Schwann Cell-Derived Desert Hedgehog Provides a Neuroprotective Effect Against the Mechanical Stimuli from Compressive Neuropathies

James S. Jung, Derek Frump, B.S., Jared Su, Minal Tapadia, MD, Tahseen Mozaffar, MD, Ranjan Gupta, MD. University of California, Irvine, Irvine, CA, USA.

Disclosures:

Introduction: Chronic nerve compression (CNC) injuries such as carpal tunnel syndrome, cubital tunnel syndrome, and spinal root stenosis cause patients significant morbidity with the ensuing loss of motor and sensory function despite optimal medical management. Carpal tunnel syndrome is managed either by surgical intervention or more conservative treatments, such as anti-inflammatory medications or injections. To improve the motor and sensory recovery in these patients, defining the molecular mechanisms responsible for the changes induced by CNC injuries is crucial so as to develop novel adjunct treatment options. Desert hedgehog (dhh) is a protein produced by Schwann cells that is critical for the formation of the nerve’s perineurium. Previous data has implicated Schwann cells as vital in the pathogenesis of CNC injuries. Therefore, we hypothesized that Schwann cell derived desert hedgehog is crucial in providing a neuroprotective effect and may possibly serve as a potential therapeutic target.

Methods: Animal Model. An in-vivo murine model of CNC injury1 was created in dhh/-/- mice (Jackson Labs) by atrumatically placing a 3mm inert tube around the sciatic nerve through a dorsal gluteal splitting approach as previously described. Genotyping was performed by Transnetyx, Inc. CNC injury was confirmed by electrophysiology performed bi-weekly. All procedures involving living animals were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Immunohistochemical and Morphometric Data Analysis. Sciatic nerves were harvested and analysis was performed to detail changes in the basal lamina and fibro-proliferative response for collagen IV, laminin-α-2, and fibronectin. Toluidine blue staining was performed for axon histology and g-ratio measurements and analyzed stereologically using VisioPharm at 2- and 6-weeks post-CNC. Moreover, nerves were further analyzed via electron microscopy (EM) for myelin debris. Additionally, nerve fibers were teased and analyzed for intermodal length in both the CNC and the contralateral normal nerve. A One-Way ANOVA and Mann-Whitney test was performed for statistical significance with p-value < 0.05 constituting significance.

Dorsal Root Ganglion (DRG) and Schwann Cell Co-Cultures. DRGs were dissected from Sprague Dawley rats at embryonic day 14 and seeded onto coverslips. Cells were maintained in medium and purified. Schwann cells were extracted from sciatic nerves of 3 day old rats and purified using the modified Brockes technique. The resulting co-cultures were maintained in medium and treated with shRNA for dhh or vector. L-ascorbic acid was added on day 7 to induce myelination. Additionally, co-cultures were treated with exogenous dhh protein or CPN (dhh inhibitor).

Results: In contrast to wildtype (WT) mice, the nerve conduction velocities (NCV) in dhh/-/- mice showed a marked and rapid decline. NCV declined from 52.15 ± 0.5 m/s at baseline to 15.06 ± 0.578 m/s at 2-weeks (p<0.0001). There was a slight improvement at 4 and 6 weeks to 25.63 ± 1.514 m/s and 26.13 ± 1.21 m/s, respectively (p<0.0001). As previously reported, wildtype animals show the slowest NCV at 6-weeks but never reached the profound slowing seen in dhh/-/- animals. IHC analysis for collagen IV, laminin-α-2, and fibronectin showed scarring in WT and dhh/-/- mice at 2 weeks. Moreover, this response was more profound in the both types of mice at 6 weeks. G-ratios were measured after 2 weeks CNC injury in normal and compressed nerves (0.64±0.004 vs. 0.67 ± 0.005; p<0.0001) as well as 6 weeks (0.63 ± 0.003 vs. 0.71 ± 0.003). After 2 weeks CNC, the percentage of large caliber axons was significantly reduced in dhh/-/- (0.404 ± 0.009 vs. 0.153 ± 0.035; p<0.0001) as well as a substantial increase in the percentage of smaller caliber fibers (0.543 ± 0.007 vs 0.727 ± 0.011; p<0.05). Interestingly, after 6 weeks CNC, a similar, but less profound, result was seen. Furthermore, teased nerve fibers after 6 weeks CNC showed a decreased intermodal length from 665.2 ± 14.40 µm vs. 340.8 ± 25.35 µm. EM revealed increased myelin debris at 2 weeks compared to 6 weeks CNC. Furthermore, in vitro myelinated co-cultures with Schwann cells transfected with dhh shRNA showed decreased myelination. Consistent with our hypothesis, exogenous dhh protein was able to rescue this demyelination and enhanced myelination to baseline levels.

Discussion: CNC injuries can be characterized by a progressive decline in NCV. After CNC injury in dhh/-/- mice, the NCV shows a more rapid and severe decline relative to wildtype mice, as well as a significant loss of large caliber myelinated axons. Moreover, the fibroproliferative response induced by CNC injury in dhh/-/- mice is more profound compared to its WT counterpart. These data point to a vital role for desert hedgehog in the physiological function of myelinated axons. Our studies suggest a possible neuroprotective role of desert hedgehog in the peripheral nerve as the lack of this protein accelerates the peripheral nerve demyelination and dysfunction secondary to the sustained mechanical stimuli of CNC injury.

Significance: Compressive neuropathies are highly prevalent, debilitating conditions with variable functional recovery following...
both surgical management as well as conservative treatment. The molecular mechanisms and signal transduction pathways involved in the pathogenesis of CNC injuries has not been well characterized and elucidated. As desert hedgehog is a produced by the Schwann cell and multiple data detail that CNC injuries are a Schwann cell-mediated disease, thus may be an essential component in preventing the morbidity and mortality seen in compressive neuropathies and offers a therapeutic adjunct to the management of compressive neuropathies.

Acknowledgments:

5 week dhh Internodal Length [IL]: Arrowheads depict internodes.
A) Normal uninjured nerve IL; B) Compressed nerve IL. C) Graphical representation of IL between normal and CNC nerves.
DRG neurons and Schwann cell myelinated cocultures. A) Vector alone B) shRNA for dhh C) Vector + CPN D) shRNA for dhh + exogenous dhh protein.

ORS 2014 Annual Meeting
Poster No: 0687