MACROPHAGE RECRUITMENT FOLLOWS THE PATTERN OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION IN A MODEL FOR CARPAL TUNNEL SYNDROME

*Bui, P; *Lin, YM; *Mozaffar, T; +Gupta, R
*University of California, Irvine, Irvine, CA

Introduction: Compressive neuropathies of the upper extremity are a growing clinical problem that limits millions of individuals with pain and loss of function. Previously established animal models have described not only histological and electrophysiological changes, but also changes in blood flow. (1,2) However, little basic science is known about the molecular pathogenesis of chronic nerve compression (CNC). Nitric oxide has been shown to be an important signaling molecule that mediates a variety of physiological and pathological processes including neurotoxicity and changes in vascularity and blood flow. (3) Previous work has demonstrated that there is a spatial and temporal up-regulation of iNOS mRNA with CNC. (4) We sought to explore whether the iNOS enzyme was expressed and if this correlated with the macrophage recruitment associated with chronic nerve compression.

Materials and Methods: Surgical Technique: A previously described animal model was used as adult male Sprague Dawley rats (200-300g) were anesthetized and the sciatic nerve was exposed dorsally. The right sciatic nerve was mobilized and a 1 cm silastic tubing (I.D. of 1.3mm) was placed around the nerve. The left sciatic nerve was mobilized and returned to its host bed to serve as a control specimen. (1,2) IRB approval was obtained for animal use from the university’s IACUC.

Electrophysiology: Prior to the harvest of nerve specimens, electrophysiological recordings were performed in-vivo under surgical anesthesia. Motor conduction in sciatic-tibial fibers was assessed by stimulating at the sciatic notch and knee while recording the M-wave (compound motor action potential) from the tibial-innervated ankle plantar flexor muscle (medial gastrocnemius). M-waves were recorded using a Cadwell Sierra LT machine (Cadwell Laboratories, Kennewick, WA) by placing monopolar needle EMG electrodes in the medial gastrocnemius muscle approximately 3 mm above the heel. The reference-recording electrode was inserted into the plantar aspect of the foot while the reference stimulating electrode was inserted into the ipsilateral lumbar paraspinal muscles.

Immunostaining for ED-1 for Macrophage Identification: Frozen sections of sciatic nerves were fixed in absolute acetone at 4°C for 10 minutes for ED-1 labeling. After rinses in with PBS, slides were immersed in 4% normal goat serum/PBS blocking solution for 1 hour. The sections were then incubated with anti-rat macrophage monoclonal antibody clone ED-1 (1:300, Chemicon) overnight at 4°C. Following thorough washes with PBS, slides were incubated in Rhodamine-labeled goat anti-mouse IgG (1:200, Chemicon) for 1 hour. After washes in PBS, slides were counterstained and mounted with DAPI and viewed under a fluorescent microscope.

Immunohistochemistry for iNOS Expression: Frozen sciatic nerve sections were initially fixed in 4% Paraformaldehyde for 20 minutes. After PBS washes, sections were treated with 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase. The sections were washed in PBS, blocked in 10% normal goat serum, and then incubated with rabbit anti-iNOS protein (Transduction Lab, 1:200) for 1 hour at room temperature. After thoroughly washes in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG (H+L) secondary antibody (Vector, 1:200) for 1 hour at room temperature. The sections were then exposed to the avidin-biotin-peroxidase complex (ABC) from the Elite kit (Vector) for 30 minutes. Peroxidase was developed with DAB Peroxidase Substrate from Sigma.

Results: Electrophysiological recordings revealed that the average conduction velocity of the motor axons to the medial gastrocnemius in a sample of normal sciatic nerves was 51.3 m/s (SEM 1.5). As illustrated in Figure 1, there were no statistically significant differences in conduction velocity one-month after the initiation of the compression and only minimal decreases in conduction velocity by the three-month time point. By the six-month time point, conduction velocity had decreased to about 65% of the control value, and remained at this level for the remainder of the survival period.

Cross sections of control and experimental nerve were examined under bright field microscopy for changes in iNOS protein expression. Intact cellular architecture was observed in all specimens analyzed. At 3 months postoperatively, there was an increase in iNOS expression in the epineurium of the peripheral nerve when compared to the contralateral control. However, there was no change in the labeling of the perineurium or endoneurium. In the 6 month experimental nerve, expression had continued to increase in the epineurium and was significantly increased in the perineurium as well. Labeling showed spatial specificity, and no labeling was observed in the endoneurium. By eight months the compressed nerve continued to show increases in iNOS localized to the epineurium and perineurium and showed increases over control in the outer endoneurium.

The ED-1 staining for macrophages showed that there was a significant increase in macrophage number in the experimental nerve compared to the contralateral normal nerve at 3, 6 and 8 months postoperatively. At the earlier time point (3 months postoperatively), positively stained macrophages (arrows) were mostly concentrated on the outer 1/3 of the sciatic nerve cross section. However, at the later time points, the macrophages were located diffusely throughout each nerve section.

Discussion and Conclusion: Chronic nerve injuries such as Carpal Tunnel Syndrome cause dysfunction through mechanical compression and microvascular changes about the compressed nerve. The molecular signals mediating changes in the nerve have been understudied. We have shown an increase in the gene expression of the enzyme iNOS which may be involved in mediating some of the histopathologic changes with compression neuropathy. The up-regulation of the gene begins in the epineurium during the early stages of compression and sustains itself throughout the injury as up-regulation continues into the perineurium and endoneurium as the CNC develops. The pattern of macrophage recruitment follows a similar pattern and suggests that these cells may be producing the iNOS enzyme associated with CNC. Furthermore, this alteration of reactive oxidative species may also mediate the Schwann cell death and axonal degeneration also seen with compression neuropathy.

References:
Acknowledgement: NIH-NINDS NS 02221-02 (RG)