Glial Fibrillary Acidic Protein (GFAP) Response in a Preclinical Rat Model of mTBI

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Glial Fibrillary Acidic Protein (GFAP) Response in a Preclinical Rat Model of mTBI

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ABSTRACT

Traumatic brain injury (TBI) impairs populations both physically and financially, explaining about 75% of all head injuries and costing over $60 billion in the United States (Ahmed et.al, 2015 and Maas et. al, 2010). Through histopathological techniques and imaging, we measured the GFAP response to mild traumatic brain injury (mTBI) in a preclinical rat model using a heterogenous experimental design to mimic the heterogeneity in the human population as closely as possible. Using an ANOVA statistical analysis, the variance between the average astrocytic area in each group will be assessed for significance with respective post-hoc analyses to follow. Due to the lab closings of COVID-19, the results and statistical analyses have not been conducted, although all the data has been collected and measured. The significance of this translational neurotrauma study lies in its aforementioned heterogenous research design to build upon the foundation for the bridge between the laboratory and the clinical settings.

Keywords: mTBI, heterogeneity, GFAP
INTRODUCTION

Traumatic brain injury (TBI) is characterized as a consequential blow to the head as a result of a range of physical forces and activities. Specifically, mild traumatic brain injury (mTBI) is denoted as one of the most common neurological conditions in the world and a severe public health problem in the US, accounting for approximately 75% of all head injuries in the United States (Ahmed et.al, 2015). The worldwide incidence of TBI is approximately 0.5% per year (Ratliff et. al, 2020). In addition to causing death and disability, the annual burden of TBI in the United States has been estimated to be more than $60 billion (Maas et. al, 2010). Multiple injuries, especially during the window of increased cerebral sensitivity, results in an increased probability of neurodegenerative diseases, stroke, and cognitive impairments (Ahmed et. al, 2015). Because of the dynamic nature of this injury, the ability to successfully translate animal studies to the clinical world is rewarding, but hard to do (Schultz et. al, 2017).

Since astrogliosis is one of the quantifiable signs of TBI, measuring this neuroprotective response to injury using an astroglial biomarker, namely GFAP, can help us better understand the interactions between glial cells, inflammatory cells, and injured axons (Agoston et. al 2017, Fitch et. al, 2008). By measuring the injury response through GFAP present in the preclinical rat model of mTBI, we grow one step closer in understanding the tissue response of mTBI in the heterogenous human population. In an attempt to accurately account for the heterogeneity present in individuals who experience mTBI, this study uses variables such as sex, genetic variety, and previous mTBI in the rat cohorts to assess glial fibrillary acid protein (GFAP) response post-mTBI.
LITERATURE REVIEW

Traumatic brain injury (TBI) is a heterogenous neurological disorder as a result of a blow to the head from a range of physical forces and activities (Wang et. al, 2018). Moderate to severe TBI is a major cause of morbidity and mortality, with around 50,000 mortalities reported annually in the United States (Heydari et. al, 2019). Another study estimates approximately 2.8-2.9 million TBI-related emergency-department visits, hospitalizations, and deaths among all ages in the US (Rutland-Brown et. al, 2003).

Out of all the TBI cases in both civilian and military populations, 80-90% are estimated to be mTBI (Cancelliere et. al, 2006-2012). It is important to note that both studies in animals and humans make it clear that mTBI is not simply a milder form of moderate-severe TBI but that it represents its own characteristic injury state (Laskowski et. al, 2015). The Glasgow Coma Scale (GCS) was originally developed to assess functional responses after TBI, such as eye, muscle, and verbal responses, but its applications fail to account for size, location and type of brain injury (Wang et. al, 2018). At the cellular level, the pathophysiology of mTBI consists of diffuse axonal injury, whereas deficits at the behavioral level result in working memory deficits (Laskowski et. al, 2015). Since patients report different symptoms, the ability to introduce consistency in this inconsistent pool of mTBI cases makes translational studies challenging but necessary (Schultz et. al, 2017). The field of TBI has some of the greatest unmet needs in medicine and public health (Maas et. al, 2010).

Heterogeneity is the result of the inherent variation in a system and its response to change. The clinical population that suffers from TBI is heterogeneous due to both the complexity of injury pathology and the different factors affecting its outcome, making no two TBI patients the same. mTBI is considered to be a “silent epidemic” because the cognitive,
motor, and somatosensory deficits that arise from it are not always explicitly apparent to observers (Laskowski et al, 2015). The extent of brain injury depends on the type of insult, age, sex, lifestyle, genetic risk factors, socioeconomic status, other co-injuries, and underlying health problems (Inampudi et. al, 2020). Thus, there are inherent and external characteristics that cause heterogeneity including the highly variable response to injury in the clinical population. This makes the heterogeneity seen in clinical populations directly contrast the extremely constant laboratory animal population in terms of animal species, age, and sex. This laboratory uniformity, or “standardization fallacy,” not only reduces reproducibility but further increases the gap between preclinical animal studies to clinical studies and trials on human patients (Martić-Kehl et. al, 2012). Addressing patient heterogeneity by also including clinically relevant outcome measures in the research design will increase the understanding of the complexity of mTBI and allow for a more efficient translation from preclinical to clinical studies (Martić-Kehl et. al, 2012).

Sex has been shown to affect the incidence, symptom progression, and pathogenesis that follow brain injury (Inampudi et. al, 2020). Males are reported to be more susceptible to brain injury compared to females. Additionally, the endogenous female sex hormone progesterone is believed to modulate the brain injury outcome in both sexes. Psychological issues are also found to be greater in males at the acute phase, but become problematic for females in the long term. This highlights the susceptibility of both sexes to brain injury with variation in symptom onset and outcome (Inampudi et. al, 2020). Therefore, it is important to incorporate both males and females in a pre-clinical model of TBI in order to account for this variation. Furthermore, it has been demonstrated that heterogenization of animal conditions, such as strain, results in more reproducibility than standardized conditions (Richter et. al, 2011). In one study, different animal
strains have been shown to respond differently to injury. Whereas Sprague Dawley rats displayed a milder histopathological injury response compared to Fischer rats following fluid percussion injury, Fischer rats performed better than Sprague Dawley rats on cognitive tests (Reid et al, 2010).

In a cohesive effort to account for the heterogeneity present in individuals who experience mTBI, we use patient relevant variables such as sex, genetic variability, and number of impacts to assess various outcome measures among the rat cohorts. Although there have been studies that try to translate preclinical animal findings to patients, the novelty behind this study is its usage of a completely heterogenous rat population and thus its related unique bioinformatics approach of data analysis, to account for all the variables. In order to analyze the progression and recovery of mTBI, histopathologic techniques to observe cellular changes with respect to the heterogeneous condition will be implemented (Blennow et al, 2012).

Other studies using rat models of TBI show that biomarkers in the serum are associated with time-dependent changes in metabolism, cell adhesion, neuronal and glial damage, axonal injury, and inflammation. Since astrogliosis is one of the quantifiable signs of TBI, measuring this neuroprotective response to injury using an astroglial biomarker, glial fibrillary acidic protein (GFAP), provides information regarding the interactions between glial cells, inflammatory cells, and injured axons that lead to the development of treatments for mTBI (Ahmed et al, 2015, Fitch et al, 2008).

The outcome measures in our study consist of an acute neurological response shown by the immediate righting response, acute cellular response measured by glial fibrillary acidic protein (GFAP), working memory shown through novel object recognition (NOR), motor dysfunction shown by measuring gait in the Noldus CatWalk XT system, and a blood biomarker
analysis measuring cellular response. A rat cohort of 144 young adults is separated based on sex, strain (Sprague Dawley and Fischer) and number of impacts (1 mTBI or 2 mTBI). Each animal is sacrificed at either 24 h, 72 h, or 1 week after their final injury. By introducing heterogeneity into our rodent population, we aim to parallel human variability while also permitting a systematic study of confounders. After each animal was sacrificed, histopathological methods are used to evaluate tissue response. There are currently no FDA-approved therapies to treat any forms of TBI, and most of the FDA approval seeking therapeutic clinical trials target severe to moderate TBI. However, the majority (>80%) of all TBI patients are mild. Therefore, it is particularly appealing to study blood-based biomarkers for TBI, because TBI biomarker tests have the potential to diagnose the presence of TBI of different severities and thus predict outcomes (Wang et. al, 2018).

Since the majority of mTBI subjects recover without treatment, this makes the therapeutic effects for this level of TBI difficult to detect and test. Although there have been many scientific strategies and advances to increase our understanding of the complex and heterogeneous pathophysiological processes associated with TBI, none have proven to be successful in TBI clinical trials. This is attributed to the lack of therapeutic intervention tracking central nervous system (CNS) biomarkers complicated by the heterogeneity of TBI and poor translatability of preclinical TBI models (Wang et. al, 2018). The pathophysiology of TBI is not merely an acute event. It consists of multiple, parallel, and interdependent cascades of biological reactions at the tissue, cellular, and subcellular levels. Due to the far-reaching anatomy of axonal fiber tracks, axons are especially vulnerable to neuro-physical trauma. Therefore, axonal injury commonly occurs in both focal as well as diffuse brain trauma and can be found in TBI of all severities (Wang et. al, 2018). Additionally, neuronal body, dendrites, and synapses are also
damaged through TBI. In addition to neurons, astroglial cells and oligodendrocytes are also at risk for injury. Therefore, a detailed and comprehensive understanding of these pathobiological processes at every cellular and subcellular level is necessary to bridge the gap between preclinical studies, post clinical testing, and ultimately potential therapy (Wang et. al, 2018).

METHODS

Measuring the injury response through GFAP in the pre-clinical rat model of mTBI brings us closer to understanding the tissue response of mTBI in the heterogenous human population. In an attempt to account for the heterogeneity present in individuals who experience mTBI, this study uses variables such as sex, genetic variety, and previous mTBI in the rat cohorts to assess glial fibrillary acid protein (GFAP) response post-mTBI. Utilizing block and stratified randomization used in clinical trials, a pre-clinical trial method was designed in order to reduce redundancy and costs associated with a clinical trial.

Animal identity

In order to account for the heterogeneity of the human population, the study used inherent and environmental heterogeneity. Inherent heterogeneity, as in sex and genetic variation was accounted for using two strains (Sprague Dawley and Fisher) of male and female rodents. Environmental heterogeneity, as in previous mTBI, was accounted for as the animals were divided among sham, 1 impact and 2 impact conditions, and sacrificed at 3 different time points: 24 hr, 72 hrs, and 1 week (N=144). Using a controlled cortical impact (CCI) protocol and
Pittsburgh Provisions Instrument with a 10 mm silicone tip, we aimed to reproduce a direct skull impact representative of mTBI, with a velocity of 5 m/s and head displacement of 5 mm. It was a closed skull injury as the head was placed on EVA foam.

**Slide Preparation and Data Analysis**

Coronal sections of the brains were cut and mounted on slides to prepare for the GFAP staining. Then, three slides were chosen to be imaged from the anterior, middle, and posterior sections of the brain. It is important to note that the researcher was blinded to the specific groupings of number of impacts and time sacrificed. Two images were taken per section with three sections per brain, totaling 6 images per brain. Of those two images taken per section, left and right sides of the section were imaged, instead of three images (such as left, right, and central) due to the ambiguity of a “central” side (Figure 1a). The slides were imaged with a Nikon camera and the Neurolucida microscopy system.

After the images were captured, a macro code in the Image J software was utilized to create a threshold for counting the total astrocytic area stained with GFAP (Figure 1b and 1c). Two similar but differing codes were used, but they both accurately measured the area representative of the astrocytes. The data from the GFAP findings is one outcome measure of the heterogeneity study as a whole, specifically the acute cellular response outcome measure. Other outcome measures include acute neurological response, measured by the immediate righting response, working memory, measured by novel object recognition (NOR), gait, modeled by the Noldus Catwalk XT system, and blood biomarkers, measured through the serum sample. An Analysis of Variance (ANOVA) statistical test will be performed to compare the variance of the
means of the different groups to see if there is any statistical significance between the sexes or injury groups.

Figure 1a. *Posterior Coronal Section with GFAP Stain of Male Fischer Rat*

Figure 1b. *Post-Image J Macro Whitening Total Astrocytic Area: Posterior Coronal Section with GFAP Stain of Male Fischer Rat*
DISCUSSION

Sex has been shown to affect the incidence, symptom progression, and pathogenesis that follow brain injury (Inampudi et al., 2020). Males are reported to be more susceptible to brain injury compared to females. In another rat model of TBI, two scientists found a neuroprotective element in female mice, specifically progesterone, gave them an advantage in regards to their behavioral response and histopathological results post-TBI (Rubin and Lipton, 2019). Further data analysis to confirm or challenge these findings is necessary due to an interruption in the image and data collection phase of this study because of COVID-19 regulations.

Furthermore, one study demonstrated that heterogenization of animal conditions, such as strain, results in greater reproducibility compared to standardized conditions (Richter et al., 2011). Different animal strains have been shown to respond differently to injury. Whereas Sprague Dawley rats displayed a milder histopathological injury response compared to Fischer
rats following fluid percussion injury, Fischer rats performed better than Sprague Dawley rats on cognitive tests (Reid et. al, 2010). Further data analysis to confirm these findings with respect to animal strain in our study is necessary due to an interruption in this study because of COVID-19 regulations.

**FUTURE DIRECTIONS**

Future research into this study is necessary to analyze the findings from the acute cellular response measured by GFAP. Initially, an ANOVA test will be used to compare the variances of the means between the groups. However, because the histopathological results are one branch of this whole study, a machine learning model of multivariate analysis can be used in the future to understand the possible interactions of the outcome measures and find out whether or not there exists any potential significance. Additionally, the behavioral analysis from the Noldus Catwalk XT system can be compared to the GFAP in a correlational manner. Since we used the total astrocytic area stained by GFAP as the metric for the injury response, running a macro code that analyzes the total number of positive cells could be a way to potentially strengthen the data from this study.
CONCLUSION

Since astrogliosis is a metric for TBI, measuring this neuroprotective response to mTBI using GFAP, an astroglial biomarker, can elucidate the interactions between glial cells, inflammatory cells, and injured axons (Agoston et. al 2017, Fitch et. al, 2008). Through the quantification and analysis of the injury response through GFAP in a pre-clinical rat model of mTBI designed to mimic the population in terms of DNA differences through different rat strains, sex differences, and injury history through differing number of impacts, we can better understand the tissue response of mTBI in the heterogenous human population. Specifically, this study uses variables such as sex, genetic variety, and previous mTBI in the rat cohorts to asses glial fibrillary acid protein (GFAP) response post-mTBI. Currently, no conclusions can be made about the results since this study is in the final stages of the data collection phase. However, once the data collection and analysis are complete, this section will be updated to reflect that.

Because of the homogeneity that characterizes controlled lab experiments, the potentially significant differences in a heterogeneous world, similar to the world we live in, provides a barrier to translational studies from a pre-clinical to clinical setting. However, it is important to note that the clinical impact of this study’s outcome measures can be potentially discounted due to the lack of other relevant factors in this model. Our study, using a heterogenous research design, aims to add to the foundation that along with other translational studies, ultimately aims to overcome this barrier.
REFERENCES


