AN ORGANOTYPIC MODEL OF TRAUMATIC BRAIN INJURY CAUSED BY ACCELERATION-INDUCED SHEAR STRAIN

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INTRODUCTION

Traumatic Brain Injury (TBI) in form of diffuse axonal injury is commonly inflicted by head acceleration, which subjects brain tissue to shear strain. This tissue level injury cascade can be simulated by *in vitro* TBI models based on organotypic brain cultures, which closely replicate the *in vivo* apparent cell heterogeneity and spatial cell architecture. However, these models typically subject cultures to stretch and fall short to employ defined shear strain as the primary mechanical insult.[1,2] This abstract presents an organotypic TBI model, which inflicts controlled, graded neuronal injury to organotypic hippocampal cultures by means of acceleration-induced shear strain.

METHODS

Hippocampi from 8-day old rat pups were harvested and sliced into 400µm thick coronal cross-sections using a tissue slicer. Hippocampal slices were plated onto Millicell culture inserts (Millipore, Bedford, MA) in a 6-well dish containing 1.1 ml of culture medium (50% MEM, 25% horse serum, 25% HBSS, 5 mg/ml glucose, and 1mM glutamine). Slices were maintained in a 37°C humidified incubator with a 5% CO enriched atmosphere for 12 days before the TBI experiment. A custom-designed linear acceleration device was utilized to produce controlled, inertia-induced shear strain in organotypic cultures (Figure 1). An electromechanical actuator impacted the acceleration module, which contained up to six Millicell culture inserts. The acceleration magnitude could be adjusted up to 12,000g. The acceleration history was measured with a piezo-resistive accelerometer (350B03, PCB Piezotronics, Depew, NY) attached to the acceleration module, and was stored with a digital oscilloscope (54603B, HP, Palo Alto, CA) at a sampling rate of 60 MHz. Following rapid linear acceleration, the acceleration module was decelerated with a constant breaking force along a 400 mm long chute with a viscoelastic foam end stop.

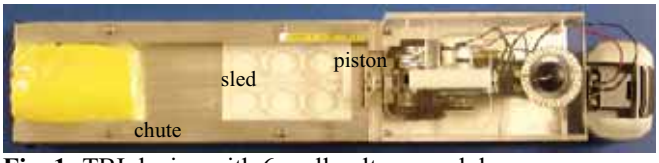

Fig. 1: TBI device with 6-well culture module.

Nine cultures each were subjected to an acceleration magnitude of 450g, 9,000g and 11,500g. Nine additional slices served as sham cultures, which were removed from the incubator, placed onto the TBI device, but were not accelerated. 21 cultures were used as control and remained in the incubator. Cellular response was measured in terms of cell death determined by propidium iodide (PI) which labels the nucleus of dead cells. Subsequently, cultures were treated with 1 mM NDMA, which caused 100% death of the neuronal cell population. This allowed expression of the initial PI labeling results as a percentage of maximum neuronal cell death. Statistical analysis was conducted using ANOVA with a Fisher's posthoc test using a confidence level of α=0.05.

RESULTS AND DISCUSSION

Viability of hippocampal cultures was not affected by placing slices onto the TBI device as shown by comparison between cell death rates in the control group (7.1±2.3%) and sham group (7.3±1.4%) (Fig. 2). Acceleration of 450g did not produce significantly elevated death of neuronal cells. Acceleration of 9,000g produced 33.6±21.9% cell death, which was significantly higher compared to sham cultures ($p<0.01$). Cell death was observed mainly in the CA1-CA3 regions and in the dentate gyrus, which are densely populated with neuronal cells (Fig. 3). 11,500g acceleration resulted in over 100% cell death, which suggests that cell death had been inflicted not only in the neuronal cell population, but extended to other cell phenotypes in the hippocampus.

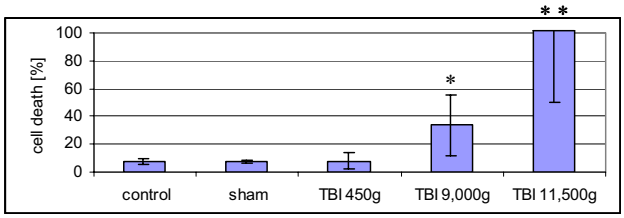

Fig. 2: Cell death response (mean +/- SD)

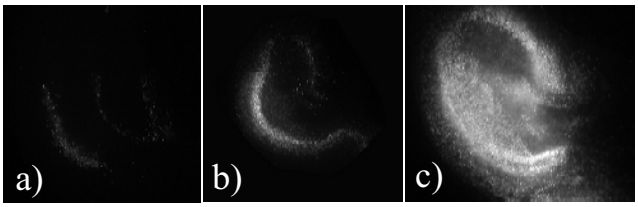

Fig. 3: PI results: a) sham; b) after 9,000 g; and c) 11,500 g.

Conclusion

The presented TBI model was able to induce acceleration-induced shear strain injury in organotypic brain slices in a graded, reproducible manner. Further characterization of this model will include measurement of the tissue–level shear strain during acceleration, a more accurate predictor of TBI.

REFERENCES

1. Morrison, B., et al., Annals Biomed Eng, 26, 1998
2. Cater, H.L., et al.. *Proceedings of Neuroscience*, San Diego,CA, 2004.

ACKNOWLEDGEMENTS

Supported by a grant of NIH/NINDS, R01NS 42946