Blasted Flies and Nanoparticles for TBI

K. S. Hockey[2], C.A .Sholar[2],V.S. Sajja[3], W.B. Hubbard[3], C. Thorpe[2], P.J. VandeVord[3], and B.A. Rzigalinski[1]

[1] Presenter, Virginia College of Osteopathic Medicine & Virginia Tech – Wake Forest Center for Injury Biomechanics; [2] Virginia College of Osteopathic Medicine; [3] Virginia Tech – Wake Forest Center for Injury Biomechanics

ABSTRACT

This presentation briefly summaries two major areas of work in our lab, development of a Drosophila model of blast injury and treatment of traumatic brain injury (TBI) with cerium oxide nanoparticles. First, we discuss the design, methodology, and results for the Drosophila blast model, and its relevance to human head injury. Briefly, we found that the Drosophila model was able to reproduce the decreased lifespan and early death seen in military personnel exposed to repetitive mild blast and NFL players exposed to repeated mild head injury. Next we discuss our *in vitro* and *in vivo* work with cerium oxide nanoparticles as neuroprotective and regenerative agents for treatment of TBI. Using a tissue culture model for TBI, we found that cerium oxide nanoparticles, delivered up to 6 hrs. post-injury, improved neuronal survival and maintained near-normal glutamate signaling in neurons of mixed organotypic brain cell cultures. *In vivo*, we found that delivery of cerium oxide nanoparticles prior to lateral fluid percussion brain injury in the rat, improved motor performance, learning and memory.

Traumatic brain injury, Drosophila, models, blast, cerium oxide nanoparticles, excitotoxicity, neuroprotection, cerium oxide nanoparticles, nanomedicine, nanotechnology

PRESENTATION SUMMARY

Drosophila Blast

Current models for blast utilize rodents or large mammals, which are both costly and awkward to implement. Further, large numbers of injuries required for statistical accuracy are often difficult to generate. The fruit fly, *Drosophila melanogaster*, is utilized for many models of human disease, including Alzheimer's disease, Parkinson's disease, and stroke. They are relatively low in cost to culture, easy to work with, and provide the ability to generate a large "n" in a timely manner. Therefore, we investigated development of a Drosophila model for blast.

To provide a flexible container for flies during blast, flies were placed in a nylon-mesh enclosure as shown in Figure 1. Male flies (20 per group) were exposed to mild blast overpressure in a blast simulator at the Center for Injury Biomechanics at Virginia Tech, as previously described (1). Controls were manipulated in the same manner, without exposure to blast. After blast, flies were returned to their standard culture conditions (5) and monitored for survival. Motor function was assessed by measurement of negative geotaxis, as previously described (1). Mild blast

resulted in death of 28% of flies exposed, while the remaining flies showed no visible or microscopic bodily injury, and were returned to their normal culture conditions. Motor function, as measured by negative geotaxis, was decreased immediately after mild blast, but returned to normal levels by 8-days, suggesting recovery (1). Despite the return of normal motor function and behavior, flies exposed to blast had a 15.4% reduction in maximum life span and a 17% reduction in median life span (Fig.2 below, reprinted from ref. 1). Although much further investigation and model characterization is needed, our results thus far suggest that the Drosophila model has the potential for development of a high-throughput screen model for mild blast or mild TBI.

Fig. 1. Drosophila Model of Mild Blast. Panel A shows the mesh enclosure in which flies are placed during blast delivery. Panel B shows the blast injury tube at the Center for Injury Biomechanics

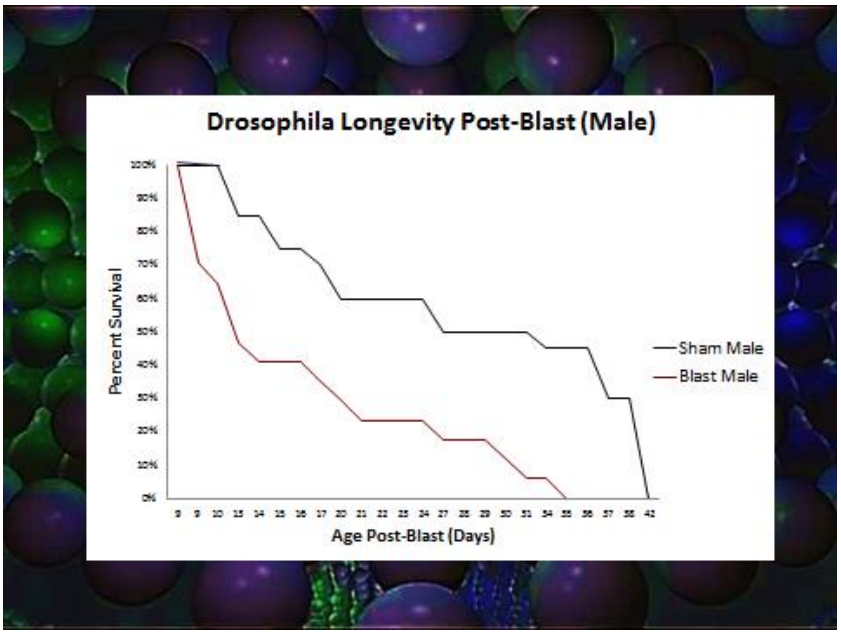

Figure 3: Lifespan is decreased in Drosophila exposed to mild blast. (Reprinted from Ref 1)

Cerium Oxide Nanoparticles in the Treatment of TBI

Our previous in vitro studies have shown that cerium oxide nanoparticles (CeONP) are regenerative free radical scavengers that protect neurons from free radical-mediated injury, extend cell lifespan, and maintain normal neuronal signaling (2-7). A single 10 nanomolar dose provided pronounced protection from oxidative stressors for the lifespan of organotypic brain cell cultures. In the fruit fly, CeONP extended lifespan by 26-30% and protected against death and motor dysfunction associated with exposure to the redox cycling agent paraquat and the mitochondrial complex I inhibitor, rotenone.

Using an established *in vitro* model for TBI that utilizes cell strain or stretch (8), we found that 10 nM CeONP, delivered up to 6 hr. post injury, protected neurons from TBI-induced cell damage. For these experiments, mixed organotypic cultures were injured and treated with either saline (control) or 10 nM CeONP at 1 and 6 hrs. post-injury. Twenty four hrs. post-injury, cells were stained with Propidium iodide and injured neurons were counted, as previously described (8). Final results are expressed as injured cells per mg of protein, shown in Fig. 3 below. Moderate (6.5 mm strain) and severe (7.5 mm strain) injury resulted in damage to the majority of neurons in the well. However treatment with CeONP at 1 and 6 hrs after injury (green and yellow bars) resulted in significantly decreased levels of injury.

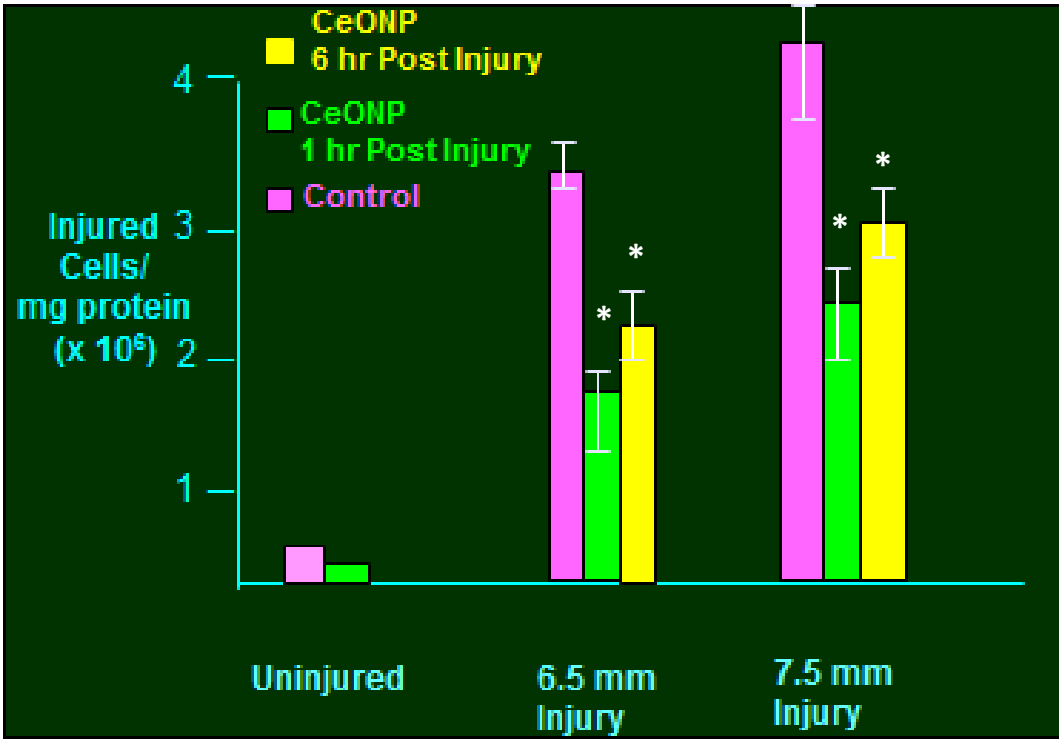

Fig. 3: Cerium oxide nanoparticle protect organotypic brain cells cultures from cell death after in vitro traumatic injury. *Significantly different from injury, p<0.01.

Our prior studies with *in vitro* TBI showed that glutamate signaling is extensively perturbed in strain-injured neurons (8, 9). To determine if such functional deficits could be ameliorated with CeONP treatment, we examined the glutamate-stimulated intracellular free calcium ($[Ca^{2+}]_i$) elevation in neurons, at 24 hrs after injury. Neurons of mixed organotypic brain cell cultures were injured and treated with 10 nM CeONP 6 hrs after injury. Twenty four hrs. after injury, neurons were loaded with Fura-2 and stimulated with glutamate as previously described (8).

As shown in Fig. 4 below, when stimulated with 100 μM glutamate, 90% of normal, uninjured neurons exhibited an increase in $[Ca^{2+}]_i$ of 100-265 nM (yellow bars). A small percentage of the cell population was either hypo- or hyper-responsive. After a moderate (6.5 mm) strain injury, the percentage of neurons responding to glutamate with the normal range of $[Ca^{2+}]_i$ elevation is dramatically decreased. The majority of injured neurons (pink bars) became either non-responsive (53%), or hyper-responsive (38%), as compared to controls. Treatment with CeONP 6 hrs after injury retained the population response to glutamate to a more normal levels, with 63% of neurons responding to glutamate in a manner similar to controls, and smaller percentages of hypo- and hyper-responders. Results are representative of 3 separate cell preparations with 200-300 neurons examined per condition.

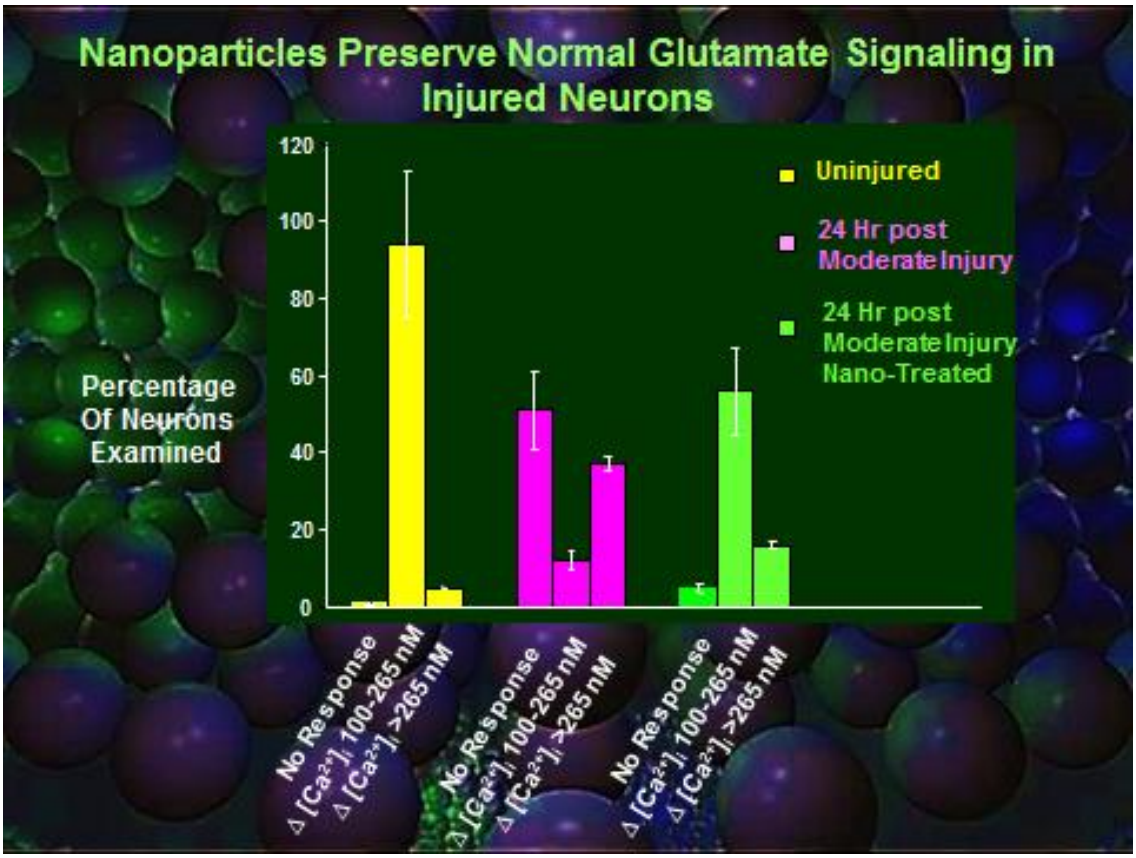

Fig. 4: Treatment with cerium oxide nanoparticles improves neuronal glutamate signaling when delivered after in vitro traumatic injury.

To determine the efficacy of CeONP *in vivo*, we pretreated rats with 0.5 μ/g CeONP via tail vein injection, followed by lateral fluid percussion brain injury. CeONP improved both motor function (balance beam) and learning and memory (Morris water maze) as shown in Figure 5 (6). These studies strongly suggest that cerium oxide nanoparticles may be useful in treatment and prevention of neurological deficits following traumatic brain injury and other forms of neurodegeneration.

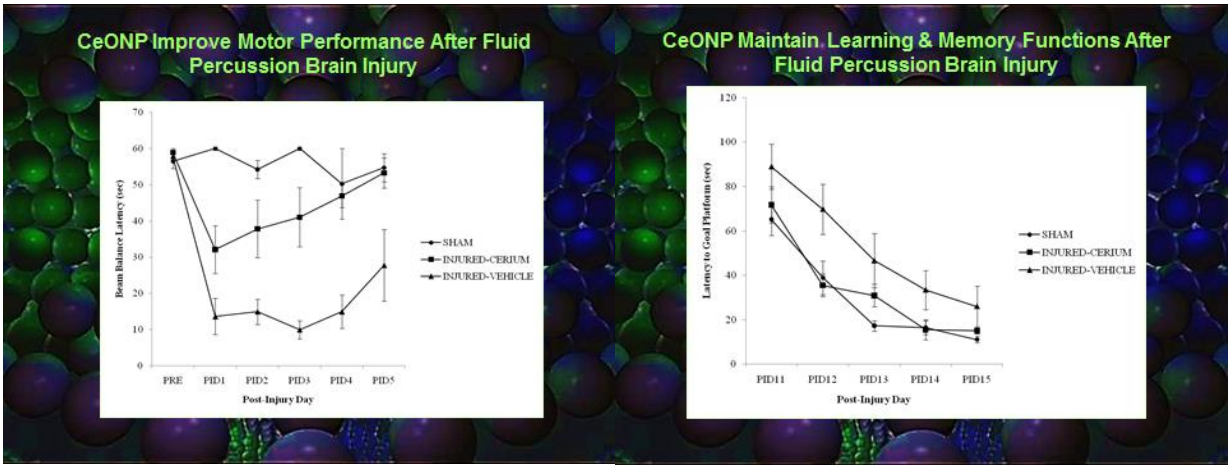

Figure 5: Cerium oxide nanoparticles improve motor performance (left panel) and learning and memory (right panel) after lateral fluid percussion brain injury

REFERENCES

1. Hockey KS, Hubbard WB, Sajja VS, Sholar CA, Thorpe C, VandeVord PJ, and Rzigalinski BA. A new model for mild blast injury utilizing Drosophila melanogaster. Biomed. Soc. Instrum. 49: 134-140, 2013

2. Rzigalinski BA, Meehan C, Davis RM, Miles WC and Cohen CA Radical Nanomedicine. Nanomedicine; 1: 399-412 (2006)

3. Bailey D, Chow L, Merchant S, Kuiry SC, Patil S, Seal S, and Rzigalinski B A Cerium oxide nanoparticles extend cell longevity and act as free radical scavengers. Nature Biotechnol. 14, 112, 2004.

4. Rzigalinski BA. Nanoparticles & cell longevity. Tech. Cancer Res. Treatment 4: 651-660, 2005.

5. Rzigalinski BA, Danelisen I., Strawn ET, Cohen CA, and Liang C. Biological Nanoparticles for Cell Engineering – A Radical Concept. In Nanotechnologies for Life Sciences, C. Kumar, editor, Wiley & Sons, in NY, 2006.

6 Whiting MD, Rzigalinski BA, Ross JS. Cerium oxide nanoparticles improve neuropathological and functional outcome following traumatic brain injury. J. Neurotrauma 26, 101, 2009.

7. Singh N, Cohen CA, and Rzigalinski BA. Treatment of neurodegenerative disorders with radical nanomedicine. Ann. NY Acad. Sci. 1122: 219-230, 2007

8. Weber JT, Rzigalinski BA, and Ellis EF. Traumatic injury of cortical neurons causes changes in intracellular calcium stores and capacitative calcium influx. J. Biol. Chem. 276: 1800-1807, 2001.
8. Zhang L, Rzigalinski BA, Ellis EF, and Satin LS. Reduction of voltage-dependent Mg2+ blockade of NMDA current in mechanically injured neurons. Science 274: 1921-1923, 1996.