ABERRANT AXONAL SPROUTING WITH THE ABSENCE OF WALLERIAN DEGENERATION IN A MODEL FOR CARPAL TUNNEL SYNDROME

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INTRODUCTION: Carpal tunnel syndrome is a prevalent problem that limits millions of individuals with pain and loss of function. It has been suggested that the chronic nerve compression (CNC) of carpal tunnel syndrome leads to changes in the structure and microvasculature of the peripheral nerve, and results in mild Wallerian degeneration (1). One of the major histopathological changes in Wallerian degeneration is granular disintegration of the axoplasm representing a breakdown of the normal axonal cytoskeleton (2). The primary purpose of this study is to evaluate the axonal architecture of nerves subjected to CNC with electron microscopy and immunohistochemistry (IHC). This is a previously described model for carpal tunnel syndrome (3,4). A rigorous quantitative analysis of the changes in compressed relative to normal nerves was performed using design-based, unbiased stereology. To further characterize the axonal pathology with compression neuropathy, IHC was used to evaluate the expression of neurofilament protein.

MATERIALS & METHODS

Surgical Technique. A previously described model of nerve compression was applied to one-month old Sprague rats (200 – 300g) (1,3,4). A dorsal gluteal-splitting approach was used to expose both sciatic nerves of each animal. The right sciatic nerves were mobilized and a sterile one-inch silastic tube (I.D. of 1.3 mm) wasatraumatically placed around each nerve. The left sciatic nerves were mobilized and returned to the host bed to serve as a control specimen. Electrodiagnostic studies were performed at the time of specimen harvest and nerve samples were harvested at both one month and eight months after surgery. IRB approval was obtained for animal use from the University’s IACUC.

Stereological Evaluation of EM Sections. Nerve segments at the site of compression were analyzed for these experiments. The specimens were postfixed in osmium tetroxide, dehydrated in acetone and embedded in Eponate 12 resin. Sixty nanometer sections were cut and placed on 200 mesh grids (bar: 37 mm; hole: 90 mm) Design-based, unbiased stereologic counting was used to obtain unbiased number and size estimates from any sample of objects within a cross section (5). The fractionator technique was utilized to directly estimate the total number of axons and cells (5,6). Photomicrographs (Phillips F10) were taken at fixed predetermined step lengths of 127 mm, in the x and y axes, throughout the cross-sectional area. Each systematically sampled photomicrograph was quantified using four counting frames to reduce the impact of clusters of axons and cells. Direct orthogonal measurements in uniform and random locations were used to determine myelin thickness (5). Each photomicrograph was studied using a grid of fixed, random test-lines with a fixed line separation. Point of intersection between the test-lines and axons perimeter were used to directly measure the myelin sheath thickness. The average of two systematically chosen locations was used to determine myelin sheath thickness of one axon.

Preparation of Tissue Samples for IHC. Following dehydration, nerve samples were embedded in a 1:1 mixture of OCT and Aquamount, cut into 10 um longitudinal sections and mounted to slides. Nerve sections were then dried and further fixed in 4% PF for 20 min. The samples were rinsed in PBS and subsequently blocked with 5% normal goat serum in PBS containing 0.25% Triton-X 100 for one hour. PBS wash was then repeated and samples were incubated overnight in mouse monoclonal anti-neurofilament 200 antibody (1:200 in PBS). Samples were then washed in PBS (X3) followed by incubation in biotinylated goat anti-mouse IgG (1:200) in PBS for one hour. Nerve sections were then rinsed in PBS and counterstained, in the absence of light, with Vectashield anti-fade solution containing DAPI. Sections were visualized under a Zeiss fluorescent microscope using a FITC filter.

RESULTS: As previous reported (3,4), there were no significant electrophysiological changes at the one-month time point. By the eight month time point, the nerve conduction velocity consistently decreased to 65% of the normal value. At the one-month time point, EM did not show significant alteration in axonal integrity, but rather demonstrated maintenance of the normal axonal cytoskeleton including neurofilament architecture. With careful study of over 240 photomicrographs, we never able to find any evidence of granular disintegration of the axoplasm. Surprisingly, at the one-month time point, there was a 58% increase in unmyelinated axons relative to normal nerve, which was not present at the eight-month time point. Although myelin thickness diminished by about 51% at both time points, these changes were detected in the outer half of the one-month nerve specimens and diffusely throughout the eight-month nerve specimens. There was a 597% and 138% increase in Schwann cell numbers at the 1 and 8-month time intervals, respectively, consistent with previously reported data (4). Fluorescent microscopy demonstrated that the neurofilament protein followed a uniform parallel pattern in the absence of compression. However, by one-month post surgery for compressed specimens, there were significant alterations indicative of axonal sprouting marked by the appearance of thinner neurofilament proteins emerging from thicker base proteins. Consistent with the EM data, such alterations are only observed at the periphery of the nerve section, while the center retained its normal architecture. By eight months, there was a return to a near normal neurofilament protein and axonal architecture.

DISCUSSION & CONCLUSION: Consistent with light microscopy data (3,4), chronic nerve compression induces a profound increase in Schwann cell number in a distinct spatial and temporal pattern. In contradistinction to previous reports in the clinical literature (1), the decrease in myelin thickness is a marker of re-innervation by Schwann cells rather than severity of CNC (7). In addition, at the one-month time point when there are significant histopathologic changes, there is no ultrastructural evidence of altered axonal integrity including granular disintegration of the cytoskeleton suggesting that CNC does not induce Wallerian degeneration. Rather, at this early time point, there is a marked increase in the number of unmyelinated axons that originate from existing axons within the affected area. These fibers, which mediate pain and temperature, are not present at the later time points. These temporal changes in unmyelinated axons follow the same time course as the expression of pain in patients with compression neuropathies. The data suggests a novel hypothesis that chronic nerve compression may provide an early stimulus for re-innervation that aberrantly results in the pain associated with compression neuropathies.

REFERENCES

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