Neuroprotective Effects of Vinaxanthone in a Rapid Stretch Injury Model of Traumatic Brain Injury

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Disclosure and Conflict of Interest:

There are no conflicts of interest or disclosures to report per AANS guidelines.
Introduction

• 1.7 million people suffer traumatic brain injury (TBI) annually, costing society an estimated $70 billion/year

• Vinaxanthone, derived from the fungus *Penicillium*, previously has been shown to improve neuronal regrowth in rodent cell lines

• We test an *in vitro* rapid stretch injury model of human stem cells to see if Vinaxanthone may be neuroprotective

On left: H19 cells under light microscopy.

On right: Cell stretch injury controller used in experiments.
Methods

- H19 cells were plated at a density of 70,000 cells per well and incubated for 48 hours with [8.675 µM] vinaxanthone, replacing the drug and media daily.
- A 50 msec, 50 psi stretch injury was administered to the cells after 48 hours.
- Lactate dehydrogenase (LDH) absorbance was measured at 4 hr and 24 hr post-stretch injury as a marker to detect cell injury.
- Statistical analyses were conducted in R Studio utilizing a two-way ANOVA with post Tukey multiple comparisons of the means to determine differences.

Figure 1: Diagram illustrating the mechanism by which the 50 msec, 50 psi stretch injury is delivered onto the cells. The airtight plug is fitted into the well and delivers the burst of nitrogen gas. Figure adapted from Cohen et al.
Results: Figure 2

Figure 2: Propidium Iodide and Hoechst Staining. Propidium iodide penetrates damaged cell membranes and intercalates between base pairs in DNA. Hoechst constitutively stains DNA in living and dead/injured cells. In the overlay image, cells that were stretched had extensive propidium iodide staining with significant differences in LDH absorbance (graph not shown). Control wells had no visible propidium iodide staining and were omitted.
Results (cont.): Figure 3A

4 Hour LDH Assay - [8.675 μM] Vinaxanthone

<table>
<thead>
<tr>
<th>Condition</th>
<th>LDH Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>CONTROL + VINA.</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>STRETCH</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>STRETCH + VINA.</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

*** indicates statistical significance.
Results (cont.):

Figure 3B

Figure 3: LDH released after rapid stretch injury with vinaxanthone treatment. The data presented and statistical analysis represent the same set of experiments. As expected, the LDH release is elevated in stretched wells compared to control (p < 0.001) at both 4 hr and 24 hr after injury. Stretched + drug cells released less LDH compared to stretch alone wells (p < 0.001) at both 4 hr and 24 hr after injury. Statistical analyses were conducted in R Studio utilizing a two-way ANOVA with post Tukey multiple comparisons of the means to determine differences.
Discussion

• 48 hour pre-treatment with 8.675 µM vinaxanthone resulted in a significant decrease in LDH released after stretch injury.

• Experiments were replicated a total of 3 times. Statistical analyses confirmed significant effect of vinaxanthone in our assay of neuroprotection.

• Future studies will examine if vinaxanthone shows similar neuroprotective activity in our in vivo TBI model. Furthermore, in vivo studies can explore correlation between lactate dehydrogenase release and clinical outcome.

• Additional exploration into an in vivo model will elucidate the untapped potential of vinaxanthone in the treatment of TBI.
Summary Points

• TBI is a devastating disease currently without an adequate pharmacologic treatment to ameliorate chronic sequelae. Current literature suggests vinaxanthone may present a solution.

• Our study utilizes a stretch injury model that simulates the stress and shear forces experienced by neurons during trauma.

• Vinaxanthone pre-treatment significantly reduced LDH release from cells at both 4 hr and 24 hr compared to controls indicating protective effects.