Implications of SARM1 Inhibitor Failed to Protect HD Dorsal Root Ganglion (DRG) Axon Induced Damage from Laser

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BACKGROUND

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease that impairs the patient's cognitive, psychiatric, and motor functions. There are medications that can lessen the severity of the psychiatric and motor disorders HD causes. However, there are no treatments or cures for this diseases as the specific damage mechanisms behind them are still unsolved. For this reason, we studied the axonal damage strength of HD. Using a protective drug for axonal damage, DRSM-3716, a Sterile Alpha and TIR motif containing 1 (SARM1) inhibitor, we examined the effects of laser induced damage on the HD cells.

METHOD

SARM1 inhibitor treatment: Dorsal Root Ganglion (DRG) sensory neurons were cultured from a HD mouse model. We treated Wild Type (WT) and HD DRG neurons with the SARM1 inhibitor for 10 minutes. The SARM1 inhibitor was diluted at 1:1000 for a final concentration of 30 μm in cell medium before replacing the medium on the cells. Cells were then incubated with the inhibitor during laser sub-axotomy experiments. A femtosecond laser was directed into a microscope at the focal point onto the target axon with 170 mW of power with a 40x objective. Images of the axons were taken at 0, 10, and 20 minutes after the laser damage. To quantify the damage, we measured the tip to tip distance of the axons before and after the experiment, to compute the reduction of axon length.

RESULTS

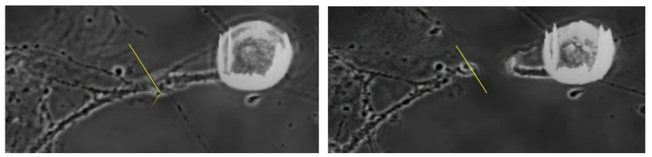

Figure 1: Segmentation laser damage: before and after laser damage for HD with the SARM1 inhibitor

The above figures shows segmentation damage, with only one tip of the axon shrinking after the laser.

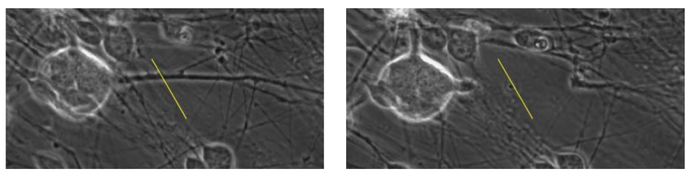

Figure 2: Shrinkage laser damage: before and after laser cutting for HD with the SARM1 inhibitor

Figure 2 shows shrinkage damage, where both sides of the segmented axon shrink after laser cutting.

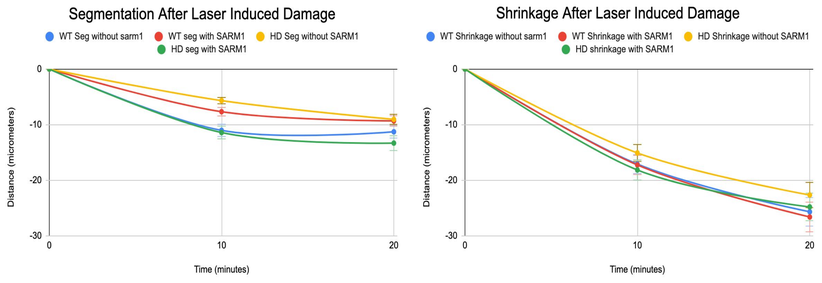

Figure 3: Comparison between Segmentation and Shrinkage with and without treatment for SARM1 inhibitor

In the WT and HD DRG axons with segmentation damage, the SARM1 inhibitor treatment decreased the average shrinkage distance for the WT DRG cells, while increasing the average distance from the laser damage for the HD DRG cells. On the other hand, in the WT and HD DRG axons that have shrinkage damage, the SARM1 inhibitor increased the overall shrinkage distance at both 10 and 20 minutes.

RESULTS

For the HD and WT DRG cells with segmentation damage, the SARM1 inhibitor treatment decreased the average shrinkage distance for the WT DRG cells. However, the treatment of the SARM1 inhibitor on the HD DRG cells actually increased the average shrinkage distance distance. This suggests that HD cells possibly more sticky and stuck to the microtubule as a type of protection compared to WT cells. In addition, when HD and WT DRG cells that experienced shrinkage damage treatment with the SARM1 inhibitor did not result in a stronger axon. These results suggest that the SARM1 inhibitor, DRSM-3716, might not be an optimal first-line treatment for Huntington's Disease.

CONCLUSIONS

1) Using a femtosecond laser on DRG sensory nerves was effective for studying the response of their axonal strength.
2) The SARM1 inhibitor, DRSM-3716, did not show improvement to the physical health and shrinkage distance of both HD and WT DRG cells axons.
3) HD DRG axons are more resistant to segmentation than WT DRG axons when reacting with segmentation damage.

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