids were administered through an ear catheter.

All animals underwent head surgical preparation. The scalp was shaved and scrubbed three times each alternating with alcohol and betadine. Bupivacaine (0.25%) was injected subcutaneously around the incision site serving as a local anesthetic while Buprenorphine (0.05 mg/kg) was given intramuscularly for analgesia. A cruciate incision was made so that six expanding custom bone screws could be inserted into the skull. These screws do not penetrate the inner table. Dental cement (Bosworth Trim®, Bosworth® Company, Illinois) was used to make a rigid connection between the bone screws and a steel slug as shown in Figure 1. This steel slug bolts into a steel cube that lies on the underside of an aluminum plate (part of both injury devices).

![Figure 1: Schematics show the rigid attachment of the minipig to the injury device and how the steel cube impacts brass tubing (Fievisohn et al., 2012).](image)

Rotational Injury:

The rotational injury device (Figure 2) is comprised of two aluminum platforms that are dropped together from different heights (angles) to produce different levels of injury. Rapid deceleration occurs when
there is impact with brass tubing. At impact, the two aluminum platforms articulate and lengthen the injury event. The minipig is attached to the aluminum platform with the head positioned away from the pivot point (as shown above). The steel cube, to which the minipig is attached through the platform, hosts the instrumentation and also impacts brass tubing causing repeatable rapid deceleration of the injury device and rigidly coupled minipig. Instrumentation includes two linear accelerometers, one angular accelerometer, and one angular rate sensor. High speed video was used for overall observation of the injury events.

**Figure 2: Rotational injury device (Fievisohn et al., 2012).**

**Linear Injury:**

The linear injury device achieves repeatable rapid deceleration by also impacting brass tubing, but motion is constrained to one plane by using linear rails. The use of linear rails allows no rotational motion thereby producing a purely linear impact (Figure 3).
Magnetic Resonance Spectroscopy

A 7T Bruker Biospin MR scanner in the Center for Biomolecular Imaging at the Wake Forest University Baptist Medical Center was used to quantify metabolites using MRS. Metabolites were quantified within a 216 mm$^3$ voxel placed in the genu of the corpus callosum. MRS scans were carried out at baseline, immediately post injury (~1 hour), and twenty-four hours post injury. Over 20 metabolites are quantified with those of particular interest being glutamate, glutamine, myo-inositol, choline, creatine, N-acetylaspartate (NAA), and N-acetylaspartylglutamate (NAAG).

Immunohistochemistry:

After post-injury scanning, the minipigs are euthanized using Beuthanasia-D (150 mg/kg Sodium Pentobarbital or greater, IV). The vasculature is flushed with heparinized saline and then perfused with 4% paraformaldehyde to fix the brain. The brains are extracted and then the genu of the corpus callosum is cut out and embedded in paraffin. Paraffin blocks are sectioned at ~ 4 µm. Immunofluorescent staining procedures were then performed to look at light and heavy neurofilament which mark axonal damage.

RESULTS

Table 1 shows the input kinematics measured for each rotational injury event. These include peak linear and rotational acceleration and angular speed for each drop height. Linear injury will be analyzed once more testing is completed.
Table 1. Peak linear acceleration, rotational acceleration, and rotational speed for rotational tests at different drop heights

<table>
<thead>
<tr>
<th>Drop Height</th>
<th>n</th>
<th>Avg. Peak Linear Acceleration (G’s)</th>
<th>Avg. Peak Rotational Speed (rad/s)</th>
<th>Avg. Peak Rotational Acceleration (rad/s²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°</td>
<td>4</td>
<td>43</td>
<td>8</td>
<td>1800</td>
</tr>
<tr>
<td>25°</td>
<td>4</td>
<td>63</td>
<td>10</td>
<td>2000</td>
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<td>1</td>
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<td>2500</td>
</tr>
<tr>
<td>40°</td>
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<td>103</td>
<td>11.5</td>
<td>3300</td>
</tr>
</tbody>
</table>

Metabolite trends in the rotational injured animals suggest increases in NAA and glutamate and a decrease in NAAG 24 hours after injury.

Preliminary immunohistochemistry results show that there is consistent neurofilament staining present in all rotationally injured animals dropped from ≥15° with both light and heavy neurofilament (Figure 4).
Figure 4: Sections of the genu immunostained for light (stained in green) and heavy neurofilament (stained in red). (A) a sham animal, (B) an animal injured from a 25° impact, and (C) is from an animal injured from a 40° drop. A magnified image is shown with a dotted line alongside an axon stained with both neurofilaments (hence the orange color).

CONCLUSIONS

Future steps include continuing to test the rotational injury and linear injury devices to obtain short term characterization of mild traumatic brain injury. Further immunofluorescent staining will be done to characterize axonal damage including iba-1 to identify microglia activation, glial fibrillary acid protein to identify astrocyte activation, cleaved caspase-3 to mark apoptosis, and Fluoro-Jade B for neurodegeneration. Metabolite concentrations will be compared to baseline values to identify possible biochemical mechanisms of injury. In addition, mapping of neuronal damage to metabolite concentrations will be done.

Once short term characterization is completed, longitudinal development of injury will be characterized by metabolic changes observed with MRS at different time points over the course of two weeks and then related to input head kinematics. High-speed biplane x-ray studies looking at head kinematics, brain response, intracranial pressure, and brain injury will support the development of an FE model of the minipig head. Once validated, the model can be used to scale graded injury metrics for prediction of brain injury in the human. Ultimately, a new head injury criterion will be developed for better prediction of the potential for traumatic brain injury.

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REFERENCES