The Damage Mechanism of Axonopathy in CMT2B and HD Using Laser Ablation as a Tool for Damage

Joy Young[1], Abigail Wei[1], Murong Su[1], Ian Chen[1], Sophia Barber[2], Yingli Gu[2], Linda Shi[1], Veronica Gomez[1], Chengbiao Wu[2]

[1] Institute of Engineering in Medicine, University of California San Diego, La Jolla, CA 92093 , [2]Department of Neurosciences, University of California San Diego, La Jolla, CA 92093

BACKGROUND

We studied the damage mechanisms of two different neurodegenerative diseases: Charcot Marie Tooth Disease type 2B (CMT2B) and Huntington's Disease (HD). There exists no current cure for either of these diseases, and the specific damage mechanisms behind them are still unclear. CMT2B is an autosomal dominant disease that causes loss of muscle mass and sensations primarily in the lower extremities. We examined the axonal damage mechanisms in CMT2B diseased neurons. We tested a mitochondrial fission inhibitor Mdiv-1 as a possible protective drug for CMT2B diseased cells. Huntington's Disease is an autosomal dominant disease that impairs cognitive, psychiatric, and motor abilities. We investigated the effects of an oxidative stress on HD cells using hydrogen peroxide (H_2O_2) as a stressor.

METHOD

Mdiv-1 strength treatment: We cultured Dorsal Root Ganglion (DRG) sensory neurons from CMT2B mouse model. The 3 genotypes we used were: wild type (wt), heterozygote (fln/+), homozygote (fln/fln). We treated the neurons with Mdiv-1 (100 nM) for 60 minutes. We then applied laser damage to those axons and captured the movement for 60 minutes. A femtosecond laser (120 mW of power) was directed into a microscope onto a target axon. We used the axonal damage levels from the Seddon classification system.

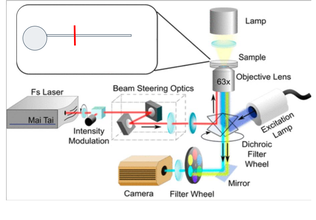

Figure 1: Laser & imaging system used to capture data

H_2O_2 stress treatment: We treated the DRG neurons from HD mouse model with H_2O_2 (100um) for 60 minutes. We then applied laser damage to those axons and captured the movement for 30 minutes. A femtosecond laser (163 mW of power) was directed into a microscope with a Zeiss 40x objective. In our experiment, we measured degeneration through change in axonal length.

RESULTS

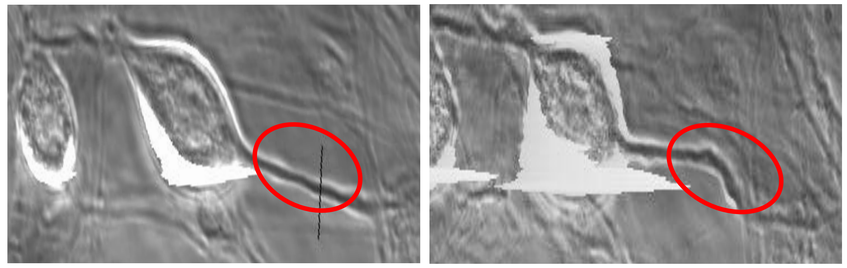

Figure 2: Axonotmesis (level 1) damage: before & after laser cutting for CMT2B
Level 0 damage (neurapraxia) represents when there is no damage to the axon. Level 1 damage (axonotmesis) is when the axon either curves or becomes thinner. Level 2 damage (neurotmesis) is when the axon completely severs and the cell may die.

Figure 3: Comparison between Fln/+ & Fln/fin with and without Mdiv-1 treatment for CMT2B
Following treatment with Mdiv-1, damage could be observed at rates of 34% (fln/+) and 68% (fln/fln). This was an overall decrease in damage observed compared to the original rates of 54% (fln/+) and 73% (fln/fln). Rates of level 0 damage in fln/+ increased from 46% to 66%, and from 27% to 32% in fln/fln. The rate of level 1 damage stayed relatively consistent -- there was a 2% increase in fln/+ and a 1% decrease in fln/fln. Level 2 damage decreased in fln/+ from 26% to 3%, and from 32% to 26% in fln/fln.

CONCLUSIONS

1) Laser ablation was an effective tool for studying how DRG sensory neurons responded to axonal injury.
2) The Mdiv-1 drug was shown effective as a protective agent against damage for axons in fln/+ (heterozygous) CMT2B cells.
3) H_2O_2 was shown effective as an oxidizing stressor on DRG sensory neurons in HD cells.
4) Axons of HD DRG neurons, not wild type neurons, were more susceptible to damages induced by H_2O_2.

RESULTS

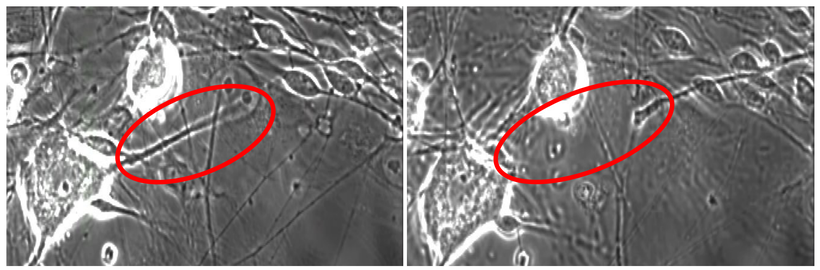

Figure 4: Shrinkage damage: before & after laser cutting for HD
In this figure, an axon experiences shrinkage towards the cell body following laser damage. The degeneration is measured through the distance from the cut point to where the axon has shrunk at each time position.

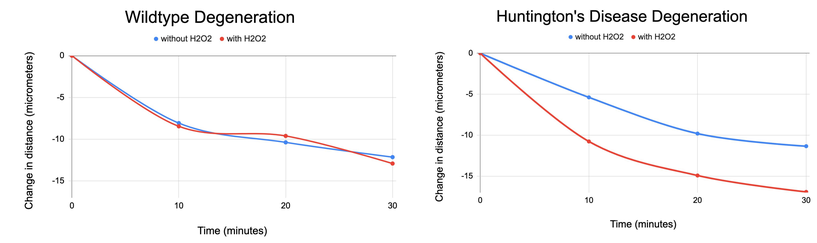

Figure 5: Comparison between wild type and HD with and without H_2O_2 treatment for HD
For the wild type cells, the results from the treatments with and without H_2O_2 show similar trends on the graph. For HD cells, the laser and H_2O_2 induced cell damage is much worse than the damage without H_2O_2. At 10 minutes, the damage with H_2O_2 is 100% more severe than the damage without H_2O_2. At 20 minutes, the damage with H_2O_2 is 52% more severe than the damage without H_2O_2. At 30 minutes, the damage with H_2O_2 is 49% more severe compared to that of without H_2O_2.

ACKNOWLEDGMENTS

This material is based upon work supported by a gift from the Beckman Foundation to M.W. Berns. The high school students (J.Y, A.W, M.S.) participated in the Outreach Program for Advancing Learning in STEM (OPALS) in Institute of Engineering in Medicine at UCSD

Zhao X, Chen XQ, Han E, Hu Y, Paik P, Ding Z, Overman J, Lau AL, Shahmoradian SH, Chiu W, Thompson L, Wu C and Mobley WC. TRiC subunits enhance BDNF axonal transport and rescue striatal atrophy in Huntington's disease. Proc Natl Acad Sci U S A. 2016 Sep 6. pii: 201603020. PubMed PMID: 27601642

Yingli Gu, Flora Guerra, Mingzheng Hu, Alexander Pope, Kijung Sung, Wanlin Yang, Simone Jetha, Thomas A. Shoff, Tessanya Gunatilake, Owen Dahlkamp, Linda Zhixia Shi, Fiore Manganelli, Maria Nolano, Yue Zhou, Jianqing Ding, Cecilia Bucci, Chengbiao Wu. Mitochondria dysfunction in Charcot Marie Tooth 2B Peripheral Sensory Neuropathy. Nature Communications Biology. 2022. in press